INNOVATIVE APPROACHES TO ASSESSING SEED QUALITY IN *BRASSICAS*

BY

JODI LYNN LARKIN

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Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

ABSTRACT

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Brassica napus is grown as an edible oil (canola) and an industrial oil (HEAR). Its fatty acid profile and chlorophyll concentration affect the quality. It is important to develop accurate and efficient methods to evaluate these traits. The first objective of this study was to improve the single nucleotide polymorphism (SNP) and sequence characterized amplified region (SCAR) molecular markers for erucic acid genotypes in the *Bn-FAE1.1* and *Bn-FAE1.2* genes in the A and C genomes in *Brassica napus* originally developed by Rahman et al. (2008). When put into practice, the error rate was unacceptably high. With the modifications that were made to the protocols, the overall accuracy remained relatively consistent indicating that further improvements are still required. The second objective was to develop a near infrared reflectance (NIR) based calibration equation for chlorophyll concentration in whole *Brassica napus* seeds. In this case, an equation was successfully created.

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1.0 INTRODUCTION

Rapeseed (*Brassica napus* L.) is one of the most widely cultivated oilseed crops in the world (Rajcan et al. 1999). There are indications that Brassica vegetables and oilseeds were some of the first types of plants to be cropped. Rapeseed production has greatly increased throughout the world in the last thirty years. Rapeseed is grown primarily for oil; however, meal leftover from extraction of the oil is particularly valuable as well. *Brassica napus* arose from the spontaneous interspecific hybridization between turnip rape (*Brassica rapa*) (A genome) and cabbage (*Brassica oleracea*) (C genome) (Snowdon et al. 2007). Rapeseed contains full chromosome sets from both *B. oleracea* and *B. rapa* and is referred to as an amphidiploid.

Most seed oils are composed of fatty acids with a sixteen to eighteen carbon chain length; however, Brassica species produce very long chain fatty acids (VLCFA) with more than eighteen carbons (Fourmann et al. 1998). Erucic acid (C22:1) is one of the main VLCFAs in rapeseed. Erucic acid has been shown to have low digestibility in humans and animals and can lead to health problems; therefore rapeseed oil has a poor reputation as a food source (Beare et al. 1963). The first low erucic acid rapeseed variety was developed in the late 1960s in Canada. This paved the way for further research and development of low erucic acid, low glucosinolate varieties thereby greatly increasing the value of the crop by making both the oil and the meal desirable. Low erucic acid, low glucosinolate varieties were later termed “canola” by the Rapeseed Crushers of Western Canada. To be suitable for human consumption, canola oil must contain less than 2% erucic acid. Canola is also used in the production of margarines, shortenings, and other

fat products. High erucic acid rapeseed varieties, termed “HEAR”, are also valuable for industrial purposes. High erucic acid rapeseed (HEAR) oil contains at least 46% erucic acid and is used in high temperature lubricants, biodegradable plastics, soaps, surfactants, paints, and inks (Snowdon et al. 2007). Development of HEAR oils may also reduce the dependency on irreplaceable fossil oils (Jourdren et al. 1996).

The biosynthesis of erucic acid in *Brassica napus* is catalyzed by the *fatty acid elongase 1* enzyme (*FAE1*) (Wu et al. 2007). The two homologs of *FAE1*, E1 and E2, have additive effects. They encode 3-ketoacyl-CoA synthases, which are involved in the elongation of oleic acid to erucic acid (Harvey and Downey 1964, Barret et al. 1998). The E1E1E2E2, E1e1E2E2 or E1E1E2e2, e1e1E2E2 or E1E1e2e2, and e1e1e2e2 genotypes are responsible for 40, 30, 20, and 0% erucic acid concentration in seeds respectively; however, both genes do not contribute equally (Jourdren et al. 1996). Canola genotypes contain a mutation that affects both the E1 and E2 loci (Harvey and Downey 1964). The two *FAE1* loci (*Bn-FAE1.1* and *Bn-FAE1.2*) are located in the A and C genomes (Wu et al. 2007).

Traditionally, erucic acid concentration in rapeseed is determined phenotypically by gas chromatography. This requires mature seed for a phenotypic assessment. With the development of molecular markers, it is possible to select plants with desired traits at a much earlier stage and at a much lower cost (Rahman et al. 2007). It is therefore helpful to utilize molecular markers in the seedling stage to distinguish between the E1 and E2 genotypes in the A and C genomes to accelerate the breeding effort.

Sequence characterized amplified region (SCAR) markers and single nucleotide polymorphism (SNP) markers target insertions and deletions in a genome and are quite

useful in marker assister selection (MAS) in plant breeding (Rahman et al. 2008). Both SCAR and SNP markers are polymerase chain reaction (PCR) based molecular markers, and are easily automated to be high throughput. They are also co-dominant, which allows for the identification of both homozygous and heterozygous genotypes. At the University of Manitoba, Rahman et al. (2008) developed SNP and SCAR markers for the E1 and E2 genes in *Brassica napus* samples; however, when put into practice, the error rate was unacceptably high thus demonstrating the need to improve these markers.

The quality of rapeseed/canola seed is determined primarily by its oil, protein, glucosinolate, and fatty acid concentration; however, chlorophyll concentration also affects the quality of the seed and the oil. Chlorophyll is a green pigment that is vital for photosynthesis; however, chlorophyll adds an undesirable green color to oil and has also been shown to affect the rate of oxidation, which causes early rancidity of the oil (Abraham and deMan 1986). It is also difficult to remove chlorophyll from oil during routine processing (Daun 1976). It is therefore desirable to have the lowest possible amount of chlorophyll in canola/rapeseed oil.

Traditionally, chlorophyll levels have been determined by extraction from the seed with an organic solvent then measuring the absorbance on a spectrophotometer at 670 nm or by comparing the percentage of green seed to the overall sample color (Appelqvist and Johannson 1967, Tkachuk et al. 1988). Neither method is preferred as there are major disadvantages to both. The first method is time consuming, requires the use of organic solvents, and destroys whole seeds; however, it does provide fairly accurate results. The second method destroys the seeds, is not very accurate, and has been shown to correlate poorly with actual extracted chlorophyll concentrations (Tkachuk et

al. 1988). As a result, there is a need to develop a less time consuming and more accurate method to determine chlorophyll concentration in whole seeds. Many labs currently use a FOSS 6500 near infrared reflectance (NIR) spectrophotometer to determine oil, protein, and glucosinolate concentration; it would therefore by advantageous to use this machine to determine chlorophyll concentration as well since it could be less expensive and less time consuming.

The overall goal of assessing the quality in Brassica seed samples is to provide efficient screening techniques and evaluation methods for the development and registration of new Brassica oilseed cultivars.

The first objective of this study was to improve the protocols for the SNP and SCAR markers for the identification of erucic acid genotypes of the *Bn-FAE1.1* gene in the A genome and the *Bn-FAE1.2* gene in the C genome for marker assisted selection published by Rahman et al. (2008) by reducing the apparent error rate (of 10 to 50%) in *Brassica napus* seedling samples.

The second objective of this study was to develop a calibration equation for chlorophyll concentration in whole *Brassica napus* seed samples using a FOSS 6500 near infrared reflectance spectrophotometer.

2.0 LITERATURE REVIEW

2.1 BRASSICA NAPUS

2.1.1 Origin of *Brassica* species

Rapeseed (*Brassica napus* L., genome AACC, 2n = 38*)* is one of the most widely cultivated oilseed crops in the world (Rajcan et al. 1999). The word "rape" in rapeseed is derived from the Latin word "rapum" for turnip. Rapeseed is a bright yellow flowering crop and is a member of the crucifer family *Brassicaceae*, which includes well-known species such as *Brassica oleracea* (cabbage, broccoli, cauliflower), *Brassica rapa* (turnip, Chinese cabbage), *Raphanus sativus* (radish), *Armoracia rusticana* (horseradish), *Arabidopsis thaliana* (model organism) and many others. *Brassica napus* arose from the spontaneous interspecific hybridization between *Brassica rapa* (genome AA, 2n = 20) and *Brassica oleracea* (genome CC, 2n = 18) followed by chromosome doubling to produce viable fertile amphidiploids (Downey 1983, Snowdon et al. 2007). Since rapeseed contains full chromosome sets from both *Brassica oleracea* and *Brassica rapa*, it is referred to as an amphidiploid species. Other species of *Brassicas* have also arisen from interspecific hybridization between other members of the *Brassicacea* family such as Indian mustard (*Brassica juncea*, genome AABB, 2n = 36) and Ethiopian or Abyssinian mustard (*Brassica carinata*, genome BBCC, 2n = 34) (Snowdon et al. 2007). These interspecific hybridizations are shown in the Triangle of U (Figure 2.1).

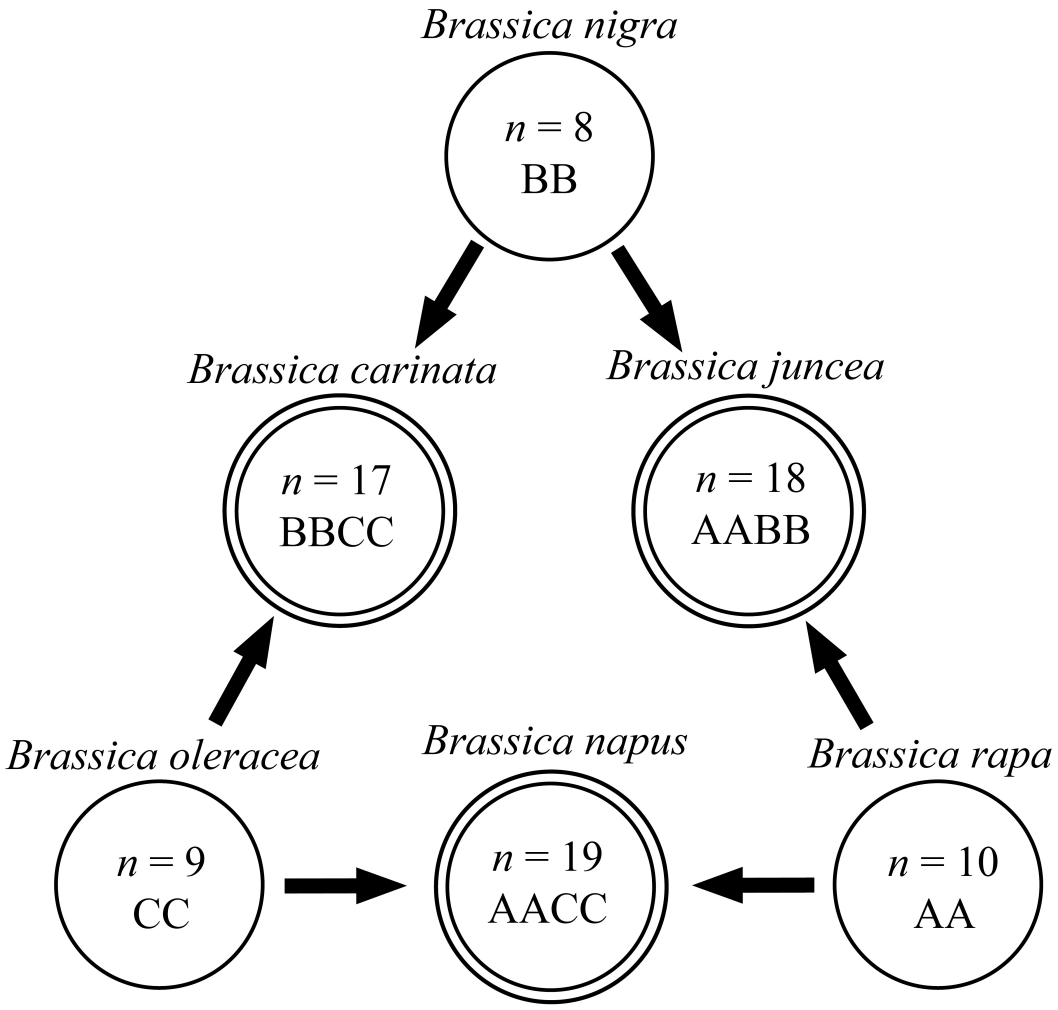

Figure 2.1. Triangle of U (Adapted from U 1935)

2.1.2 History and economic importance of rapeseed

There are indications that *Brassica* vegetables and oilseeds were some of the first types of plants to be cropped and may have been cultivated as early as ten thousand years ago. Records from India indicate that oil from rapeseed was used as early as 4000 BC and its use spread into China and Japan nearly 2000 years ago (Bell 1982). By the 16th century in Europe, rapeseed oil was the main source of lamp fuel (Snowdon et al. 2007). A significant acreage increase of rapeseed crops occurred in Europe during the 18th

century when the oil was discovered to be a valuable lubricant due to its ability to adhere to steam and water-washed metal surfaces better than any other lubricant (Snowdon et al. 2007). Early varieties of rapeseed produced oil that contained high amounts of the very long chain fatty acid (VLCFA) erucic acid (C22:1) making it an excellent industrial lubricant; however, oil that is high in erucic acid is not suitable for human consumption. When consumed in large quantities, erucic acid has been shown be detrimental to mammalian health as it causes cardiac lesions (Charlton et al. 1975). By the 19th century, rapeseed oil superseded petroleum as the main source of lamp fuel. With the increasing shortages of petroleum during the Second World War, the demand for rapeseed oil increased, promoting its growth in Canada (Snowdon et al. 2007). During times of crisis and poverty, rapeseed oil was often used in the production of margarine even though it leads to health problems. After the war, the production of rapeseed fell. However, during the 20th century, rapeseed production was once again in demand solely for that fact that use of its oil as an industrial lubricant increased (Snowdon et al. 2007). Today, rapeseed is predominantly grown in North America, particularly Canada, western Europe, and China. It is the most commonly produced oilseed in Europe and only soybean supersedes its production worldwide (Snowdon et al. 2007). In Canada, the acreage of rapeseed has been increasing year after year. For example, in 1948, the number of acres of rapeseed grown in Canada was less than 1000 acres, this jumped to 750,000 acres of canola in 1975, all the way to 16.8 million acres in 2010, proving that it is an economically important crop (Statistics Canada).

2.1.3 Development of canola

Rapeseed oil had a poor reputation as a food source until the development of low erucic acid varieties. The first low erucic acid variety, Oro, was released in 1968 by Dr. Keith Downey at the Agriculture Canada Research Station in Saskatoon, Saskatchewan. Oro was derived from a cross between the German spring rapeseed cultivar, Liho, and the Argentinian rapeseed cultivar, Nugget (Snowdon et al. 2007, Canola Council of Canada). Since the meal contained high amounts of glucosinolates, the value of rapeseed crops was still not very high. Glucosinolates have been shown to cause thyroid, liver, kidney, and lymph complications in livestock and delivers a bitter, unappetizing taste (Snowdon et al. 2007). Rapeseed meal, left over after oil extraction, is high in protein and can be a good source of animal feed in the absence of high concentrations of glucosinolates.

The first low glucosinolate variety, Bronowski, was discovered in Poland in 1969 (Snowdon et al. 2007). This variety was used in a backcrossing program, which in 1974 resulted in the release of the zero erucic acid, low concentration *Brassica napus* cultivar, Tower, by Dr. Baldur Stefansson at the University of Manitoba in Winnipeg, Manitoba (Steffanson and Kondra 1975). Low erucic acid, low glucosinolate varieties were later termed “canola” (Canadian oil, low acid) by the Rapeseed Crushers of Western Canada in order to distinguish them from high erucic acid rapeseed varieties.

2.1.4 Development of HEAR

Today, *Brassica napus* crops are grown primarily as a food source; however, there remains a vast array of uses of rapeseed oil with erucic acid concentrations above 40% ranging from biodiesel to industrial lubricant to biodegradable plastics to use in

detergents and soaps (Snowdon et al. 2007). These cultivars are termed high erucic acid rapeseed or HEAR. The most valuable HEAR cultivars are also low in glucosinolates allowing the meal to be used as livestock feed.

In 1982, the world's first HEAR cultivar, Reston, was released (Alberta Agriculture 1982) followed by Hero in 1991 (Scarth et al. 1991). Since then, many new HEAR cultivars have been released by the University of Manitoba. The University of Manitoba HEAR breeding program primarily focuses on developing new cultivars with improved agronomic and quality characteristics with herbicide tolerance to glyphosate (Roundup Ready), glufosinate (Liberty Link), or imidazolinone (Clearfield).

2.1.5 Importance of canola

Canola yields a very high quality oil which is light, stable, colorless, odorless, and does not smoke when heated. It also has a beneficial fatty acid profile and contains no cholesterol. Canola oil contains the lowest levels of saturated fats of any vegetable oil along with high levels of essential fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) and is therefore favorable for human health. Canola oil is the most popular vegetable oil in Canada and is largely exported to the United States, Japan, and Mexico, making it an important industry in Canada. Canola is consumed as a vegetable oil and is used primarily in the production of margarines, shortening, and other fat products.

2.1.6 Importance of HEAR

Some uses of HEAR oil products include use as a lubricant for rolled sheet steel manufacturing, use as a slip agent, use in plasticizers, use as an environmentally friendly

lubricating oil for agricultural and forestry industries, use in printing inks, use in cosmetics, use in paints, use in pharmaceuticals, and it can be a source of biodiesel. HEAR oil has many advantageous properties including a high smoke point, a high flash point, its stability at high temperatures, its durability, its ability to remain fluid at low temperatures, and its hydrophobic nature (Snowdon et al. 2007). The use of rapeseed oil as an industrial lubricant has many advantages including environmental benefits in the fact that it is biodegradable, it has low toxicity in humans, animals, and the environment, and does not provide any net carbon dioxide pollution to the environment (Snowdon et al. 2007). Advantages of HEAR oil as a biodiesel are primarily environmental in that they produce less smoke and particulates, produce less emissions, and are biodegradable and non toxic (Snowdon et al. 2007). However, HEAR as biodiesel is not practical economically because it is would be necessary to grow very large quantities whereas the land is better used to grow economically important canola and HEAR crops.

Non-direct uses of HEAR oil include use of the derivatives erucamide, behenic acid, brassilic acid, and pelagonic acid. Erucamide is most commonly used as a slip additive in polyethylene and polypropylene manufacturing to reduce friction and adhesion between plastic film surfaces such as in grocery bags; behenic acid is used as a stabilizer in peanut butter; brassilic acid is used as a plasticizer, in perfumes, and in Nylon 13-13; and pelargonic acid is also used as a plasticizer and in perfumes, coatings, lubricants, and Nylon 9-9. Erucamide is very expensive to produce synthetically; therefore, HEAR is very important economically.

Today, HEAR production pales in comparison to canola production; however, both remain economically important to Canada and the world.

2.2 QUALITY TRAITS IN BRASSICAS

2.2.1 Oil concentration

Oil is defined as a thick, viscous compound that is insoluble in water but soluble in organic solvents and is typically derived from animals, plants, or minerals. Plants and animals produce oil through natural metabolic processes. Approximately 80% of seed oil is found in the cells of the cotyledons, 7 to 12% is found in the endosperm layer, and the remainder is found in the seed coat (Downey et al. 1975). Oil content is the most valuable seed component in rapeseed. Once the seed is harvested, it is sold to processing companies to be crushed to extract the oil which can then be sold. The meal, leftover after oil extraction, is also sold as livestock feed. Oil is much more valuable than meal, worth approximately two to four times the value of meal, therefore the goal of breeders is to increase oil content rather than protein content.

Traditionally, oil content in *Brassicas* has been measured by Nuclear Magnetic Resonance (NMR) following the procedure set out by the International Organization for Standardization, reference number ISO 10565:1992(E) Oilseeds—Simultaneous determination of oil and moisture contents—Method using pulsed nuclear magnetic resonance spectroscopy; however, labs that have a NIR spectrophotometer currently prefer this method.

2.2.2 Protein concentration

Proteins are biological compounds that consist of chains of amino acids joined by peptide bonds to form polypeptides. Proteins are arranged in varying configurations based on the pattern of polypeptides making the function of each protein unique. Proteins

are essential parts of organisms and are an important dietary requirement to support life. Approximately 20 to 40% of the weight of oilseeds is due to proteins with the majority of that weight being storage proteins. Storage proteins play an important role in seedling germination and early development in *Brassica* species. Protein concentration is also important in rapeseed meal that is left over after extraction of the oil as it is used as a source of livestock feed.

Traditionally, protein concentration was determined by the Kjeldahl method of acid digestion, steam distillation of nitrogen, and back titration, or more recently, by nitrogen combustion techniques; however, NIR has proven to be an accurate, less expensive, and less time consuming method to determine protein concentration in *Brassicas*.

2.2.3 Glucosinolate concentration

Glucosinolates are a class of organic compound which occur as a secondary metabolite in almost all plants in the *Brassicacea* family. They contain both sulfur and nitrogen and are derived from glucose and an amino acid that varies depending on the species. Glucosinolates have been shown to be toxic at high doses in humans and animals. Glucosinolates appear to serve as a form of defense for plants since when the plant cells are destroyed, the myrosinase enzyme catalyzes their degradation into isothiocyantes, thiocynates, and nitriles, which cause an unpleasant bitter taste and health problems such as thyroid complications and damage to the liver and kidneys (Mithen 2001). The presence of glucosinolates in the seed meal significantly reduces the quality. The Western Canada Canola/Rapeseed Recommending Committee (WCC/RRC) requires

the glucosinolate concentrations to be below 12 μmoles gram^{-1} seed at 8.5% moisture for a new cultivar to be registered (Canola Council of Canada 2011).

Total glucosinolate concentration has traditionally been analyzed in *Brassicas* using gas chromatography and high performance liquid chromatography; however, currently NIR is the preferred method as it is faster and less expensive than traditional methods.

2.2.4 Fatty Acid composition

Fatty acid composition is also a very important measure of quality in *Brassica* seed oil. Vegetables oils are made up primarily of triglycerides. A triglyceride is made up of a glycerol backbone with three fatty acids molecules of varying carbon chain lengths. A fatty acid is a carboxylic acid with a long hydrocarbon chain that is either saturated, with single bonds, or unsaturated, with double bonds. Mono-unsaturated fatty acids contain a single double bond, whereas poly-unsaturated fatty acids contain multiple double bonds. Fatty acids generally have chains with an even number of carbon atoms ranging between 4 and 28; however, those with less than 14 or more than 20 carbon atoms are less common. Since vegetable oils are made up of fatty acids, their fatty acid profile determines whether the oil is to be used for edible or industrial purposes (Table 2.1).

Table 2.1. Percent fatty acid composition of Canadian oilseed rape - HEAR and canola

Fatty Acid	Formula	HEAR	Canola
Palmitic	C16:0	4.0%	4.7%
Steric	C18:0	1.5%	1.8%
Oleic	C18:1	17.0%	63.0%
Linoleic	C18:2	13.0%	20.0%
Linolenic	C18:3	9.0%	8.6%
Eicosenoic	C20:1	14.5%	1.9%
Erucic	C22:1	41.0%	0.0%

Adapted from Downey 1990

Most animals, including humans, can synthesize almost all of the fatty acids they require for survival; however, there are certain fatty acids that cannot be synthesized and must be obtained through a proper diet. These are termed essential fatty acids. Canola oil contains high levels of the essential fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) making it favorable for human health. On the other hand, HEAR oil contains high concentrations of the fatty acid erucic acid (C22:1), making it an unsuitable edible oil but extremely favorable industrial oil. It is also beneficial to have lower levels of saturated fatty acids (palmitic and steric) as high levels have been shown to cause increased blood cholesterol levels and a higher risk of coronary heart disease (Eskin et al. 1996).

Traditionally fatty acid composition in *Brassicas* is determined by the process of transesterification of triglycerides to fatty acid methyl esters, which are then measured using gas chromatography.

2.2.4.1 Fatty acid biosynthesis

The fatty acid composition of rapeseed oil is primarily determined by the genetic makeup of the developing embryo rather than by the maternal parent (Downey and

Harvey 1963). The fatty acid biosynthesis process begins in the plastids and is completed in the endoplasmic reticulum of plant cells (Harwood 1988). It is a four step repeating cyclic process in which two carbon atoms are added per cycle; however, malonyl-CoA must first be produced by a reaction of acetyl-CoA and bicarbonate. The first step in the cycle is to form 3-ketoacyl-CoA by a condensation reaction of oleoyl-CoA to malonyl-CoA, followed by a reduction of 3-ketoacyl-CoA to produce 3-hydroxyacyl-CoA, then a dehydration reaction of 3-hydroxyacyl-CoA to form trans-(2,3)-enoyl-CoA, followed by another reduction of trans-(2,3)-enoyl-CoA. Seven repetitions of this cycle results in the formation of palmitic acid (C16:0). The enzymes involved in this process are packed together in a complex called fatty acid synthase (FAS). All fatty acids are derived from palmitic acid. For longer fatty acids, palmitic acid is converted to palmityl-CoA and an elongation enzyme complex is used to add two carbons at a time using malonyl-CoA as the donor. Coenzyme A (CoA) desaturase enzymes are used to create unsaturated fatty acids as they are specific to double bond positions (Figure 2.2).

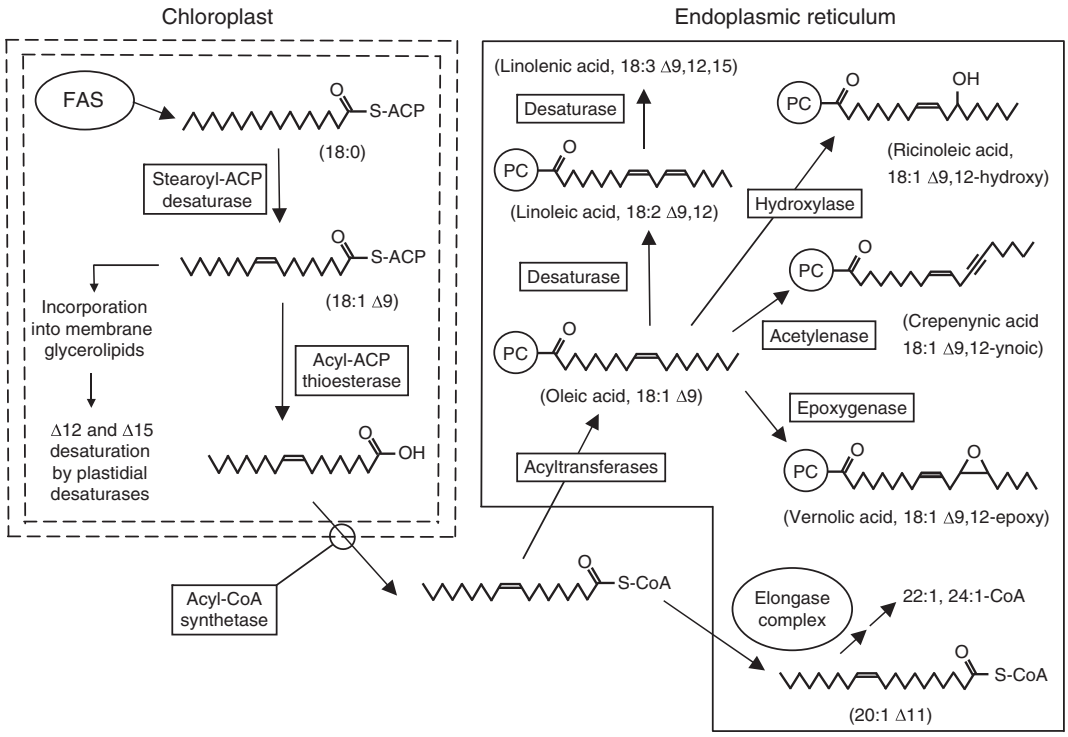

Figure 2.2. Fatty acid biosynthesis in higher plants (Brown et al. 2009)
Abbreviations: FAS - fatty acid synthase complex, ACP – acyl carrier protein, PC – phosphatidylcholine, CoA – CoenzymeA.

2.2.4.2 Erucic acid

Most seed oils are composed of fatty acids with a sixteen to eighteen carbon chain length; however, *Brassica* species produce very long chain fatty acids (VLCFA) with more than eighteen carbons (Fourmann et al. 1998). Erucic acid, C22:1, or *cis*-13-docosenoic acid, is one of the main VLCFAs in rapeseed with a twenty-two carbon chain (Figure 2.3).

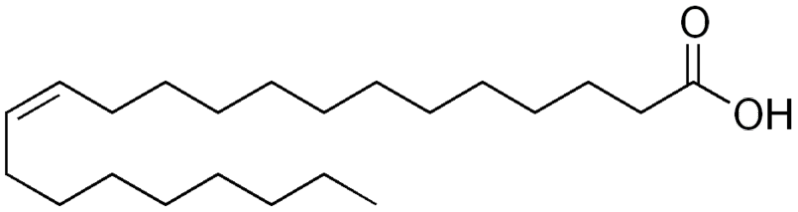

Figure 2.3. Structure of erucic acid

Erucic acid has been shown to have low digestibility in mammals and can lead to health problems (Beare et al. 1963). High erucic acid rapeseed (HEAR) oil contains at least 46% erucic acid and has many industrial applications. On the other hand, to be suitable for human consumption, canola oil must contain less than 2% erucic acid.

Erucic acid biosynthesis is catalyzed by the Fatty Acid Elongase 1 (FAE1) enzyme (Wu et al. 2007). The two homologs of FAE1, E1 and E2, have additive effects and are involved in the elongation process of VLCFA biosynthesis (Harvey and Downey 1964, Stefansson 1983). They encode the 3-ketoacyl-CoA synthase enzyme, which is involved in the elongation of oleic acid (oleoyl-CoA) to erucic acid (Puyaubert et al. 2005). The two homologous sequences of the *FAE1* gene are termed *Bn-FAE1.1* for the E1 locus and *Bn-FAE1.2* for the E2 locus in *Brassica napus* and are located in the A and C genomes (Wu et al. 2007). *Bn-FAE1.1* is a linked to the E1 locus since the position of the quantitative trait loci (QTL) covers 2.7 cM and the two homologues of the *FAE1* gene share 98% of the amino acid sequence of the encoded proteins (Barret et al. 1998).

Knowledge of the genetics of erucic acid is necessary for successful plant breeding to improve the quality of the crop. Low erucic acid rapeseed (canola) genotypes contain a mutation that affects both the E1 and E2 loci (Harvey and Downy 1964). A two base deletion in the *Bn-FAE1.2* gene in the C genome and an amino acid substitution from serine to phenyl-alanine found at position 282 due to a single base pair change in the *Bn-FAE1.1* gene in the A genome results in the low erucic acid concentration of canola (Fourmann et al. 1998, Katavic et al. 2002). The E1E1E2E2, E1e1E2E2 or E1E1E2e2, e1e1E2E2 or E1E1e2e2, and e1e1e2e2 genotypes are responsible for 40, 30,

20, and 0% erucic acid concentration in seeds respectively; however, both genes do not contribute equally (Jourdren et al. 1996).

Traditionally fatty acid composition including erucic acid concentration in *Brassicas* is determined by the process of transesterification of triglycerides to fatty acid methyl esters, which are then measured using gas chromatography. This requires mature seed for a phenotypic assessment.

2.2.5 Chlorophyll concentration

Chlorophyll is a green pigment that is located within the chloroplasts of plants. It is responsible for the green color in the leaves of plants. Chlorophyll a is a type of blue-green photosynthetic pigment that participates directly in light reactions, whereas chlorophyll b is a type of yellow-green accessory pigment that is involved in the transfer of energy to chlorophyll a. Both chlorophyll a and chlorophyll b have a light absorbing head called the porphyrin ring with a magnesium atom in the center and a hydrocarbon tail. They differ by only one functional group attached to the porphyrin ring (Figure 2.4). Chlorophyll a and b are present in plants; however, rapeseed contains mainly chlorophyll a in a 3:1 ratio to chlorophyll b (Tkachuk et al. 1988).

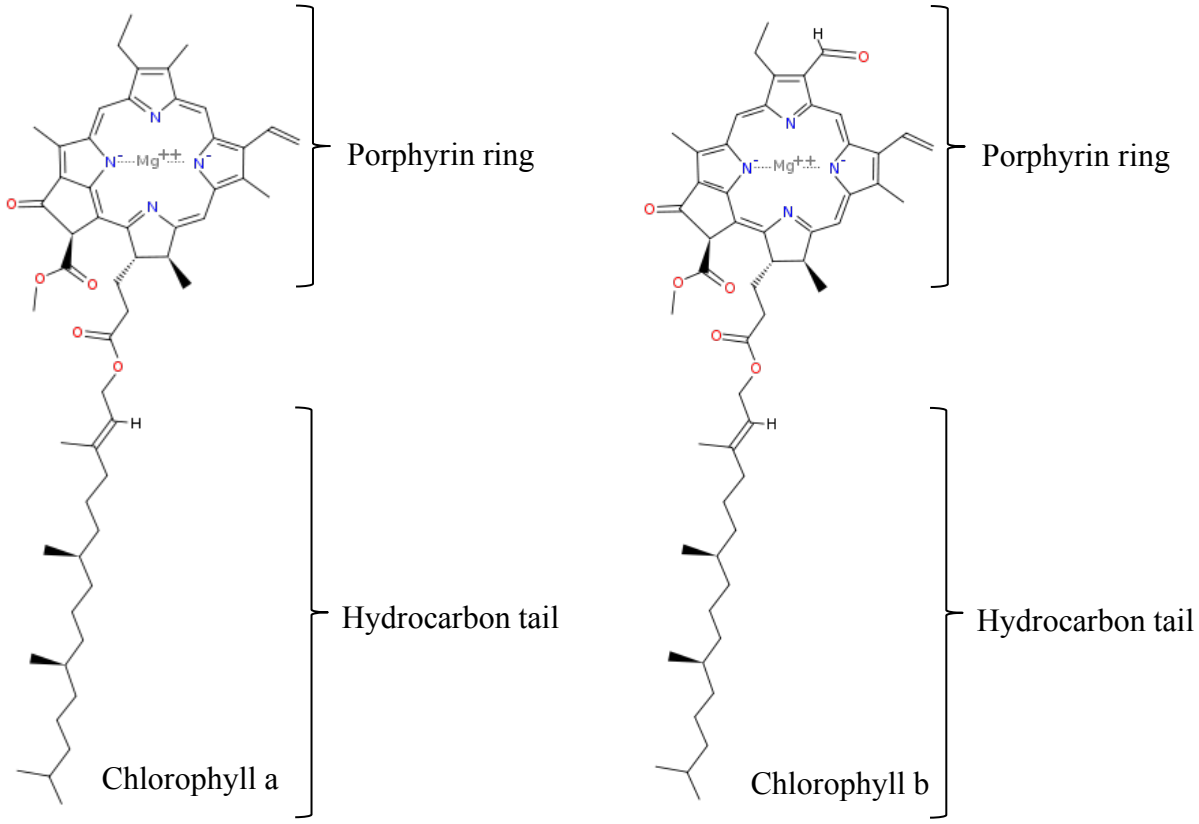

Figure 2.4. Structure of chlorophyll a and chlorophyll b.

As necessary as chlorophyll is for plants during photosynthesis, it is quite a nuisance when it comes to oilseed crops. In Canada, to maintain quality, seeds are assigned a grade based on various factors, one of these being the amount of green color present in canola/rapeseed samples. The amount of green seeds is related to higher a concentration of chlorophyll which greatly decreases the grade thereby decreasing the available profit. Depending on maturity, rapeseed can contain varying amounts of chlorophyll. Immature seeds contain large amounts of chlorophyll; however, as the seed matures, the chlorophyll is metabolized until there is very little left in a fully ripe seed (Tkachuk and Kuzina 1982). There are several agronomic factors that affect the level of

chlorophyll in *Brassica napus* including seeding date, seeding rate, frost, and growing period.

When oil is extracted from green seeds, the oil appears green in color if chlorophyll is extracted along with the oil (Daun 1976). This greenish color is unappealing to consumers. It is also difficult to remove chlorophyll from oil (Daun 1976). Chlorophyll pigments are difficult to remove using conventional alkali or bleaching treatments and bleaching clay has also been shown to retain anywhere from one third to three quarters its weight in oil thereby causing greater loss of valuable oil (Tkachuk et al. 1988). The removal of chlorophyll pigments therefore increases the refining costs and reduces profit (Tkachuk et al. 1988). It has also been shown that even after removal of chlorophyll, oil has a reduced shelf life when compared to higher-grade seed. Chlorophyll pigments also affect the rate of hydrogenation, making it more difficult to turn the oil into margarines and shortenings, and accelerates oxidation, causing the oil to go rancid prematurely (Tkachuk et al. 1988).

Traditionally chlorophyll concentration is measured by extraction from the oil with an organic solvent and measuring the absorbance on a spectrophotometer (Appelqvist and Johansson 1967).

2.3 QUALITY ANALYSIS

The main characteristics which determine the quality of rapeseed/canola are oil concentration, protein concentration, chlorophyll concentration, glucosinolate concentration, and fatty acid composition. These traits are analyzed when considering a new cultivar for licensing and therefore quality analysis techniques for theses traits are

quite useful to breeders. These traits are also analyzed throughout the transportation, processing and marketing of rapeseed/canola cultivars. Ideally, the analysis of these characteristics should be accurate and precise in addition to being rapid, simple, and economical. It is also beneficial to have nondestructive analytical methods.

Quality control is an important aspect in plant breeding. It is necessary to use good laboratory practices to consistently generate valid results. The Canadian Grain Commission has a Proficiency Testing Program in which laboratories in Canada, including the University of Manitoba, follow the same procedures, generally those set out by the International Standards Organization (ISO), when analyzing quality traits. The Proficiency Testing Program provides a non-biased assessment of the lab's competency. Each year, each lab is provided with a set of unknown samples, which they must determine the concentration of the particular quality parameter (oil concentration, protein concentration, fatty acid composition, glucosinolate concentration, and chlorophyll concentration) by following the standard procedures to have their labs certified by the Canadian Grain Commission. This ensures that all the labs are generating valid results.

The overall goal of assessing the quality in *Brassica* seed samples is to provide efficient screening techniques and evaluation methods for the development and registration of new *Brassica* oilseed cultivars.

2.3.1 Erucic acid concentration analysis

2.3.1.1 Molecular markers in marker assisted selection (MAS)

Marker assisted selection (MAS) is a process in which markers are used for indirect selection of a trait of interest. There are three kinds of markers, morphological

markers, biochemical markers, and molecular markers (Jain and Brar 2010). Markers are commonly used in plant breeding for selection of a trait of interest including quality, disease resistance, and stress tolerance. In order for molecular markers to be effective in marker assister selection, they must be closely linked to a QTL (Dudley 1993). Morphological markers are based on plant traits and are highly influenced by environmental factors. Biochemical markers are based on proteins and isozymes and results can vary depending on the developmental stage at which sampling occurred. Molecular markers are based on unique DNA sequences. Molecular markers are superior to both morphological and biochemical markers in that they are consistent throughout all plant developmental stages since DNA remains constant in all cells through the entire life cycle of a plant (Jain and Brar 2010).

There are many different molecular markers available. An ideal marker should easily allow for the identification of all possible phenotypes. Molecular markers are useful in cases where the selection trait is expressed late in development or at maturity. Molecular markers can be categorized into two separate groups: (1) hybridization-based markers including restriction fragment length polymorphism (RFLP) and (2) PCR-based markers including random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) or microsatellite, sequence related amplified polymorphism (SRAP), sequence characterized amplified region (SCAR) and single nucleotide polymorphism (SNP) (Jain and Brar 2010). Markers systems are generally differentiated based on technical requirements, cost per sample, labor requirements, and level of difficulty (Table 2.2).

Table 2.2. Comparison of molecular marker systems commonly used in crop development

	RFLP	RAPD	AFLP	SSR	SRAP	SNP	SCAR
Description	Restriction enzyme digestion, Southern blot hybridization	Amplification of DNA sequence using random primers	Restriction enzyme digestion of DNA, adaptor ligation, uses specific primers	Amplification of microsatellite sequence using specific primers	Amplification of DNA sequence using random primers	Single nucleotide difference in DNA sequence	Amplification of DNA fragment using specific primers
Level of difficulty	High	Low	High	Low	Low	Medium-High	Low
Reproducibility	High	Low	High	High	High	High	High
Automation possible	No	No	Yes	Yes	Yes	Yes	Yes
Inheritance	Co-dominant	Dominant	Dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant
Cost per analysis	Medium	Low	High	High	Low	High	Low
DNA quality	High	High	Moderate	Low	Low	Moderate	Moderate
Number of loci assayed	1-5	1-50	10-100	1-3	5-50	1	?

Adapted from Jain and Brar 2010

Molecular markers are an efficient way to complement traditional breeding programs. DNA based molecular markers were first introduced in the 1980s. RFLPs were the first DNA based markers that were used successfully in plant breeding (Helentjaris et al. 1985). Since RFLPs are time consuming and require large amounts of DNA, they were gradually replaced with more convenient PCR-based molecular markers. PCR-based molecular markers are quicker and cheaper to use than any of the other kinds of markers. PCR-based molecular markers have been applied to many crop species including cereals and oilseeds.

The use of molecular markers allows for the selection of desirable traits in a non-destructive manner with no environmental influence and at any stage in plant development. In the past, plants with desired traits were selected based on phenotypic traits. It has been shown that a breeder using phenotypic selection must select up to 16.7 times more progeny than a breeder using MAS to be sure that the proper genotype has been selected (Knapp 1998). Since large numbers of plants must be screened in a single session, it is beneficial to automate the molecular marker system. With the development of molecular markers, it is also possible to select plants with desired traits at a much earlier stage, saving space in the greenhouse thereby reducing cost. MAS is commonly used in plant breeding programs for selecting traits in which the plant or seeds must be destroyed to score its phenotype, such as in determining erucic acid concentration.

2.3.1.2 History of erucic acid analysis

Traditionally, fatty acid composition including erucic acid concentration in *Brassicas* is measured by the process of transesterification of triglycerides to fatty acid

methyl esters, which are then measured using gas chromatography. The Canadian Grain Commission recommends following the procedure set out by the International Organization for Standardization method reference number ISO 5508:1990 (E), Animal and vegetable fats and oils-Analysis by gas chromatography of methyl esters of fatty acids to determine fatty acid composition in oilseeds. This is a time consuming process that requires mature seeds.

Marker assisted applications in *Brassicas* first began to appear in the late 1980s with the development of the first RFLP linkage maps for *Brassica oleracea* (Slocum et al. 1990), *Brassica rapa* (Song et al. 1991), and *Brassica napus* (Landry et al. 1991). DNA sequences were used as markers to construct these linkage maps indicating that the results were relevant for applications in Brassica breeding. With the development of polymerase chain reaction (PCR) by Mullis and Faloona (1987), the potential for marker assisted breeding increased dramatically.

Jourdren et al. (1996) developed RAPD markers linked to the loci controlling erucic acid level in *Brassica napus*. Since the genetic markers were closely linked to the genes, it allowed for homozygous and heterozygous genotypes with high levels of erucic acid to be more easily distinguished. Using doubled-haploid (DH) progeny, they also found that both genes do not contribute equally to the level of erucic acid found in each plant. In the meantime, Barret et al. (1998) isolated, from a *Brassica napus* immature embryo cDNA library, two DNA sequences homologous to the *FAE1* gene, which is involved in erucic acid synthesis. They found that the sequences of the two cDNA homologues shared 97% of their nucleotide sequence and 98% of the their amino acid sequence. They also discovered that one of the genes was tightly linked to one of the two

loci, which controls erucic acid concentration in *Brassica napus.* Later that year, Fourmann et al. (1998) developed polymorphic markers for the *Brassica napus FAE1* loci from the A and C genomes. From this, they were able to show that the two genes co-segregated with the erucic acid loci that were originally identified by Ecke et al. (1995). Finally, Das et al. (2002) cloned and characterized the *FAE1* gene from high and low erucic acid lines of *Brassica campestris* and *Brassica oleracea*.

Rahman et al. (2008) used bacterial artificial chromosome (BAC) clones containing the *Bn-FAE1.1* genes from the A genome and *Bn-FAE1.2* genes from the C genome libraries to extend the sequence to the outside of these two genes to develop genome specific molecular markers. The purpose of these markers was to be high throughput to significantly accelerate breeding effort in the selection of the genes that control erucic acid concentration.

2.3.1.3 Sequence characterized amplified region (SCAR) markers

Sequence characterized amplified region (SCAR) markers target insertions and deletions in a genome and are quite useful in MAS in plant breeding. SCAR markers are usually developed from RAPD fingerprints (Koveza et al. 2001) or from AFLP fingerprints (Negi et al. 2000). SCAR is a PCR based marker system that uses two specific primers. There are many advantages to SCAR markers including their specificity, low cost, timeliness and ease of use. SCAR markers have been successfully used in identifying genotypes in plant and animal species. SCAR markers are co-dominant, which allows for identification of both homozygous and heterozygous

genotypes. SCAR markers are also easily automated to be high throughput. In this study, a SCAR marker is used to identify the E2 allele in the C genome of *Brassica napus.*

2.3.1.4 Single nucleotide polymorphism (SNP) markers

Single nucleotide polymorphism (SNP) markers target changes in a genome and are quite useful in MAS in plant breeding. SNPs occur when a single nucleotide (A, T, C or G) is substituted in the organism's genome. SNPs are the most abundant form of DNA polymorphisms. SNP markers are co-dominant, which allows for identification of both homozygous and heterozygous genotypes. SNP markers are also easily automated to be high throughput. In this study, the SNP marker is used to identify the E1 allele in the A genome of *Brassica napus.*

2.3.2 Chlorophyll analysis

2.3.2.1 History of chlorophyll analysis

Even though chlorophyll concentration is not considered as a quality factor in the registration of new canola/rapeseed varieties, it is still an important quality factor in oil processing. Prior to shipment to processing plants, seeds are graded at grain elevators following a grading system set out by the Canadian Grain Commission. Grades are based on inclusion of foreign material, damage to seeds, maturity, and overall colour. The highest grade, No. 1 Canada, must contain less than 2% distinctly green seeds; No. 2 Canada grade may contain up to 6% distinctly green seeds; and No. 3 Canada grade may contain up to 20% distinctly green seed (Canadian Grain Commission 2011). Prices are set based on the grade, with the smallest market and least profit for No. 3 grade;

therefore, it is most beneficial to have the least amount of chlorophyll in seeds. Over the years, there have been many different grading and analytical procedures for determining the concentration of chlorophyll in *Brassica napus* seed samples.

The Canadian Oilseed Industry uses the percentage of distinctly green seed compared to the overall seed color to determine acceptable levels of chlorophyll for trade purposes. This method involves transferring one hundred seeds to the sticky side of a piece of tape using a special applicator, crushing the seeds with a roller, and visually assessing the number of green seeds. Approximately 2% distinctly green seeds corresponds to around 24 mg/kg (ppm) chlorophyll. The Canadian Grain Commission specifications for trading allow canola oil to have 25 mg/kg to 30 mg/kg chlorophyll. This is not an accurate method since the percentage of green seed does not correlate well with the actual chlorophyll concentration in seeds or extracted oil (Tkachuk et al. 1988).

For analytical purposes, the Canadian Grain Commission follows a different procedure in which the oil is extracted from the seed and the chlorophyll concentration is measured on a spectrophotometer following the official methods determined by the International Organization for Standardization method reference number ISO 10519:1997(E), Rapeseed-Determination of chlorophyll content-Spectrophotometric method. In this method, two grams of ground rapeseed per sample is extracted using thirty millilitres of heptane:ethanol in a 3:1 ratio. The samples are shaken in a metal tube with steel balls for one hour, filtered, and then the absorbance is measured on a spectrophotometer at 625 nm, 665nm, and 705 nm. This method corrects for background noise on both sides of the chlorophyll peak. This method is relatively accurate and has high repeatability. The main problems with this method are that it is time consuming,

uses unfavourable organic solvents, and destroys whole seeds. Other factors such as sampling procedures, clarity of the extracted solution, spectral bandwidth, and degradation of the sample may affect the overall chlorophyll concentration results. These disadvantages prove that there is a growing need to find an easier and less time consuming method.

Many labs have used high performance liquid chromatography (HPLC) to measure chlorophyll derivatives that are then used to calculate total chlorophyll levels. HPLC allows for the separation of a mixture of chlorophyll pigments and their detection using a visible fluorescence detector. The main problems with this method are that whole seeds are destroyed; there is no standard method so results vary between labs based on the solvents and detectors used; and chlorophyll derivatives are measured rather than measuring chlorophyll directly so when compared to the AOCS method, the HPLC chlorophyll concentration are generally much higher.

In 1976, Daun suggested the use of reflectance spectroscopy to overcome these disadvantages. It was concluded that chlorophyll cannot truly be measured using NIR technology since chlorophyll is present in such small amounts that the signal from the bond used for NIR measurements is hidden by the signal from other compounds; however, it is possible to measure chlorophyll directly using the visible region of NIR instruments. In 1976, this process involved grinding seed samples in a specially designed grinder and measuring them on a Beckman Color DB-G reflectance spectrophotometer at 710, 670, and 630 nm. The results from the visible spectrum of crude rapeseed oil showed a sharp absorption band at λ_{max} = 670 nm due to chlorophyll. The reflectance spectrum of ground rapeseed also shows the same band. A calibration curve was created

by plotting the results from a series of samples analyzed by the absorbance procedure for chlorophyll concentration over the corrected absorbance at 670 nm on the ground samples. The precision and accuracy of this method was estimated in two experiments. In the first experiment, ten samples from the same seed lot were analyzed by the absorbance and reflectance procedures. The absorbance procedure results were 7.3 ± 0.3 ppm and the reflectance procedure results were 5.7 ± 0.7 ppm. The root mean square was ± 0.9 ppm which takes into account the differences in means determined by both procedures. In the second experiment, nine samples ranging between 4 and 12 ppm chlorophyll were analyzed five times each by absorbance procedure and twice by reflectance procedure. Results from the absorbance procedure were averaged for each sample and were considered accurate in the consideration of the accuracy of the reflectance procedure. Each measurement from the reflectance procedure was considered separately. The difference in root mean square values between both procedures was ± 1.2 ppm. Overall, the accuracy and precision of this experiment is suitable for surveys on large numbers of samples. It is a rapid procedure in which 60-80 samples can be analyzed in an eight hour period, however, this procedure is not preferred for small sample sizes as the accuracy and precision are lower. This procedure also destroys whole seeds.

In 1982, Tkachuk and Kuzina developed a method for determining chlorophyll concentration in whole rapeseed seeds using a near infrared reflectance (NIR) Cary 171 spectrophotometer. There were some challenges with this methods as well.

In 1988, Tkachuk et al. developed a reflectance calibration equation for analyzing chlorophyll in ground rapeseed using six Dickey-John Instalab 600 NIR instruments modified by replacing the filters with filters in the visible range at wavelengths of 674

and 696 nm. Stepwise multiple linear regression analysis showed that a combination of 674, 696, and 2100 nm wavelengths gave the most accurate estimation of chlorophyll concentration. The calibration equations which incorporated all three wavelengths were shown to have an average R of 0.980 and a standard error of the estimate (SEE) of 3.1 ppm indicating that the equation can be used with confidence. A major disadvantage with this method results from the long term stability of the 696 nm interference filter and that the seeds must be ground as well. This technique was unique since it used an NIR instrument in the visible range.

As a result of the problems with the above-mentioned methods, there is a need to develop a less time consuming and more accurate method to determine chlorophyll levels in whole seeds.

2.3.2.2 Near infrared reflectance (NIR) spectroscopy

NIR is the study of the absorption of near infrared light energy by molecules. It is part of the study of the interaction of electromagnetic waves and matter. It encompasses the wavelengths between 780 and 2500 nm in the electromagnetic spectrum (EMS), whereas the visual region of the electromagnetic spectrum ranges from 380 to 780 nm. The NIR region of the EMS is used as in analytical procedures for the evaluation of biological and synthetic materials.

Biology is dependent on the electromagnetic spectrum. The visible spectrum (violet, blue, green, yellow, orange, and red) contains the primary information for life. It makes leaves on plants green through chlorophyll molecules, for example. Light supplies the energy to support life on planet earth. Colors are produced by electrons as they move

from one ring to the next around atoms in a particular substance. Visible light is measured by light reflected from the surface of an object that is then quantified by our eyes; however, visible light provides very little information about the chemical composition of the object.

Near-infrared radiation, just above the red band of color in the EMS, not visible to the eye, is emitted by the sun and is absorbed by all biological compounds. It provides information about the building blocks of all biological material, including the stretching, bending, and oscillation of molecular bonds (C-H, N-H, O-H) and other elemental compounds and molecules that support the matrix of the compound. A near-infrared reflectance spectrophotometer, such as the FOSS 6500, would prove useful for measuring the concentration of these chemical bonds, and computer software, attached to the NIR instrument, would be useful for interpreting this information.

The potential for NIR spectroscopy as a quality analysis method was first discovered in 1965 by Norris and Hart. It was found that when combined with correlation transform spectroscopy, reflectance spectroscopy provides a simple, rapid method for analyzing agricultural products.

When electromagnetic radiation encounters a substance, it will either be absorbed or transmitted depending upon its frequency and the molecular structure of the substance. A spectrophotometer measures the relative amount of light at different wavelengths that is absorbed or transmitted by a sample solution. Inside a spectrophotometer, white light is separated into colors by a prism, which are then passed one by one through the sample. The light then strikes a photoelectric tube, which converts the light into energy, which can then be measured by the instrument. Since molecules naturally vibrate, if there is a

match between the disturbing energy frequency (from a spectrophotometer) and the natural vibration frequency, the molecule absorbs this energy, which can then visualized as a spectrum. Computer software (WinISI) is then used to interpret the spectrum and predict the concentration of the constituent being measured. For this, a calibration equation is required.

A calibration is the process of matching spectral readings to lab reference data. A calibration equation is used to determine an unknown concentration of a substance in a sample by comparing it to a set of standard samples of known concentration. The process of developing a calibration equation contains several important steps. The first step is to obtain a large number of samples, scan them through the NIR instrument, and determine their reference values by wet chemistry. The next step is to apply math treatments such as scatter corrections to reduce the influence of light scatter and derivatives to clarify peaks in the spectra. Next, regression techniques are employed to relate the constituent data to the spectra. Finally, the equation must be tested against a separate set of samples to predict its efficiency.

Many labs currently use a FOSS 6500 near infrared reflectance spectrophotometer to determine oil, protein, and glucosinolate concentrations; it would therefore by advantageous to use the machine to determine chlorophyll concentration as well since it could be less expensive and less time consuming.

This particular application of the NIR spectrophotometer is unique in that the measurements are made in the visible range of the electromagnetic spectrum. NIR instruments are able to detect and measure light in the visible range since their sodium light emits visible radiation; therefore it provides a simple and convenient method for

scanning samples with reflected light. The main advantages of using NIR instrumentation is that little or no samples preparation is required, it is non destructive, it does not require any chemicals, it is operator friendly, it is fast, and it is reliable and precise.

3.0 IMPROVEMENTS TO THE HIGH THROUGHPUT GENOME-SPECIFIC MOLECULAR MARKER PROTOCOL FOR ERUCIC ACID GENES IN *BRASSICA NAPUS*

3.1 Introduction

Brassica napus (genome AACC, 2n = 38*)* arose from the spontaneous interspecific hybridization between *Brassica rapa* (genome AA, 2n = 20) and *Brassica oleracea* (genome CC, 2n = 18) (Downey 1983).

Fatty acid composition such as erucic acid concentration is a very important measure of quality in oilseeds. Fatty acid composition in vegetable oils determines whether the oil is to be used for edible or industrial purposes.

Brassica napus (canola/rapeseed) produces very long chain fatty acids (VLCFA) such as erucic acid (C22:1). High levels of erucic acid make HEAR (high erucic acid rapeseed) oil an excellent industrial oil. On the other hand, little or no erucic acid, makes canola oil an excellent edible oil since high levels of erucic acid leads to health problems (Beare et al. 1963). HEAR oil contains at least 46% erucic acid, whereas, to be suitable for human consumption, canola oil must contain less than 2% erucic acid.

Fatty acid biosynthesis involves a four step cyclic process in which two carbon atoms are added per cycle. The first step in the cycle is a condensation reaction, followed by a reduction reaction, then a dehydration reaction, and finally by another reduction reaction. Seven repetitions of this cycle results in the formation of palmitic acid (C16:0). For longer fatty acids such as erucic acid, palmitic acid undergoes additional cycles of elongation. The enzyme involved in this elongation process is termed Fatty Acid Elongase 1 (FAE1) (Wu et al. 2007). The two homologs of *FAE1*, E1 and E2, have

additive effects and encode the 3-ketoacyl-CoA synthase enzyme, which is responsible for the elongation of oleic acid (C18:1) to erucic acid (Harvey and Downey 1964, Stefansson 1983, Puyaubert et al. 2005). The two homologous sequences of the *FAE1* gene are termed *Bn-FAE1.1* for the E1 locus and *Bn-FAE1.2* for the E2 locus in *Brassica napus* and are located in the A and C genomes (Wu et al. 2007).

Low erucic acid rapeseed (canola) genotypes contain a mutation that affects both the E1 and E2 loci (Harvey and Downy 1964). A two base deletion in the *Bn-FAE1.2* gene in the C genome and a single base pair change resulting in an amino acid substitution in the *Bn-FAE1.1* gene in the A genome results in low erucic acid concentration (Fourmann et al. 1998, Katavic et al. 2002). The E1E1E2E2, E1e1E2E2 or E1E1E2e2, e1e1E2E2 or E1E1e2e2, and e1e1e2e2 genotypes are responsible for 40, 30, 20, and 0% erucic acid concentration in seeds respectively (Jourdren et al. 1996).

Traditionally fatty acid composition, including erucic acid concentration, in *Brassicas* is determined by the process of transesterification of triglycerides to fatty acid methyl esters, which are then measured using gas chromatography. This requires mature seed for a phenotypic assessment. The Canadian Grain Commission recommends following the procedure set out by the International Organization for Standardization method reference number ISO 5508:1990 (E), Animal and vegetable fats and oils-Analysis by gas chromatography of methyl esters of fatty acids to determine fatty acid composition in oilseeds.

Marker assisted selection (MAS) is a process in which morphological, biochemical, or molecular markers are used for indirect selection of a trait of interest. An ideal marker should easily allow for the identification of all possible phenotypes.

Molecular markers are based on unique DNA sequences and are useful in cases where the selection trait is expressed late in development or at maturity. Markers are commonly used in plant breeding for selection of trait of interest including quality, disease resistance, and stress tolerance and are therefore an efficient way to complement traditional breeding programs.

The use of molecular markers allows for the selection of desirable traits in a non-destructive manner with no environmental influence and at any stage in plant development. In the past, plants with desired traits were selected based on phenotypic traits. Since large numbers of plants must be screened in a single session, it is beneficial to automate the molecular marker system. With the development of molecular markers, it is also possible to select plants with desired traits at a much earlier stage, saving space in the greenhouse thereby reducing cost. MAS is commonly used in plant breeding programs for selecting traits in which the plant or seeds must be destroyed to score phenotype, such as in determining erucic acid concentration.

There are many different molecular markers available. PCR-based molecular markers are quicker and cheaper to use than any of the other forms of markers. PCR-based molecular markers have been applied to many crop species including cereals and oilseeds (Jain and Brar 2010).

Sequence characterized amplified region (SCAR) markers target insertions and deletions in a genome and are quite useful in MAS in plant breeding. SCAR is a PCR based marker system that uses two specific primers. There are many advantages to SCAR markers including their specificity, low cost, timeliness, and ease of use. They are also easily automated to be high throughput. SCAR markers are co-dominant as well,

allowing for the identification of both homozygous and heterozygous genotypes (Rahman et al. 2008).

Single nucleotide polymorphism (SNP) markers target changes in a genome and are quite useful in MAS in plant breeding as well. SNPs occur when a single nucleotide (A, T, C or G) is substituted in an organism's genome. SNP markers are co-dominant, which allows for the identification of both homozygous and heterozygous genotypes. SNP markers are also easily automated to be high throughput (Rahman et al. 2008).

At the University of Manitoba, Rahman et al. (2008) used bacterial artificial chromosome (BAC) clones containing the *Bn-FAE1.1* genes from the A genome and *Bn-FAE1.2* genes from the C genome libraries to develop genome specific molecular markers by extending the sequence to the outside of these two genes. The purpose of these markers was to be high throughput to significantly accelerate breeding effort in the selection of the genes that control erucic acid concentration. Rahman et al. (2008) were able to develop these markers and the protocol for their use; however, when used as high throughput, the error rate was too high to be considered useful.

The purpose of this study was to improve the SNP and SCAR marker analysis for erucic acid of the *Bn-FAE1.1* gene in the A genome and the *Bn-FAE1.2* gene in the C genome in *Brassica napus* seedling samples published by Rahman et al. (2008).

3.2 Materials and Methods

3.2.1 Plant materials

Twelve Canola x HEAR backcrosses, along with the pure breeding parental line, 08C344, and the pure breeding parental cultivar, Red River 1997, were grown in the 2010

spring greenhouse cycle (January to May 2010) (Table 3.2.1), followed by ten canola x HEAR crosses in the 2011 spring greenhouse cycle (January to May 2011) (Table 3.2.2) at the University of Manitoba. Between 31 and 96 plants were grown for each cross. A pure breeding canola cultivar, Sentry, and a pure breeding HEAR cultivar, MillenniUM 03, were grown as controls. All plant materials were grown in 15 cm plastic pots in standard potting soil which consisted of two parts soil, one part sand, and one part peat mix. After three weeks, during the seedling stage, approximately 100 mg of leaf tissue samples were collected from each plant. The genotypes for the BC_1F_1 generation progeny are of four types: E1E1E2E2, E1E1E2e2, E1e1E2E2, and E1e1E2e2 and are responsible for approximately 40%, 30%, 30% or 20% erucic acid concentration. After tissue sampling occurred, all plants were left to grow to maturity, harvested, and seeds were sent to the Brassica seed quality lab at the University of Manitoba for analysis.

Table 3.2.1. List of Canola x HEAR BC_1F_1 progeny from the 2010 spring greenhouse cycle

Cross	Number of Plants
08C344 x 1852H RR	95
08C344 x 71-45 RR	96
08C344 x 1841 RR	96
08C344 x 4414 RR	96
1841 RR x Red River 1997	32
1852 RR x RRHR6818	31
30412-B6RR x Red River 1997	32
30507-B6RR x Red River 1997	31
30609 B6RR x Red River 1997	32
SP621RR x Red River 1997	32
SW-PL-7888 x Red River 1997	32
SP-Favourable x Red River 1997	32

Table 3.2.2. List of Canola x HEAR BC_1F_1 progeny from the 2011 spring greenhouse cycle

Cross	Number of Plants
08C344 x 30221 – D8RR	96
08C344 x 30220 – D8RR	96
08C344 x 30216 – C7RR	96
08C344 x 30422 – C7RR	96
08C344 x 30408 – C7RR	96
Red River 1997 x 30216 – C7RR	96
Red River 1997 x 30220 – D8RR	96
Red River 1997 x 30422 – C7RR	96
Red River 1997 x 30408 – C7RR	96
Red River 1997 x 30221 – D8RR	96

3.2.2 DNA extraction

DNA was extracted from the leaf tissue samples using a modification of the CTAB method described by Li and Quiros (2001). DNA was extracted from each leaf sample from all plants in all crosses. Liquid nitrogen was added to each well of the 96 deep well plates containing approximately 100 mg of leaf tissue and the contents ground into a fine powder using a plastic pestle plate. Then, 400 µl of 2X CTAB buffer (see below) was added to each well of the 96 well plates which were then incubated at 65°C for 90 minutes. After incubation, 400 µl of chloroform was added to each well and mixed vigorously on the shaker for 15 minutes to remove proteins. The plates were centrifuged at 6,200 rpm for 10 minutes. Then, 90 µl of clean supernatant containing DNA was transferred into a new 96 well plate using a multichannel pipette with new tips for each sample. DNA was precipitated by adding 45 µl of 2-propanol to the 96 well plate, covering with an aluminum foil cover, shaking vigorously by hand for approximately 15-30 seconds, then centrifuged for 8 minutes at 6,200 rpm. The supernatant was discarded and DNA was washed with 100 µl of 70% ethanol and centrifuged for 5 minutes at 6,200

rpm. The pellet containing DNA was then dried overnight and resuspended in 40 µl distilled water the following day. DNA samples were stored at -20°C until needed.

2X CTAB Buffer:
100 ml of 1 M tris, pH 8.0 (100 mM)
280 ml of 5 M NaCl (1.4 M)
40 ml of 0.5 M EDTA, pH 8.0 (20 mM)
20 g of Cetyltrimethyl ammonium bromide, 2% (CTAB)
Add dH_2O to make final volume of 1 L.

1 M Tris, pH 8.0:
121.1 g of tris (Fisher catalogue #: BP152-5)
700 ml of ddH_2O
Dissolve tris and bring to 900 ml.
Adjust pH to 8.0 with concentrated HCl (~50 ml)
Add dH_2O to final volume of 1 L.

0.5 M EDTA, pH 8.0:
186.12 g of EDTA (Fisher catalogue #: BP120-1)
750 ml of ddH_2O
Add about 20 g of NaOH pellets.
Slowly add more NaOH until pH is 8.0. EDTA will dissolve at about pH 8.0.
Add dH_2O to a final volume of 1 L.

5 M NaCl:
292.2 g of NaCl (Fisher catalogue #: BP358-10)
700 ml dH_2O
Dissolve and add dH_2O to a final volume of 1 L.

3.2.3 Assessment of the validity of the markers

The validity of the makers was assessed to confirm that the markers were properly detecting allelic differences at the erucic acid gene loci using plant material with known genotypes. The plant material included the pure breeding canola cultivar, Sentry, with the known genotype e1e1e2e2, and the pure breeding HEAR cultivar, MillenniUM 03, with the known genotype E1E1E2E2. The pure breeding parental line/cultivar, 08C344 and Red River 1997, of the BC_1F_1 progeny with the E1E1E2E2 genotype were assessed as well. The original protocol without any modifications published by Rahman et al. (2008)

for the sequence characterized amplified region (SCAR) marker for the *Bn-FAE1.2* gene (E2e2) in the C genome and the single nucleotide polymorphism (SNP) marker for the *Bn-FAE1.1* gene (E1e1) in the A genome was followed for this assessment.

3.2.4 First round of MAS following original protocols

Once the validity of the markers was determined, a first round of marker assisted selection (MAS) was performed on the canola by HEAR BC_1F_1 progeny from the 2010 spring greenhouse cycle (Table 3.2.1). Modifications to the CTAB method for DNA extraction as set out by Li and Quiros (2001) were followed. The published technique for the SCAR and SNP markers, as set out by Rahman et al. (2008), was followed without any modifications.

3.2.5 Second round of MAS following modifications to the original protocol after DNA quality and quantity assessment

Once the results from the first round of MAS were analyzed, a second round of MAS was performed on the canola by HEAR BC_1F_1 progeny from the 2011 spring greenhouse cycle (Table 3.2.2) to further improve the marker systems. Modifications to the CTAB method for DNA extraction published by Li and Quiros (2001) were followed. Following extraction, DNA samples were analyzed using a NanoDrop spectrophotometer to determine whether DNA quality and concentration would affect the MAS results. See section 3.2.5.1 for the protocol for the DNA quantification using the NanoDrop spectrophotometer. The protocol for the SCAR and SNP markers, as published by Rahman et al. (2008), was followed with refinements as indicated below in sections 3.2.5.2 and 3.2.5.3. Once the results from the second round of MAS were determined, a

SNP marker for the E2e2 gene of the C genome was developed to replace the C genome SCAR marker in progeny of crosses where a four base pair change in the SCAR marker result appears when a two base pair change is expected and to see if it was possible to further improve/simplify the marker system. See section 3.2.5.4 for the C genome (E2e2) SNP marker system protocol.

3.2.5.1 Protocol for DNA quantification using the NanoDrop Spectrophotometer

The NanoDrop Spectrophotometer (Thermo Scientific model 2000) was used to measure the concentration and quality of the genomic DNA. A 2 µl volume of DNA was pipetted onto the measurement pedestal containing the receiving fiber optic cable. The arm with the source fiber optic cable was then brought into contact with the sample causing the liquid to form a bridge between the two fibers. The lamp source consists of a xenon flash lamp and the array consists of a linear CCD array, which analyzes the light that passes through the sample. The measurement pedestal was wiped clean after each sample using a dry KimWipe. Since the genomic DNA was dissolved in distilled water, distilled water was used as the instrument blank. The spectrum of the blank was used to calculate the absorbance of the sample. The instrument is controlled by NanoDrop 2000/2000c software, which exports the data into workbook files, which can then be exported into Microsoft Excel files.

The reading taken at 260 nm was used in the calculation of the concentration of the DNA in the sample. The reading taken at 280 nm was used in the calculation of the amount of protein in the sample. The ratio of the absorbance at 260 nm and 280 nm was used to determine the quality of the DNA. Samples with a 260/280 ratio equal to or

greater than 1.8 are considered to be pure DNA. If the ratio is less than 1.8, it may indicate incomplete removal of proteins during the extraction process. The ratio of absorbance at 260 nm and 230 nm was used as a secondary measure of purity. Samples with a 260/230 ratio equal to or greater than 2.0, are considered to be pure. If the ratio is less than 2.0, it may indicate the presence of contaminants. Compounds such as phenol absorb strongly at 230 nm; therefore, their presence at this wavelength may indicate the carry-over of phenol from the extraction process into the sample.

The absorbance was calculated by the software using the following formula:

$$\text{Absorbance} = -\log\frac{[\text{intensity}_{\text{sample}}]}{[\text{intensity}_{\text{blank}}]}$$

The DNA concentration was calculated by the software using the following Beer-Lambert equation:

$c = (A \times \varepsilon)/b$, where

c = the DNA concentration in ng/µl

A = the absorbance in AU

ε = the wavelength-dependent extinction coefficient in ng/µl

b = the pathlength in cm

3.2.5.2 Protocol for the A genome (E1e1) SNP marker system

The protocol for the single nucleotide polymorphism (SNP) marker system followed the method published by Rahman et al. (2008). The FE15 (GTCAATAGCAAGTTTGAAA) and FEA2a (CGTATGCTCTTGTGGTGAGCA) primers used for the SNP marker system are specific to the A genome *Bn-FAE1.1* gene

(E1e1). One primer is located in the upstream flanking region and the other primer is located inside the gene.

The first step was to perform PCR to produce genome specific PCR products that contained the SNP position. The reaction constituents were: 100 ng of genomic DNA; 1X PCR buffer (50 mM KCl, 10 mM tris, 0.1% triton, 1.5 mM $MgCl_2$, pH 9.3); 0.375 mM dNTPs; 1 unit *Thermus aqaticus* (Taq) DNA polymerase; 0.15 uM forward primer FEA2a; and 0.15uM reverse primer FE15 (Table 3.2.3). Distilled water was added to bring the PCR reaction volume to 8 µl, which was then added to each well of a 384 well plate. 2 µl of genomic DNA was added to each well using a multichannel pipette to bring the total reaction volume in each well to 10 µl.

Table 3.2.3. Components for PCR reactions used for SNP marker system for the E1e1 gene

Stock	Volume per well (10 µl)	Final Concentration
dH_2O	6.4 µl	-
10x PCR buffer	1.0 µl	1x
25 mM dNTPs	0.15 µl	0.375 mM
6 U/µl Taq polymerase	0.15 µl	1 U/10 µl
10 µM Forward primer	0.15 µl	0.15 µM
10 µM Reverse primer	0.15 µl	0.15 µM
Template DNA	2.0 µl	~100 ng

The PCR cycling program was as follows: 94°C for 3 minutes for initial denaturing of DNA; 30 cycles of denaturing at 94 °C for 50 seconds, annealing at 55 °C for 50 seconds, and extension at 72 °C for 50 seconds; followed by the final extension at 72 °C for 10 minutes, and cool down at 10°C for 5 minutes.

After the PCR program was completed, PCR products were checked by adding 3 µl of loading buffer and running an agarose gel for 30 minutes at 120 V. The gel was checked with the gel image analyzer.

Next, dNTPs and primers were removed by adding 2.5 µl of a mixture of 0.2 units of shrimp alkaline phosphatase (SAP) (1 µl/1U) and 0.02 units of Exo I DNase (1 µl/20U) to 2.0 µl of the PCR product in a new plate using the multichannel pipette. The samples were then incubated at 37°C for 40 minutes and 75°C for 15 minutes to inactivate the enzymes.

The SNaPshot reactions were set up by adding 2.0 µl of a mixture of 0.2 µl SNaPshot mix, 0.5 µl detection primers (Table 3.2.4), and 1.3 µl 1x PCR buffer to each well that contained PCR products using the multichannel pipette. The PCR cycling program was as follows: 96°C for 3 minutes, followed by a rapid thermal ramp to 96°C for 10 seconds, then rapid thermal ramp to 50°C for 5 seconds, followed by rapid thermal ramp to 60°C for 30 seconds for 25 cycles.

Table 3.2.4. Detection primers for SNP marker system for the E1e1 gene

Primer Name	Primer Sequence
FEAF1	TTTTTGCCGCTATTTTGCTCT
FEAF2	TTTTTTTTTTGCCGCTATTTTGCTCT
FEAF3	TTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF4	TTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF5	TTTTTTTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF6	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF7	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF8	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF9	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF10	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF11	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCGCTATTTTGCTCT
FEAF12	TTTTTTCGATCTCCAGGCTTGTTG
FEAF13	TTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF14	TTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF15	TTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF16	TTTTTTTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF17	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF18	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF19	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF20	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF21	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG
FEAF22	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATCTCCAGGCTTGTTG

Next, ddNTPs were removed by adding 2.0 µl of diluted SAP (1 µl/1U, 0.02 units diluted with 1x PCR buffer) using a multichannel pipette, then incubated at 37°C for 40 minutes and 75°C for 15 minutes.

Then, a 384 pin replicator was used to add the PCR products to a new 384 well plate containing 7 µl of formamide with LIZ-labeled GeneScan 120 (ABI) size standard. The samples were then denatured for 5 minutes at 94°C, chilled on ice for 10 minutes, then separated with the ABI 3100 Genetic Analyzer (Applied Biosystems Institute, California). The data was analyzed and scored using ABI GeneScan software. The ABI GeneScan software assigned the color black to the nucleotide 'C', red to 'T', blue to 'G', and green to 'A'. When viewed with the GeneScan software, the homozygous dominant (E1E1) genotype appeared as one peak, whereas the heterozygous (E1e1) genotype appeared as two peaks. The color of the peaks depends upon which primer set was used. The data was transferred into a Microsoft Excel spreadsheet for analysis.

3.2.5.3 Protocol for the C genome (E2e2) SCAR marker system

Sequence characterized amplified region (SCAR) is a PCR based marker system that uses two primers specific to the C genome *Bn-FAE1.2* gene (E2e2) which amplifies the position of the two base deletion within the gene. The reverse primer, FE42A (GACCATCTTTAACCCTAAAACC) is labeled. Twenty different forward primers that amplify fragments ranging from 270 to 484 bp are available for multiplex use (Table 3.2.5).

Table 3.2.5. List of forward primers used for SCAR marker system for the E2e2 gene

Primer Name	Primer Sequence
FE42D	CAATGTCAAAGCTTCAA
FE42D1	GGCTCTAAACAATGTCAAAGC
FE42E1	TGCAGTTTGGGTGGCTCT
FE42F	GTGTAACAGTGCAGTTTGGG
FE42F1	GGCTTTAAGTGTAACAGTGC
FE42G1	TTAGGGTCAGGCTTTAAG
FE42J	GGTAATAAAGTTTGGCAG
FE42K	GCAAAAGGAAGGATGAAG
FE41	GGCATACATAGAAGCAAAAG
FE42L	TGGTATGAGTTGGCATAC
FE42M1	CTAGCTCAATATGGTATGAG
FE42M	GGAAACACTTCATCTAGCTC
FE42N1	CATAGATTTGGAAACACTTC
FE42N2	CAACGTTACATAGATTTGG
FE42N	GGCATCAAGATCAACGTTAC
FE42O	TCGATGTAGAGGCATCAAG
FE42P	CCTAGCACCGATCGATGTAG
FE42Q	AGAACCTAGGCCTAGCACCG
FE42R	GTGCTAGAGAAGAACCTAGG
FE42S	AGCCGTGATTGATGTGCTAG

The protocol for the SCAR marker system followed the method published by Rahman et al. (2008). The first step was to perform PCR to produce genome specific PCR products. The PCR reaction constituents were: 100 ng of genomic DNA; 1X PCR buffer (50 mM KCl, 10 mM Tris, 0.1% Triton, 1.5 mM $MgCl_2$, pH 9.3); 0.375 mM dNTPs; 1 unit *Thermus aqaticus* (Taq) DNA polymerase; 0.15uM reverse labeled primer FE42A (GACCATCTTTAACCCTAAAACC); and 0.15 uM forward unlabeled primer (Table 3.2.5, Table 3.2.6). Distilled water was added to bring the PCR reaction volume to 8 µl, which was then added to each well of a 384 well plate. Then, 2 µl of genomic DNA was added to each well using a multichannel pipette to bring the total reaction volume in each well to 10 µl.

Table 3.2.6. Components for PCR reactions used for SCAR marker system for the E2e2 gene

Stock	Volume per well (10 µl)	Final Concentration
dH_2O	6.4 µl	-
10x PCR buffer	1.0 µl	1x
25 mM dNTPs	0.15 µl	0.375 mM
6 U/µl Taq polymerase	0.15 µl	1 U/10 µl
10 µM Forward primer	0.15 µl	0.15 µM
10 µM Reverse primer (labeled)	0.15 µl	0.15 µM
Template DNA	2.0 µl	~100 ng

The PCR cycling program was as follows: 94°C for 3 minutes for initial denaturing of DNA; 30 cycles of denaturing at 94°C for 50 seconds, annealing at 55°C for 50 seconds and extension at 72°C for 50 seconds; followed by the final extension at 72°C for 10 minutes.

Once the PCR reaction was complete, a 384 pin replicator was used to add the PCR products to a new 384 well plate containing 7 µl of formamide with LIZ-labeled GeneScan 500 (ABI) size standard. The samples were then denatured for 5 minutes at 94°C, chilled on ice for 10 minutes, then separated with the ABI 3100 Genetic Analyzer (Applied Biosystems Institute, California). The data was analyzed using ABI GeneScan software and the genotypes were scored using Genographer and ABI GeneScan software. When exported into Genographer, the data appeared as a gel image, which allowed for the genotypes to be scored as homozygous dominant (E2E2) with one band or heterozygous (E2e2) with two bands. When viewed with the GeneScan software, the homozygous dominant genotype appeared as one peak, whereas the heterozygous genotype appeared as two peaks. The data was transferred into a Microsoft Excel spreadsheet for analysis.

3.2.5.4 Protocol for the C genome (E2e2) SNP marker system

To increase the efficiency of the C genome results, a SNP marker system specific to the C genome was developed. This process uses a two step nested polymerase chain reaction (PCR). The purpose of nested PCR is to reduce the appearance of unexpected PCR products caused by primers that bind to, and amplify incorrect regions of the DNA sequence. The first step of nested PCR was to perform a specific PCR program using a specific set of primers unique to the C genome SNP to produce genome specific PCR products that contained the SNP position. The second round of PCR uses a second set of primers, which amplify a secondary target within the PCR products from the first round of PCR.

For the first round of PCR, the reaction constituents were: 100 ng of genomic DNA; 1X PCR buffer (50 mM KCl, 10 mM tris, 0.1% triton, 1.5 mM $MgCl_2$, pH 9.3); 0.375 mM dNTPs; 1 unit *Thermus aqaticus* (Taq) DNA polymerase; 0.15 uM of forward primer FE42A (GACCATCTTTAACCCTAAAACC); and 0.15uM reverse primer FE42J (GGTAATAAAGTTTGGCAG) (Table 3.2.7). Distilled water was added to bring the PCR reaction volume to 10 µl, which was then added to each well of a 384 well plate. Genomic DNA was added to each well using a 96 pin replicator.

Table 3.2.7. Components for first round of PCR reactions used for SNP marker system for the E2e2 gene

Stock	Volume per well (10 µl)	Final Concentration
dH_2O	9 µl	-
10x PCR buffer	1.0 µl	1x
25 mM dNTPs	0.15 µl	0.375 mM
6 U/µl Taq polymerase	0.15 µl	1 U/10 µl
10 µM Forward primer FE42J	0.15 µl	0.15 µM
10 µM Reverse primer FE42A	0.15 µl	0.15 µM
Template DNA	Use pin replicator	~10 ng

The PCR cycling program was as follows: 94°C for 3 minutes for initial denaturing of DNA; followed by 20 cycles of denaturing at 94°C for 50 seconds, annealing at 57°C for 50 seconds, and extension at 72°C for 50 seconds. Increasing the annealing temperature to 57°C and reducing the number of cycles to 20 reduced the background noise in the gel image from the ABI 3100 Genetic Analyzer displaying more distinguished peaks.

For the second round of PCR, the reaction constituents were: 1X PCR buffer (50 mM KCl, 10 mM tris, 0.1% triton, 1.5 mM $MgCl_2$, pH 9.3); 0.375 mM dNTPs; 1 unit *Thermus aqaticus* (Taq) DNA polymerase; 0.15uM of forward primer FE42A (GACCATCTTTAACCCTAAAACC); and 0.15uM reverse primer FE42D1 (GGCTCTAAACAATGTCAAAGC) (Table 3.2.8). Distilled water was added to bring the PCR reaction volume to 10 µl, which was then added to each well of a new 384 well plate. PCR products from the first round of PCR were added to each well using a 384 pin replicator.

Table 3.2.8. Components for second round of PCR reactions used for SNP marker system for the E2e2 gene

Stock	Volume per well (10 µl)	Final Concentration
dH_2O	9 µl	-
10x PCR buffer	1.0 µl	1x
25 mM dNTPs	0.15 µl	0.375 mM
6 U/µl Taq polymerase	0.15 µl	1 U/10 µl
10 µM Forward primer FE42D1	0.15 µl	0.15 µM
10 µM Reverse primer FE42A	0.15 µl	0.15 µM
Template DNA	Use pin replicator	~10 ng

The PCR cycling program was as follows: 94°C for 3 minutes for initial denaturing of DNA; 20 cycles of denaturing at 94°C for 50 seconds, annealing at 55°C

for 50 seconds, and extension at 72°C for 50 seconds; followed by the final extension at 72°C for 10 minutes.

After the PCR programs were completed, the PCR products were checked by adding 3 µl of loading buffer and running an agarose gel for 30 minutes at 120 V. The gel was checked for the presence of clear bands with the gel image analyzer.

Next, dNTPs and primers were removed by adding 2.5 µl of a mixture of 0.2 units of shrimp alkaline phosphatase (SAP) (1 µl/1U) and 0.02 units of Exo I DNase (1 µl/20U) to 2.0 µl of the PCR product in a new plate using a multichannel pipette. The samples were then incubated at 37°C for 40 minutes and 75°C for 15 minutes to inactivate the enzymes.

The SNaPshot reactions were set up by adding 2.0 µl of a mixture of 0.2 µl SNaPshot mix, 0.5 µl detection primers FESNP2 (TTTTTTTTTGTTCCCAAGGACTATTTG) and FESNP4 (TTTTTTTTTTTTTTGTTCCCAAGGACT), and 1.3 µl 1x PCR buffer to each well that contained PCR products using a multichannel pipette. The PCR cycling program was as follows: 96°C for 3 minutes, followed by a rapid thermal ramp to 96°C for 10 seconds, then rapid thermal ramp to 50°C for 5 seconds, followed by rapid thermal ramp to 60°C for 30 seconds for 25 cycles.

Next, ddNTPs were removed by adding 2.0 µl of diluted SAP (1 µl/1U, 0.02 units diluted with 1x PCR buffer) using a multichannel pipette, then incubated at 37°C for 40 minutes and 75°C for 15 minutes.

Then, a 384 pin replicator was used to add the PCR products to a new 384 well plate containing 7 µl of formamide with LIZ-labeled GeneScan 120 (ABI) size standard.

The samples were then denatured for 5 minutes at 94°C, chilled on ice for 10 minutes, then separated with the ABI 3100 Genetic Analyzer (Applied Biosystems Institute, California). The data was analyzed and scored using ABI GeneScan software. The ABI GeneScan software assigned the color black to the nucleotide 'C', red to 'T', blue to 'G', and green to 'A'. When viewed with the GeneScan software, the homozygous dominant (E2E2) genotype appeared as one peak, whereas the heterozygous (E2e2) genotype appeared as two peaks. The color of the peaks depends upon which primer set was used. The data was transferred into a Microsoft Excel spreadsheet for analysis.

3.2.6 Sample preparation and gas chromatography (GC) procedure for determining erucic acid concentration in mature seeds

Fatty acid concentration in the oil was determined following the procedure set out by the International Organization for Standardization reference number ISO 5508:1990 (E), Animal and vegetable fats and oils-Analysis by gas chromatography of methyl esters of fatty acids. This procedure is used to determine the fatty acid profile ranging in chainlength from sixteen to twenty-four carbons of *Brassica sp.* in mature seed including that of C22:1 erucic acid; and other components of interest such as: C16:0 (palmitic), C16:1 (palmitoleic), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), C18:3 (linolenic), C20:0 (arachidic), C20:1 (eicosenoic), C20:2 (eicosadienoic), C22:0 (behenic), C22:2 (docosadienoic), C24:0 (lignoceric), and C24:1 (nervonic).

Approximately 30 mg of seed was obtained from each plant. Each seed sample was placed in an eppendorf tube containing 0.5 ml of heptane. The seed samples were then crushed with a glass rod and left overnight to extract the oil. The transesterification of triglycerides to fatty acid methyl esters (FAME) was completed by adding 100 µl of

0.5 N sodium methoxide reagent (13.5 g of sodium methylate powder in 500 ml anhydrous methanol) to each sample. The samples were then shaken vigorously for 20 seconds and left to stand for 20 minutes, then shaken again for 20 seconds and left stand for another 20 minutes. Next, 100 µl of of acidified water (0.3% acetic acid) was added to each sample, gently mixed and left to stand for 1-2 hours in the fridge to clear. Finally, approximately 250 µl of reaction mixture from each sample was pipetted into 2 ml autosample vials fitted with a 250 µl polypropylene insert for gas chromatography (GC) analysis.

Gas chromatography was performed using a Varian (model 3900) chromatograph equipped with a CP-Wax 52 CB capillary column and a flame ionization detector. A 15 m column with a 0.32 mm interior diameter coated with fused silica and a 0.025 micron polyethylene glycol phase (Varian, Walnut Creek, USA) was used for all analyses. Ultra High Purity (UHP) helium with a flow rate of 2.0 ml/min was used as the carrier gas. The split vent ratio was set at 100:1, with the septum purge flow set at 4 ml/min. The total hydrogen flow rate to the detector was 30 ml/min, the make-up gas (UHP helium) flow rate was 30 ml/min, and the detector gas was supplied at a flow rate of 300 ml/min from breathing grade air. The GC was equipped with a Varian (model CP-8400) autosampler which carried out the injections using a Varian (model CP-1117) injector with a cup splitter inlet liner with 10% OV-1 on Chromsorb-W HP and was operated at 250°C. The detector was set at 280°C and the column oven temperature ranged from 190°C to 240°C. The area of the peaks were measured using the Varian Star Workstation software. The reference standard, GLC # 421, (Nu-Check Prep, Elysian, Minnesota) was used to ensure proper GC operation. A lab sample was inserted after every 20 injections in order to

ensure the fatty acid methyl ester sample preparation occurred with accuracy and precision.

3.2.7 Analysis

The results from the genomic assessment for erucic acid were compared to the results from the fatty acid gas chromatography profiling. The results from the fatty acid gas chromatography profile are considered to be accurate and reliable. Any discrepancies between results were considered to be errors with the genomic assessment. ANOVA analyses were used to determine differences in results between sample sets and categories.

Fatty acid results in transition ranges were excluded from the genotype-phenotype comparison since the genotype for the erucic acid genes in these ranges cannot be identified with certainty. Ranges were chosen based on Mendelian genetics and environmental differences. Mendelian genetics genotypic segregation in BC_1F_1 generation follows a 1 E1E1E2E2: 2 E1e2E2E2 or E1E1E2e2: 1 E1e1E2e2 ratio.

3.3 Results and Discussion

3.3.1 Plant materials

All plant material was grown in the greenhouse. Tissue sampling was done at the 2 to 3 leaf stage. After that point, plants were left to grow to maturity, bagged during flowering, harvested, and seeds sent to the Brassica seed quality lab at the University of Manitoba for erucic acid analysis using gas chromatography following the procedure set out by the International Organization for Standardization reference number ISO

5508:1990 (E), Animal and vegetable fats and oils-Analysis by gas chromatography of methyl esters of fatty acids.

3.3.2 DNA extraction

DNA was extracted from the leaf tissue samples using a modification of the CTAB method published by Li and Quiros (2001). It was found that many factors can contribute to contamination of DNA samples thus leading to false results. These factors must be avoided during the DNA extraction process. Gloves must also be worn to help prevent contamination between samples. It is important to maintain the correct orientation of the 96 deep well plate as well. It is good lab practice to write on one side of the plate only and always have that side pointing in the same direction when performing any steps in the extraction process. In the first step, tissue sampling, it is important to avoid touching the cut edge of the leaf tissue with fingers or forceps. This can lead to contamination between the samples as the DNA can leak out of the cut edge and be transfered to the next sample. It may be beneficial to sterilize the forceps or other instruments between samples. The disadvantage with sterilization is that it is time consuming and when trying to operate in a high throughput manner, it would considerably slow down the process. When taking tissue samples, it is important to take approximately the same size sample from each plant. This ensures that each sample will contain the same quantity and quality of DNA. It is not necessary to use a punch to get the same size leaf tissue samples since a punch can cause contamination if not properly sterilized. The 96 deep well plates used should be free of DNA contamination and they must not have any broken wells. After the addition of chloroform, it is extremely

important to have a good seal between the plate and the lid since when the plate is put on its side, leaks cause cross contamination between the samples. If this occurs, the entire DNA extraction process must be repeated. Once the extraction process has begun, it is important to use new pipet tips to avoid contamination between samples when removing the supernatant following incubation of the crushed sample with the CTAB buffer. When transferring the supernatant to a new 96 well plate, it is also important to make sure to place the pipet tips the same depth into each well when picking up the liquid. Finally, once the DNA extraction process is complete, it is important to freeze the DNA when not in use to avoid evaporation thus altering the concentration of DNA.

3.3.3 Assessment of the validity of the markers

Once DNA was extracted from the leaf tissue, it was important to assess the validity of the markers. This was done by following the original protocol for the C genome (E2e2) SCAR marker system and the A genome (E1e1) SNP marker system published by Rahman et al. (2008) on a pure breeding HEAR cultivar, MillenniUM 03, a pure breeding canola cultivar, Sentry, the pure breeding HEAR parental line, 08C344, and the pure breeding HEAR cultivar, Red River 1997 (Table 3.3.1, Appendix Table A1).

Table 3.3.1. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes for pure breeding canola cultivar 'Sentry', pure breeding HEAR cultivars 'MillenniUM 03' and 'Red River 1997', and pure breeding HEAR parental line '08C344' following regular MAS procedures for determining erucic acid genotypes

Cultivar/Line	Sample Number	Number						Frequency (%)					
		Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Sentry	88	44	0	13	25	6	44	100.00	0.00	14.77	28.41	6.82	50.00
MillenniUM 03	81	36	0	8	32	5	45	100.00	0.00	9.88	39.51	6.17	55.56
Red River 1997	92	26	0	34	14	18	66	100.00	0.00	36.96	15.22	19.57	71.74
08C344	58	13	0	28	4	13	45	100.00	0.00	48.28	6.90	22.41	77.59
Total	319	119	0	83	75	42	200	100.00	0.00	26.02	23.51	13.17	62.70
Mean	-	-	-	-	-	-	-	100.00	0.00	27.47	22.51	13.74	63.72
Minimum	-	-	-	-	-	-	-	100.00	0.00	9.88	6.90	6.17	50.00
Maximum	-	-	-	-	-	-	-	100.00	0.00	48.28	39.51	22.41	77.59
Total Plants Evaluated (%)	37.30												
Overall Accuracy (%)	100.00												

It can be noted that only 37.30% of the plants were evaluated; however, of all the plants that were evaluated, all genotypes were correct. The main reason samples were not evaluated was due to low signal intensity from the ABI 3100 Genetic Analyzer output. This is often due to low concentration or poor quality DNA. Incomplete enzyme digestion in the SNP procedure also makes it difficult to evaluate the genotypes of samples.

The frequency of total missing results was consistent between cultivars and lines. The total missing results between E1 and E2 alleles was also consistent. Based on the results from the ANOVA analysis, the frequency of missing E1 and E2 results was similar ($P<0.6836$) between parental lines/cultivars at an average frequency of 27.47% missing E1 results and 22.51% missing E2 results (Table 3.3.2). Since both markers appear to be detecting allelic differences at the erucic acid gene loci on plant material with known genotypes, it would seem that the markers are valid. A problem does exist in the fact that it was impossible to score many samples due to missing E1 or E2 results. This would indicate that the markers are not very practical for use in a plant breeding program without first correcting these issues.

Table 3.3.2. ANOVA analysis summary table for the frequency of missing results in the parental line/cultivars, Sentry, MillenniUM 03, Red River 1997, and 08C344

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
E1 vs E2	49.2650	1	49.2650	0.1831	0.6836	5.9874
Residual	1614.2595	6	269.0432			
Total	1663.5245	7				

3.3.4 First round of MAS following original protocols

3.3.4.1 Statistical comparison within the 2010 conventional HEAR sample set

The first round of MAS was done on BC_1F_1 progeny from conventional HEAR crosses 08C344 x 1841 RR, 08C344 x 1852H RR, 08C344 x 4414 RR, and 08C344 x 71-45 RR from the 2010 spring greenhouse cycle following the original MAS protocol for determining erucic acid genotypes as published by Rahman et al. (2008) (Table 3.3.3, Appendix Table A2). Erucic acid results in the 43.3-40.2% and 35.2-32.1% C22:1 ranges were excluded from the genotype-phenotype comparison since the genotype for the erucic acid genes in these ranges cannot be identified with certainty (Appendix Table A3).

Table 3.3.3. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes excluding results ranging from 43.3%-40.2% and 35.2%-32.1% C22:1 from BC1F1 progeny from conventional HEAR crosses 08C344 x 1841 RR, 08C344 x 1852H RR, 08C344 x 4414 RR, and 08C344 x 71-45 RR from the 2010 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 1841 RR	≥43.3%	24	8	15	0	1	0	1	34.78	65.22	0.00	4.17	0.00	4.17
08C344 x 1852H RR	≥43.3%	31	20	9	0	2	0	2	68.97	31.03	0.00	6.45	0.00	6.45
08C344 x 4414 RR	≥43.3%	22	16	5	1	0	0	1	76.19	23.81	4.55	0.00	0.00	4.55
08C344 x 71-45 RR	≥43.3%	15	7	6	0	2	0	2	53.85	46.15	0.00	13.33	0.00	13.33
Total	≥43.3%	92	51	35	1	5	0	6	59.30	40.70	1.09	5.43	0.00	6.52
Mean	≥43.3%		13	9	0	1	0	2	58.45	41.55	1.14	5.99	0.00	7.12
Minimum	≥43.3%		7	5	0	0	0	1	34.78	23.81	0.00	0.00	0.00	4.17
Maximum	≥43.3%		20	15	1	2	0	2	76.19	65.22	4.55	13.33	0.00	13.33
08C344 x 1841 RR	40.1%-35.3%	26	15	8	0	3	0	3	65.22	34.78	0.00	11.54	0.00	11.54
08C344 x 1852H RR	40.1%-35.3%	5	4	0	1	0	0	1	100.00	0.00	20.00	0.00	0.00	20.00
08C344 x 4414 RR	40.1%-35.3%	30	27	1	0	2	0	2	96.43	3.57	0.00	6.67	0.00	6.67
08C344 x 71-45 RR	40.1%-35.3%	26	10	14	0	2	0	2	41.67	58.33	0.00	7.69	0.00	7.69
Total	40.1%-35.3%	87	56	23	1	7	0	8	70.89	29.11	1.15	8.05	0.00	9.20
Mean	40.1%-35.3%		14	6	0	2	0	2	75.83	24.17	5.00	6.47	0.00	11.47
Minimum	40.1%-35.3%		4	0	0	0	0	1	41.67	0.00	0.00	0.00	0.00	6.67
Maximum	40.1%-35.3%		27	14	1	3	0	3	100.00	58.33	20.00	11.54	0.00	20.00
08C344 x 1841 RR	≤32.2%	14	3	9	0	2	0	2	25.00	75.00	0.00	14.29	0.00	14.29
08C344 x 1852H RR	≤32.2%	13	10	3	0	0	0	0	76.92	23.08	0.00	0.00	0.00	0.00
08C344 x 4414 RR	≤32.2%	15	13	0	1	1	0	2	100.00	0.00	6.67	6.67	0.00	13.33
08C344 x 71-45 RR	≤32.2%	20	7	13	0	0	0	0	35.00	65.00	0.00	0.00	0.00	0.00
Total	≤32.2%	62	33	25	1	3	0	4	56.90	43.10	1.61	4.84	0.00	6.45
Mean	≤32.2%		8	6	0	1	0	1	59.23	40.77	1.67	5.24	0.00	6.90
Minimum	≤32.2%		3	0	0	0	0	0	25.00	0.00	0.00	0.00	0.00	0.00
Maximum	≤32.2%		13	13	1	2	0	2	100.00	75.00	6.67	14.29	0.00	14.29
Total Plants Evaluated (%)		63.90												
Overall Accuracy (%)		62.78												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

The frequency of missing E1 and E2 results was similar (P<0.6973) between each cross in the 2010 conventional HEAR sample set at an average frequency of 2.60% missing E1 results and 5.90% missing E2 results overall. The frequency of missing E1 and E2 results was also similar (P<0.1954) between each C22:1 category in the 2010 conventional HEAR sample set (Table 3.3.4).

Table 3.3.4. ANOVA analysis summary table for the frequency of missing results in the 2010 conventional HEAR sample set

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
E1 vs E2 (cross)	26.5684	2	13.2842	0.3678	0.6973	3.5545
E1 vs E2 (C22:1 category)	65.3047	1	65.3047	1.8083	0.1954	4.4138
Residual	650.029	18	36.1127			
Total	741.9021	21				

The overall accuracy for the conventional HEAR sample set from the 2010 spring greenhouse cycle was only 62.78%. It should be noted that only 63.90% of samples were analyzed overall. Of the samples that weren't analyzed, these results were scored as missing either the E1 results, the E2 results, or both the E1 and E2 results. Missing results were mostly due to poor quality DNA or incomplete enzyme digestion in the SNP protocol resulting in genotypes that could not be properly scored.

3.3.4.2 Statistical comparison within the 2010 Roundup Ready HEAR sample set

The first round of MAS was also done on BC_1F_1 progeny from Roundup Ready HEAR crosses 1841 RR x Red River 1997, 1852H RR x Red River 1997, 30412-B6 RR x Red River 1997, 30507-B6 RR x Red River 1997, 30609-B6 x Red River 1997, SP Favourable RR x Red River 1997, SP621 RR x Red River 1997, and SW-PL-7888 RR x

Red River 1997 from the 2010 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes as published by Rahman et al. (2008) (Table 3.3.5, Appendix Table A4). Erucic acid results in the 39.4-36.4% and 29.5-26.5% C22:1 ranges were excluded from the genotype-phenotype comparison since the genotype for the erucic acid genes in these ranges cannot be identified with certainty (Appendix Table A5).

Table 3.3.5. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes excluding results ranging from 39.4%-36.4% and 29.5%-26.5% C22:1 for BC1F1 progeny from Roundup Ready HEAR crosses 1841 RR x Red River 1997, 1852H RR x Red River 1997, 30412-B6 RR x Red River 1997, 30507-B6 RR x Red River 1997, 30609-B6 x Red River 1997, SP Favourable RR x Red River 1997, SP621 RR x Red River 1997, and SW-PL-7888 RR x Red River 1997 from the 2010 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
1841 RR x Red River 1997	≥39.5%	7	5	0	1	1	0	2	100.00	0.00	14.29	14.29	0.00	28.57
1852H RR x Red River 1997	≥39.5%	4	2	0	0	2	0	2	100.00	0.00	0.00	50.00	0.00	50.00
30412-B6 RR x Red River 1997	≥39.5%	7	6	1	0	0	0	0	85.71	14.29	0.00	0.00	0.00	0.00
30507-B6 RR x Red River 1997	≥39.5%	6	5	1	0	0	0	0	83.33	16.67	0.00	0.00	0.00	0.00
30609-B6 RR x Red River 1997	≥39.5%	10	9	0	0	1	0	1	100.00	0.00	0.00	10.00	0.00	10.00
SP Favourable RR x Red River 1997	≥39.5%	7	6	1	0	0	0	0	85.71	14.29	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	≥39.5%	12	7	3	1	1	0	2	70.00	30.00	8.33	8.33	0.00	16.67
SW-PL-7888 RR x Red River 1997	≥39.5%	14	4	6	1	3	0	4	40.00	60.00	7.14	21.43	0.00	28.57
Total	≥39.5%	67	44	12	3	8	0	11	78.57	21.43	4.48	11.94	0.00	16.42
Mean	≥39.5%		6	2	0	1	0	1	83.10	16.90	3.72	13.01	0.00	16.73
Minimum	≥39.5%		2	0	0	0	0	0	40.00	0.00	0.00	0.00	0.00	0.00
Maximum	≥39.5%		9	6	1	3	0	4	100.00	60.00	14.29	50.00	0.00	50.00
1841 RR x Red River 1997	36.3%-29.6%	14	11	1	1	0	1	2	91.67	8.33	7.14	0.00	7.14	14.29
1852H RR x Red River 1997	36.3%-29.6%	11	7	2	1	1	0	2	77.78	22.22	9.09	9.09	0.00	18.18
30412-B6 RR x Red River 1997	36.3%-29.6%	13	12	0	1	0	0	1	100.00	0.00	7.69	0.00	0.00	7.69
30507-B6 RR x Red River 1997	36.3%-29.6%	14	11	0	1	2	0	3	100.00	0.00	7.14	14.29	0.00	21.43
30609-B6 RR x Red River 1997	36.3%-29.6%	11	10	0	0	1	0	1	100.00	0.00	0.00	9.09	0.00	9.09
SP Favourable RR x Red River 1997	36.3%-29.6%	11	10	1	0	0	0	0	90.91	9.09	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	36.3%-29.6%	6	2	4	0	0	0	0	33.33	66.67	0.00	0.00	0.00	0.00
SW-PL-7888 RR x Red River 1997	36.3%-29.6%	4	2	1	1	0	0	1	66.67	33.33	25.00	0.00	0.00	25.00
Total	36.3%-29.6%	84	65	9	5	4	1	10	87.84	12.16	5.95	4.76	1.19	11.90
Mean	36.3%-29.6%		8	1	1	1	0	1	82.54	17.46	7.01	4.06	0.89	11.96
Minimum	36.3%-29.6%		2	0	0	0	0	0	33.33	0.00	0.00	0.00	0.00	0.00
Maximum	36.3%-29.6%		12	4	1	2	1	3	100.00	66.67	25.00	14.29	7.14	25.00
1841 RR x Red River 1997	≤26.4%	7	5	1	1	0	0	1	83.33	16.67	14.29	0.00	0.00	14.29
1852H RR x Red River 1997	≤26.4%	6	2	3	1	0	0	1	40.00	60.00	16.67	0.00	0.00	16.67
30412-B6 RR x Red River 1997	≤26.4%	6	6	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
30507-B6 RR x Red River 1997	≤26.4%	4	4	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
30609-B6 RR x Red River 1997	≤26.4%	7	5	0	1	1	0	2	100.00	0.00	14.29	14.29	0.00	28.57
SP Favourable RR x Red River 1997	≤26.4%	2	2	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	≤26.4%	1	1	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
SW-PL-7888 RR x Red River 1997	≤26.4%	5	4	0	0	1	0	1	100.00	0.00	0.00	20.00	0.00	20.00
Total	≤26.4%	38	29	4	3	2	0	5	87.88	12.12	7.89	5.26	0.00	13.16
Mean	≤26.4%		4	1	0	0	0	1	90.42	9.58	5.65	4.29	0.00	9.94

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Minimum	≤26.4%		1	0	0	0	0	0	40.00	0.00	0.00	0.00	0.00	0.00
Maximum	≤26.4%		6	3	1	1	0	2	100.00	60.00	16.67	20.00	0.00	28.57
Total Plants Evaluated (%)		66.80												
Overall Accuracy (%)		84.66												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

The frequency of missing E1 and E2 results was similar (P<0.5612) between each cross in the 2010 Roundup Ready HEAR sample set at an average frequency of 5.46% missing E1 results and 7.12% missing E2 results overall. The frequency of missing E1 and E2 results was also similar (P<0.5495) between each C22:1 category in the 2010 Roundup Ready HEAR sample set (Table 3.3.6).

Table 3.3.6. ANOVA analysis summary table for the frequency of missing results in the 2010 Roundup Ready HEAR sample set

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
E1 vs E2 (cross)	105.7878	2	52.8939	0.5855	0.5612	3.21993
E1 vs E2 (C22:1 category)	32.8880	1	32.8880	0.3640	0.5495	4.07266
Residual	3794.0644	42	90.3348	1.9611	0.1533	3.21993
Total	3932.7402	45				

The overall accuracy for the Roundup Ready HEAR sample set from the 2010 spring greenhouse cycle was 84.66%. While much better than the overall accuracy of the conventional HEAR sample set from the 2010 spring greenhouse cycle, it is still too low to be considered useful in a breeding program. It should also be noted that only 66.80% of samples were analyzed overall. Again, missing results were mostly due to poor quality DNA or incomplete enzyme digestion in the SNP protocol resulting in genotypes that could not be properly scored.

3.3.4.3 Statistical comparison between the 2010 conventional HEAR and 2010 Roundup Ready HEAR sample sets

The frequency of correct results was different (P<0.0355) between the conventional HEAR sample set and the Roundup Ready sample set from the 2010 spring greenhouse cycle with the Roundup Ready sample set performing better with an average

of 84.66% correct results than the conventional HEAR sample set with an average of 62.78% correct results. The frequency of correct results was similar (P<0.3337) between each C22:1 category between the conventional HEAR and the Roundup Ready HEAR samples sets from the 2010 spring greenhouse cycle (Table 3.3.7).

Table 3.3.7. ANOVA analysis summary table for the overall frequency of correct results and the frequency of correct results within each C22:1 category between conventional HEAR sample set and the Roundup Ready HEAR samples set from the 2010 spring greenhouse cycle

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	112.9150	2	56.4575	1.9964	0.3337	19.0000
Conv vs RR	752.6400	1	752.6400	26.6148	0.0355	18.5127
Residual	56.5579	2	28.2789			
Total	922.1129	5				

The frequency of missing E1 and E2 results was similar (P<0.0563) between the conventional HEAR sample set and the Roundup Ready sample set from the 2010 spring greenhouse cycle with an average frequency of 3.70% missing E1 results and an average frequency of 6.71% missing E2 results. The frequency of missing E1 and E2 results was also similar (P<0.0563) in each C22:1 category between the conventional HEAR sample set and the Roundup Ready HEAR samples set from the 2010 spring greenhouse cycle (Table 3.3.8).

Table 3.3.8. ANOVA analysis summary table for the overall frequency of missing E1 and E2 results and the frequency of missing E1 and E2 results within each C22:1 category between conventional HEAR sample set and the Roundup Ready HEAR samples set from the 2010 spring greenhouse cycle

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	27.3310	1	27.3310	4.9700	0.0563	5.3176
Conv vs RR	27.3310	1	27.3310	4.9700	0.0563	5.3176
Residual	43.9932	8	5.4991			
Total	98.6552	10				

3.3.5 Second round of MAS following modifications to the original protocol after DNA quality and quantity assessment

3.3.5.1 Statistical comparison within the 2011 conventional HEAR sample set

The second round of MAS was completed on BC_1F_1 progeny from conventional HEAR crosses 08C344 x 30216-C7RR, 08C344 x 30220-D8RR, 08C344 x 30221-D8RR, 08C344 x 30408-C7RR, and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following modifications to the original MAS protocol for determining erucic acid genotypes published by Rahman et al. (2008) including the results from the DNA quantity and quality analysis using NanoDrop technology (Table 3.3.9, Appendix Table A6). Erucic acid results in the 40.7-37.7% and 31.3-28.3% C22:1 ranges were excluded from the genotype-phenotype comparison since the genotype for the erucic acid genes in these ranges cannot be identified with certainty (Appendix Table A7).

Table 3.3.9. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes excluding results ranging from 40.7%-37.7% and 31.3%-28.3% C22:1 from BC1F1 progeny from conventional HEAR crosses 08C344 x 30216-C7RR, 08C344 x 30220-D8RR, 08C344 x 30221-D8RR, 08C344 x 30408-C7RR, and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 30216-C7RR	≥40.8%	24	17	5	1	1	0	2	77.27	22.73	4.17	4.17	0.00	8.33
08C344 x 30220-D8RR	≥40.8%	27	20	3	0	4	0	4	86.96	13.04	0.00	14.81	0.00	14.81
08C344 x 30221-D8RR	≥40.8%	24	10	13	1	0	0	1	43.48	56.52	4.17	0.00	0.00	4.17
08C344 x 30408-C7RR	≥40.8%	21	17	1	0	3	0	3	94.44	5.56	0.00	14.29	0.00	14.29
08C344 x 30422-C7RR	≥40.8%	23	7	7	3	4	2	9	50.00	50.00	13.04	17.39	8.70	39.13
Total	≥40.8%	119	71	29	5	12	2	19	71.00	29.00	4.20	10.08	1.68	15.97
Mean	≥40.8%		14	6	1	2	0	4	70.43	29.57	4.28	10.13	1.74	16.15
Minimum	≥40.8%		7	1	0	0	0	1	43.48	5.56	0.00	0.00	0.00	4.17
Maximum	≥40.8%		20	13	3	4	2	9	94.44	56.52	13.04	17.39	8.70	39.13
08C344 x 30216-C7RR	37.6%-31.4%	31	23	2	2	3	1	6	92.00	8.00	6.45	9.68	3.23	19.35
08C344 x 30220-D8RR	37.6%-31.4%	23	17	3	1	2	0	3	85.00	15.00	4.35	8.70	0.00	13.04
08C344 x 30221-D8RR	37.6%-31.4%	38	23	15	0	0	0	0	60.53	39.47	0.00	0.00	0.00	0.00
08C344 x 30408-C7RR	37.6%-31.4%	37	24	3	0	10	0	10	88.89	11.11	0.00	27.03	0.00	27.03
08C344 x 30422-C7RR	37.6%-31.4%	28	22	3	1	2	0	3	88.00	12.00	3.57	7.14	0.00	10.71
Total	37.6%-31.4%	157	109	26	4	17	1	22	80.74	19.26	2.55	10.83	0.64	14.01
Mean	37.6%-31.4%		22	5	1	3	0	4	82.88	17.12	2.87	10.51	0.65	14.03
Minimum	37.6%-31.4%		17	2	0	0	0	0	60.53	8.00	0.00	0.00	0.00	0.00
Maximum	37.6%-31.4%		24	15	2	10	1	10	92.00	39.47	6.45	27.03	3.23	27.03
08C344 x 30216-C7RR	≤28.2%	22	18	3	1	0	0	1	85.71	14.29	4.55	0.00	0.00	4.55
08C344 x 30220-D8RR	≤28.2%	12	10	0	1	1	0	2	100.00	0.00	8.33	8.33	0.00	16.67
08C344 x 30221-D8RR	≤28.2%	10	6	3	1	0	0	1	66.67	33.33	10.00	0.00	0.00	10.00
08C344 x 30408-C7RR	≤28.2%	14	3	2	0	9	0	9	60.00	40.00	0.00	64.29	0.00	64.29
08C344 x 30422-C7RR	≤28.2%	23	15	1	3	4	0	7	93.75	6.25	13.04	17.39	0.00	30.43
Total	≤28.2%	81	52	9	6	14	0	20	85.25	14.75	7.41	17.28	0.00	24.69
Mean	≤28.2%		10	2	1	3	0	4	81.23	18.77	7.18	18.00	0.00	25.19
Minimum	≤28.2%		3	0	0	0	0	1	60.00	0.00	0.00	0.00	0.00	4.55
Maximum	≤28.2%		18	3	3	9	0	9	100.00	40.00	13.04	64.29	0.00	64.29
Total Plants Evaluated (%)		62.45												
Overall Accuracy (%)		78.38												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

The frequency of missing E1 and E2 results was similar (P<0.5149) between each cross in the 2011 conventional HEAR sample set at an average frequency of 4.78% missing E1 results and 12.88% missing E2 results overall. The frequency of missing E1 and E2 results was also similar (P<0.0889) between each C22:1 category in the 2011 conventional HEAR sample set (Table 3.3.10).

Table 3.3.10. ANOVA analysis summary table for the frequency of missing results in the 2011 conventional HEAR sample set

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
E1 vs E2 (cross)	213.8122	2	106.9061	0.6822	0.5149	3.4028
E1 vs E2 (C22:1 category)	492.4141	1	492.4141	3.1426	0.0889	4.2596
Residual	3760.4941	24	156.6872			
Total	4748.0933	27				

The overall accuracy for the conventional HEAR sample set from the 2011 spring greenhouse cycle was 78.38%. While slightly better than the overall accuracy of the conventional HEAR sample set from the 2010 spring greenhouse cycle, it is still too low to be considered useful in a breeding program. It should also be noted at only 62.45% of samples were analyzed overall. Again, missing results were mostly due to poor quality DNA or incomplete enzyme digestion in the SNP protocol resulting in genotypes that could not be properly scored.

3.3.5.2 Statistical comparison within the 2011 Roundup Ready HEAR sample set

The second round of MAS was also completed on BC_1F_1 progeny from Roundup Ready HEAR crosses Red River 1997 x 30216-C7RR, Red River 1997 x 30220-D8RR, Red River 1997 x 30221-D8RR, Red River 1997 x 30408-C7RR, and Red River 1997 x

30422-C7RR from the 2011 spring greenhouse cycle following modifications to the original MAS protocol for determining erucic acid genotypes published by Rahman et al. in 2008 including the results from the DNA quantity and quality analysis using NanoDrop technology. (Table 3.3.11, Appendix Table A8). Erucic acid results in the 36.7-33.7% and 26.7-23.7% C22:1 ranges were excluded from the genotype-phenotype comparison since the genotype for the erucic acid genes in these ranges cannot be identified with certainty (Appendix Table A9).

Table 3.3.11. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes excluding results ranging from 36.7%-33.7% and 26.7%-23.7% C22:1 from BC1F1 progeny from Roundup Ready HEAR crosses Red River 1997 x 30216-C7RR, Red River 1997 x 30220-D8RR, Red River 1997 x 30221-D8RR, Red River 1997 x 30408-C7RR, and Red River 1997 x 30422-C7RR from the 2011 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Red River 1997 x 30216-C7RR	≥36.8%	26	22	3	0	1	0	1	88.00	12.00	0.00	3.85	0.00	3.85
Red River 1997 x 30220-D8RR	≥36.8%	30	20	8	0	2	0	2	71.43	28.57	0.00	6.67	0.00	6.67
Red River 1997 x 30221-D8RR	≥36.8%	19	11	0	0	8	0	8	100.00	0.00	0.00	42.11	0.00	42.11
Red River 1997 x 30408-C7RR	≥36.8%	20	13	3	0	4	0	4	81.25	18.75	0.00	20.00	0.00	20.00
Red River 1997 x 30422-C7RR	≥36.8%	36	17	14	0	5	0	5	54.84	45.16	0.00	13.89	0.00	13.89
Total	≥36.8%	131	83	28	0	20	0	20	74.77	25.23	0.00	15.27	0.00	15.27
Mean	≥36.8%		17	6	0	4	0	4	79.10	20.90	0.00	17.30	0.00	17.30
Minimum	≥36.8%		11	0	0	1	0	1	54.84	0.00	0.00	3.85	0.00	3.85
Maximum	≥36.8%		22	14	0	8	0	8	100.00	45.16	0.00	42.11	0.00	42.11
Red River 1997 x 30216-C7RR	33.6%-26.8%	30	26	3	0	1	0	1	89.66	10.34	0.00	3.33	0.00	3.33
Red River 1997 x 30220-D8RR	33.6%-26.8%	29	19	6	0	4	0	4	76.00	24.00	0.00	13.79	0.00	13.79
Red River 1997 x 30221-D8RR	33.6%-26.8%	35	21	1	0	13	0	13	95.45	4.55	0.00	37.14	0.00	37.14
Red River 1997 x 30408-C7RR	33.6%-26.8%	33	11	10	0	12	0	12	52.38	47.62	0.00	36.36	0.00	36.36
Red River 1997 x 30422-C7RR	33.6%-26.8%	16	8	6	0	2	0	2	57.14	42.86	0.00	12.50	0.00	12.50
Total	33.6%-26.8%	143	85	26	0	32	0	32	76.58	23.42	0.00	22.38	0.00	22.38
Mean	33.6%-26.8%		17	5	0	6	0	6	74.13	25.87	0.00	20.63	0.00	20.63
Minimum	33.6%-26.8%		8	1	0	1	0	1	52.38	4.55	0.00	3.33	0.00	3.33
Maximum	33.6%-26.8%		26	10	0	13	0	13	95.45	47.62	0.00	37.14	0.00	37.14
Red River 1997 x 30216-C7RR	≤23.6%	11	9	1	0	1	0	1	90.00	10.00	0.00	9.09	0.00	9.09
Red River 1997 x 30220-D8RR	≤23.6%	12	11	0	0	1	0	1	100.00	0.00	0.00	8.33	0.00	8.33
Red River 1997 x 30221-D8RR	≤23.6%	16	7	2	0	7	0	7	77.78	22.22	0.00	43.75	0.00	43.75
Red River 1997 x 30408-C7RR	≤23.6%	9	9	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
Red River 1997 x 30422-C7RR	≤23.6%	23	7	7	3	4	2	9	50.00	50.00	13.04	17.39	8.70	39.13
Total	≤23.6%	71	43	10	3	13	2	18	81.13	18.87	4.23	18.31	2.82	25.35
Mean	≤23.6%		9	2	1	3	0	4	83.56	16.44	2.61	15.71	1.74	20.06
Minimum	≤23.6%		7	0	0	0	0	0	50.00	0.00	0.00	0.00	0.00	0.00
Maximum	≤23.6%		11	7	3	7	2	9	100.00	50.00	13.04	43.75	8.70	43.75
Total Plants Evaluated (%)		58.51												
Overall Accuracy (%)		76.73												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

The frequency of missing E1 and E2 results was different (P<0.0004) between each cross in the 2011 Roundup Ready HEAR sample set at an average frequency of 0.87% missing E1 results and 17.88% missing E2 results overall. The frequency of missing E1 and E2 results was similar (P<0.9460) between each C22:1 category in the 2011 Roundup Ready HEAR sample set (Table 3.3.12).

Table 3.3.12. ANOVA analysis summary table for the frequency of missing results in the 2011 Roundup Ready HEAR sample set

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
E1 vs E2 (C22:1 category)	14.5084	2	7.2542	0.0556	0.9460	3.4028
E1 vs E2 (cross)	2170.2543	1	2170.2543	16.6369	0.0004	4.2596
Residual	3130.7428	24	130.4476			
Total	5315.5055	27				

In this case, it was discovered that progeny from certain crosses, namely Red River 1997 x 30221-D8RR and Red River 1997 x 30408-C7RR from the 2011 Roundup Ready HEAR material produced a four base pair change in the SCAR marker when a two base pair change is expected. This would explain why the frequency of missing E2 results was higher than the frequency of missing E1 results since when a four base pair change was detected, it was scored as a missing results. For this, a SNP marker for the E2e2 gene of the C genome was developed to replace the C genome SCAR marker in progeny of crosses where a four base pair change in the SCAR marker appears when a two base pair change is expected and to see if it was possible to further improve/simplify the marker system.

The overall accuracy for the Roundup Ready HEAR sample set from the 2011 spring greenhouse cycle was 76.73% which is similar to the overall accuracy of the conventional HEAR sample set from the 2011 spring greenhouse cycle; however, both

are still too low to be considered useful in a breeding program. It should also be noted at only 58.51% of samples were analyzed overall. Again, missing results were mostly due to poor quality DNA, incomplete enzyme digestion in the SNP protocol, or the 4 base pair change in the C genome SCAR marker when a 2 base pair change was expected resulting in genotypes that could not be properly scored.

3.3.5.3 Statistical comparison between the 2011 conventional HEAR and 2011 Roundup Ready HEAR sample sets

The frequency of correct results was similar (P<0.6260) between the conventional HEAR sample set and the Roundup Ready sample set from the 2011 spring greenhouse cycle with an average of 78.38% correct results in the conventional HEAR sample set and an average of 76.73% correct results in the Roundup Ready HEAR sample set. The frequency of correct results was also similar (P<0.1634) between each C22:1 category between the conventional HEAR and the Roundup Ready HEAR samples sets from the 2011 spring greenhouse cycle (Table 3.3.13).

Table 3.3.13. ANOVA analysis summary table for the overall frequency of correct results and the frequency of correct results within each C22:1 category between conventional HEAR sample set and the Roundup Ready HEAR samples set from the 2011 spring greenhouse cycle

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	106.7097	2	53.3548	5.1163	0.1634	19.0000
Conv vs RR	3.3900	1	3.3900	0.3250	0.6260	18.5127
Residual	20.8564	2	10.4282			
Total	130.9561	5				

The frequency of missing E1 and E2 results was different (P<0.0261) between the conventional HEAR sample set and the Roundup Ready sample set from the 2011 spring

greenhouse cycle with an average frequency of 6.01% missing E1 results and an average frequency of 12.67% missing E2 results. Again, this difference is due to the fact certain progeny in the 2011 Roundup Ready HEAR material produced a four base pair change in the SCAR marker when a two base pair change is expected. This would explain why the frequency of missing E2 results was higher than the frequency of missing E1 results since when a four base pair change was detected, it was scored as a missing result. The frequency of missing E1 and E2 results ($P<0.5876$) in each C22:1 category was similar between the conventional HEAR sample set and the Roundup Ready HEAR samples set from the 2011 spring greenhouse cycle (Table 3.3.14).

Table 3.3.14. ANOVA analysis summary table for the overall frequency of missing E1 and E2 results and the frequency of missing E1 and E2 results within each C22:1 category between conventional HEAR sample set and the Roundup Ready HEAR samples set from the 2011 spring greenhouse cycle

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	5.7132	1	5.7132	0.3190	0.5876	5.3176
Conv vs RR	132.6675	1	132.6675	7.4079	0.0261	5.3176
Residual	143.2713	8	17.9089	143.2713		
Total	281.6520	10				

The frequency of correct results was similar ($P<0.4761$) between C22:1 categories in the conventional HEAR from the 2010 and the 2011 spring greenhouse cycles. The overall accuracy also similar ($P<0.1056$) between the conventional HEAR results from the 2010 and the 2011 spring greenhouse cycles at 62.78% and 78.38% indicating that the modifications made to the protocols in the second round of MAS did not provide significant improvements to the overall method (Table 3.3.15).

Table 3.3.15. ANOVA analysis summary table for the overall frequency of correct results and the frequency of correct results within each C22:1 category between conventional HEAR sample sets from the 2010 and 2011 spring greenhouse cycles

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	114.2103	2	57.1051	1.1001	0.4761	19.0000
2010 vs 2011	415.0016	1	415.0016	7.9949	0.1056	18.5127
Residual	103.8158	2	51.9079			
Total	633.0278	5				

The frequency of missing E1 and E2 results was similar (P<0.0508) between the conventional HEAR sample sets from the 2010 and the 2011 spring greenhouse cycles with an average frequency of 5.95% missing E1 results and an average frequency of 6.39% missing E2 results. The frequency of missing E1 and E2 results was similar (P<0.8436) in each C22:1 category between the conventional HEAR sample set from the 2010 and the 2011 spring greenhouse cycles (Table 3.3.16).

Table 3.3.16. ANOVA analysis summary table for the overall frequency of missing E1 and E2 results and the frequency of missing E1 and E2 results within each C22:1 category from the conventional HEAR sample sets and from the 2010 and 2011 spring greenhouse cycles

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	73.7056	1	73.7056	5.2698	0.0508	5.3176
2010 vs 2011	0.5808	1	0.5808	0.0415	0.8436	5.3176
Error	111.8906	8	13.9863			
Total	186.1770	10				

The frequency of correct results was similar (P<0.1786) between the Roundup Ready HEAR sample sets from the 2010 and 2011 spring greenhouse cycles at 84.66% and 76.73% respectively. The frequency of correct results was also similar (P<0.0786) in each C22:1 category from the Roundup Ready HEAR sample sets from the 2010 and 2011 spring greenhouse cycles (Table 3.3.17).

Table 3.3.17. ANOVA analysis summary table for the overall frequency of correct results and the frequency of correct results within each C22:1 category between Roundup Ready HEAR sample sets from the 2010 and 2011 spring greenhouse cycles

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	64.8972	2	32.4486	4.5975	0.1786	19.0000
2010 vs 2011	79.2793	1	79.2793	11.2327	0.0786	18.5127
Residual	14.1157	2	7.0578			
Total	158.2922	5				

The frequency of missing E1 and E2 results was different ($P<0.0008$) between the Roundup Ready HEAR sample sets from the 2010 and the 2011 spring greenhouse cycles with an average frequency of 3.76% missing E1 results and an average frequency of 12.99% missing E2 results. The frequency of missing E1 and E2 results was also different ($P<0.0025$) in each C22:1 category between the Roundup Ready HEAR sample set from the 2010 and the 2011 spring greenhouse cycles (Table 3.3.18).

Table 3.3.18. ANOVA analysis summary table for the overall frequency of missing E1 and E2 results and the frequency of missing E1 and E2 results within each C22:1 category from the Roundup Ready HEAR sample sets and from the 2010 and 2011 spring greenhouse cycles

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
C22:1 category	33.0340	1	33.0340	3.5061	0.0980	5.3176
2010 vs 2011	255.4864	1	255.4864	27.1166	0.0008	5.3176
Error	75.3739	8	9.4217			
Total	363.8943	10				

3.3.6 Development of a SNP marker to replace the SCAR marker for the E2 allele in the C genome

The third round of MAS was completed on BC_1F_1 progeny from conventional HEAR crosses 08C344 x 30408-C7RR and 08C344 x 30422-C7RR, and the Roundup

Ready HEAR crosses Red River 1997 x 30221-D8RR and Red River 1997 x 30408-C7RR from the 2011 spring greenhouse cycle following the newly developed SNP protocol to replace the SCAR marker for E2 allele in C genome to account for the four base pair change in the SCAR marker when a two base pair change is expected. The genotype of the E1 allele was determined following the same modifications to the original MAS protocol for determining erucic acid genotypes as published by Rahman et al. (2008) in the second round of MAS (Table 3.3.19, Table 3.3.20, Appendix Table A10, Appendix Table A12). Erucic acid results in the 40.7-37.7% and 31.3-28.3% C22:1 range for the conventional HEAR and 36.7-33.7% and 26.7-23.7% C22:1 ranges for the Roundup Ready HEAR were excluded since the genotype for the erucic acid genes in these ranges cannot be identified with certainty (Appendix Table A11, Appendix Table A13).

Table 3.3.19. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes excluding results ranging from 40.7%-37.7% and 31.3%-28.3% C22:1 from BC1F1 progeny from conventional HEAR crosses 08C344 x 30408-C7RR and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following the regular and newly developed SNP MAS procedures for determining erucic acid genotypes

			Regular SCAR Procedure											
			Number						Frequency (%)					
Pedigree (BC1F1)	Category[z]	Sample Number	Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 30408-C7RR	≥40.8%	21	17	1	0	3	0	3	94.44	5.56	0.00	14.29	0.00	14.29
08C344 x 30422-C7RR	≥40.8%	23	7	7	3	4	2	9	50.00	50.00	13.04	17.39	8.70	39.13
Total	≥40.8%	44	24	8	3	7	2	12	75.00	25.00	6.82	15.91	4.55	27.27
Mean	≥40.8%		12	4	2	4	1	6	72.22	27.78	6.52	15.84	4.35	26.71
Minimum	≥40.8%		7	1	0	3	0	3	50.00	5.56	0.00	14.29	0.00	14.29
Maximum	≥40.8%		17	7	3	4	2	9	94.44	50.00	13.04	17.39	8.70	39.13
08C344 x 30408-C7RR	37.6%-31.4%	37	24	3	0	10	0	10	88.89	11.11	0.00	27.03	0.00	27.03
08C344 x 30422-C7RR	37.6%-31.4%	28	22	3	1	2	0	3	88.00	12.00	3.57	7.14	0.00	10.71
Total	37.6%-31.4%	65	46	6	1	12	0	13	88.46	11.54	1.54	18.46	0.00	20.00
Mean	37.6%-31.4%		23	3	1	6	0	7	88.44	11.56	1.79	17.08	0.00	18.87
Minimum	37.6%-31.4%		22	3	0	2	0	3	88.00	11.11	0.00	7.14	0.00	10.71
Maximum	37.6%-31.4%		24	3	1	10	0	10	88.89	12.00	3.57	27.03	0.00	27.03
08C344 x 30408-C7RR	≤28.2%	14	3	2	0	9	0	9	60.00	40.00	0.00	64.29	0.00	64.29
08C344 x 30422-C7RR	≤28.2%	23	15	1	3	4	0	7	93.75	6.25	13.04	17.39	0.00	30.43
Total	≤28.2%	37	18	3	3	13	0	16	85.71	14.29	8.11	35.14	0.00	43.24
Mean	≤28.2%		9	2	2	7	0	8	76.88	23.13	6.52	40.84	0.00	47.36
Minimum	≤28.2%		3	1	0	4	0	7	60.00	6.25	0.00	17.39	0.00	30.43
Maximum	≤28.2%		15	2	3	9	0	9	93.75	40.00	13.04	64.29	0.00	64.29
Total Plants Evaluated (%)		54.97												
Overall Accuracy (%)		83.81												

Pedigree (BC1F1)	Category[z]	Sample Number	New SNP Procedure											
			Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 30408-C7RR	≥40.8%	21	9	9	0	3	0	3	50.00	50.00	0.00	14.29	0.00	14.29
08C344 x 30422-C7RR	≥40.8%	23	6	11	4	2	0	6	35.29	64.71	17.39	8.70	0.00	26.09
Total	≥40.8%	44	15	20	4	5	0	9	42.86	57.14	9.09	11.36	0.00	20.45
Mean	≥40.8%		8	10	2	3	0	5	42.65	57.35	8.70	11.49	0.00	20.19
Minimum	≥40.8%		6	9	0	2	0	3	35.29	50.00	0.00	8.70	0.00	14.29
Maximum	≥40.8%		9	11	4	3	0	6	50.00	64.71	17.39	14.29	0.00	26.09
08C344 x 30408-C7RR	37.6%-31.4%	37	16	15	0	6	0	6	51.61	48.39	0.00	16.22	0.00	16.22
08C344 x 30422-C7RR	37.6%-31.4%	28	23	2	1	2	0	3	92.00	8.00	3.57	7.14	0.00	10.71
Total	37.6%-31.4%	65	39	17	1	8	0	9	69.64	30.36	1.54	12.31	0.00	13.85
Mean	37.6%-31.4%		20	9	1	4	0	5	71.81	28.19	1.79	11.68	0.00	13.47
Minimum	37.6%-31.4%		16	2	0	2	0	3	51.61	8.00	0.00	7.14	0.00	10.71
Maximum	37.6%-31.4%		23	15	1	6	0	6	92.00	48.39	3.57	16.22	0.00	16.22
08C344 x 30408-C7RR	≤28.2%	14	5	1	0	4	4	8	83.33	16.67	0.00	28.57	28.57	57.14
08C344 x 30422-C7RR	≤28.2%	23	19	0	3	1	0	4	100.00	0.00	13.04	4.35	0.00	17.39
Total	≤28.2%	37	24	1	3	5	4	12	96.00	4.00	8.11	13.51	10.81	32.43
Mean	≤28.2%		12	1	2	3	2	6	91.67	8.33	6.52	16.46	14.29	37.27
Minimum	≤28.2%		5	0	0	1	0	4	83.33	0.00	0.00	4.35	0.00	17.39
Maximum	≤28.2%		19	1	3	4	4	8	100.00	16.67	13.04	28.57	28.57	57.14
Total Plants Evaluated (%)		60.73												
Overall Accuracy (%)		67.24												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

Table 3.3.20. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes excluding results ranging from 36.7%-33.7% and 26.7%-23.7% C22:1 from BC1F1 progeny from Roundup Ready HEAR crosses Red River 1997 x 30221-D8RR and Red River 1997 x 30408-C7RR from the 2011 spring greenhouse cycle following the regular and newly developed SNP MAS procedure for determining erucic acid genotypes

			Regular SCAR Procedure											
			Number						Frequency (%)					
Pedigree (BC1F1)	Category[z]	Sample Number	Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Red River 1997 x 30221-D8RR	≥36.8%	19	11	0	0	8	0	8	100.00	0.00	0.00	42.11	0.00	42.11
Red River 1997 x 30408-C7RR	≥36.8%	20	13	3	0	4	0	4	81.25	18.75	0.00	20.00	0.00	20.00
Total	≥36.8%	39	24	3	0	12	0	12	88.89	11.11	0.00	30.77	0.00	30.77
Mean	≥36.8%		12	2	0	6	0	6	90.63	9.38	0.00	31.05	0.00	31.05
Minimum	≥36.8%		11	0	0	4	0	4	81.25	0.00	0.00	20.00	0.00	20.00
Maximum	≥36.8%		13	3	0	8	0	8	100.00	18.75	0.00	42.11	0.00	42.11
Red River 1997 x 30221-D8RR	33.6%-26.8%	35	21	1	0	13	0	13	95.45	4.55	0.00	37.14	0.00	37.14
Red River 1997 x 30408-C7RR	33.6%-26.8%	33	11	10	0	12	0	12	52.38	47.62	0.00	36.36	0.00	36.36
Total	33.6%-26.8%	68	32	11	0	25	0	25	74.42	25.58	0.00	36.76	0.00	36.76
Mean	33.6%-26.8%		16	6	0	13	0	13	73.92	26.08	0.00	36.75	0.00	36.75
Minimum	33.6%-26.8%		11	1	0	12	0	12	52.38	4.55	0.00	36.36	0.00	36.36
Maximum	33.6%-26.8%		21	10	0	13	0	13	95.45	47.62	0.00	37.14	0.00	37.14
Red River 1997 x 30221-D8RR	≤23.6%	16	7	2	0	7	0	7	77.78	22.22	0.00	43.75	0.00	43.75
Red River 1997 x 30408-C7RR	≤23.6%	9	9	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
Total	≤23.6%	25	16	2	0	7	0	7	88.89	11.11	0.00	28.00	0.00	28.00
Mean	≤23.6%		8	1	0	4	0	4	88.89	11.11	0.00	21.88	0.00	21.88
Minimum	≤23.6%		7	0	0	0	0	0	77.78	0.00	0.00	0.00	0.00	0.00
Maximum	≤23.6%		9	2	0	7	0	7	100.00	22.22	0.00	43.75	0.00	43.75
Total Plants Evaluated (%)		50.87												
Overall Accuracy (%)		81.82												

Pedigree (BC1F1)	Category[z]	Sample Number	New SNP Procedure											
			Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Red River 1997 x 30221-D8RR	≥36.8%	19	9	5	0	2	0	2	44.44	55.56	0.00	10.00	0.00	10.00
Red River 1997 x 30408-C7RR	≥36.8%	20	9	4	0	2	0	2	55.56	44.44	0.00	18.18	0.00	18.18
Total	≥36.8%	39	18	9	0	4	0	4	66.67	33.33	0.00	10.26	0.00	10.26
Mean	≥36.8%		9	5	0	2	0	2	50.00	50.00	0.00	14.09	0.00	14.09
Minimum	≥36.8%		9	4	0	2	0	2	44.44	44.44	0.00	10.00	0.00	10.00
Maximum	≥36.8%		9	5	0	2	0	2	55.56	55.56	0.00	18.18	0.00	18.18
Red River 1997 x 30221-D8RR	33.6%-26.8%	34	22	13	0	0	0	0	64.86	35.14	0.00	5.13	0.00	5.13
Red River 1997 x 30408-C7RR	33.6%-26.8%	33	17	14	0	2	0	2	61.11	38.89	0.00	5.26	0.00	5.26
Total	33.6%-26.8%	67	39	27	0	2	0	2	59.09	40.91	0.00	2.99	0.00	2.99
Mean	33.6%-26.8%		20	14	0	1	0	1	62.99	37.02	0.00	5.20	0.00	5.20
Minimum	33.6%-26.8%		17	13	0	0	0	0	61.11	35.14	0.00	5.13	0.00	5.13
Maximum	33.6%-26.8%		22	14	0	2	0	2	64.86	38.89	0.00	5.26	0.00	5.26
Red River 1997 x 30221-D8RR	≤23.6%	23	7	18	0	2	0	2	33.33	66.67	0.00	10.00	0.00	10.00
Red River 1997 x 30408-C7RR	≤23.6%	16	10	21	0	0	0	0	40.00	60.00	0.00	0.00	0.00	0.00
Total	≤23.6%	39	17	39	0	2	0	2	30.36	69.64	0.00	5.13	0.00	5.13
Mean	≤23.6%		9	20	0	1	0	1	36.67	63.34	0.00	5.00	0.00	5.00
Minimum	≤23.6%		7	18	0	0	0	0	33.33	60.00	0.00	0.00	0.00	0.00
Maximum	≤23.6%		10	21	0	2	0	2	40.00	66.67	0.00	10.00	0.00	10.00
Total Plants Evaluated (%)		73.26												
Overall Accuracy (%)		54.01												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

The frequency of missing E2 results was similar (P<0.1500) between the original SCAR protocol when compared to the newly developed SNP marker for the E2 loci in the C genome for the 2011 conventional HEAR sample set at an average frequency of 23.17% missing E2 results from the SCAR maker and 12.39% for the SNP marker. On the other hand, the frequency of missing E2 results was different (P<0.0015) between the original SCAR protocol when compared to the newly developed SNP marker for the E2 loci in the C genome for the 2011 Roundup Ready HEAR sample set with the average frequency of missing E2 results at 31.84% and 6.13% respectively. This proves that the newly developed SNP marker is better at determining genotypes than the SCAR method (Table 3.3.21).

Table 3.3.21. ANOVA analysis summary table for the overall frequency of missing E2 results between the original SCAR protocol and the newly developed SNP protocol for the E2e2 gene in the C genome for the BC_1F_1 progeny from the conventional HEAR crosses 08C344 x 30408-C7RR and 08C344 x 30422-C7RR and the Roundup Ready HEAR crosses Red River 1997 x 30221-D8RR and Red River 1997 x 30408-C7RR from the 2011 spring greenhouse cycle

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
SCAR vs SNP (conv)	174.2048	1	174.2048	3.1602	0.1500	7.7086
SCAR vs SNP (RR)	992.0204	1	992.0204	58.3427	0.0015	7.7086
Residual	2386.4543	8	298.3067			
Total	3552.6795	10				

The overall frequency of correct results was similar (P<0.4333) between the original SCAR protocol and the newly developed SNP protocol. The overall frequency of correct results was also similar (P<0.0516) between the original SCAR protocol and the newly developed SNP protocol between the conventional HEAR crosses and the Roundup Ready crosses (Table 3.3.22). This indicates that the newly developed SNP protocol provides neither better nor worse accuracy than the original SCAR protocol.

Since the SCAR protocol is less time consuming and less complicating, it would be beneficial to continue using it rather than replacing it with a SNP protocol providing improvements are made to increase the accuracy and to decrease the number of missing results. If the SNP protocol for the C genome could be successfully combined with the SNP protocol for the A genome and only one reaction could be completed per sample using different primers for each SNP, it may be even more beneficial in the fact that it would require less time and less reagents. Further testing would be required to validate the "two SNP method" however.

Table 3.3.22. ANOVA analysis summary table for the frequency of correct results between the original SCAR protocol and the newly developed SNP protocol for the E2e2 gene in the C genome for the BC_1F_1 progeny from the conventional HEAR crosses 08C344 x 30408-C7RR and 08C344 x 30422-C7RR and the Roundup Ready HEAR crosses Red River 1997 x 30221-D8RR and Red River 1997 x 30408-C7RR from the 2011 spring greenhouse cycle

Source	Sums of squares	Degrees of freedom	Mean square	F-value	P-value	F-critical
SCAR vs SNP	202.9518	1	202.9518	0.6803	0.4333	5.3176
SCAR vs SNP (conv/RR)	1558.3802	1	1558.3802	5.2240	0.0516	5.3176
Residual	2386.4543	8	298.3067			
Total	4147.7863	10				

3.3.7 Conclusions

Overall it appears that there was no difference between the overall frequency of correct genotype identification between the conventional HEAR and the Roundup Ready HEAR samples. No significant improvements were made between 2010 and 2011 after modifications to the protocol. One possible cause could be that the primers that were originally developed by Rahman et al. (2008) are parent specific so perhaps the parental lines/cultivars should be sequenced each time a new parent is chosen and new primers may need to be designed each time; however, this would not be very efficient.

There are many steps in this entire process in which errors can occur resulting in incorrect genotype identification. Contamination during the DNA extraction process is a major factor when trying to operate in a high throughput manner. The SNP protocol is complex and has many steps in which precision measuring of reagents is required. It is necessary to find a way to correct for these problems to be able to depend on these markers to properly identify erucic acid genotypes.

It can be noted that the overall accuracy is not high enough to be able to use these markers reliably in a breeding program. The frequency of missing results was also too high to be acceptable and it did not seem to improve substantially from one year to the next. It would also be beneficial to improve these markers to be able to score all erucic acids genotypes in all C22:1 categories including the ranges that were currently excluded from the analysis since the genotypes for erucic acid cannot be identified with certainty. Further research is needed to perfect the molecular markers for erucic acid genes in *Brassica napus* seedling samples. Until the error rate can be significantly reduced and the number of missing results can be greatly decreased, it is not practical to apply these markers in a breeding program.

4.0 NIR BASED CHLOROPHYLL CALIBRATION EQUATION DEVELOPMENT FOR WHOLE *BRASSICA NAPUS* SEED SAMPLES

4.1 Introduction

The worldwide production of *Brassica napus* has widely increased in the last thirty years. It is now one of the most widely cultivated oilseed crops in the world. It is grown primarily as an edible oil and to a lesser amount as an industrial oil. *Brassica napus* is most commonly known as canola or rapeseed.

In Canada, rapeseed/canola seeds are assigned a grade based on their oil concentration, protein concentration, glucosinolate concentration, and chlorophyll concentration as well as their fatty acid profile. Chlorophyll, a green pigment, that is vital for photosynthesis, also affects seed quality. When chlorophyll is extracted along with the oil, it receives a lower grade thereby reducing the profit. Other disadvantages of chlorophyll in the oil include an undesirable green color, early rancidity, and difficulty removing during routine processing (Abraham and deMan 1986, Daun 1976). It is therefore desirable to have the lowest possible concentration of chlorophyll in canola/rapeseed oil.

Traditionally, chlorophyll concentration has been determined by extraction from the seed with an organic solvent and measuring the absorbance on a spectrophotometer at 670 nm or by comparing the percentage of green seed to the overall sample color (Appelqvist and Johannson 1967, Tkachuk et al. 1988). Neither method is preferred as there are major disadvantages to both. While the first method produces fairly accurate results, it is time consuming, requires the use of organic solvents, and destroys whole seeds. The second method also destroys whole seeds, is not very accurate, and has been

shown to correlate poorly with actual extracted chlorophyll concentrations (Tkachuk et al. 1988).

There is a growing need to find a more accurate, easier, and less time consuming method to determine chlorophyll concentration in whole *Brassica napus* seeds. Many labs currently use a FOSS 6500 near infrared reflectance (NIR) spectrophotometer to determine oil, protein, and glucosinolate concentration; it would therefore be advantageous to use this machine to determine chlorophyll concentration as well since it would be less expensive and less time consuming.

NIR spectroscopy detects bonds such as carbon-hydrogen bonds (CH), nitrogen-hydrogen bonds (NH), and oxygen-hydrogen bonds (OH) in biological substances and is therefore quite useful in the analysis of biological materials such as protein, fat, moisture, chlorophyll, etc. When electromagnetic radiation encounters a substance, it will either be absorbed or transmitted depending upon its frequency and the molecular structure of the substance. A spectrophotometer measures the relative amount of light at different wavelengths that is absorbed or transmitted by a sample solution and produces a spectrum. Then, computer software such as WinISI interprets the spectrum and predicts the concentration of the constituent being measured. For this, a calibration equation that is specific to the material that is being measured is required.

This particular application of the NIR spectrophotometer is unique in that the measurements for chlorophyll are taken in the visible range of the electromagnetic spectrum. The NIR instrument simply acts as a convenient method for scanning a sample with reflected light.

The purpose of this study was to develop a calibration equation for chlorophyll concentration in whole *Brassica napus* seeds using a selection of samples with a wide range of chlorophyll concentration for a FOSS 6500 near infrared reflectance (NIR) spectrophotometer.

4.2 Materials and Methods

4.2.1 Seed samples

A set of 112 whole *Brassica napus* seed samples with a wide range of chlorophyll concentrations were obtained from the Canadian Grain Commission (CGC) (Table 4.2.1) and a set of 119 whole *Brassica napus* seed samples with a wide range of chlorophyll concentrations were obtained from the University of Manitoba (Table 4.2.2). The samples obtained from the CGC and the University of Manitoba comprised the calibration set and were used in the development of the chlorophyll calibration equation. A set of twenty samples was randomly chosen from the calibration set to serve as the validation set to monitor the equation (Table 4.2.3). Chlorophyll concentration in each of the above sets was determined by the CGC or by the author and the staff in the Brassica seed quality lab at the University of Manitoba using traditional extraction methods.

The Grain Research Laboratory (GRL) at the CGC also supplied the 2011 Laboratory Certification Set of 12 whole seed samples as a part of the Laboratory Proficiency Program (Table 4.2.4). These samples were used to verify the performance of the equation and the results were submitted to the GRL to allow the Brassica seed quality lab to receive new certification for NIR determination of whole seed chlorophyll concentration.

Table 4.2.1. List of samples along with chlorophyll concentration (chll) in parts per million (ppm) obtained from the Canadian Grain Commission

ID	chll	ID	chll	ID	chll
1204020	23.6	1328355	37.3	201	22.7
1204027	26.9	1328529	30.4	228	16.2
1204028	32.2	1328660	17.7	234	28.7
1204041	37.5	1331242	23.1	239	43.8
1204088	43.7	1346054	36.8	255	38.0
1204174	27.7	CN00CL11	25.2	276	28.2
1204231	11.5	CN01CL13	55.8	283	16.7
1217125	28.4	CN02HF31	20.9	324	9.9
1217193	26.1	CN02HF32	38.7	333	30.8
1217407	20.1	CN02HF33	58.8	376	45.3
1217446	17.6	CN02HF35	91.3	393	22.0
1217731	33.4	CN03CL10	28.9	414	24.0
1222132	19.8	CN03CL12	49.8	432	27.2
1222928	28.4	CN04CL08	27.6	450	43.3
1223922	24.9	CN04CL09	32.4	457	35.7
1224925	52.9	CN04CL10	43.1	501	32.0
1225465	4.4	CN04CL11	66.3	525	25.5
1227785	12.6	CN04CL15	27.9	599	43.2
1227839	41.3	CN04CL15	32.6	608	13.1
1227851	5.8	CN05CL09	31.5	610	25.6
1227968	13.7	CN06CL02	14.1	623	17.9
1228059	2.6	CN06CL04	10.6	628	11.2
1228084	16.2	CN06CL14	12.5	700	31.9
1228114	21.5	CN07CL08	6.5	714	10.4
1317041	26.3	CN07CL12	13.2	720	41.4
1317044	28.6	CN07CL17	33.1	747	21.4
1317101	21.6	CN07CL18	49.5	777	32.5
1317610	24.9	CN6CL017	36.9	789	18.6
1317806	32.5	101	46.2	801	28.1
1317808	16.2	107	34.4	802	29.5
1317809	82.1	110	24.0	809	40.5
1317810	72.6	111	31.6	816	12.6
1317811	33.4	112	16.1	869	25.1
1317812	53.3	121	26.5	876	12.5
1317813	28.6	161	31.1	912	23.2
1322623	29.7	171	33.4	931	40.9
1328042	18.5	199	38.5	998	17.6
1328130	23.3				

Table 4.2.2. List of samples along with chlorophyll concentration (chll) in parts per million (ppm) obtained from the University of Manitoba

ID	chll	ID	chll	ID	chll
2004 - OP268	13.8	2006 - C898	9.6	2008 - C351	19.2
2004 - OP109	11.5	2006 - C648	8.7	2008 - C899	11.0
2004 - OP444	10.7	2006 - C913	13.6	2009 - C673	5.7
2004 - GF461	14.0	2006 - C802	24.5	2009 - C708	10.5
2004 - GF452	12.9	2007 - GF242	5.1	2009 - C165	14.4
2004 - GF133	2.5	2007 - GF221	10.6	2009 - C182	17.6
2004 - C229	14.6	2007 - OP502	11.2	2009 - C035	27.2
2004 - C092	44.6	2007 - OP126	9.5	2009 - OP213	9.4
2004 - C893	15.0	2007 - OP656	24.5	2009 - OP386	8.9
2004 - C698	13.6	2007 - OP171(07)	34.5	2009 - OP724	10.7
2005 - GF121	4.8	2007 - GF936	10.0	2009 - OP325	10.5
2005 - OP706	13.3	2007 - GF849	12.2	2009 - OP848	8.8
2005 - OP529	15.9	2007 - GF752	12.5	2009 - OP494	8.6
2005 - OP862	15.1	2007 - GF406	8.4	2009 - GF163	11.8
2005 - OP171(05)	16.2	2007 - OP533	8.4	2009 - GF912	12.2
2005 - OP394	14.3	2007 - C988	8.9	2009 - GF008	10.3
2005 - OP613	12.6	2007 - C846	24.5	2009 - GF806	26.1
2005 - GF608	27.6	2007 - C449	32.0	2010 - C389	5.1
2005 - GF907	24.5	2007 - C572	8.2	2010 - C164	7.9
2005 - GF216	27.9	2008 - GF028	25.5	2010 - C502	7.3
2005 - C306	29.3	2008 - GF075	7.7	2010 - C297	15.4
2005 - C205	14.9	2008 - GF195	13.2	2010 - C327	29.7
2005 - C912	37.9	2008 - GF310	14.9	2010 - C190	33.2
2005 - C859	24.5	2008 - GF569	13.5	2010 - OP617	12.1
2005 - C528	61.7	2008 - OP041a	33.1	2010 - OP169	7.5
2006 - GF212	8.8	2008 - OP226	11.1	2010 - OP831	9.4
2006 - OP172	11.4	2008 - OP231	10.8	2010 - OP554	9.1
2006 - OP117	11.9	2008 - OP497	10.8	2010 - OP911	8.7
2006 - OP665	12.2	2008 - OP436	12.1	2010 - GF471	13.7
2006 - GF369	31.5	2008 - C337	6.2	2010 - GF619	10.2
2006 - GF604	11.7	2008 - C082	20.3	2010 - GF342	10.2
2006 - C725	38.8	2008 - C735	43.1	2010 - GF791	12.9
2006 - C672	13.2	2008 - C093	14.4		

Table 4.2.3. List of samples in the validation set along with chlorophyll concentration (chll) in parts per million (ppm) obtained from the University of Manitoba

ID	chll
2004 - C905	42.7
2004 - GF516	4.9
2004 - GF340	11.9
2004 - C496	26.4
2004 - OP688	10.8
2004 - GF377	20.5
2004 - OP041b	18.9
2005 - GF105	17.5
2005 - C711	39.6
2006 - OP344	9.8
2006 - GF422	60.2
2006 - GF948	14.4
2006 - OP599	17.8
2007 - OP346	10.7
2007 - C762	20.5
2007 - C619	12.8
2008 - OP352	8.5
2009 - C151	25.7
2009 - GF481	8.1
2010 - OP491	12.1

Table 4.2.4. List of samples in the 2011 Laboratory Certification Set obtained from the Grain Research Laboratory. Chlorophyll concentration (chll) is unknown and will be determined by NIR and by traditional extraction methods

ID	chll
2011 - C221	n/a
2011 - C261	n/a
2011 - C349	n/a
2011 - C353	n/a
2011 - C446	n/a
2011 - C514	n/a
2011 - C571	n/a
2011 - C678	n/a
2011 - C701	n/a
2011 - C704	n/a
2011 - C878	n/a
2011 - C968	n/a

4.2.2 Protocol for traditional extraction method for determining chlorophyll concentration

Chlorophyll concentration was determined by extraction following a modified version of the protocol set out by the International Organization for Standardization - ISO 10519:1997 (E), Rapeseed— Determination of chlorophyll content—Spectrometric method.

Approximately 10 g of whole seeds from each sample was weighed and placed into a wide glass tube. To reduce the moisture content of the seeds to below 8%, the samples were dried overnight in the oven at 45°C. All glassware/steel extraction tubes were dried in the oven overnight as well to remove all traces of water. The following day, the tubes were capped quickly upon removal from the oven to prevent the seeds from

absorbing moisture from the air. The extraction solvent, which consists of three parts anhydrous technical n-heptane to one part anhydrous ethanol, was prepared on the day of the extractions. The presence of more than one phase of extraction solvent indicates the presence of excessive moisture. If moisture is present, dry molecular sieve, size 3A, 8-12 mesh, must be added to the ethanol and left to stand overnight. The extraction solvent must be remade the following day in that case.

To prepare a sample for extraction, the seeds were ground in a coffee grinder for a total of 14 seconds and stirred once during the grinding process to ensure even grinding throughout the sample. Once ground, exactly 2.00 grams of ground material was added to the stainless steel extraction tubes containing two steel Dangoumau ball mills. Precision weighing is critical since small differences in weight can greatly affect the end results. The coffee grinder was wiped clean with a KimWipe after each sample. Next, 25 ml of extraction solvent was added to each extraction tube which were then tightly capped. The extraction tubes were added to a specially designed wooden container, which securely holds 18 tubes. The container was then placed on its side into the Eberbach shaker and shaken at 180 rpm for 2 hours. After two hours, the wooden container was removed from the shaker, placed in the upright position and left to stand for 20 to 30 minutes to allow the contents to settle. Next, two-thirds of the extract was pipette out and filtered into test tubes. The test tubes were capped immediately to prevent evaporation, placed into a cardboard box, and covered to prevent light from reaching the samples since chlorophyll degrades in the light.

To determine the amount of chlorophyll in parts per million (ppm) in each sample following the extraction, approximately 3 ml of the extract was added to a quartz cuvette

and the absorbance was read on a spectrophotometer at the following wavelengths: 625 nm, 665 nm, and 705 nm. The cuvette was thoroughly rinsed in between each sample using a small amount of extraction solvent. The extraction solvent also served as the instrument blank. The absorbance values were converted to chlorophyll concentration in ppm using the following formula:

$$\text{Chlorophyll ppm} = \frac{k \times A_{corr} \times V}{m \times l}$$

where, "k" is the absorption coefficient of chlorophyll and is equal to 13, "A_{corr}" refers to the corrected absorbance ($A_{665} - (A_{705} + A_{625})/2$), "V" is the volume of extraction solvent which is equal to 25 ml, "m" is the weight of ground material which is equal to 2.00 g, and "l" is the path length of the cuvette which is equal to 1.0 cm in this case.

Extractions on each chosen sample were performed in triplicate and the results were averaged to obtain the overall chlorophyll concentration of the sample. The results from the wet chemistry analysis were used as the reference data in the development of the chlorophyll calibration equation for the FOSS 6500 NIR spectrophotometer.

4.2.3 NIR methods

The results from the traditional wet chemistry chlorophyll extraction method are relatively accurate and highly repeatable; however, the main disadvantages with this method include the fact that it is time consuming; it uses unfavourable organic solvents; and it destroys whole seeds. These disadvantages prove that there is a growing need to find an easier, less time consuming method that uses whole seeds. Since many labs

currently use a NIR spectrophotometer to measure other seed quality traits, it would be beneficial to employ its use to determine chlorophyll concentration as well. The main advantages of using NIR instrumentation is that little or no sample preparation is required, it is non destructive, it does not require any chemicals, it is operator friendly, it is fast, reliable and precise.

NIR spectroscopy works based on the fact that chemical bonds in molecules naturally vibrate. Molecules that absorb energy are active molecules that contain vibrating dipoles, where each dipole vibrates with a specific frequency (number of vibrations per unit of time) and amplitude (distance covered at the extremes of the vibrating dipole). When there is a match between the energy of light and the energy of the vibrating molecule, the molecule absorbs the light as it transitions to a higher energy vibrational state. This causes an increase in amplitude; however, the frequency remains constant.

An NIR spectrophotometer uses a lamp as the energy source, where the light from the lamp is dispersed into individual wavelengths by a holographic grating. The dispersed light energy travels to the sample where it is either absorbed by the molecules in the sample or reflected back to the detectors.

The FOSS 6500 NIR spectrophotometer is attached to a computer which operates using two software applications, ISIScan and WinISI. ISIScan is involved in NIR instrument operations and diagnostics and for routine analyses. WinISI is used for calibration development and has no system operation capabilities.

The computer uses the reference scan to determine the wavelengths, how much light energy was sent to the sample, how much light energy was returned to the detectors,

and how much light energy was absorbed by the sample. From this, the software can then display a spectrum that shows how much light energy was absorbed by the sample and at what wavelengths. To be able to predict the concentration of the constituent of interest, a calibration equation within the software is required.

The process of developing a calibration equation contains several important steps. The first step is to obtain a large number of samples, scan them through the NIR instrument. The next step is to apply math treatments such as scatter corrections to reduce the influence of light scatter, and derivatives to clarify peaks in the spectra. Next, regression techniques are employed to relate the constituent data to the spectral data. Finally, the equation must be tested against a separate set of samples to predict its efficiency.

4.2.4 NIR instrument maintenance

Within the ISIscan software, maintenance activities must be performed to ensure the accuracy of the readings. Maintenance activities include diagnostic testing, performance testing, fan cleaning or replacement, lamp replacement, and cleaning the sample cells. Sample cells are cleaned using a KimWipe moistened with anhydrous ethanol.

The University of Manitoba, Department of Plant Science Brassica seed quality lab completes instrument standardization on the FOSS 6500 at the beginning of each routine analysis session and performs performance testing once per week. In addition to performance testing, whenever any changes or repairs to the instrument are made, advanced diagnostics must be completed.

The instrument standardization or "check cell" is a natural sample that is used to ensure instrument stability and provides valuable information about instrument performance. The results are stored and indicate trends over time. If the check cell fails, instrument diagnostics must be run to determine the cause of the problem.

The performance tests include the instrument response test, wavelength accuracy test, and NIR repeatability test. The instrument response test analyses the performance of the detectors by measuring the absolute reflectance from the ceramic plate within the instrument to evaluate the total amount of energy output. The wavelength accuracy test measures the wavelength alignment of the instrument using two standards, dydimium for the visible region, and polystyrene for the near infrared region. If this test fails, the wavelength linearization advanced diagnostic test must be performed. The NIR repeatability test is a measure of the deviations in optical data at each wavelength, also referred to as "noise". This test may fail due to excessive humidity in the lab, light leakage in the instrument, or low lamp intensity.

The advanced diagnostic testing includes three different tests: wavelength linearization, gain test, and self test. The wavelength linearization uses internal standards to align the monochromator which ensures accurate predictions and calibrations. The gain test measures the response of the detector to the lamp and indicates if the lamp needs to be changed or if there is too much humidity to run an analysis. The self test tests the motherboard and circuits for potential problems. Should one or more of the diagnostic tests fail, it should be repeated. If repeated failure occurs, it may be necessary to replace the lamp; however, if that does not fix the problem, FOSS should be contacted to repair the instrument.

4.2.5 Scanning sample spectra

Approximately 2 g per sample of thoroughly cleaned whole seeds was added to the small sample cell and analyzed using the ISIScan software on the FOSS 6500 near infrared reflectance spectrophotometer operating in the visible range. The seed samples were checked for moisture content using the NIR spectrophotometer and were confirmed to have a moisture content of less than 8%. The samples obtained from the CGC (Table 4.2.1) were scanned three separate times throughout the course of the year, in the fall, winter, and spring. The files were titled "grl1.nir", "grl3.nir", and "grl5.nir". The laboratory reference values were then added, changing the ".nir" files into calibration ".cal" files using the "Spectra Files" option in the "View & Modify Files" section of the WinISI version 4 software. Calibration files are required for the development of the calibration equation. The samples obtained from the University of Manitoba (Table 4.2.2) had been previously scanned and converted to calibration files. These files were titled "deb102.cal" and deb118.cal".

Next, the "grl1.cal", "grl3.cal", and "grl5.cal" files were merged together. Then, the "deb102.cal" and deb118.cal" were merged together and a subset of twenty samples was randomly selected and removed from the calibration set to serve as the validation set. This file was titled "valset.cal". The validation file is used to determine the suitability of the equation. The subset of samples chosen should contain a number that is approximately equal to ten percent of the overall samples used in the equation. After the validation file was created, all calibration files were merged together. This file was titled "combined.cal". The spectra had to be trimmed in the calibration file, as there was too much background noise below 650 nm. All wavelengths below 650 nm were removed

using the WinISI version 4 software. The wavelengths ranged from 650 nm and increased by 2 nm increments up to 2498 nm. This file was titled “trimmed.cal”.

4.2.6 Population structuring

NIR spectroscopy relies on the collection of a suitable population of samples and use of the best mathematical treatments to reach the most accurate calibration. The sample spectra must be properly grouped to enable accurate results to be produced. To accomplish this objective, the spectral data must be converted into scores. A score is a mathematical reduction of the spectra into single points (scores) based on their principle components. It is the amount of pattern in a particular spectrum. Population structuring allows for the comparison of the variations in the spectra in a group of samples to enable the identification of the similarities and differences between the samples. The population structuring that the WinISI software conducts, involves breaking down the spectra to their principle components, creating scores, and comparing them to each other as well as to the group.

Since each sample was scanned three times throughout the course of the year, the spectra (and laboratory reference values) were averaged. This file was titled “average.cal”. This is beneficial since scans may vary slightly over time. In this case, the laboratory reference values were the same and therefore did not need to be averaged. Samples must have the same sample name to be averaged. If the samples were not averaged, it would invalidate the cross validation, making it impossible to develop a functioning equation.

The next step in the population structuring was to define the spectral boundaries to identify the outliers in the spectral population. Within the WinISI version 4 software, the "Create Score File from Spectral File" option was selected. Within this option, the "Loading Type" was set to "PCA". A Principle Component Analysis (PCA) identifies patterns in a group of spectra that provide the most variation. The "PCA Loading Type" option evaluates the samples based only on the sample spectra. The best samples add spectral variation to the file.

"Measure distance from "Mean"" was selected since it compares each sample spectrum score to the average spectrum score for the file. Under "Cutoff by "H or R"", the "H value" was set to 3.0, which represents a standard deviation from the mean spectrum of the file. Under "H or R measurement", "H-statistic" was selected because it bases the calculation on Mahalonobis distance (standard deviation) instead of a correlation factor (R-statistic) that is independent of the variation in the file.

The "Wavelengths and Math Treatments" options considers the spectral regions, the scatter correction, and the math treatments being considered. The purpose of these selection parameters is to accent the spectral variations and minimize environmental influences. It is important to be consistent with these selection parameters between files. Derivative math is most commonly used for the enhancement of near infrared spectra. It enhances spectral features, minimizes baseline variations, eliminates slopes, and reduces particle size effects. Derivatives also take into account differences between absorbances at adjacent wavelengths and are normally combined with some smoothing. In this case, several math treatments were tested and it was found that the 1,4,4,1 math treatment was best (Table 4.2.4). The 1,4,4,1 math treatment signifies the first derivative with a 4 point

(8 nanometer) gap and 4 data point (8 nanometer) smooth applied to it. The derivative is calculated using absorbance (y axis) data. By placing a 1 beside “Smooth2” indicates that there is no second smooth. This setting is a carry over to accommodate older, less stable instruments. The “Gap” is the number of data points over which the derivative is calculated. The “Smooth” is the number of points to be averaged for data smoothing; it should not be larger than the “Gap”. No scatter correction was applied to the spectra therefore “none” was selected. Scatter corrections account for the fact that the light that is scattered could exhibit a non-linear function and may distort the relationship between the NIR scan and the laboratory value. The options included “none”, “SNV” (Standard Normal Variant) and “Detrend”. “SNV” scales each spectrum to have a standard deviation of 1.0 from the baseline. This helps to reduce particle size effects. “Detrend” removes linear and quadratic curvature from each spectrum.

Table 4.2.5. Math treatments used in NIR based chlorophyll calibration equation development

Number of outlier elimination passes	Scatter	Math Treatment
2	none	1,4,4,1
2	none	2,4,4,1
2	none	0,0,1,1
2	SNV, Detrend	1,4,4,1
2	SNV, Detrend	2,4,4,1
2	SNV, Detrend	0,0,1,1
1	none	1,4,4,1
1	none	2,4,4,1
1	none	0,0,1,1
1	SNV, Detrend	1,4,4,1
1	SNV, Detrend	2,4,4,1
1	SNV, Detrend	0,0,1,1
0	none	1,4,4,1
0	none	2,4,4,1
0	none	0,0,1,1
0	SNV, Detrend	1,4,4,1
0	SNV, Detrend	2,4,4,1
0	SNV, Detrend	0,0,1,1

Under “Fraction of explainable variance in the spectra”, “Enter components used to meaure GH” is the calculated number of principal components needed to explain a statistically significant amount of variation in the file. The second time this appears is the amount of principal components with the outliers removed. The “global H” outliers are more than three standard deviations from the mean. The “global H” identifies spectral outliers; it is a measure of how similar a sample spectrum is to the calibration samples.

Next, redundant spectra were removed. Sample selection is derived from the same score file that was developed above. This file was titled “selected.cal”. The purpose of this step is to identify any samples that are so spectrally similar to one another that they do not contribute any new information to the calibration. The new file that is created has

unique samples with spectral variability. This process uses the “neighborhood concept”. The space near a sample is called a “neighbor”; therefore the distance between a sample and its neighbor is defined as the “H-distance”; this distance is called the “neighborhood H”. Only one sample is needed per neighborhood. Under the “Select Sample from a Spectral File” option in the WinISI version 4 software, basically the same options as stated above were selected, however, the “H or R value” cutoff was set to 0.6 rather than 3.0 so any sample with a distance that is less than 0.6 to it nearest neighbor is considered similar and spectrally redundant and will therefore be removed from the new calibration file.

Duplicate spectra were also removed since duplicate spectra would invalidate the cross validation, making it impossible to develop a functioning equation. This was done by following the same procedure as above, however, the “H-cutoff” was set to 0.0001 rather than to 0.6.

4.2.7 Equation development

Once the score file (“selected.cal”) was developed, it was possible to create the equation. Within the WinISI version 4 software “Calibrate” option, “Global” and then “Develop Equations with Full Spectrum” were selected. The “Modified Partial Least Squares” (MPLS) method was chosen. This method is a modified form of principal component regression where the residuals are standardized after each iteration. This method uses laboratory reference data in the regression. MPLS uses spectral regions instead of discrete wavelengths. This method also combines the use of principal components with the regression analysis to give the best fit to the laboratory reference

data. The factors are calculated to model the variation. This method is most commonly used in agricultural applications.

Many settings must be configured within the MPLS option, including, the “outlier types”, “maximum number of terms”, cross validation groups”, “number of outlier elimination passes”, “missing data value”, and “math treatments”. Each setting is explained as followed.

Within the “outlier types”, all three options including “Critical ‘T’ outlier”, “Critical ‘GH’ outlier”, and “Critical ‘X’ outlier” were selected. The “Critical ‘T’ outlier” is set at a factory default value of 2.5. It is used to eliminate samples that show a large difference between the laboratory reference value and the NIR predicted value. It is two and a half times the standard error of the equation. The “Critical ‘GH’ outlier” is used in the elimination process of samples whose spectrum is more than 10 (if 10 was entered) standard deviations away from the mean after the math treatments and scatter corrections have been applied. The “Critical ‘X’ outlier” is used to eliminate sample spectra that are unusual. Generally a value of 10 is used here.

The “maximum number of terms” refers to the maximum number of principal component factors that the software calculates for the data set. This is automatically determined by the software based on the number of samples in the file and the regression technique that was used.

The “cross validations groups” is a method that predicts each sample in the calibration. It is a true indication of the accuracy of the predictions from the calibration equation. It depends upon the number of samples in the calibration and thereby divides each population into groups. The software automatically displays a default value;

however, a minimum of 3 is recommended. Samples with large residuals, i.e. “T values” larger than 2.5, are generally disregarded. The cross validation error will be higher than the calibration error and it leaves out samples according to segment size.

The “number of outlier elimination passes” is the number of times the software will process the data to remove outliers.

The “selected samples to delete” option allows for the elimination of samples from the regression without deleting them. It should be set to 0.

The “maximum number of terms” is the maximum number of principal components that will be used in the regression. It is calculated by the software based on the number of samples in the file and the regression technique that was used. It is usually the number of samples divided by 10 plus 2 or 3.

The “missing data value” tells the software to assume that any reference value of ‘0’ is due to the fact that the reference value is not available and is not actually a value of 0. This was not necessary in this case as all laboratory reference values were present.

As explained above, the “Wavelengths and Math Treatments” options consider the spectral regions, the scatter correction, and the math treatments being considered and it is important to be consistent with these selection parameters between files. In this case, several math treatments were tested and it was found that the 1,4,4,1 math treatment with no scatter correction was the best. The “best” math treatment and scatter correction varies based on the equation being developed. The above process resulted in the development of an equation “.eqa” file. The above process was repeated many times using a different math treatment each time. One equation file was created per math treatment applied. Once created, each calibration equation provides an output file with the calibration

statistics making it possible to select the equation with the best math treatment based. The output file displays the following statistics: “N”, “mean”, “SEC”, “RSQ”, “SECV”, and “1-VR”; where, the “N’ refers to the number of samples used in the regression, “mean” refers to the average of the laboratory reference values, “SEC” refers to the standard error of calibration which is the average difference between the laboratory reference values and the predicted values for the samples used to develop the equation, the “RSQ” is the R-squared or correlation of the data with the predicted values, the “SECV” is the standard error of cross validation which is the average difference between the laboratory reference values and the predicted values of samples when they were not used in the development of the equation, and the “1-VR” is 1 minus the variance which is an estimate of the fraction of explained variance – a correlation between the lab data and the cross validation results. The equation that gives the lowest “SECV” and the highest “1-VR” value is considered the “best” equation as it is the most like the results that that will be seen when applying the equation to other samples. Once the best equation was selected, all others with varying math treatments and scatter corrections were deleted.

4.2.8 Equation validation

After an equation has been developed, it must be validated using a set of samples that were not used in the creation of the equation. The validation set should be similar to the samples used in the development of the equation and cover the range of the constituent; however, they must not be so spectrally similar that they are redundant because it will not give a true indication of the reliability of the equation. The validation set must include the sample spectra with reliable laboratory reference data. The validation

set should include a total of 10% of the overall number of samples that we used in the equation development.

The “Monitor Results” program in the WinISI version 4 software performs a statistical comparison between the laboratory reference values and the NIR predicted values for the data set. It is display as the standard error of prediction (“SEP”, “Bias”, and “SEP(C)”) statistic. The “bias limit” is 0.6 times the “SEP(C)” of the equation. The “SEP limit” is 1.3 times the “SEP(C)” of the equation. If the standard deviation of the two sets of data have a greater than 20% difference, the software will highlight the “Std Dev. Results”. Outliers must be removed from the calculations.

Once the equation has been developed and validated, it can be used in the lab provisionally when checked against standard lab wet chemistry based protocols. After a suitable period of time, it may be implemented in routine operations. The equation should be periodically tested against wet chemistry based laboratory results. The equation should also be periodically updated/expanded due to the fact that natural product variation occurs over time.

4.2.9 Testing the equation

To test the performance of the equation, each sample in the validation set (Table 4.2.3) was run through the NIR spectrophotometer three times using the newly developed chlorophyll calibration equation. The NIR values were averaged and entered into a Microsoft Excel spreadsheet. Chlorophyll extractions were also performed three times on each sample. The average chlorophyll extraction values were then compared to the average NIR values. According to the standards set out by the CGC, to be considered

accurate, the values must be within 3 ppm from one another. The NIR values along with the extraction values were submitted to the GRL so the Brassica seed quality lab at the University of Manitoba can retain its certification for chlorophyll concentration using the extraction method and receive new certification for NIR determination of whole seed chlorophyll concentration as a part of the Laboratory Proficiency Program.

The purpose of the Laboratory Proficiency Program is to ensure that consistent information can be collected from various seed quality laboratories across Canada and to allow them to develop precise and accurate testing methods for all laboratories. Since the University of Manitoba periodically registers new HEAR/canola varieties, it is imperative that their testing methods provide accurate and precise results, not only for chlorophyll concentration but for oil, protein, and glucosinolate concentrations as well as fatty acid profiles.

4.3 Results and Discussion

4.3.1 Modifications to traditional extraction protocol to determine chlorophyll concentration

Chlorophyll concentration was determined by extraction following a modified version of the protocol set out by the International Organization for Standardization (ISO 10519:1997 (E), Rapeseed— Determination of chlorophyll content—Spectrometric method).

Chlorophyll concentration values from the University of Manitoba Brassica seed quality lab as determined by traditional extraction method have generally been lower than other labs that participate in the GRL Laboratory Proficiency Program. To correct for this, several modifications were made to the original protocol for determining chlorophyll

concentration using the traditional extraction method. It was determined that grinding the samples for four seconds longer than indicated in the original protocol increased the final chlorophyll concentration. A new shaker was purchased as well to mix the samples more vigorously and the shaking time was increased to 120 minutes from 90 minutes. Factors such as filtering and settling overnight were also considered and tested. Eight samples, 2006 - GF346, 2006 - GF421, 2006 - GF345, 2007 - GF436, CN04CL11, 1317806, 1227785, and 1225465, with varying chlorophyll concentrations were selected at random from the entire sample population. The first group of eight samples was tested following the original protocol, without filtering or leaving to settle overnight. The second group was filtered and measured on a spectrophotometer on the same day. The third group was filtered, left to settle overnight, then measured the next day. The fourth group was left to settle overnight, then measured the next day (Appendix Table B1). Based on the statistical analysis (Table 4.3.1), it can be concluded with 95% confidence that there are no statistical differences between any of the groups. From this, it was decided to filter the samples to prevent ground seed material in the sample solution from interfering with the clarity of the solution and to measure the absorbance on the same day to save time.

Table 4.3.1. t-test statistics to determine differences between test groups to determine if any modifications are needed to improve chlorophyll extraction protocol, α = 0.05

ID	Sample Mean (μ_X)	Standard Deviation (δ)	Sample size (n)	Degrees of freedom (df = n-1)	Population mean (μ_0)	t-Statistic $(\mu_X - \mu_0)/(\delta/\sqrt{n})$	P-value (two-tailed; H_0: $\mu_X=\mu_0$)
125465	1.9906	0.7068	4	3	0	5.63305224	0.011067202
2007 - GF436	3.2500	1.1021	4	3	0	5.897678246	0.00973217
1227785	0.4058	0.0021	4	3	0	393.6353194	3.61558E-08
2006 - GF345	0.4575	0.0127	4	3	0	72.03756768	5.89512E-06
2006 - GF241	0.4848	0.0118	4	3	0	82.03542442	3.9924E-06
1317806	0.4673	0.0100	4	3	0	93.64529802	2.68432E-06
2006 - GF364	0.4890	0.0076	4	3	0	128.1418206	1.04786E-06
CN04CL11	0.5043	0.0330	4	3	0	30.53258166	7.71801E-05

Based on the above modifications to the protocol, several more samples were selected and chlorophyll concentration was determined. Each sample was repeated in triplicate and results were averaged then compared to the results obtained by the GRL (Table 4.3.2). Absorbance values at 665 nm, 625 nm, and 705 nm were used to calculate chlorophyll concentration (Appendix Table B2). It is important to have accurate chlorophyll concentration results as these values will be used as the reference data in the development of the NIR based calibration equation for chlorophyll concentration. The GRL states that chlorophyll concentrations must be within 3 ppm from the expected results to be accepted. When compared to the overall results from the GRL, it appears that the test results are significantly lower. The average difference in concentration was greater than 3.0 ppm at 8.5 ppm; therefore, the results provided by the GRL will be considered correct and will be used as the reference data.

Table 4.3.2. Chlorophyll concentration (chll) in ppm determined following modifications to the traditional extraction method compared to chll results obtained from the GRL

ID	Chll		Difference
	Test[z]	GRL[y]	($chll_{GRL}$ - $chll_{Test}$)
333	25.6	30.8	5.2
623	13.9	17.9	4.0
1204174	18.6	27.7	9.1
1224925	37.6	52.9	15.3
1228059	2.7	2.6	-0.1
CN02HF35	81.7	91.3	9.6
2004 - C496	9.5	26.4	16.9
2004 - C905	8.4	42.7	34.3
2004 - GF340	12.7	11.9	-0.8
2004 - GF516	2.7	4.9	2.2
2004 - OP041b	13.7	33.1	19.4
2006 - GF422	43.3	60.2	16.9
2006 - GF948	10.6	14.4	3.8
2006 - OP344	5.9	9.8	4.0
2006 - OP599	12.8	17.8	5.0
2007 - C762	16.1	20.5	4.4
2007 - OP346	7.3	10.7	3.4
2008 - OP352	6.5	8.5	2.0
2010 - OP491	7.0	12.1	5.1
Average Difference			8.5

[z]Calculated based on corrected absorbance (A_{Corr})

[y]Determined by GRL, considered accurate.

4.3.2 Scan spectra

All samples were successfully scanned through the FOSS 6500 NIR spectrophotometer. Reference data supplied by the GRL was successfully added to the calibration file and all files were checked to ensure all sample numbers and reference values were entered correctly. All calibration files were merged together and duplicate samples were averaged. The spectra in the calibration file were trimmed to reduce background noise below 650 nm (Appendix Table A3). The validation set was successfully created at random from the calibration file and trimmed to reduce background noise below 650 nm as well (Appendix Table B4).

4.3.3 Population structuring

To prepare for the development of the calibration equation, spectral boundaries were defined and spectral outliers were removed from the calibration file (Appendix Table B5). This process is used to determine which samples are so spectrally similar to each other that they contribute no new information to the calibration and to identify unique samples with spectral variability. The purpose of selecting the appropriate parameters is to accent the spectral variations and minimize environmental influences within the spectra. The WinISI Version 4 software determined that 2005-C912, 2004-C092, and 2004-GF133 were spectral outliers and were therefore removed from the calibration file.

4.3.4 Equation development

Once the population structuring was successfully completed, each calibration file was used to create an equation to see which set provided the best calibration statistics. Once created, each calibration equation provides an output file with calibration statistics (Table 4.3.3). "SEC" refers to the standard error of calibration which is the average difference between the laboratory reference values and the predicted values for the samples used to develop the equation; "RSQ" is the R-squared or correlation of the data with the predicted values; "SECV" is the standard error of cross validation which is the average difference between the laboratory reference values and the predicted values of samples when they were not used in the development of the equation; and "1-VR" is 1 minus the variance which is an estimate of the fraction of explained variance.

Table 4.3.3. Calibration statistics for each calibration file on its own or with the addition of other calibration files to determine which calibration file provided the best calibration statistics

Calibration File	SEC	RSQ	SECV	1-VR
grl1	4.8110	0.8399	5.3957	0.8021
grl3	5.3844	0.8011	5.8688	0.7641
grl5	5.4456	0.7988	6.2193	0.7430
deb102	3.1412	0.9066	4.5622	0.7995
deb118	2.8838	0.9202	4.4051	0.8111
grl1 & deb102	4.2196	0.8841	4.6519	0.8611
grl1 & deb118	3.9675	0.8962	4.4578	0.8704
grl1, deb102, & deb118	4.0735	0.8897	4.3343	0.8754
grl1, grl3, grl5, deb102, & deb118	3.4875	0.9011	3.7580	0.8840

It should be noted that although the "grl1", "grl3", and "grl5" sets include all the exact same samples, the calibration statistics vary with "grl1" having the best statistics. This is likely due to environmental changes, such as varying temperatures and humidity,

in the laboratory. The “grl1” files was initially prepared by scanning the samples through the NIR spectrophotometer in the fall, whereas “grl3” and “grl5” were scanned in the winter and spring. It appears that moisture contents decreased slightly between the “grl1”, “grl3”, and “grl5” sets as the seeds were left out at room temperature in the lab. Since the changes in moisture were so low, there was likely no affect on the chlorophyll concentration in the seeds (Appendix Table B6). To account for this variability, all three files were merged together, and then averaged. Although a positive correlation exists between chlorophyll concentration and moisture content in *Brassica napus* seeds, researchers have found that little or no chlorophyll loss occurs below 35% moisture in seeds (Johnson-Flanagan and Thiagarajah 1990).

The equation that gives the lowest “SECV” and the highest “1-VR” value is considered the “best” equation as it is the most like the results that that will be seen when applying the equation to other samples. In this case, the final equation with all files (“grl1”, “grl3”, “grl5”, “deb102”, and “deb118”) is considered the “best” equation. The “SECV” value at 3.7580 and the “1-VR” value at 0.8840 are by far the highest than any of the other cases. This is reasonable since it contains the highest number of samples, therefore containing the greatest spectral variability.

FOSS North America creates calibration equations for all sorts of material and quality traits and sells them to laboratories across North America. To be suitable for sale, FOSS requires the “RSQ” value to be as close to 1.0 as possible; the “SEQV” value to be as close to 3.0 as possible; and the “1-VR” should be as close to 1.0 as possible. The equation developed in this project will not have the same accuracy as the FOSS equations since the number of samples is limited; therefore to be suitable for use in the Brassica

seed quality lab in the Department of Plant Science at the University of Manitoba, FOSS recommends the "RSQ" value to be higher than 0.90 and as close to 1.0 as possible; the "SEQV" value to be under 4.0; and the "1-VR" to be higher than 0.90 and as close to 1.0 as possible. The NIR based chlorophyll calibration equation developed in this project was sent to Mr. Ole Rassmussen at FOSS North America for verification as well. Mr. Rasmussen determined that the calibration statistics were valid and that the equations should perform well in the Brassica seed quality lab at the University of Manitoba. He also suggested that the equation should be expanded periodically when new samples are available.

4.3.5 Equation validation

Since a NIR based chlorophyll calibration equation with a "SECV" value below 4.0, a "1-VR" value near 0.90, and an "RSQ" value above 0.90 was successfully created, it was then important to validate the equation, using a set of samples that were not used in the calibration equation, to ensure it would perform properly under laboratory conditions.

To validate the equation, the WinISI software performed a statistical comparison between the laboratory reference values and the NIR predicted values for a data set. These results are displayed as the standard error of prediction ("SEP", "Bias", and "SEP(C)") statistics. The "bias limit" is 0.6 times the "SEP(C)" of the equation. The "SEP limit" is 1.3 times the "SEP(C)" of the equation. In this case, the "bias limit" is 2.225 and the "SEP(C) limit" is 4.885. The SEP(C) value was 5.057 and the bias was -1.763. Since the SEP(C) was outside the limit, the WinISI software determined that 2006-GF422, 2004-C905, 2006-GF948, 2006-OP599, 2004-GF340, 2005-GF105, 2009-

GF481, and 2004-GF377 were outliers. These outliers were not considered in the validation of the equation; therefore, they were removed and another validation was performed. The SEP(C) and the bias were within the limits after the second validation at 4.680 and -1.228 respectively. The "SEP" for the first validation was 5.235 and for the second validation, after the outliers were removed, it decreased to 4.662 as well.

All samples in the validation set were also measured on the NIR spectrophotometer to test the performance of the equation. Each sample was measured in triplicate and the results were averaged then compared to the results obtained by traditional extraction methods at the GRL (Table 4.3.4). The average difference in concentration was greater than 3.0 ppm at 5.1 ppm, indicating that the equation needs some improvement. With the removal of the outliers, the average difference in concentration between the NIR results and the results obtained by traditional chlorophyll extraction by the GRL was much less at 3.7 ppm (Table 4.3.5). Even though this value is still above the acceptable value of 3.0 ppm, the equation was confirmed to be valid by the WinISI software. Since the validation statistics are close the to the limits as well, it would be beneficial to expand the equation in the future to increase the proficiency.

Table 4.3.4. Validation set with chlorophyll concentration (chll) in ppm determined using newly developed NIR based chll calibration equation. Samples were measured in triplicate then averaged. NIR results were compared to the results obtained by traditional extraction methods at the GRL

ID	Trial 1	Trial 2	Trial 3	Chll		Difference
				NIR	GRL	($chll_{GRL}$ - $chll_{NIR}$)
2006 - OP344	7.8	9.0	5.8	7.5	9.8	2.3
2007 - OP346	14.5	10.7	8.0	11.1	10.7	-0.4
2010 - OP491	23.1	20.4	20.3	21.3	12.1	-9.2
2008 - OP352	13.6	14.6	11.6	13.3	8.5	-4.8
2006 - GF422	44.0	43.8	51.0	46.3	60.2	13.9
2004 - C905	15.9	14.9	18.3	16.4	42.7	26.3
2004 - GF516	6.0	2.3	4.4	4.2	4.9	0.7
2004 - OP041b	22.8	19.8	22.2	21.6	18.9	-2.7
2006 - GF948	11.0	15.0	9.5	11.8	14.4	2.6
2006 - OP599	17.5	21.8	20.2	19.8	17.8	-2.0
2004 - GF340	24.2	14.8	18.6	19.2	11.9	-7.3
2007 - C762	25.7	25.4	32.2	27.8	20.5	-7.3
2004 - C496	12.3	15.3	14.6	14.1	26.4	12.3
2004 - OP688	13.9	10.7	12.6	12.4	10.8	-1.6
2005 - C711	39.5	36.6	45.3	40.5	39.6	-0.9
2009 - GF105	18.8	22.1	17.8	19.6	17.5	-2.1
2009 - C151	25.2	29.3	27.1	27.2	25.7	-1.5
2009 - GF481	10.2	10.9	7.9	9.7	8.1	-1.6
2004 - GF377	23.0	19.1	22.7	21.6	20.5	-1.1
2007 - C619	13.4	15.3	13.8	14.1	12.8	-1.3
Average Difference						5.1

Table 4.3.5. Validation set with outliers removed with chlorophyll concentration (chll) in ppm determined using newly developed NIR based chll calibration equation. Samples were measured in triplicate. NIR results were compared to the results obtained by traditional extraction methods at the GRL

				Chll		Difference
ID	Trial 1	Trial 2	Trial 3	NIR	GRL	($chll_{GRL}$ - $chll_{NIR}$)
2006 - OP344	7.8	9.0	5.8	7.5	9.8	2.3
2007 - OP346	14.5	10.7	8.0	11.1	10.7	-0.4
2010 - OP491	23.1	20.4	20.3	21.3	12.1	-9.2
2008 - OP352	13.6	14.6	11.6	13.3	8.5	-4.8
2004 - GF516	6.0	2.3	4.4	4.2	4.9	0.7
2004 - OP041b	22.8	19.8	22.2	21.6	18.9	-2.7
2007 - C762	25.7	25.4	32.2	27.8	20.5	-7.3
2004 - C496	12.3	15.3	14.6	14.1	26.4	12.3
2004 - OP688	13.9	10.7	12.6	12.4	10.8	-1.6
2005 - C711	39.5	36.6	45.3	40.5	39.6	-0.9
2009 - C151	25.2	29.3	27.1	27.2	25.7	-1.5
2007 - C619	13.4	15.3	13.8	14.1	12.8	-1.3
Average Difference						3.7

4.3.6 NIR certification

The results of the 2011 Laboratory Certification Set from the chlorophyll extractions along with the results from the NIR analysis from the newly developed chlorophyll calibration equation were submitted to the GRL as a part of the 2011 Laboratory Proficiency Program. The Canola Proficiency Report was returned and the NIR based calibration equation for determining chlorophyll concentration was determined to be valid. The Brassica seed quality lab in the Department of Plant Science at the University of Manitoba is now certified to use NIR spectroscopy to determine chlorophyll concentration in whole *Brassica napus* seed samples.

The 2011 Canola Proficiency Program Reference Sample Report from the GRL shows chlorophyll concentration results as determined by traditional extraction or by NIR spectroscopy (Table 4.3.6). The NIR determination of chlorophyll concentration failed on the CN2010Chk reference sample and was therefore not included in the overall mean. The bias value must be below 3.0 ppm for a sample result to be accepted. The bias value for the CN2010Chk reference sample was 4.2 indicating that the difference between the NIR sample mean and the overall mean was greater than 3.0 ppm.

Table 4.3.6. 2011 GRL Canola Proficiency Program Report - Reference sample chlorophyll concentration results as determined by traditional extraction or by NIR spectroscopy. Note: Lab numbers were changed to letters for confidentiality reasons. Brassica seed quality lab in the Department of Plant Science at the University of Manitoba is listed as Lab "A"

		CN2010Chk						CN2011Chk					
Lab	Method	1	2	Outliers	z-score	Mean	Bias	1	2	Outliers	z-score	Mean	Bias
A	Extraction	6.2	6.0		1.7	6.1	1.3	10.4	10.6		1.1	10.5	1.7
A	NIR	8.8	9.1	x	5.4	9.0	4.2	12.3	12.9		2.6	12.6	3.8
B	Extraction	4.9	5.1		0.3	5.0	0.2	9.2	9.0		0.2	9.1	0.3
C	NIR	18.3	19.0	x	17.9	18.7	13.9	21.1	19.8	x	7.9	20.5	11.7
D	Extraction	4.1	4.1		-0.9	4.1	-0.7	8.2	7.6		-0.6	7.9	-0.9
D	NIR	4.9	4.1		-0.4	4.5	-0.3	7.3	6.5		-1.3	6.9	-1.9
E	NIR	5.3	4.8		0.3	5.1	0.3	9.8	10.1		0.8	10.0	1.2
F	Extraction	4.7	4.6		-0.2	4.7	-0.1	9.3	9.3		0.3	9.3	0.5
G	Extraction	5.8	6.2		1.6	6.0	1.2	7.9	7.7		-0.7	7.8	-1.0
H	Extraction	3.9			-1.2	3.9	-0.9	8.1			-0.5	8.1	-0.7
H	Extraction	3.9			-1.2	3.9	-0.9	7.5			-0.9	7.5	-1.3
I	Extraction	6.1	5.9		1.6	6.0	1.2	8.1	8.1		-0.5	8.1	-0.7
I	Extraction	4.8	5.0		0.1	4.9	0.1	8.0	8.6		-0.4	8.3	-0.5
I	NIR	5.1	5.0		0.4	5.1	0.3	7.8	9.0		-0.3	8.4	-0.4
I	NIR	5.1	5.1		0.4	5.1	0.3	7.2	8.0		-0.8	7.6	-1.2
J	Extraction	4.2	3.9		-0.9	4.1	-0.8	7.0	7.6		-1.0	7.3	-1.5
J	Extraction	4.2	3.9		-0.9	4.1	-0.8	7.0	7.6		-1.0	7.3	-1.5
K	Extraction	5.0	4.8		0.2	4.9	0.1	9.2	9.4		0.3	9.3	0.5
K	NIR	5.1	5.0		0.3	5.1	0.3	9.3	9.2		0.3	9.3	0.4
L	Extraction	5.1	4.8		0.2	5.0	0.1	10.0	10.3		0.9	10.2	1.4
L	NIR	16.9	15.6	x	14.8	16.3	11.5	19.6	19.1	x	7.1	19.4	10.6
M	NIR	4.9	2.9		-1.1	3.9	-0.9	8.0	8.9		-0.3	8.5	-0.4
M	NIR	5.5	3.4		-0.4	4.5	-0.4	8.6	9.4		0.1	9.0	0.2
N	NIR	8.1	8.5	x	4.6	8.3	3.5	11.5	11.6		1.8	11.6	2.8
Mean				4.8						8.8			
S(r)				0.5						0.4			
S(R)				0.8						1.5			
RSD(r)				11.0						4.4			
RSD(R)				16.1						16.7			
r				1.5						1.1			
R				2.2						4.1			

Legend:
Mean = Overall mean of the laboratory values
S(r) = Repeatability (blind duplicates) standard deviation
S(R) = Reproducibility (between labs) standard deviation
RSD(r) = Repeatability relative standard deviation
RSD(R) = Reproducibility relative standard deviation
r = Repeatability value = 2.8s(r)
R = reproducibility = 2.8s(R)

The 2011 Canola Proficiency Program Chlorophyll Report from the GRL shows chlorophyll concentration results as determined by traditional extraction or NIR by spectroscopy on blind duplicates (Table 4.3.7). For simplicity, only the results from the Brassica seed quality lab in the Department of Plant Science at the University of Manitoba, "Lab A", were shown. The overall mean and statistical analysis include all labs that participate in the Laboratory Proficiency Program. For some labs, the z-score was greater than +/- 3 which means there is a significant bias in the results. This did not occur in the results submitted by Lab A. A chi-square (x^2) value above 16.0 indicates a problem with repeatability between the duplicate samples. The chi-square value for the extraction method from Lab A was 2.26, indicating that the repeatability was excellent. On the other hand, the chi-square value for the NIR method was 14.76, indicating that improvements to the equation are needed.

Table 4.3.7. 2011 GRL Canola Proficiency Program Report - Chlorophyll concentration results as determined by traditional extraction or by NIR spectroscopy. Note: no data was shown for any labs other than Lab A for simplicity

		C349, C446						C261, C678						C221, C353					
Lab	Method	1	2	Outliers	z-score	Mean	Bias (%)	1	2	Outliers	z-score	Mean	Bias (%)	1	2	Outliers	z-score	Mean	Bias (%)
A[z]	Extraction	5.8	5.7		1.3	5.8	23.3	6.6	7.1		0.5	6.9	12.4	11.4	10.1		1.0	10.8	13.3
A	NIR	7.8	5.8		2.6	6.8	46.2	8.8	8.9		1.9	8.8	45.1	12.0	13.1		2.3	12.5	32.0
Mean[y]				4.67						6.10						9.49			
S(r)				0.20						0.32						9.39			
S(R)				0.83						1.42						1.30			
RSD(r)				4.31						5.22						4.07			
RSD(R)				17.72						23.25						13.74			
r				0.56						0.89						1.08			
R				2.32						3.97						3.65			

		C704, C968						C514, C701						C571, C878					
Lab	Method	1	2	Outliers	z-score	Mean	Bias (%)	1	2	Outliers	z-score	Mean	Bias (%)	1	2	Outliers	z-score	Mean	Bias (%)
A	Extraction	15.3	15.8		0.2	15.6	3.2	21.0	21.3		0.6	21.2	7.2	30.1	29.3		0.3	29.7	3.9
A	NIR	18.9	16.0		1.0	17.4	15.8	23.8	25.7		2.0	24.7	25.4	35.3	34.3		1.9	34.8	21.7
Mean[y]				15.06						19.73						28.57			
S(r)				0.74						0.90						0.98			
S(R)				2.36						2.49						3.26			
RSD(r)				4.94						4.57						3.43			
RSD(R)				15.67						12.61						11.41			
r				2.09						2.52						2.74			
R				6.61						6.97						9.12			

		All samples					
Lab	Method	Mean Bias (%)	Mean z-scores	Mean Difference	Standard Deviation	Repeatability	x^2
A	Extraction	10.5	0.7	0.6	0.7	1.93	2.26
A	NIR	31	2.0	1.5	1.8	4.93	14.76

[z]Lab A - Brassica seed quality lab, Department of Plant Science, University of Manitoba

[y]Signifies overall mean of laboratory data. Note: not all data is shown, only data from lab A is shown for simplicity

Legend:

S(r) = Repeatability (blind duplicates) standard deviation

S(R) = Reproducibility (between labs) standard deviation

RSD(r) = Repeatability relative standard deviation

RSD(R) = Reproducibility relative standard deviation

r = Repeatability value = 2.8s(r)

R = reproducibility = 2.8s(R)

4.3.7 Conclusions

The NIR based calibration equation was successfully created for the analysis of chlorophyll concentration in whole *Brassica napus* seed samples. Expanding the equation by adding more samples would improve both the repeatability and the z-scores for the Laboratory Proficiency Program. It would be beneficial to continue participating in this program to retain laboratory certification and to ensure the equation is functioning properly. The calibration statistics within the WinISI software would also improve with the addition of more spectrally different samples. The calibration errors (SECV of SEP) generally improve as the product database is being developed since expanding the database increases the spectral diversity and widens the range of reference values beyond the limits of linearity. The equation should also be periodically tested against wet chemistry based laboratory results.

5.0 GENERAL DISCUSSION AND CONCLUSIONS

Since *Brassica napus* is such an economically important crop in the world, it is important to have accurate methods for evaluating its quality. Quality control is an important aspect in plant breeding. Over the years, quality analysis techniques are gradually being improved and new techniques are often being developed. New techniques should be less time consuming, less expensive, and more accurate than previous techniques to be able to successfully replace older techniques. It is also beneficial to have nondestructive analytical methods.

The main characteristics which determine the quality of rapeseed/canola are oil concentration, protein concentration, chlorophyll concentration, glucosinolate concentration, and fatty acid composition. These traits are analyzed when considering a new cultivar for licensing and throughout the transportation, processing, and marketing of rapeseed/canola cultivars. The overall goal of assessing the quality in *Brassica* seed samples is to provide efficient screening techniques and evaluation methods for the development and registration of new *Brassica* oilseed cultivars.

This study focused on two different quality analysis techniques. The first objective was to determine the erucic acid genotypes of canola/rapeseed seedling samples using the SCAR and SNP marker protocol for marker assisted selection published by Rahman et al. (2008) and to reduce the apparent error rate (of 10 to 50%) of the SNP and SCAR marker analysis for erucic acid genotypes of the *Bn-FAE1.1* gene in the A genome and the *Bn-FAE1.2* gene in the C genome. The overall results from this analysis indicate

that further improvements are necessary to adopt this method of evaluating erucic acid genotypes in *Brassica napus* samples.

With the modifications that were made to the molecular marker protocol, the overall frequency of correct results did not change between the 2010 and 2011 sample sets. It is still to low to be able to put these markers into use in a breeding program. The frequency of missing results was also too high to be acceptable and it did not seem to improve substantially from one year to the next. Further improvements to the molecular marker protocol are necessary to be able to successfully replace the traditional method that uses gas chromatography for determining erucic acid content and therefore genotypes of *Brassica napus* samples. It would also be beneficial to improve these markers to be able to score all erucic acids genotypes in all C22:1 categories including those which were currently excluded from the analysis since could not be identified with certainty. It may also be the case that these markers are parent specific. The original primers developed by Rahman et al. (2008) may have been based on different parents than those used in the project. This shows that perhaps the parental lines/cultivars should be sequenced each time a new parent is chosen and new primers may need to be designed each time; however, this would not be very efficient. Until the error rate can be significantly reduced and the number of missing results can be greatly decreased, it is not practical to apply these markers in a breeding program.

The second objective of this study was to develop a calibration equation for chlorophyll concentration in whole *Brassica napus* seed samples using a FOSS 6500 near infrared reflectance spectrophotometer. In this case, the NIR based calibration equation

was successfully created for the analysis of chlorophyll concentration in whole *Brassica napus* seed samples.

Even though it appears as if this equation is operating successfully and that is has been certified by the Grain Research Laboratory (GRL), improvements over time should be considered. Expanding the equation by adding more samples would improve both the repeatability and the z-scores for the Laboratory Proficiency Program. It would be beneficial to continue participating in this program to retain laboratory certification and to ensure the equation is functioning properly. The calibration statistics within the WinISI software would also improve with the addition of more spectrally different samples. The calibration errors (SECV of SEP) generally improve as the product database is being developed since expanding the database increases the spectral diversity and widens the range of reference values beyond the limits of linearity. The equation should also be periodically tested against wet chemistry based laboratory results.

Successful breeding programs rely on successful quality analysis techniques. It is therefore important to periodically evaluate and improve current techniques or to develop new techniques over time.

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7.0 APPENDIX

7.1 Appendix A

Appendix Table A1. Erucic acid (C22:1) genotype for pure breeding canola cultivar 'Sentry', pure breeding HEAR cultivars 'MillenniUM 03' and 'Red River 1997', and pure breeding HEAR parental line '08C344' following regular MAS procedures for determining erucic acid genotypes

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	-	--	-	E1&E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	-	--	-	E1&E2
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	-	-	--	-	E1&E2
Sentry	≤2%	-	-	--	-	E1&E2
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	-	--	-	E1&E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	1	-	e1e1-	-	E2

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	-	-	--	-	E1&E2
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	-	1	-e2e2	-	E1
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	1	e1e1e2e2	Y	-
Sentry	≤2%	1	-	e1e1-	-	E2
Sentry	≤2%	1	1	e1e1e2e2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	-	1	-E2E2	-	E1
MillenniUM 03	≥40%	-	-	--	-	E1&E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	-	-	--	-	E1&E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	-	1	-E2E2	-	E1
MillenniUM 03	≥40%	-	1	-E2E2	-	E1
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	-	1	-E2E2	-	E1
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	-	1	-E2E2	-	E1

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	-	-	--	-	E1&E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	-	-	--	-	E1&E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	1	E1E1E2E2	Y	-
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	-	1	-E2E2	-	E1
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	-	1	-E2E2	-	E1
MillenniUM 03	≥40%	-	-	--	-	E1&E2
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
MillenniUM 03	≥40%	-	1	-E2E2	-	E1
MillenniUM 03	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	-	E1E1-	-	E2

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	1	-	E1E1-	-	E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	-	--	-	E1&E2
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	1	1	E1E1E2E2	Y	-
Red River 1997	≥40%	-	1	-E2E2	-	E1
Red River 1997	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	1	-	E1E1-	-	E2

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
08C344	≥40%	1	-	E1E1-	-	E2
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	1	-	E1E1-	-	E2
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	1	-	E1E1-	-	E2
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	1	1	E1E1E2E2	Y	-
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	-	--	-	E1&E2

Cultivar/Line	C22:1 Phenotype	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result	
					Correct Y/N?	Missing E1/E2?
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	-	--	-	E1&E2
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1
08C344	≥40%	-	1	-E2E2	-	E1

Appendix Table A2. Phenotypic and genotypic results for erucic acid (C22:1) content for BC1F1 progeny from conventional HEAR crosses 08C344 x 1841 RR, 08C344 x 1852H RR, 08C344 x 4414 RR, and 08C344 x 71-45 RR from the 2010 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 1841 RR	51.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	51.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	51.7	≥43.3%	1	0	E1E1-	-	-	E2
08C344 x 1841 RR	50.8	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	49.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	49.0	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	48.9	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	48.6	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	48.3	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	48.0	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	46.7	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	45.9	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	45.3	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	44.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	44.6	≥43.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 1841 RR	44.6	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	44.6	≥43.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 1841 RR	43.9	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	43.9	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	43.8	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	43.7	≥43.3%	2	1	E1e1E2E2	N	E1	-
08C344 x 1841 RR	43.3	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	43.1	≥43.3%	2	1	E1e1E2E2	N	E1	-
08C344 x 1841 RR	43.0	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1841 RR	42.4	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	42.2	43.3%-40.2%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 1841 RR	41.9	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	41.9	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	41.7	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	41.5	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	41.1	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	41.0	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	40.8	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	40.6	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	40.4	43.3%-40.2%	0	2	-E2e2	-	-	E1
08C344 x 1841 RR	40.4	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	40.3	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	40.2	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	40.2	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	40.0	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 1841 RR	39.7	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1841 RR	39.7	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	39.4	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	39.2	40.1%-35.3%	1	0	E1E1-	-	-	E2
08C344 x 1841 RR	39.2	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	38.7	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1841 RR	38.7	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1841 RR	38.6	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 1841 RR	38.6	40.1%-35.3%	1	0	E1E1-	-	-	E2
08C344 x 1841 RR	38.5	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 1841 RR	38.5	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	38.5	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	38.3	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 1841 RR	37.3	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	37.3	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1841 RR	37.2	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	36.9	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	36.8	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	36.5	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	36.3	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 1841 RR	36.2	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	35.8	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	35.8	40.1%-35.3%	2	0	E1e1-	-	-	E2
08C344 x 1841 RR	35.8	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1841 RR	35.5	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1841 RR	35.1	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	34.9	35.2%-32.1%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	34.8	35.2%-32.1%	1	2	E1E1E2e2	N	E2	-
08C344 x 1841 RR	34.7	35.2%-32.1%	1	0	E1E1-	-	-	E2
08C344 x 1841 RR	34.7	35.2%-32.1%	1	2	E1E1E2e2	N	E1	-
08C344 x 1841 RR	34.6	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	34.2	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 1841 RR	33.8	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	33.4	35.2%-32.1%	1	2	E1E1E2e2	N	E1	-
08C344 x 1841 RR	32.8	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 1841 RR	32.8	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	32.5	35.2%-32.1%	1	2	E1E1E2e2	N	E1	-
08C344 x 1841 RR	32.2	35.2%-32.1%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1841 RR	32.0	≤32.0%	1	0	E1E1-	-	-	E2
08C344 x 1841 RR	30.2	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	30.1	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	29.9	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	29.5	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	29.1	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	27.3	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	26.6	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1841 RR	26.6	≤32.0%	2	0	E1e1-	-	-	E2
08C344 x 1841 RR	26.4	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	26.1	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1841 RR	25.8	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1841 RR	25.8	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1841 RR	24.6	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1852H RR	52.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	51.5	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	50.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	49.6	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	49.3	≥43.3%	0	1	-E2E2	-	-	E1
08C344 x 1852H RR	49.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	49.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	49.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	49.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	48.7	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	48.6	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	48.0	≥43.3%	1	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 1852H RR	47.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	47.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	47.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	47.1	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	46.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	45.9	≥43.3%	0	1	-E2E2	-	-	E1
08C344 x 1852H RR	45.6	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	45.5	≥43.3%	2	1	E1e1E2E2	N	E1	-
08C344 x 1852H RR	45.5	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1852H RR	45.3	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	44.5	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1852H RR	44.3	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1852H RR	44.3	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1852H RR	44.0	≥43.3%	2	1	E1e1E2E2	N	E1	-
08C344 x 1852H RR	44.0	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	43.9	≥43.3%	2	1	E1e1E2E2	N	E1	-
08C344 x 1852H RR	43.8	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1852H RR	43.6	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 1852H RR	43.3	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 1852H RR	43.0	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	42.8	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	42.6	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	42.5	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	42.4	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	42.4	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	42.3	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	42.3	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	42.3	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	42.2	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1852H RR	41.9	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	41.9	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	41.7	43.3%-40.2%	0	1	-E2E2	-	-	E1
08C344 x 1852H RR	41.7	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	41.6	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	41.4	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	41.4	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	41.2	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	41.0	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	40.6	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	40.6	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 1852H RR	40.4	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	40.4	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	40.4	43.3%-40.2%	2	0	E1e1-	-	-	E2
08C344 x 1852H RR	40.3	43.3%-40.2%	0	0	--	-	-	E1&E2
08C344 x 1852H RR	40.2	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	40.2	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	40.0	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	39.9	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	39.8	40.1%-35.3%	0	2	-E2e2	-	-	E1
08C344 x 1852H RR	38.9	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 1852H RR	38.8	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 1852H RR	34.9	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	34.8	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	34.1	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 1852H RR	33.1	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 1852H RR	32.8	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	32.4	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 1852H RR	31.7	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	31.4	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1852H RR	31.1	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	30.8	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	30.5	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	30.2	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1852H RR	30.0	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	29.6	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 1852H RR	29.6	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	29.4	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	29.3	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	29.0	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 1852H RR	27.7	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	51.5	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	50.5	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	48.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	48.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	48.4	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	48.1	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	47.9	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	47.5	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	47.0	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	46.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	46.0	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	45.0	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	44.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	44.3	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	44.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	44.2	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 4414 RR	43.7	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 4414 RR	43.6	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 4414 RR	43.5	≥43.3%	2	1	E1e1E2E2	N	E1	-
08C344 x 4414 RR	43.4	≥43.3%	0	1	-E2E2	-	-	E1
08C344 x 4414 RR	43.4	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 4414 RR	43.3	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 4414 RR	42.9	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 4414 RR	42.7	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	42.5	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	42.3	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 4414 RR	42.0	43.3%-40.2%	0	1	-E2E2	-	-	E1
08C344 x 4414 RR	42.0	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	41.9	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	41.8	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	41.6	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 4414 RR	41.4	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	41.2	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 4414 RR	41.1	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	40.8	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	40.6	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	40.6	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	40.5	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 4414 RR	40.2	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 4414 RR	40.1	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	40.1	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	40.1	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	40.0	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	39.8	40.1%-35.3%	1	0	E1E1-	-	-	E2
08C344 x 4414 RR	39.5	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	39.2	40.1%-35.3%	1	0	E1E1-	-	-	E2
08C344 x 4414 RR	39.1	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	39.0	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 4414 RR	39.0	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	38.8	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	38.3	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	38.2	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	38.0	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	37.8	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	37.7	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	37.7	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	37.6	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	37.3	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	37.1	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	37.0	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	37.0	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	37.0	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	36.7	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	36.2	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	36.2	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	36.0	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	35.9	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 4414 RR	35.8	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	35.3	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 4414 RR	35.1	35.2%-32.1%	1	0	E1E1-	-	-	E2
08C344 x 4414 RR	35.0	35.2%-32.1%	1	2	E1E1E2e2	N	E1	-
08C344 x 4414 RR	34.4	35.2%-32.1%	1	2	E1E1E2e2	N	E1	-
08C344 x 4414 RR	34.1	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	33.7	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 4414 RR	33.3	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 4414 RR	32.8	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	32.8	35.2%-32.1%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	31.6	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	30.3	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	30.3	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	29.1	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	28.8	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	28.3	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	27.7	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	27.6	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	26.9	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	26.2	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	25.0	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	25.0	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 4414 RR	24.3	≤32.0%	0	2	-E2e2	-	-	E1
08C344 x 4414 RR	23.9	≤32.0%	2	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 4414 RR	23.6	≤32.0%	2	0	E1e1-	-	-	E2
08C344 x 71-45 RR	51.8	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 71-45 RR	50.6	≥43.3%	1	0	E1E1-	-	-	E2
08C344 x 71-45 RR	49.1	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 71-45 RR	48.6	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 71-45 RR	48.5	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 71-45 RR	48.3	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 71-45 RR	47.3	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 71-45 RR	47.2	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 71-45 RR	47.2	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 71-45 RR	45.1	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 71-45 RR	44.7	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 71-45 RR	44.6	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 71-45 RR	44.6	≥43.3%	1	1	E1E1E2E2	Y	-	-
08C344 x 71-45 RR	44.2	≥43.3%	1	2	E1E1E2e2	N	E2	-
08C344 x 71-45 RR	43.5	≥43.3%	2	0	E1e1-	-	-	E2
08C344 x 71-45 RR	43.2	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	43.1	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	43.1	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	42.7	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	42.6	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	42.3	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	42.0	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 71-45 RR	41.4	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	41.2	43.3%-40.2%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	41.1	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	40.8	43.3%-40.2%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 71-45 RR	40.7	43.3%-40.2%	2	1	E1e1E2E2	Y	-	-
08C344 x 71-45 RR	40.7	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	40.6	43.3%-40.2%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	39.9	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 71-45 RR	39.9	40.1%-35.3%	1	0	E1E1-	-	-	E2
08C344 x 71-45 RR	38.7	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	38.6	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	38.3	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	38.2	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	38.2	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	37.9	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	37.2	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	37.2	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 71-45 RR	37.1	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 71-45 RR	37.1	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 71-45 RR	37.0	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	36.9	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 71-45 RR	36.8	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 71-45 RR	36.4	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	36.3	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	36.3	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 71-45 RR	36.1	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 71-45 RR	36.0	40.1%-35.3%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	36.0	40.1%-35.3%	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 71-45 RR	35.8	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-
08C344 x 71-45 RR	35.6	40.1%-35.3%	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 71-45 RR	35.4	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 71-45 RR	35.4	40.1%-35.3%	2	1	E1e1E2E2	Y	-	-
08C344 x 71-45 RR	35.3	40.1%-35.3%	2	0	E1e1-	-	-	E2
08C344 x 71-45 RR	35.1	35.2%-32.1%	1	2	E1E1E2e2	N	E1	-
08C344 x 71-45 RR	35.1	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	34.7	35.2%-32.1%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	34.3	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	33.8	35.2%-32.1%	1	2	E1E1E2e2	N	E1	-
08C344 x 71-45 RR	33.5	35.2%-32.1%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	32.8	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	32.1	35.2%-32.1%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	31.8	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	31.7	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	31.4	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 71-45 RR	31.3	≤32.0%	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 71-45 RR	30.9	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 71-45 RR	29.3	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 71-45 RR	28.1	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	28.0	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	27.2	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	27.1	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	26.2	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	25.9	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	25.8	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	25.5	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	25.0	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 71-45 RR	24.4	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	24.2	≤32.0%	2	1	E1e1E2E2	N	E2	-
08C344 x 71-45 RR	24.0	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 71-45 RR	23.4	≤32.0%	2	2	E1e1E2e2	Y	-	-
08C344 x 71-45 RR	22.2	≤32.0%	2	2	E1e1E2e2	Y	-	-

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

Appendix Table A3. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes from BC1F1 progeny from conventional HEAR crosses 08C344 x 1841 RR, 08C344 x 1852H RR, 08C344 x 4414 RR, and 08C344 x 71-45 RR from the 2010 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 1841 RR	≥43.3%	24	8	15	0	1	0	1	34.78	65.22	0.00	4.17	0.00	4.17
08C344 x 1852H RR	≥43.3%	31	20	9	0	2	0	2	68.97	31.03	0.00	6.45	0.00	6.45
08C344 x 4414 RR	≥43.3%	22	16	5	1	0	0	1	76.19	23.81	4.55	0.00	0.00	4.55
08C344 x 71-45 RR	≥43.3%	15	7	6	0	2	0	2	53.85	46.15	0.00	13.33	0.00	13.33
Total	≥43.3%	92	51	35	1	5	0	6	59.30	40.70	1.09	5.43	0.00	6.52
Mean	≥43.3%		13	9	0	1	0	2	58.45	41.55	1.14	5.99	0.00	7.12
Minimum	≥43.3%		7	5	0	0	0	1	34.78	23.81	0.00	0.00	0.00	4.17
Maximum	≥43.3%		20	15	1	2	0	2	76.19	65.22	4.55	13.33	0.00	13.33
08C344 x 1841 RR	43.3%-40.2%	15	9	5	0	1	0	1	64.29	35.71	0.00	6.67	0.00	6.67
08C344 x 1852H RR	43.3%-40.2%	27	22	3	1	1	0	2	88.00	12.00	3.70	3.70	0.00	7.41
08C344 x 4414 RR	43.3%-40.2%	17	11	5	1	0	0	1	68.75	31.25	5.88	0.00	0.00	5.88
08C344 x 71-45 RR	43.3%-40.2%	14	8	6	0	0	0	0	57.14	42.86	0.00	0.00	0.00	0.00
Total	43.3%-40.2%	73	50	19	2	2	0	4	72.46	27.54	2.74	2.74	0.00	5.48
Mean	43.3%-40.2%		13	5	1	1	0	1	69.54	30.46	2.40	2.59	0.00	4.99
Minimum	43.3%-40.2%		8	3	0	0	0	0	57.14	12.00	0.00	0.00	0.00	0.00
Maximum	43.3%-40.2%		22	6	1	1	0	2	88.00	42.86	5.88	6.67	0.00	7.41
08C344 x 1841 RR	40.1%-35.3%	26	15	8	0	3	0	3	65.22	34.78	0.00	11.54	0.00	11.54
08C344 x 1852H RR	40.1%-35.3%	5	4	0	1	0	0	1	100.00	0.00	20.00	0.00	0.00	20.00
08C344 x 4414 RR	40.1%-35.3%	30	27	1	0	2	0	2	96.43	3.57	0.00	6.67	0.00	6.67
08C344 x 71-45 RR	40.1%-35.3%	26	10	14	0	2	0	2	41.67	58.33	0.00	7.69	0.00	7.69
Total	40.1%-35.3%	87	56	23	1	7	0	8	70.89	29.11	1.15	8.05	0.00	9.20
Mean	40.1%-35.3%		14	6	0	2	0	2	75.83	24.17	5.00	6.47	0.00	11.47
Minimum	40.1%-35.3%		4	0	0	0	0	1	41.67	0.00	0.00	0.00	0.00	6.67
Maximum	40.1%-35.3%		27	14	1	3	0	3	100.00	58.33	20.00	11.54	0.00	20.00
08C344 x 1841 RR	35.2%-32.1%	13	2	9	0	2	0	2	18.18	81.82	0.00	15.38	0.00	15.38
08C344 x 1852H RR	35.2%-32.1%	6	4	1	0	0	1	1	80.00	20.00	0.00	0.00	16.67	16.67
08C344 x 4414 RR	35.2%-32.1%	8	3	4	0	1	0	1	42.86	57.14	0.00	12.50	0.00	12.50
08C344 x 71-45 RR	35.2%-32.1%	8	0	8	0	0	0	0	0.00	100.00	0.00	0.00	0.00	0.00
Total	35.2%-32.1%	35	9	22	0	3	1	4	29.03	70.97	0.00	8.57	2.86	11.43
Mean	35.2%-32.1%		2	6	0	1	0	1	35.26	64.74	0.00	6.97	4.17	11.14
Minimum	35.2%-32.1%		0	1	0	0	0	0	0.00	20.00	0.00	0.00	0.00	0.00
Maximum	35.2%-32.1%		4	9	0	2	1	2	80.00	100.00	0.00	15.38	16.67	16.67

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 1841 RR	≤32.2%	14	3	9	0	2	0	2	25.00	75.00	0.00	14.29	0.00	14.29
08C344 x 1852H RR	≤32.2%	13	10	3	0	0	0	0	76.92	23.08	0.00	0.00	0.00	0.00
08C344 x 4414 RR	≤32.2%	15	13	0	1	1	0	2	100.00	0.00	6.67	6.67	0.00	13.33
08C344 x 71-45 RR	≤32.2%	20	7	13	0	0	0	0	35.00	65.00	0.00	0.00	0.00	0.00
Total	≤32.2%	62	33	25	1	3	0	4	56.90	43.10	1.61	4.84	0.00	6.45
Mean	≤32.2%		8	6	0	1	0	1	59.23	40.77	1.67	5.24	0.00	6.90
Minimum	≤32.2%		3	0	0	0	0	0	25.00	0.00	0.00	0.00	0.00	0.00
Maximum	≤32.2%		13	13	1	2	0	2	100.00	75.00	6.67	14.29	0.00	14.29
Total Plants Evaluated (%)		92.55												
Overall Accuracy (%)		61.61												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

Appendix Table A4. Phenotypic and genotypic results for erucic acid (C22:1) content for BC1F1 progeny from Roundup Ready HEAR crosses 1841 RR x Red River 1997, 1852H RR x Red River 1997, 30412-B6 RR x Red River 1997, 30507-B6 RR x Red River 1997, 30609-B6 x Red River 1997, SP Favourable RR x Red River 1997, SP621 RR x Red River 1997, and SW-PL-7888 RR x Red River 1997 from the 2010 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
1841 RR x Red River 1997	45.4	≥39.5%	1	0	E1E1-	-	-	E2
1841 RR x Red River 1997	42.6	≥39.5%	0	1	-E2E2	-	-	E1
1841 RR x Red River 1997	42.3	≥39.5%	1	1	E1E1E2E2	Y	-	-
1841 RR x Red River 1997	41.7	≥39.5%	1	1	E1E1E2E2	Y	-	-
1841 RR x Red River 1997	41.0	≥39.5%	1	1	E1E1E2E2	Y	-	-
1841 RR x Red River 1997	40.2	≥39.5%	1	1	E1E1E2E2	Y	-	-
1841 RR x Red River 1997	40.1	≥39.5%	1	1	E1E1E2E2	Y	-	-
1841 RR x Red River 1997	36.7	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	35.5	36.3%-29.6%	0	1	-E2E2	-	-	E1
1841 RR x Red River 1997	35.1	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1841 RR x Red River 1997	34.7	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	34.2	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	34.2	36.3%-29.6%	1	1	E1E1E2E2	N	E1/E2	-
1841 RR x Red River 1997	33.9	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	33.7	36.3%-29.6%	0	0	--	-	-	E1&E2
1841 RR x Red River 1997	33.5	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1841 RR x Red River 1997	32.7	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	32.4	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	32.3	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1841 RR x Red River 1997	31.9	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	30.8	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1841 RR x Red River 1997	30.1	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1841 RR x Red River 1997	29.2	29.5%-26.5%	1	2	E1E1E2e2	N	E1	-
1841 RR x Red River 1997	29.1	29.5%-26.5%	2	1	E1e1E2E2	N	E2	-
1841 RR x Red River 1997	28.8	29.5%-26.5%	2	1	E1e1E2E2	N	E2	-
1841 RR x Red River 1997	24.2	≤26.4%	2	2	E1e1E2e2	Y	-	-
1841 RR x Red River 1997	23.1	≤26.4%	1	2	E1E1E2e2	N	E1	-
1841 RR x Red River 1997	23.0	≤26.4%	2	2	E1e1E2e2	Y	-	-
1841 RR x Red River 1997	22.7	≤26.4%	2	2	E1e1E2e2	Y	-	-
1841 RR x Red River 1997	22.2	≤26.4%	2	2	E1e1E2e2	Y	-	-
1841 RR x Red River 1997	21.8	≤26.4%	0	2	-E2e2	-	-	E1
1841 RR x Red River 1997	21.4	≤26.4%	2	2	E1e1E2e2	Y	-	-
1852H RR x Red River 1997	43.6	≥39.5%	1	1	E1E1E2E2	Y	-	-
1852H RR x Red River 1997	41.7	≥39.5%	1	0	E1E1-	-	-	E2
1852H RR x Red River 1997	40.2	≥39.5%	1	1	E1E1E2E2	Y	-	-
1852H RR x Red River 1997	40.1	≥39.5%	1	0	E1E1-	-	-	E2
1852H RR x Red River 1997	38.8	39.4%-36.4%	1	1	E1E1E2E2	N	E1/E2	-
1852H RR x Red River 1997	37.6	39.4%-36.4%	1	1	E1E1E2E2	N	E1/E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
1852H RR x Red River 1997	37.3	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
1852H RR x Red River 1997	37.0	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
1852H RR x Red River 1997	36.8	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
1852H RR x Red River 1997	36.5	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
1852H RR x Red River 1997	36.4	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
1852H RR x Red River 1997	35.5	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1852H RR x Red River 1997	34.9	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1852H RR x Red River 1997	34.8	36.3%-29.6%	2	0	E1e1-	-	-	E2
1852H RR x Red River 1997	34.8	36.3%-29.6%	1	1	E1E1E2E2	N	E1/E2	-
1852H RR x Red River 1997	34.5	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1852H RR x Red River 1997	34.3	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1852H RR x Red River 1997	33.5	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
1852H RR x Red River 1997	33.4	36.3%-29.6%	1	1	E1E1E2E2	N	E1/E2	-
1852H RR x Red River 1997	32.6	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1852H RR x Red River 1997	30.3	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
1852H RR x Red River 1997	30.0	36.3%-29.6%	0	2	-E2e2	-	-	E1
1852H RR x Red River 1997	29.4	29.5%-26.5%	2	1	E1e1E2E2	N	E2	-
1852H RR x Red River 1997	27.3	29.5%-26.5%	2	1	E1e1E2E2	N	E2	-
1852H RR x Red River 1997	25.2	≤26.4%	2	1	E1e1E2E2	N	E2	-
1852H RR x Red River 1997	25.1	≤26.4%	2	2	E1e1E2e2	Y	-	-
1852H RR x Red River 1997	24.6	≤26.4%	2	1	E1e1E2E2	N	E2	-
1852H RR x Red River 1997	23.7	≤26.4%	2	2	E1e1E2e2	Y	-	-
1852H RR x Red River 1997	23.4	≤26.4%	2	1	E1e1E2E2	N	E2	-
1852H RR x Red River 1997	23.4	≤26.4%	2	1	E1e1E2E2	N	E2	-
30412-B6 RR x Red River 1997	45.2	≥39.5%	1	1	E1E1E2E2	Y	-	-
30412-B6 RR x Red River 1997	43.5	≥39.5%	1	1	E1E1E2E2	Y	-	-
30412-B6 RR x Red River 1997	43.0	≥39.5%	1	1	E1E1E2E2	Y	-	-
30412-B6 RR x Red River 1997	42.5	≥39.5%	1	1	E1E1E2E2	Y	-	-
30412-B6 RR x Red River 1997	41.0	≥39.5%	1	1	E1E1E2E2	Y	-	-
30412-B6 RR x Red River 1997	40.5	≥39.5%	1	1	E1E1E2E2	Y	-	-
30412-B6 RR x Red River 1997	39.5	≥39.5%	1	2	E1E1E2e2	N	E2	-
30412-B6 RR x Red River 1997	37.5	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	37.4	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	37.0	39.4%-36.4%	0	1	-E2E2	-	-	E1
30412-B6 RR x Red River 1997	36.6	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
30412-B6 RR x Red River 1997	36.0	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30412-B6 RR x Red River 1997	35.5	36.3%-29.6%	0	1	-E2E2	-	-	E1
30412-B6 RR x Red River 1997	35.5	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	35.1	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	35.1	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	35.0	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	34.9	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
30412-B6 RR x Red River 1997	34.8	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30412-B6 RR x Red River 1997	34.7	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30412-B6 RR x Red River 1997	34.2	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	33.1	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	32.9	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30412-B6 RR x Red River 1997	31.9	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30412-B6 RR x Red River 1997	29.8	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
30412-B6 RR x Red River 1997	27.0	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
30412-B6 RR x Red River 1997	26.1	≤26.4%	2	2	E1e1E2e2	Y	-	-
30412-B6 RR x Red River 1997	25.2	≤26.4%	2	2	E1e1E2e2	Y	-	-
30412-B6 RR x Red River 1997	24.4	≤26.4%	2	2	E1e1E2e2	Y	-	-
30412-B6 RR x Red River 1997	23.8	≤26.4%	2	2	E1e1E2e2	Y	-	-
30412-B6 RR x Red River 1997	23.6	≤26.4%	2	2	E1e1E2e2	Y	-	-
30412-B6 RR x Red River 1997	23.0	≤26.4%	2	2	E1e1E2e2	Y	-	-
30507-B6 RR x Red River 1997	45.4	≥39.5%	1	1	E1E1E2E2	Y	-	-
30507-B6 RR x Red River 1997	45.1	≥39.5%	1	1	E1E1E2E2	Y	-	-
30507-B6 RR x Red River 1997	44.6	≥39.5%	1	1	E1E1E2E2	Y	-	-
30507-B6 RR x Red River 1997	44.4	≥39.5%	1	1	E1E1E2E2	Y	-	-
30507-B6 RR x Red River 1997	44.0	≥39.5%	1	1	E1E1E2E2	Y	-	-
30507-B6 RR x Red River 1997	39.9	≥39.5%	1	2	E1E1E2e2	N	E2	-
30507-B6 RR x Red River 1997	38.4	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
30507-B6 RR x Red River 1997	38.2	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	37.5	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	36.3	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30507-B6 RR x Red River 1997	36.0	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	35.4	36.3%-29.6%	1	0	E1E1-	-	-	E2
30507-B6 RR x Red River 1997	34.9	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	34.6	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	34.6	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	34.4	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	34.4	36.3%-29.6%	1	0	E1E1-	-	-	E2
30507-B6 RR x Red River 1997	34.3	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	34.3	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	34.0	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	33.9	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30507-B6 RR x Red River 1997	33.1	36.3%-29.6%	0	1	-E2E2	-	-	E1
30507-B6 RR x Red River 1997	32.9	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30507-B6 RR x Red River 1997	27.7	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
30507-B6 RR x Red River 1997	27.2	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
30507-B6 RR x Red River 1997	26.5	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
30507-B6 RR x Red River 1997	26.0	≤26.4%	2	2	E1e1E2e2	Y	-	-
30507-B6 RR x Red River 1997	25.3	≤26.4%	2	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
30507-B6 RR x Red River 1997	25.0	≤26.4%	2	2	E1e1E2e2	Y	-	-
30507-B6 RR x Red River 1997	24.3	≤26.4%	2	2	E1e1E2e2	Y	-	-
30609-B6 RR x Red River 1997	48.2	≥38.7%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	45.2	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	43.3	≥39.5%	1	0	E1E1-	-	-	E2
30609-B6 RR x Red River 1997	42.3	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	42.1	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	42.0	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	41.8	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	41.3	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	41.1	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	40.8	≥39.5%	1	1	E1E1E2E2	Y	-	-
30609-B6 RR x Red River 1997	37.9	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	37.1	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	36.0	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	34.9	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	34.8	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	34.5	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	34.0	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	33.4	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30609-B6 RR x Red River 1997	33.3	36.3%-29.6%	1	0	E1E1-	-	-	E2
30609-B6 RR x Red River 1997	33.2	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	33.0	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	32.7	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
30609-B6 RR x Red River 1997	31.1	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
30609-B6 RR x Red River 1997	27.1	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
30609-B6 RR x Red River 1997	27.1	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
30609-B6 RR x Red River 1997	24.8	≤26.4%	2	2	E1e1E2e2	Y	-	-
30609-B6 RR x Red River 1997	24.8	≤26.4%	2	2	E1e1E2e2	Y	-	-
30609-B6 RR x Red River 1997	24.3	≤26.4%	0	2	-E2e2	-	-	E1
30609-B6 RR x Red River 1997	23.7	≤26.4%	2	2	E1e1E2e2	Y	-	-
30609-B6 RR x Red River 1997	23.4	≤26.4%	2	2	E1e1E2e2	Y	-	-
30609-B6 RR x Red River 1997	23.2	≤26.4%	1	0	E1E1-	-	-	E2
30609-B6 RR x Red River 1997	23.1	≤26.4%	2	2	E1e1E2e2	Y	-	-
SP Favourable RR x Red River 1997	49.0	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP Favourable RR x Red River 1997	45.6	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP Favourable RR x Red River 1997	44.8	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP Favourable RR x Red River 1997	44.2	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP Favourable RR x Red River 1997	40.8	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP Favourable RR x Red River 1997	39.8	≥39.5%	1	2	E1E1E2e2	N	E2	-
SP Favourable RR x Red River 1997	39.5	≥39.5%	1	2	E1E1E2e2	N	E2	-
SP Favourable RR x Red River 1997	38.9	39.4%-36.4%	1	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
SP Favourable RR x Red River 1997	38.5	39.4%-36.4%	1	1	E1E1E2E2	N	E1/E2	-
SP Favourable RR x Red River 1997	37.7	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
SP Favourable RR x Red River 1997	37.0	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
SP Favourable RR x Red River 1997	36.3	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP Favourable RR x Red River 1997	36.1	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
SP Favourable RR x Red River 1997	35.9	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
SP Favourable RR x Red River 1997	34.9	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
SP Favourable RR x Red River 1997	34.5	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP Favourable RR x Red River 1997	33.9	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
SP Favourable RR x Red River 1997	33.8	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP Favourable RR x Red River 1997	32.7	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP Favourable RR x Red River 1997	32.6	36.3%-29.6%	2	1	E1e1E2E2	Y	-	-
SP Favourable RR x Red River 1997	30.5	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP Favourable RR x Red River 1997	30.1	36.3%-29.6%	2	2	E1e1E2e2	N	E1/E2	-
SP Favourable RR x Red River 1997	27.4	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SP Favourable RR x Red River 1997	26.8	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SP Favourable RR x Red River 1997	26.2	≤26.4%	2	2	E1e1E2e2	Y	-	-
SP Favourable RR x Red River 1997	23.4	≤26.4%	2	2	E1e1E2e2	Y	-	-
SP621 RR x Red River 1997	49.4	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP621 RR x Red River 1997	48.7	≥39.5%	1	0	E1E1-	-	-	E2
SP621 RR x Red River 1997	45.8	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP621 RR x Red River 1997	45.7	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP621 RR x Red River 1997	44.8	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP621 RR x Red River 1997	44.7	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP621 RR x Red River 1997	43.3	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP621 RR x Red River 1997	41.6	≥39.5%	1	2	E1E1E2e2	N	E2	-
SP621 RR x Red River 1997	41.4	≥39.5%	2	1	E1e1E2E2	N	E1	-
SP621 RR x Red River 1997	40.2	≥39.5%	1	2	E1E1E2e2	N	E2	-
SP621 RR x Red River 1997	40.1	≥39.5%	1	1	E1E1E2E2	Y	-	-
SP621 RR x Red River 1997	39.7	≥39.5%	1	2	E1E1E2e2	N	E2	-
SP621 RR x Red River 1997	39.1	39.4%-36.4%	0	1	-E2E2	-	-	E1
SP621 RR x Red River 1997	38.8	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
SP621 RR x Red River 1997	38.5	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
SP621 RR x Red River 1997	38.1	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
SP621 RR x Red River 1997	37.7	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
SP621 RR x Red River 1997	37.2	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
SP621 RR x Red River 1997	36.5	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
SP621 RR x Red River 1997	36.2	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP621 RR x Red River 1997	35.7	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP621 RR x Red River 1997	34.7	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SP621 RR x Red River 1997	33.9	36.3%-29.6%	2	2	E1e1E2e2	N	E1/E2	-
SP621 RR x Red River 1997	33.5	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
						Correct Y/N?	Error E1/E2?	Missing E1/E2?
SP621 RR x Red River 1997	31.0	36.3%-29.6%	2	2	E1e1E2e2	N	E1/E2	-
SP621 RR x Red River 1997	28.6	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SP621 RR x Red River 1997	27.8	29.5%-26.5%	1	2	E1E1E2e2	N	E1	-
SP621 RR x Red River 1997	27.7	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SP621 RR x Red River 1997	27.0	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SP621 RR x Red River 1997	26.8	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SP621 RR x Red River 1997	24.6	≤26.4%	2	2	E1e1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	47.9	≥39.5%	1	2	E1E1E2e2	N	E2	-
SW-PL-7888 RR x Red River 1997	45.6	≥39.5%	1	1	E1E1E2E2	Y	-	-
SW-PL-7888 RR x Red River 1997	44.7	≥39.5%	1	1	E1E1E2E2	Y	-	-
SW-PL-7888 RR x Red River 1997	44.5	≥39.5%	1	0	E1E1-	-	-	E2
SW-PL-7888 RR x Red River 1997	44.4	≥39.5%	1	1	E1E1E2E2	Y	-	-
SW-PL-7888 RR x Red River 1997	43.7	≥39.5%	1	1	E1E1E2E2	Y	-	-
SW-PL-7888 RR x Red River 1997	42.1	≥39.5%	1	2	E1E1E2e2	N	E2	-
SW-PL-7888 RR x Red River 1997	42.1	≥39.5%	0	1	-E2E2	-	-	E1
SW-PL-7888 RR x Red River 1997	41.2	≥39.5%	2	1	E1e1E2E2	N	E1	-
SW-PL-7888 RR x Red River 1997	40.8	≥39.5%	1	0	E1E1-	-	-	E2
SW-PL-7888 RR x Red River 1997	39.9	≥39.5%	2	1	E1e1E2E2	N	E1	-
SW-PL-7888 RR x Red River 1997	39.7	≥39.5%	2	1	E1e1E2E2	N	E1	-
SW-PL-7888 RR x Red River 1997	39.7	≥39.5%	2	1	E1e1E2E2	N	E1	-
SW-PL-7888 RR x Red River 1997	39.5	≥39.5%	1	0	E1E1-	-	-	E2
SW-PL-7888 RR x Red River 1997	39.1	39.4%-36.4%	2	1	E1e1E2E2	N	E1	-
SW-PL-7888 RR x Red River 1997	37.8	39.4%-36.4%	2	1	E1e1E2E2	Y	-	-
SW-PL-7888 RR x Red River 1997	37.6	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	37.5	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	37.1	39.4%-36.4%	1	2	E1E1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	36.1	36.3%-29.6%	0	2	-E2e2	-	-	E1
SW-PL-7888 RR x Red River 1997	35.6	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	35.6	36.3%-29.6%	1	2	E1E1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	30.6	36.3%-29.6%	2	2	E1e1E2e2	N	E1/E2	-
SW-PL-7888 RR x Red River 1997	29.7	29.5%-26.5%	2	0	E1e1-	-	-	E2
SW-PL-7888 RR x Red River 1997	28.9	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	27.5	29.5%-26.5%	2	2	E1e1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	26.2	≤26.4%	2	2	E1e1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	26.1	≤26.4%	2	2	E1e1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	25.0	≤26.4%	2	2	E1e1E2e2	Y	-	-
SW-PL-7888 RR x Red River 1997	24.1	≤26.4%	1	2	E1E1E2e2	N	E1	-
SW-PL-7888 RR x Red River 1997	22.7	≤26.4%	2	2	E1e1E2e2	Y	-	-

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

Appendix Table A5. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes for BC1F1 progeny from Roundup Ready HEAR crosses 1841 RR x Red River 1997, 1852H RR x Red River 1997, 30412-B6 RR x Red River 1997, 30507-B6 RR x Red River 1997, 30609-B6 x Red River 1997, SP Favourable RR x Red River 1997, SP621 RR x Red River 1997, and SW-PL-7888 RR x Red River 1997 from the 2010 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
1841 RR x Red River 1997	≥39.5%	7	5	0	1	1	0	2	100.00	0.00	14.29	14.29	0.00	28.57
1852H RR x Red River 1997	≥39.5%	4	2	0	0	2	0	2	100.00	0.00	0.00	50.00	0.00	50.00
30412-B6 RR x Red River 1997	≥39.5%	7	6	1	0	0	0	0	85.71	14.29	0.00	0.00	0.00	0.00
30507-B6 RR x Red River 1997	≥39.5%	6	5	1	0	0	0	0	83.33	16.67	0.00	0.00	0.00	0.00
30609-B6 RR x Red River 1997	≥39.5%	10	9	0	0	1	0	1	100.00	0.00	0.00	10.00	0.00	10.00
SP Favourable RR x Red River 1997	≥39.5%	7	6	1	0	0	0	0	85.71	14.29	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	≥39.5%	12	7	3	1	1	0	2	70.00	30.00	8.33	8.33	0.00	16.67
SW-PL-7888 RR x Red River 1997	≥39.5%	14	4	6	1	3	0	4	40.00	60.00	7.14	21.43	0.00	28.57
Total	≥39.5%	67	44	12	3	8	0	11	78.57	21.43	4.48	11.94	0.00	16.42
Mean	≥39.5%		6	2	0	1	0	1	83.10	16.90	3.72	13.01	0.00	16.73
Minimum	≥39.5%		2	0	0	0	0	0	40.00	0.00	0.00	0.00	0.00	0.00
Maximum	≥39.5%		9	6	1	3	0	4	100.00	60.00	14.29	50.00	0.00	50.00
1841 RR x Red River 1997	39.4%-36.4%	1	1	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
1852H RR x Red River 1997	39.4%-36.4%	7	5	2	0	0	0	0	71.43	28.57	0.00	0.00	0.00	0.00
30412-B6 RR x Red River 1997	39.4%-36.4%	4	3	0	1	0	0	1	100.00	0.00	25.00	0.00	0.00	25.00
30507-B6 RR x Red River 1997	39.4%-36.4%	3	3	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
30609-B6 RR x Red River 1997	39.4%-36.4%	2	2	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
SP Favourable RR x Red River 1997	39.4%-36.4%	4	3	1	0	0	0	0	75.00	25.00	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	39.4%-36.4%	7	6	-1	1	1	0	2	120.00	-20.00	14.29	14.29	0.00	28.57
SW-PL-7888 RR x Red River 1997	39.4%-36.4%	5	4	1	0	0	0	0	80.00	20.00	0.00	0.00	0.00	0.00
Total	39.4%-36.4%	33	27	3	2	1	0	3	90.00	10.00	6.06	3.03	0.00	9.09
Mean	39.4%-36.4%		3	0	0	0	0	0	93.30	6.70	4.91	1.79	0.00	6.70
Minimum	39.4%-36.4%		1	-1	0	0	0	0	71.43	-20.00	0.00	0.00	0.00	0.00
Maximum	39.4%-36.4%		6	2	1	1	0	2	120.00	28.57	25.00	14.29	0.00	28.57
1841 RR x Red River 1997	36.3%-29.6%	14	11	1	1	0	1	2	91.67	8.33	7.14	0.00	7.14	14.29
1852H RR x Red River 1997	36.3%-29.6%	11	7	2	1	1	0	2	77.78	22.22	9.09	9.09	0.00	18.18
30412-B6 RR x Red River 1997	36.3%-29.6%	13	12	0	1	0	0	1	100.00	0.00	7.69	0.00	0.00	7.69
30507-B6 RR x Red River 1997	36.3%-29.6%	14	11	0	1	2	0	3	100.00	0.00	7.14	14.29	0.00	21.43
30609-B6 RR x Red River 1997	36.3%-29.6%	11	10	0	0	1	0	1	100.00	0.00	0.00	9.09	0.00	9.09
SP Favourable RR x Red River 1997	36.3%-29.6%	11	10	1	0	0	0	0	90.91	9.09	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	36.3%-29.6%	6	2	4	0	0	0	0	33.33	66.67	0.00	0.00	0.00	0.00
SW-PL-7888 RR x Red River 1997	36.3%-29.6%	4	2	1	1	0	0	1	66.67	33.33	25.00	0.00	0.00	25.00
Total	36.3%-29.6%	84	65	9	5	4	1	10	87.84	12.16	5.95	4.76	1.19	11.90
Mean	36.3%-29.6%		8	1	1	1	0	1	82.54	17.46	7.01	4.06	0.89	11.96

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Minimum	36.3%-29.6%		2	0	0	0	0	0	33.33	0.00	0.00	0.00	0.00	0.00
Maximum	36.3%-29.6%		12	4	1	2	1	3	100.00	66.67	25.00	14.29	7.14	25.00
1841 RR x Red River 1997	29.5%-26.5%	3	0	3	0	0	0	0	0.00	100.00	0.00	0.00	0.00	0.00
1852H RR x Red River 1997	29.5%-26.5%	2	0	-1	1	1	1	3	0.00	100.00	50.00	50.00	50.00	150.00
30412-B6 RR x Red River 1997	29.5%-26.5%	2	2	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
30507-B6 RR x Red River 1997	29.5%-26.5%	3	3	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
30609-B6 RR x Red River 1997	29.5%-26.5%	2	2	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
SP Favourable RR x Red River 1997	29.5%-26.5%	2	2	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	29.5%-26.5%	5	4	1	0	0	0	0	80.00	20.00	0.00	0.00	0.00	0.00
SW-PL-7888 RR x Red River 1997	29.5%-26.5%	3	2	0	0	1	0	1	100.00	0.00	0.00	33.33	0.00	33.33
Total	29.5%-26.5%	22	15	3	1	2	1	4	83.33	16.67	4.55	9.09	4.55	18.18
Mean	29.5%-26.5%		2	0	0	0	0	1	72.50	27.50	6.25	10.42	6.25	22.92
Minimum	29.5%-26.5%		0	-1	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	29.5%-26.5%		4	3	1	1	1	3	100.00	100.00	50.00	50.00	50.00	150.00
1841 RR x Red River 1997	≤26.4%	7	5	1	1	0	0	1	83.33	16.67	14.29	0.00	0.00	14.29
1852H RR x Red River 1997	≤26.4%	6	2	3	1	0	0	1	40.00	60.00	16.67	0.00	0.00	16.67
30412-B6 RR x Red River 1997	≤26.4%	6	6	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
30507-B6 RR x Red River 1997	≤26.4%	4	4	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
30609-B6 RR x Red River 1997	≤26.4%	7	5	0	1	1	0	2	100.00	0.00	14.29	14.29	0.00	28.57
SP Favourable RR x Red River 1997	≤26.4%	2	2	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
SP621 RR x Red River 1997	≤26.4%	1	1	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
SW-PL-7888 RR x Red River 1997	≤26.4%	5	4	0	0	1	0	1	100.00	0.00	0.00	20.00	0.00	20.00
Total	≤26.4%	38	29	4	3	2	0	5	87.88	12.12	7.89	5.26	0.00	13.16
Mean	≤26.4%		4	1	0	0	0	1	90.42	9.58	5.65	4.29	0.00	9.94
Minimum	≤26.4%		1	0	0	0	0	0	40.00	0.00	0.00	0.00	0.00	0.00
Maximum	≤26.4%		6	3	1	1	0	2	100.00	60.00	16.67	20.00	0.00	28.57
Total Plants Evaluated (%)		86.48												
Overall Accuracy (%)		85.31												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

Appendix Table A6. Phenotypic and genotypic results for erucic acid (C22:1) content for BC1F1 progeny from conventional HEAR crosses 08C344 x 30216-C7RR, 08C344 x 30220-D8RR, 08C344 x 30221-D8RR, 08C344 x 30408-C7RR, and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30216-C7RR	50.9	≥40.8%	204.7	2.44	0.56	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	48.8	≥40.8%	268.7	2.08	0.73	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	47.6	≥40.8%	317.3	2.13	0.87	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	46.7	≥40.8%	88.8	1.35	0.82	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	44.7	≥40.8%	270.6	1.45	0.62	0.0	1	-E2E2	-	-	E1
08C344 x 30216-C7RR	44.7	≥40.8%	42.1	2.31	0.76	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	44.3	≥40.8%	1284.3	2.05	1.61	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	44.3	≥40.8%	57.0	1.97	1.21	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	43.9	≥40.8%	99.3	1.19	0.31	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30216-C7RR	43.7	≥40.8%	28.8	2.00	0.97	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30216-C7RR	43.5	≥40.8%	27.7	2.09	1.56	2.0	1	E1e1E2E2	N	-	-
08C344 x 30216-C7RR	43.4	≥40.8%	549.7	1.20	0.33	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	43.1	≥40.8%	252.7	2.16	0.39	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	43.1	≥40.8%	122.5	2.15	0.96	1.0	2	E1E1E2e2	N	-	-
08C344 x 30216-C7RR	43.0	≥40.8%	542.9	2.09	1.12	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	42.9	≥40.8%	619.8	1.87	0.61	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	42.6	≥40.8%	52.5	2.14	1.19	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	42.5	≥40.8%	359.8	2.13	0.84	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	42.5	≥40.8%	255.0	1.73	0.57	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	42.1	≥40.8%	88.3	1.98	1.74	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	41.9	≥40.8%	303.1	2.02	0.83	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30216-C7RR	41.9	≥40.8%	80.7	1.19	0.81	1.0	0	E1E1-	-	-	E2

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30216-C7RR	41.9	≥40.8%	22.9	1.68	1.02	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	41.5	≥40.8%	94.3	2.07	1.02	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30216-C7RR	40.2	40.7%-37.7%	68.2	2.09	1.17	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	39.9	40.7%-37.7%	207.7	2.00	0.93	1.0	0	E1E1-	-	-	E2
08C344 x 30216-C7RR	39.5	40.7%-37.7%	126.9	2.04	1.23	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	39.5	40.7%-37.7%	23.5	2.16	0.76	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	39.4	40.7%-37.7%	124.0	2.08	1.21	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	38.6	40.7%-37.7%	148.2	3.10	0.98	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	38.5	40.7%-37.7%	194.0	1.86	0.49	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	38.3	40.7%-37.7%	272.3	2.01	1.05	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	38.0	40.7%-37.7%	38.7	2.06	1.20	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	37.9	40.7%-37.7%	105.9	2.02	1.76	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	37.6	37.6%-31.4%	1241.8	1.94	1.56	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	37.6	37.6%-31.4%	194.3	2.09	1.11	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	37.5	37.6%-31.4%	180.2	2.04	1.92	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30216-C7RR	37.4	37.6%-31.4%	425.8	1.95	0.35	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	37.2	37.6%-31.4%	470.8	1.97	0.85	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	37.2	37.6%-31.4%	379.4	1.96	1.03	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	37.2	37.6%-31.4%	335.6	1.97	1.58	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	37.1	37.6%-31.4%	209.7	2.22	1.14	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30216-C7RR	37.1	37.6%-31.4%	109.2	2.03	0.75	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	37.0	37.6%-31.4%	47.3	2.08	1.19	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	36.6	37.6%-31.4%	233.6	1.84	0.47	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	36.2	37.6%-31.4%	871.1	2.24	1.32	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	36.2	37.6%-31.4%	226.7	1.40	0.59	0.0	1	-E2E2	-	-	E2

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30216-C7RR	36.2	37.6%-31.4%	190.5	2.07	1.22	0.0	0	--	-	-	E1&E2
08C344 x 30216-C7RR	36.0	37.6%-31.4%	229.7	1.99	1.43	2.0	0	E1e1-	-	-	E2
08C344 x 30216-C7RR	35.7	37.6%-31.4%	112.4	2.01	0.65	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	35.5	37.6%-31.4%	760.3	1.96	0.84	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	35.4	37.6%-31.4%	501.7	1.94	0.43	0.0	1	-E2E2	-	-	E1
08C344 x 30216-C7RR	35.4	37.6%-31.4%	69.0	2.10	0.55	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	34.9	37.6%-31.4%	117.6	1.74	0.45	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	34.9	37.6%-31.4%	101.1	2.01	0.77	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	34.7	37.6%-31.4%	475.3	1.98	0.73	1.0	0	E1E1-	-	-	E2
08C344 x 30216-C7RR	34.6	37.6%-31.4%	121.0	2.13	0.43	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	34.3	37.6%-31.4%	19.9	2.09	1.05	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	34.0	37.6%-31.4%	113.5	2.05	1.77	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	33.7	37.6%-31.4%	161.2	1.77	0.44	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	33.7	37.6%-31.4%	139.2	1.68	0.50	0.0	2	-E2e2	-	-	E1
08C344 x 30216-C7RR	32.7	37.6%-31.4%	352.2	1.86	0.91	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	32.6	37.6%-31.4%	9.2	0.86	0.39	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30216-C7RR	32.4	37.6%-31.4%	239.0	2.10	1.00	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	31.8	37.6%-31.4%	89.5	2.15	0.89	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30216-C7RR	30.9	31.3%-28.3%	4.2	2.14	1.44	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30216-C7RR	30.4	31.3%-28.3%	131.5	2.12	0.86	1.0	2	E1E1E2e2	N	E1	-
08C344 x 30216-C7RR	30.2	31.3%-28.3%	145.1	1.91	1.24	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30216-C7RR	30.1	31.3%-28.3%	130.0	1.90	1.61	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30216-C7RR	29.9	31.3%-28.3%	100.8	2.17	1.49	1.0	2	E1E1E2e2	N	E1	-
08C344 x 30216-C7RR	29.6	31.3%-28.3%	26.7	2.14	0.97	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	28.9	31.3%-28.3%	205.5	2.01	0.65	2.0	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30216-C7RR	28.4	31.3%-28.3%	437.2	1.98	0.57	2.0	0	E1e1-	-	-	E2
08C344 x 30216-C7RR	28.1	≤28.2%	156.2	2.09	0.95	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	27.9	≤28.2%	94.5	2.00	0.91	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	27.3	≤28.2%	402.2	2.22	1.29	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	27.1	≤28.2%	525.6	2.07	0.57	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	26.8	≤28.2%	130.6	2.21	0.24	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	26.7	≤28.2%	309.9	0.36	1.52	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30216-C7RR	26.5	≤28.2%	177.3	1.12	0.78	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	26.2	≤28.2%	100.8	2.07	1.08	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	26.1	≤28.2%	46.0	2.53	0.60	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	25.8	≤28.2%	276.6	2.15	0.78	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	25.8	≤28.2%	216.7	2.15	1.35	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	25.8	≤28.2%	143.7	2.10	1.02	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	25.7	≤28.2%	1183.1	1.84	0.37	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30216-C7RR	25.7	≤28.2%	220.2	2.16	1.76	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	25.1	≤28.2%	139.0	2.22	1.63	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30216-C7RR	24.3	≤28.2%	153.9	1.82	1.17	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	24.2	≤28.2%	196.6	2.12	1.28	0.0	2	-E2e2	-	-	E1
08C344 x 30216-C7RR	24.1	≤28.2%	853.0	2.04	0.39	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	23.8	≤28.2%	159.4	2.04	0.87	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	22.9	≤28.2%	168.2	2.16	0.71	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	22.7	≤28.2%	463.8	2.06	0.43	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30216-C7RR	22.6	≤28.2%	107.1	1.93	0.98	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	51.6	≥40.8%	186.5	1.95	1.98	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	51.0	≥40.8%	1834.6	2.12	1.65	1.0	0	E1E1-	-	-	E2

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30220-D8RR	48.7	≥40.8%	757.5	2.06	0.64	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	48.3	≥40.8%	1769.3	2.02	1.94	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	47.9	≥40.8%	10.1	1.40	0.10	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	46.4	≥40.8%	475.7	1.37	0.78	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	46.3	≥40.8%	53.9	1.82	1.60	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	46.1	≥40.8%	7.2	2.17	2.16	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	46.0	≥40.8%	140.4	2.21	1.14	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	45.5	≥40.8%	299.7	2.11	1.34	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	45.5	≥40.8%	270.8	1.88	1.01	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	45.5	≥40.8%	65.4	2.11	0.78	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	45.2	≥40.8%	299.3	1.99	0.41	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	45.2	≥40.8%	251.4	2.10	1.23	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30220-D8RR	45.1	≥40.8%	17204.0	2.32	1.44	1.0	0	E1E1-	-	-	E2
08C344 x 30220-D8RR	44.3	≥40.8%	76.6	2.03	1.12	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	44.2	≥40.8%	113.0	1.98	1.35	1.0	0	E1E1-	-	-	E2
08C344 x 30220-D8RR	43.9	≥40.8%	196.1	2.18	1.64	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	43.8	≥40.8%	-21.6	2.20	1.41	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	43.7	≥40.8%	239.1	2.10	1.26	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	43.5	≥40.8%	318.8	0.79	0.80	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	42.5	≥40.8%	8531.7	2.12	0.72	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	41.9	≥40.8%	299.8	2.05	1.38	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30220-D8RR	41.4	≥40.8%	1194.2	2.94	1.19	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30220-D8RR	41.3	≥40.8%	202.0	1.97	0.70	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	41.1	≥40.8%	173.3	1.79	0.20	2.0	0	E1e1-	-	-	E2
08C344 x 30220-D8RR	41.0	≥40.8%	1054.6	2.05	1.04	1.0	2	E1E1E2e2	N	E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30220-D8RR	40.5	40.7%-37.7%	213.6	1.96	0.54	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	40.4	40.7%-37.7%	99.1	2.10	0.81	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	40.3	40.7%-37.7%	140.0	1.29	0.78	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	40.3	40.7%-37.7%	131.0	2.09	1.40	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	40.2	40.7%-37.7%	760.6	2.04	1.26	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30220-D8RR	40.1	40.7%-37.7%	90.0	0.84	0.66	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	40.0	40.7%-37.7%	557.7	2.15	1.98	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	40.0	40.7%-37.7%	488.6	2.08	0.95	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	40.0	40.7%-37.7%	156.3	2.23	1.16	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	39.7	40.7%-37.7%	260.4	2.19	1.78	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	39.5	40.7%-37.7%	689.2	2.18	0.97	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	39.5	40.7%-37.7%	191.2	2.05	1.11	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	39.1	40.7%-37.7%	90.2	1.97	0.79	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30220-D8RR	38.8	40.7%-37.7%	3447.2	2.13	1.19	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	38.6	40.7%-37.7%	6.2	2.03	1.08	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	38.4	40.7%-37.7%	146.0	2.06	0.82	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	38.4	40.7%-37.7%	25.6	2.04	0.66	1.0	0	E1E1-	-	-	E2
08C344 x 30220-D8RR	38.3	40.7%-37.7%	204.4	2.00	1.02	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	38.1	40.7%-37.7%	288.9	2.19	0.70	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	38.1	40.7%-37.7%	183.2	1.58	0.20	1.0	0	E1E1-	-	-	E2
08C344 x 30220-D8RR	38.0	40.7%-37.7%	29.4	2.13	0.88	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	37.9	40.7%-37.7%	195.1	2.18	1.88	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	37.6	37.6%-31.4%	327.5	2.13	0.99	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	37.6	37.6%-31.4%	220.5	2.11	0.78	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	37.5	37.6%-31.4%	128.8	2.15	1.73	1.0	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30220-D8RR	37.4	37.6%-31.4%	262.2	1.81	0.87	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	37.4	37.6%-31.4%	78.7	1.94	0.85	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	37.3	37.6%-31.4%	137.3	1.39	1.38	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	37.1	37.6%-31.4%	355.1	2.10	0.75	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	37.0	37.6%-31.4%	472.5	2.13	0.91	2.0	0	E1e1-	-	-	E2
08C344 x 30220-D8RR	36.9	37.6%-31.4%	586.6	1.98	1.59	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	36.8	37.6%-31.4%	87.0	1.98	1.19	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	36.7	37.6%-31.4%	367.1	1.98	1.45	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30220-D8RR	36.6	37.6%-31.4%	264.8	-0.39	0.10	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	36.6	37.6%-31.4%	250.5	2.13	1.32	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	36.6	37.6%-31.4%	161.8	2.23	2.09	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30220-D8RR	35.0	37.6%-31.4%	205.9	2.12	1.54	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	34.6	37.6%-31.4%	401.8	1.82	1.67	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	33.2	37.6%-31.4%	151.5	1.69	0.60	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	33.1	37.6%-31.4%	163.7	2.12	1.32	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	32.9	37.6%-31.4%	65.9	2.07	1.51	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30220-D8RR	32.6	37.6%-31.4%	207.7	2.10	1.05	2.0	0	E1e1-	-	-	E2
08C344 x 30220-D8RR	32.3	37.6%-31.4%	279.5	1.65	1.07	0.0	1	-E2E2	-	-	E1
08C344 x 30220-D8RR	32.0	37.6%-31.4%	126.1	1.70	1.40	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30220-D8RR	31.6	37.6%-31.4%	509.8	2.10	0.87	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30220-D8RR	31.3	31.3%-28.3%	80.9	2.07	0.98	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	30.7	31.3%-28.3%	113.1	1.84	1.18	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	30.6	31.3%-28.3%	355.4	2.11	1.81	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	30.6	31.3%-28.3%	171.6	1.96	0.81	1.0	2	E1E1E2e2	N	E1	-
08C344 x 30220-D8RR	29.6	31.3%-28.3%	213.2	-1.02	0.58	2.0	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30220-D8RR	29.5	31.3%-28.3%	215.6	0.96	0.97	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	29.4	31.3%-28.3%	173.4	2.13	0.69	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	29.1	31.3%-28.3%	358.5	2.00	0.45	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	29.0	31.3%-28.3%	766.8	2.22	0.33	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	28.6	31.3%-28.3%	54.3	1.43	0.74	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30220-D8RR	28.5	31.3%-28.3%	134.6	2.06	2.10	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	27.7	≤28.2%	302.0	2.13	1.76	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	27.2	≤28.2%	4770.5	2.06	1.03	0.0	2	-E2e2	-	-	E1
08C344 x 30220-D8RR	27.0	≤28.2%	350.7	2.20	1.66	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	26.8	≤28.2%	2419.1	1.50	0.91	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	26.8	≤28.2%	313.2	2.11	1.58	2.0	0	E1e1-	-	-	E2
08C344 x 30220-D8RR	26.6	≤28.2%	278.7	0.63	0.74	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	25.8	≤28.2%	269.8	0.72	-0.46	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	25.4	≤28.2%	556.1	2.10	1.25	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	25.2	≤28.2%	338.8	2.03	0.74	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	24.4	≤28.2%	4251.2	2.08	1.90	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	22.6	≤28.2%	159.5	2.09	1.89	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30220-D8RR	20.4	≤28.2%	1915.4	2.07	0.91	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	52.3	≥40.8%	237.3	2.13	1.80	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	49.4	≥40.8%	43.0	2.08	0.69	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	49.2	≥40.8%	753.9	2.13	1.96	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	47.2	≥40.8%	367.6	1.16	0.32	0.0	1	-E2E2	-	-	E1
08C344 x 30221-D8RR	45.8	≥40.8%	492.4	1.30	2.34	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	45.7	≥40.8%	689.6	1.94	1.52	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	45.4	≥40.8%	933.1	2.11	2.13	1.0	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30221-D8RR	45.2	≥40.8%	193.0	2.03	0.71	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	45.1	≥40.8%	388.0	1.81	0.84	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	44.8	≥40.8%	209.7	2.09	1.40	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	44.7	≥40.8%	59.8	1.78	0.23	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	43.9	≥40.8%	33.3	2.12	1.24	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	43.8	≥40.8%	470.1	2.13	1.10	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	43.7	≥40.8%	760.1	2.06	2.00	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	43.7	≥40.8%	28.5	2.07	0.85	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	43.6	≥40.8%	74.5	2.05	1.66	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	43.3	≥40.8%	196.7	2.10	1.15	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	43.0	≥40.8%	1251.5	2.15	1.98	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30221-D8RR	43.0	≥40.8%	215.7	2.00	0.39	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	42.5	≥40.8%	179.6	2.14	0.63	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	42.3	≥40.8%	186.4	2.06	0.95	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	41.6	≥40.8%	284.2	2.80	0.28	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	41.3	≥40.8%	31.2	2.08	0.99	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30221-D8RR	40.8	≥40.8%	37.3	2.10	0.70	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30221-D8RR	40.7	40.7%-37.7%	130.1	2.11	1.11	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	40.5	40.7%-37.7%	162.0	2.09	0.73	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	40.4	40.7%-37.7%	117.9	2.08	0.94	0.0	2	-E2e2	-	-	E1
08C344 x 30221-D8RR	40.2	40.7%-37.7%	126.7	2.11	1.35	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	40.1	40.7%-37.7%	231.8	2.09	0.84	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	40.1	40.7%-37.7%	19.3	2.10	1.15	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	39.9	40.7%-37.7%	236.5	2.12	1.16	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	39.4	40.7%-37.7%	16.1	2.05	1.68	1.0	1	E1E1E2E2	N	E1/E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30221-D8RR	39.3	40.7%-37.7%	197.3	1.30	0.67	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	39.0	40.7%-37.7%	884.5	2.31	0.34	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	39.0	40.7%-37.7%	574.0	2.21	0.24	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	38.3	40.7%-37.7%	500.3	1.97	1.69	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	37.9	40.7%-37.7%	25.3	2.08	0.62	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	37.4	37.6%-31.4%	400.0	1.34	0.12	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	37.0	37.6%-31.4%	412.2	2.06	1.19	1.0	2	E1E1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	36.9	37.6%-31.4%	773.5	1.93	0.64	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	36.9	37.6%-31.4%	383.2	2.06	0.97	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	36.9	37.6%-31.4%	128.9	2.13	1.49	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	36.8	37.6%-31.4%	605.0	1.90	0.54	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	36.8	37.6%-31.4%	128.7	1.89	0.39	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	36.6	37.6%-31.4%	2215.6	1.98	0.57	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	36.5	37.6%-31.4%	43.7	2.25	0.31	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	36.4	37.6%-31.4%	12.6	2.09	1.16	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	36.3	37.6%-31.4%	755.3	1.31	0.14	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	36.3	37.6%-31.4%	109.7	1.99	0.96	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	36.0	37.6%-31.4%	824.6	2.11	1.77	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	36.0	37.6%-31.4%	68.2	2.02	0.76	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	35.8	37.6%-31.4%	131.8	1.30	0.14	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	35.8	37.6%-31.4%	76.4	2.02	1.28	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	35.7	37.6%-31.4%	31.0	2.06	1.34	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	35.7	37.6%-31.4%	10.9	1.96	0.30	2.0	0	E1e1-	-	-	E2
08C344 x 30221-D8RR	35.5	37.6%-31.4%	441.2	2.00	1.15	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	35.4	37.6%-31.4%	6.6	4.63	-0.28	0.0	1	-E2E2	-	-	E1

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30221-D8RR	34.8	37.6%-31.4%	384.2	2.04	1.51	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	34.8	37.6%-31.4%	14.1	2.13	1.28	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	34.6	37.6%-31.4%	195.2	2.08	1.56	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	34.4	37.6%-31.4%	27.0	1.80	0.30	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	34.3	37.6%-31.4%	265.0	2.13	1.44	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	34.3	37.6%-31.4%	81.4	1.61	1.42	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	34.1	37.6%-31.4%	531.2	14.29	1.00	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	33.7	37.6%-31.4%	343.0	2.08	1.01	0.0	2	-E2e2	-	-	E1
08C344 x 30221-D8RR	33.7	37.6%-31.4%	16.1	2.01	0.50	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	33.6	37.6%-31.4%	166.1	2.11	1.29	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	33.6	37.6%-31.4%	24.3	2.40	1.75	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	33.2	37.6%-31.4%	374.8	2.09	1.03	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	33.2	37.6%-31.4%	198.4	2.02	0.77	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30221-D8RR	32.5	37.6%-31.4%	1283.9	1.84	0.71	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	32.3	37.6%-31.4%	17.4	-4.81	0.48	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30221-D8RR	32.1	37.6%-31.4%	1.5	2.09	1.16	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	31.9	37.6%-31.4%	175.3	1.67	0.93	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30221-D8RR	31.9	37.6%-31.4%	51.1	-0.12	-0.27	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30221-D8RR	31.1	31.3%-28.3%	161.9	1.80	0.90	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	30.3	31.3%-28.3%	143.4	2.16	1.80	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	30.1	31.3%-28.3%	26.9	1.95	0.80	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	30.0	31.3%-28.3%	337.4	2.18	1.69	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	29.5	31.3%-28.3%	3211.7	2.05	1.31	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	29.3	31.3%-28.3%	308.9	0.75	1.50	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	29.3	31.3%-28.3%	248.5	1.57	0.57	2.0	1	E1e1E2E2	N	E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30221-D8RR	28.8	31.3%-28.3%	311.5	1.99	1.33	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	28.1	≤28.2%	129.0	2.07	1.41	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	27.5	≤28.2%	82.2	2.13	1.47	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	26.8	≤28.2%	146.6	2.10	1.00	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	26.1	≤28.2%	67.4	2.03	0.95	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	25.2	≤28.2%	43.4	1.82	0.95	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	24.8	≤28.2%	139.0	2.09	0.54	0.0	2	-E2e2	-	-	E1
08C344 x 30221-D8RR	24.1	≤28.2%	372.9	1.33	0.99	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	23.4	≤28.2%	229.3	1.24	0.77	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30221-D8RR	22.9	≤28.2%	74.4	1.51	0.18	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30221-D8RR	21.9	≤28.2%	138.0	1.38	0.16	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	50.1	≥40.8%	246.4	2.20	1.22	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	49.5	≥40.8%	191.1	1.72	1.34	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	47.2	≥40.8%	171.4	2.23	0.91	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	46.5	≥40.8%	498.9	1.96	0.92	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	46.3	≥40.8%	69.7	2.21	1.03	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	45.6	≥40.8%	176.2	2.13	1.11	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	44.8	≥40.8%	180.2	1.35	0.76	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	44.5	≥40.8%	363.6	2.14	1.11	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	44.4	≥40.8%	260.2	2.14	1.11	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.8	≥40.8%	429.6	2.10	1.01	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.7	≥40.8%	109.2	2.14	0.99	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.6	≥40.8%	112.2	2.32	1.10	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.3	≥40.8%	102.3	2.14	0.98	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.3	≥40.8%	78.1	2.05	0.62	1.0	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	43.2	≥40.8%	107.3	1.93	0.31	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	42.7	≥40.8%	209.0	1.94	0.96	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	42.6	≥40.8%	18.7	1.81	0.26	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	42.0	≥40.8%	336.6	2.19	1.37	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	41.9	≥40.8%	287.1	2.11	1.05	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	41.9	≥40.8%	204.3	2.09	1.83	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	41.0	≥40.8%	56.4	2.17	1.16	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	40.4	40.7%-37.7%	11.1	2.36	1.32	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	40.2	40.7%-37.7%	582.2	2.01	1.12	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	40.2	40.7%-37.7%	313.3	2.26	1.01	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	39.9	40.7%-37.7%	181.8	2.12	1.15	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	39.4	40.7%-37.7%	835.9	1.99	0.70	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	39.0	40.7%-37.7%	384.5	2.13	0.92	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	38.7	40.7%-37.7%	601.7	1.48	0.80	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	38.6	40.7%-37.7%	550.1	2.18	1.11	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	38.5	40.7%-37.7%	601.0	1.50	0.92	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	38.5	40.7%-37.7%	112.2	2.14	0.60	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	38.5	40.7%-37.7%	5.1	0.86	0.59	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	38.4	40.7%-37.7%	254.2	2.45	0.74	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	38.3	40.7%-37.7%	84.4	1.56	0.86	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	38.0	40.7%-37.7%	751.1	2.19	0.86	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.9	40.7%-37.7%	234.6	2.01	1.14	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.8	40.7%-37.7%	13185.8	2.07	0.77	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.7	40.7%-37.7%	297.8	1.54	0.44	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	37.5	37.6%-31.4%	274.9	2.28	1.05	2.0	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	37.3	37.6%-31.4%	79.9	2.11	1.20	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.2	37.6%-31.4%	377.0	2.07	1.06	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.2	37.6%-31.4%	133.4	2.10	0.58	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.1	37.6%-31.4%	31.3	2.09	0.96	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	36.9	37.6%-31.4%	445.7	2.15	0.74	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	36.6	37.6%-31.4%	668.9	2.12	1.02	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	36.5	37.6%-31.4%	248.7	2.24	1.18	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	36.3	37.6%-31.4%	176.0	2.24	0.92	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	36.3	37.6%-31.4%	89.5	2.28	0.73	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	36.2	37.6%-31.4%	72.1	2.01	1.46	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.8	37.6%-31.4%	191.3	2.07	0.92	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.7	37.6%-31.4%	193.3	2.29	1.38	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.7	37.6%-31.4%	99.9	2.12	0.50	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	35.6	37.6%-31.4%	283.4	2.15	1.36	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	35.6	37.6%-31.4%	113.4	2.72	0.94	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.5	37.6%-31.4%	150.6	2.12	1.25	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.4	37.6%-31.4%	381.8	2.46	1.38	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.4	37.6%-31.4%	261.9	1.44	0.96	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	35.2	37.6%-31.4%	430.9	2.38	1.08	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.2	37.6%-31.4%	85.7	2.06	1.09	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.0	37.6%-31.4%	85.5	2.25	0.72	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	34.9	37.6%-31.4%	713.3	2.30	1.03	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	34.8	37.6%-31.4%	233.6	2.00	0.68	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	34.8	37.6%-31.4%	225.0	2.13	1.35	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	34.6	37.6%-31.4%	76.8	2.14	0.87	1.0	0	E1E1-	-	-	E2

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	34.3	37.6%-31.4%	71.3	2.09	0.96	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	34.1	37.6%-31.4%	72.0	2.05	1.14	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	34.1	37.6%-31.4%	32.9	1.90	0.77	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	34.0	37.6%-31.4%	184.4	2.18	1.00	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	34.0	37.6%-31.4%	161.5	2.08	1.41	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	33.7	37.6%-31.4%	117.9	2.07	0.84	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	33.6	37.6%-31.4%	250.0	2.05	1.08	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	33.2	37.6%-31.4%	353.6	2.21	1.24	1.0	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	32.4	37.6%-31.4%	93.6	2.29	0.96	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	32.2	37.6%-31.4%	66.1	2.18	1.25	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	31.8	37.6%-31.4%	142.0	1.86	0.42	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	31.3	31.3%-28.3%	245.8	2.08	0.89	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	31.3	31.3%-28.3%	244.6	2.29	0.51	0	2	-E2e2	-	-	E1
08C344 x 30408-C7RR	30.1	31.3%-28.3%	275.2	1.70	0.47	0.0	1	-E2E2	-	-	E1
08C344 x 30408-C7RR	29.9	31.3%-28.3%	90.4	2.07	1.01	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	29.4	31.3%-28.3%	209.5	1.83	1.79	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	29.2	31.3%-28.3%	138.8	2.00	0.75	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	28.0	≤28.2%	435.7	2.17	1.07	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	27.5	≤28.2%	73.9	2.14	0.75	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	27.3	≤28.2%	306.0	2.04	0.44	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	26.9	≤28.2%	492.4	2.05	0.83	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	26.9	≤28.2%	421.4	2.32	0.89	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	26.6	≤28.2%	449.9	2.14	0.79	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	26.6	≤28.2%	84.1	1.96	0.78	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	26.3	≤28.2%	447.9	2.27	2.29	2.0	1	E1e1E2E2	N	E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	26.2	≤28.2%	64.5	2.19	0.87	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	25.9	≤28.2%	331.7	1.46	0.31	0.0	0	--	-	-	E1&E2
08C344 x 30408-C7RR	25.5	≤28.2%	487.4	1.95	1.30	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	24.3	≤28.2%	151.0	2.11	1.11	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	23.8	≤28.2%	192.5	2.15	1.13	2.0	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	23.0	≤28.2%	198.8	2.18	1.37	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	48.4	≥40.8%	2835.1	1.93	0.88	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	46.4	≥40.8%	127.1	1.77	0.67	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	46.3	≥40.8%	79.0	2.10	1.20	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	46.3	≥40.8%	9.6	2.12	1.53	0.0	1	-E2E2	-	-	E1
08C344 x 30422-C7RR	46.0	≥40.8%	243.5	2.11	1.39	0.0	0	--	-	-	E1&E2
08C344 x 30422-C7RR	44.9	≥40.8%	485.0	2.08	0.99	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	44.6	≥40.8%	332.1	1.90	0.54	2.0	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	44.6	≥40.8%	328.7	2.04	0.87	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	44.3	≥40.8%	251.0	2.06	1.09	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30422-C7RR	44.0	≥40.8%	406.2	1.73	0.68	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	43.5	≥40.8%	209.0	2.10	1.22	0.0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	43.4	≥40.8%	161.2	1.97	0.74	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	43.2	≥40.8%	1.8	1.28	0.30	0.0	0	--	-	-	E1&E2
08C344 x 30422-C7RR	43.1	≥40.8%	285.8	1.93	0.58	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	42.8	≥40.8%	44.4	0.36	0.05	0.0	1	-E2E2	-	-	E1
08C344 x 30422-C7RR	42.7	≥40.8%	14.9	1.94	1.21	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30422-C7RR	42.6	≥40.8%	322.7	2.17	1.26	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	42.3	≥40.8%	114.5	1.41	0.83	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	41.6	≥40.8%	163.7	1.95	0.96	1.0	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	41.5	≥40.8%	291.9	1.67	0.83	1.0	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	41.4	≥40.8%	586.4	1.65	1.67	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30422-C7RR	41.3	≥40.8%	1216.6	2.00	0.93	1.0	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	41.2	≥40.8%	425.1	2.04	1.32	2.0	1	E1e1E2E2	N	E1	-
08C344 x 30422-C7RR	40.7	40.7%-37.7%	69.6	1.81	1.43	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	40.5	40.7%-37.7%	120.8	2.15	0.97	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	40.0	40.7%-37.7%	292.5	1.96	1.04	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	39.8	40.7%-37.7%	181.7	1.86	0.33	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	39.6	40.7%-37.7%	227.8	2.16	1.32	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	39.2	40.7%-37.7%	171.9	1.88	0.66	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	39.2	40.7%-37.7%	108.7	2.02	0.40	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	38.9	40.7%-37.7%	297.2	3.25	1.18	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	38.9	40.7%-37.7%	29.6	2.09	0.64	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	38.7	40.7%-37.7%	266.6	1.71	0.72	0.0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	38.6	40.7%-37.7%	386.1	1.88	1.24	2.0	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	38.1	40.7%-37.7%	316.7	1.84	0.37	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	38.1	40.7%-37.7%	1.1	2.08	1.43	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	38.0	40.7%-37.7%	167.0	1.96	0.53	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	37.9	40.7%-37.7%	122.1	1.87	0.78	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	37.8	40.7%-37.7%	440.5	1.89	0.70	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	37.5	37.6%-31.4%	158.7	1.79	1.37	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	37.4	37.6%-31.4%	14.5	1.95	0.89	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	37.0	37.6%-31.4%	248.5	2.19	1.66	1.0	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	36.9	37.6%-31.4%	194.8	1.58	0.38	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.7	37.6%-31.4%	289.5	2.15	1.88	1.0	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	36.6	37.6%-31.4%	426.3	2.09	1.00	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	36.6	37.6%-31.4%	48.0	1.60	1.54	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.6	37.6%-31.4%	13.8	2.03	1.28	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.5	37.6%-31.4%	122.6	1.68	0.33	0.0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	36.5	37.6%-31.4%	49.2	1.87	0.64	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.4	37.6%-31.4%	157.3	1.76	0.47	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.2	37.6%-31.4%	94.3	0.85	-52.60	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.2	37.6%-31.4%	40.1	1.77	0.63	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.9	37.6%-31.4%	222.8	2.10	1.37	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.8	37.6%-31.4%	116.9	1.64	1.50	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.8	37.6%-31.4%	43.7	1.86	0.65	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.7	37.6%-31.4%	621.2	2.07	1.48	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.3	37.6%-31.4%	219.6	2.05	0.81	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	35.0	37.6%-31.4%	76.0	2.06	0.92	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	34.7	37.6%-31.4%	209.2	1.21	0.73	2.0	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	34.5	37.6%-31.4%	54.1	1.91	1.00	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	34.2	37.6%-31.4%	167.2	1.66	0.92	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	34.2	37.6%-31.4%	37.9	2.05	0.42	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	33.8	37.6%-31.4%	179.7	1.18	0.70	2.0	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	33.8	37.6%-31.4%	167.8	2.05	0.80	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	33.0	37.6%-31.4%	70.9	1.79	0.65	1.0	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	32.8	37.6%-31.4%	143.6	2.00	0.91	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	32.6	37.6%-31.4%	284.1	1.84	0.62	1.0	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	31.2	31.3%-28.3%	81.5	1.92	0.95	1.0	2	E1E1E2e2	N	E1	-
08C344 x 30422-C7RR	30.9	31.3%-28.3%	242.2	3.52	1.18	1.0	2	E1E1E2e2	N	E1	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	30.0	31.3%-28.3%	19.3	1.93	0.54	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	29.4	31.3%-28.3%	299.3	1.72	1.63	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	29.0	31.3%-28.3%	74.2	2.04	1.00	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	28.5	31.3%-28.3%	357.0	2.20	1.16	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	28.0	≤28.2%	17.3	2.08	0.82	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.8	≤28.2%	224.5	1.58	0.75	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.5	≤28.2%	7819.6	1.98	0.93	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.2	≤28.2%	244.9	1.96	0.88	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.1	≤28.2%	206.7	-0.15	-0.19	0.0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	26.8	≤28.2%	793.7	2.23	0.93	2.0	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	26.8	≤28.2%	196.4	2.13	0.51	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.7	≤28.2%	54.0	2.01	0.57	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.5	≤28.2%	181.3	1.98	0.82	0.0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	26.4	≤28.2%	144.7	1.78	0.59	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.1	≤28.2%	171.0	2.04	1.13	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.0	≤28.2%	233.3	1.51	1.17	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.8	≤28.2%	142.8	2.03	1.09	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.8	≤28.2%	34.3	2.42	1.21	0.0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	25.6	≤28.2%	292.5	2.16	1.43	2.0	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	25.6	≤28.2%	270.4	1.91	0.57	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.6	≤28.2%	42.1	2.01	0.96	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.5	≤28.2%	124.7	1.93	1.12	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.5	≤28.2%	52.8	1.99	0.74	2.0	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	24.9	≤28.2%	6111.3	1.94	0.79	2.0	1	E1e1E2E2	N	E2	-
08C344 x 30422-C7RR	24.8	≤28.2%	198.5	1.82	1.04	2.0	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	24.8	≤28.2%	28.8	2.05	0.79	2.0	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	24.4	≤28.2%	207.6	2.54	0.75	2.0	0	E1e1-	-	-	E2

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Indicator of quality of DNA. Should be greater than 1.8 to be considered pure. Less than 1.8, indicates incomplete removal of proteins during the extraction process.

[x]Secondary measurement of purity of DNA. Should be greater than 2.0 to be considered pure. Less than 2.0 indicates the presence of contaminants.

Appendix Table A7. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes from BC1F1 progeny from conventional HEAR crosses 08C344 x 30216-C7RR, 08C344 x 30220-D8RR, 08C344 x 30221-D8RR, 08C344 x 30408-C7RR, and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 30216-C7RR	≥40.8%	24	17	5	1	1	0	2	77.27	22.73	4.17	4.17	0.00	8.33
08C344 x 30220-D8RR	≥40.8%	27	20	3	0	4	0	4	86.96	13.04	0.00	14.81	0.00	14.81
08C344 x 30221-D8RR	≥40.8%	24	10	13	1	0	0	1	43.48	56.52	4.17	0.00	0.00	4.17
08C344 x 30408-C7RR	≥40.8%	21	17	1	0	3	0	3	94.44	5.56	0.00	14.29	0.00	14.29
08C344 x 30422-C7RR	≥40.8%	23	7	7	3	4	2	9	50.00	50.00	13.04	17.39	8.70	39.13
Total	≥40.8%	119	71	29	5	12	2	19	352.15	147.85	21.38	50.66	8.70	80.73
Mean	≥40.8%		14	6	1	2	0	4	70.43	29.57	4.28	10.13	1.74	16.15
Minimum	≥40.8%		7	1	0	0	0	1	43.48	5.56	0.00	0.00	0.00	4.17
Maximum	≥40.8%		20	13	3	4	2	9	94.44	56.52	13.04	17.39	8.70	39.13
08C344 x 30216-C7RR	40.7%-37.7%	10	9	0	0	1	0	1	100.00	0.00	0.00	10.00	0.00	10.00
08C344 x 30220-D8RR	40.7%-37.7%	22	18	2	0	2	0	2	90.00	10.00	0.00	9.09	0.00	9.09
08C344 x 30221-D8RR	40.7%-37.7%	13	4	8	1	0	0	1	33.33	66.67	7.69	0.00	0.00	7.69
08C344 x 30408-C7RR	40.7%-37.7%	17	9	1	0	7	0	7	90.00	10.00	0.00	41.18	0.00	41.18
08C344 x 30422-C7RR	40.7%-37.7%	16	5	6	1	4	0	5	45.45	54.55	6.25	25.00	0.00	31.25
Total	40.7%-37.7%	78	45	17	2	14	0	16	358.79	141.21	13.94	85.27	0.00	99.21
Mean	40.7%-37.7%		9	3	0	3	0	3	71.76	28.24	2.79	17.05	0.00	19.84
Minimum	40.7%-37.7%		4	0	0	0	0	1	33.33	0.00	0.00	0.00	0.00	7.69
Maximum	40.7%-37.7%		18	8	1	7	0	7	100.00	66.67	7.69	41.18	0.00	41.18
08C344 x 30216-C7RR	37.6%-31.4%	31	23	2	2	3	1	6	92.00	8.00	6.45	9.68	3.23	19.35
08C344 x 30220-D8RR	37.6%-31.4%	23	17	3	1	2	0	3	85.00	15.00	4.35	8.70	0.00	13.04
08C344 x 30221-D8RR	37.6%-31.4%	38	23	15	0	0	0	0	60.53	39.47	0.00	0.00	0.00	0.00
08C344 x 30408-C7RR	37.6%-31.4%	37	24	3	0	10	0	10	88.89	11.11	0.00	27.03	0.00	27.03
08C344 x 30422-C7RR	37.6%-31.4%	28	22	3	1	2	0	3	88.00	12.00	3.57	7.14	0.00	10.71
Total	37.6%-31.4%	157	109	26	4	17	1	22	414.42	85.58	14.37	52.54	3.23	70.14
Mean	37.6%-31.4%		22	5	1	3	0	4	82.88	17.12	2.87	10.51	0.65	14.03
Minimum	37.6%-31.4%		17	2	0	0	0	0	60.53	8.00	0.00	0.00	0.00	0.00
Maximum	37.6%-31.4%		24	15	2	10	1	10	92.00	39.47	6.45	27.03	3.23	27.03
08C344 x 30216-C7RR	31.3%-28.3%	8	2	5	0	1	0	1	28.57	71.43	0.00	12.50	0.00	12.50
08C344 x 30220-D8RR	31.3%-28.3%	11	9	2	0	0	0	0	81.82	18.18	0.00	0.00	0.00	0.00
08C344 x 30221-D8RR	31.3%-28.3%	8	2	6	0	0	0	0	25.00	75.00	0.00	0.00	0.00	0.00
08C344 x 30408-C7RR	31.3%-28.3%	6	1	1	2	2	0	4	50.00	50.00	33.33	33.33	0.00	66.67
08C344 x 30422-C7RR	31.3%-28.3%	6	4	2	0	0	0	0	66.67	33.33	0.00	0.00	0.00	0.00

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Total	31.3%-28.3%	39	18	16	2	3	0	5	252.06	247.94	33.33	45.83	0.00	79.17
Mean	31.3%-28.3%		4	3	0	1	0	1	50.41	49.59	6.67	9.17	0.00	15.83
Minimum	31.3%-28.3%		1	1	0	0	0	0	25.00	18.18	0.00	0.00	0.00	0.00
Maximum	31.3%-28.3%		9	6	2	2	0	4	81.82	75.00	33.33	33.33	0.00	66.67
08C344 x 30216-C7RR	≤28.2%	22	18	3	1	0	0	1	85.71	14.29	4.55	0.00	0.00	4.55
08C344 x 30220-D8RR	≤28.2%	12	10	0	1	1	0	2	100.00	0.00	8.33	8.33	0.00	16.67
08C344 x 30221-D8RR	≤28.2%	10	6	3	1	0	0	1	66.67	33.33	10.00	0.00	0.00	10.00
08C344 x 30408-C7RR	≤28.2%	14	3	2	0	9	0	9	60.00	40.00	0.00	64.29	0.00	64.29
08C344 x 30422-C7RR	≤28.2%	23	15	1	3	4	0	7	93.75	6.25	13.04	17.39	0.00	30.43
Total	≤28.2%	81	52	9	6	14	0	20	406.13	93.87	35.92	90.01	0.00	125.93
Mean	≤28.2%		10	2	1	3	0	4	81.23	18.77	7.18	18.00	0.00	25.19
Minimum	≤28.2%		3	0	0	0	0	1	60.00	0.00	0.00	0.00	0.00	4.55
Maximum	≤28.2%		18	3	3	9	0	9	100.00	40.00	13.04	64.29	0.00	64.29
Total Plants Evaluated (%)		82.70												
Overall Accuracy (%)		75.26												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

Appendix Table A8. Phenotypic and genotypic results for erucic acid (C22:1) content for BC1F1 progeny from Roundup Ready HEAR crosses 08C344 x 30216-C7RR, 08C344 x 30220-C7RR, 08C344 x 30221-D8RR, 08C344 x 30408-C7RR, and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30216-C7RR	46.3	≥36.8%	97.5	2.92	0.88	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	44.2	≥36.8%	56.0	2.40	0.82	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	44.1	≥36.8%	183.6	2.69	1.29	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	43.6	≥36.8%	346.4	1.92	1.15	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	43.0	≥36.8%	246.0	1.94	1.23	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	42.9	≥36.8%	1635.8	1.91	1.43	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	42.5	≥36.8%	241.5	2.47	1.30	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	42.3	≥36.8%	902.3	2.01	1.30	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	41.8	≥36.8%	85.5	3.11	0.50	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	41.7	≥36.8%	95.1	2.83	0.98	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	41.0	≥36.8%	508.3	1.40	0.88	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	40.7	≥36.8%	456.4	2.28	1.39	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	40.7	≥36.8%	153.8	2.73	1.25	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	40.7	≥36.8%	153.8	2.36	1.33	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	40.7	≥36.8%	40.5	2.39	1.09	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	40.3	≥36.8%	222.7	1.79	0.99	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	39.4	≥36.8%	823.4	1.76	1.34	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30216-C7RR	39.1	≥36.8%	158.6	2.04	0.48	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	39.0	≥36.8%	500.6	2.19	1.66	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	38.8	≥36.8%	338.7	2.20	1.23	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	38.6	≥36.8%	105.5	2.02	1.22	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	38.5	≥36.8%	106.9	1.33	0.43	1	0	E1E1-	-	-	E2
Red River 1997 x 30216-C7RR	38.4	≥36.8%	319.4	2.05	1.61	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	38.4	≥36.8%	96.9	2.99	1.01	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	36.9	≥36.8%	105.4	2.36	0.95	1	2	E1E1E2e2	N	E1/E2	-
Red River 1997 x 30216-C7RR	36.8	≥36.8%	186.4	2.18	1.15	1	2	E1E1E2e2	N	E1/E2	-
Red River 1997 x 30216-C7RR	36.2	36.7%-33.7%	248.6	2.14	1.35	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	36.0	36.7%-33.7%	273.9	1.91	1.42	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30216-C7RR	35.9	36.7%-33.7%	171.8	2.02	1.24	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	35.7	36.7%-33.7%	470.0	2.50	1.31	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	35.4	36.7%-33.7%	51.4	3.17	0.95	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	35.4	36.7%-33.7%	49.4	2.20	0.87	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	35.3	36.7%-33.7%	247.8	2.06	1.10	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	35.3	36.7%-33.7%	35.9	2.32	0.79	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	35.1	36.7%-33.7%	63.0	3.22	0.94	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	35.0	36.7%-33.7%	32.3	1.98	0.75	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	34.9	36.7%-33.7%	615.0	1.59	1.33	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	34.6	36.7%-33.7%	32.0	2.12	0.82	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	34.4	36.7%-33.7%	173.5	2.32	1.06	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	34.0	36.7%-33.7%	384.0	1.90	1.08	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	34.0	36.7%-33.7%	182.5	2.04	1.47	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	33.9	36.7%-33.7%	3381.5	1.99	1.88	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30216-C7RR	33.9	36.7%-33.7%	271.2	2.99	1.28	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.7	36.7%-33.7%	219.7	2.05	1.43	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.7	36.7%-33.7%	77.3	1.95	0.89	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.5	33.6%-26.8%	104.4	2.70	0.91	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	33.5	33.6%-26.8%	94.9	3.19	0.47	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	33.3	33.6%-26.8%	253.6	2.35	1.29	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.2	33.6%-26.8%	149.3	2.64	0.95	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.2	33.6%-26.8%	127.8	2.43	1.04	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.2	33.6%-26.8%	101.4	2.16	0.60	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.0	33.6%-26.8%	194.5	2.84	0.93	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	33.0	33.6%-26.8%	139.6	3.18	1.06	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	32.9	33.6%-26.8%	73.3	1.54	0.63	1	0	E1E1-	-	-	E2
Red River 1997 x 30216-C7RR	32.6	33.6%-26.8%	653.1	1.28	0.86	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	32.4	33.6%-26.8%	174.1	2.06	1.36	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	32.2	33.6%-26.8%	102.2	2.86	0.87	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	32.2	33.6%-26.8%	80.0	2.34	1.08	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	32.1	33.6%-26.8%	11475.8	1.71	1.53	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	32.1	33.6%-26.8%	42.7	2.35	0.38	2	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30216-C7RR	32.0	33.6%-26.8%	199.8	2.21	1.06	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	31.4	33.6%-26.8%	4.1	1.21	0.36	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30216-C7RR	31.3	33.6%-26.8%	533.5	2.18	1.25	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	31.2	33.6%-26.8%	352.1	2.59	1.42	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	31.2	33.6%-26.8%	142.9	2.60	0.95	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	31.2	33.6%-26.8%	76.1	2.68	0.78	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	31.1	33.6%-26.8%	3442.7	1.67	1.48	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	30.8	33.6%-26.8%	316.6	2.04	1.19	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	30.3	33.6%-26.8%	377.5	2.19	1.15	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	30.3	33.6%-26.8%	122.1	2.05	1.26	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	29.8	33.6%-26.8%	195.3	3.15	0.93	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	29.8	33.6%-26.8%	126.5	1.97	0.94	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30216-C7RR	28.6	33.6%-26.8%	179.4	2.71	1.11	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	27.4	33.6%-26.8%	146.3	2.00	1.11	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30216-C7RR	26.8	33.6%-26.8%	761.7	2.30	1.15	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30216-C7RR	25.0	26.7%-23.7%	141.9	1.75	0.61	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	24.6	26.7%-23.7%	146.2	2.07	1.04	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30216-C7RR	24.5	26.7%-23.7%	140.4	2.54	0.92	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	24.5	26.7%-23.7%	91.0	2.00	0.69	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	24.3	26.7%-23.7%	97.8	2.09	1.01	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	24.2	26.7%-23.7%	145.7	2.06	0.99	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	24.0	26.7%-23.7%	346.8	1.91	1.32	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	23.9	26.7%-23.7%	85.0	2.64	1.04	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	23.9	26.7%-23.7%	69.7	3.13	0.79	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	23.7	26.7%-23.7%	85.7	2.13	0.95	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	23.4	≤23.6%	129.8	2.10	0.66	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	23.2	≤23.6%	170.2	2.65	1.06	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	23.1	≤23.6%	134.1	2.02	0.85	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	23.1	≤23.6%	133.6	2.14	0.99	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	22.5	≤23.6%	214.1	2.34	1.24	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	22.2	≤23.6%	307.1	1.84	1.15	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	22.1	≤23.6%	138.2	2.58	1.01	2	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30216-C7RR	21.8	≤23.6%	533.3	1.39	0.77	2	0	E1e1-	-	-	E2
Red River 1997 x 30216-C7RR	21.2	≤23.6%	341.9	2.29	1.36	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30216-C7RR	20.5	≤23.6%	242.9	1.44	0.77	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30216-C7RR	20.0	≤23.6%	97.5	2.19	0.91	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	45.9	≥36.8%	80.4	3.85	0.88	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	44.7	≥36.8%	221.0	2.56	1.14	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	43.9	≥36.8%	406.8	1.10	0.84	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	43.8	≥36.8%	210.8	2.40	1.29	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	43.0	≥36.8%	251.0	2.26	1.31	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	42.8	≥36.8%	185.8	4.86	1.09	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	42.4	≥36.8%	487.6	2.17	1.37	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30220-D8RR	42.4	≥36.8%	338.6	2.08	1.39	1	0	E1E1-	-	-	E2
Red River 1997 x 30220-D8RR	42.2	≥36.8%	114.3	3.02	0.88	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	42.0	≥36.8%	165.2	2.67	1.09	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	41.0	≥36.8%	559.3	2.18	1.27	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	41.0	≥36.8%	314.3	2.26	1.23	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30220-D8RR	41.0	≥36.8%	239.0	2.17	1.02	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	40.9	≥36.8%	176.8	3.08	0.98	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	40.7	≥36.8%	5909.4	1.81	1.76	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	40.3	≥36.8%	164.8	2.94	1.10	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	39.5	≥36.8%	360.2	2.26	1.29	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	39.4	≥36.8%	213.6	1.28	0.79	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	39.1	≥36.8%	1044.1	1.95	1.58	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	38.8	≥36.8%	317.3	2.46	1.31	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	38.7	≥36.8%	263.4	2.65	1.23	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	38.4	≥36.8%	941.7	1.52	1.30	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30220-D8RR	38.1	≥36.8%	321.4	1.75	1.02	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30220-D8RR	38.0	≥36.8%	94.6	2.04	1.03	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30220-D8RR	37.7	≥36.8%	12859.1	2.01	1.99	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	37.7	≥36.8%	686.9	2.09	1.54	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	37.4	≥36.8%	80.5	1.91	0.64	1	0	E1E1-	-	-	E2
Red River 1997 x 30220-D8RR	37.3	≥36.8%	143.8	2.12	1.01	1	2	E1E1E2e2	N	E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30220-D8RR	37.3	≥36.8%	112.0	2.21	0.71	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30220-D8RR	37.2	≥36.8%	530.0	2.03	1.39	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30220-D8RR	36.6	36.7%-33.7%	237.7	3.52	0.88	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	36.5	36.7%-33.7%	390.1	2.29	1.43	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	36.5	36.7%-33.7%	254.6	2.27	1.16	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	36.5	36.7%-33.7%	235.2	2.98	1.03	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	35.7	36.7%-33.7%	149.0	2.06	1.18	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	34.9	36.7%-33.7%	136.1	2.02	1.20	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	34.0	36.7%-33.7%	1207.7	1.27	1.10	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30220-D8RR	33.9	36.7%-33.7%	72.1	2.50	1.13	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	33.8	36.7%-33.7%	322.4	2.44	1.35	2	0	E1e1-	-	-	E2
Red River 1997 x 30220-D8RR	33.6	33.6%-26.8%	169.0	2.56	1.00	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	33.4	33.6%-26.8%	290.5	2.74	1.34	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	33.3	33.6%-26.8%	112.7	4.27	1.04	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	33.1	33.6%-26.8%	858.3	2.14	1.63	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30220-D8RR	33.1	33.6%-26.8%	119.2	1.95	0.79	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	32.8	33.6%-26.8%	230.6	2.88	1.01	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	32.5	33.6%-26.8%	802.0	1.81	1.37	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	32.4	33.6%-26.8%	1667.6	1.86	1.72	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	32.4	33.6%-26.8%	197.9	2.60	1.19	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	32.4	33.6%-26.8%	176.5	2.17	1.16	2	0	E1e1-	-	-	E2
Red River 1997 x 30220-D8RR	32.0	33.6%-26.8%	218.8	3.13	1.02	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	32.0	33.6%-26.8%	52.7	2.36	0.95	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	31.9	33.6%-26.8%	229.2	2.35	0.99	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	31.7	33.6%-26.8%	297.6	2.11	1.18	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	31.6	33.6%-26.8%	3828.9	1.73	1.67	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	31.6	33.6%-26.8%	409.3	2.26	1.24	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	31.5	33.6%-26.8%	855.9	2.11	1.55	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	30.4	33.6%-26.8%	1057.6	1.57	1.35	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	30.3	33.6%-26.8%	1004.4	1.46	1.26	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30220-D8RR	30.3	33.6%-26.8%	164.7	2.99	1.29	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	29.9	33.6%-26.8%	271.4	1.28	0.67	1	0	E1E1-	-	-	E2

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30220-D8RR	29.6	33.6%-26.8%	72.7	2.01	1.15	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	29.0	33.6%-26.8%	270.1	2.77	1.31	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30220-D8RR	28.1	33.6%-26.8%	2412.7	1.29	1.26	2	0	E1e1-	-	-	E2
Red River 1997 x 30220-D8RR	28.1	33.6%-26.8%	32.9	3.27	0.49	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30220-D8RR	27.1	33.6%-26.8%	508.8	2.36	1.57	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30220-D8RR	27.0	33.6%-26.8%	269.4	1.97	1.05	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30220-D8RR	26.8	33.6%-26.8%	1551.8	2.00	1.71	2	0	E1e1-	-	-	E2
Red River 1997 x 30220-D8RR	26.8	33.6%-26.8%	233.5	2.05	1.28	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30220-D8RR	26.6	26.7%-23.7%	50.7	2.93	0.53	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	26.2	26.7%-23.7%	197.9	2.75	1.08	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	25.8	26.7%-23.7%	1517.2	2.09	1.60	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	25.8	26.7%-23.7%	634.8	1.96	1.39	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	25.7	26.7%-23.7%	184.0	1.47	0.84	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	25.6	26.7%-23.7%	212.7	2.30	1.28	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	24.7	26.7%-23.7%	261.0	2.72	1.07	2	0	E1e1-	-	-	E2
Red River 1997 x 30220-D8RR	24.7	26.7%-23.7%	92.6	1.93	0.95	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	24.3	26.7%-23.7%	311.9	2.16	1.57	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30220-D8RR	24.3	26.7%-23.7%	147.7	2.11	1.10	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	24.3	26.7%-23.7%	119.1	2.19	0.65	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	24.2	26.7%-23.7%	478.3	1.62	1.12	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	23.8	26.7%-23.7%	929.4	1.72	1.38	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30220-D8RR	23.5	≤23.6%	140.1	2.50	1.09	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	23.3	≤23.6%	164.8	1.77	0.85	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	23.0	≤23.6%	124.0	2.19	1.02	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	22.3	≤23.6%	247.9	2.56	1.01	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	22.2	≤23.6%	258.3	2.11	1.04	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	22.1	≤23.6%	1811.6	1.94	1.52	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	21.9	≤23.6%	41.3	1.14	0.14	1	0	E1E1-	-	-	E2
Red River 1997 x 30220-D8RR	21.6	≤23.6%	805.5	1.79	1.44	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	21.5	≤23.6%	100.6	2.67	1.01	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	20.9	≤23.6%	355.6	2.11	0.95	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30220-D8RR	20.4	≤23.6%	10334.2	1.95	1.81	2	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30220-D8RR	20.4	≤23.6%	341.2	2.59	1.21	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	45.9	≥36.8%	64.1	2.62	0.64	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	45.5	≥36.8%	86.7	2.96	0.93	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	44.9	≥36.8%	65.7	2.71	0.72	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	42.3	≥36.8%	126.0	2.73	1.03	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	42.2	≥36.8%	93.3	2.13	0.69	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	41.1	≥36.8%	39.6	2.89	0.79	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	40.9	≥36.8%	161.8	1.92	0.91	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	40.9	≥36.8%	38.6	2.32	0.75	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	40.3	≥36.8%	149.6	2.55	0.97	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	40.2	≥36.8%	160.8	2.28	1.12	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	39.5	≥36.8%	889.8	1.82	1.45	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	38.9	≥36.8%	389.0	1.97	1.29	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	38.9	≥36.8%	128.2	4.26	0.88	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	37.9	≥36.8%	38.2	2.33	0.82	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	37.7	≥36.8%	2519.9	1.92	1.79	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	37.6	≥36.8%	269.4	2.48	1.30	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	37.2	≥36.8%	60.8	2.52	0.62	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	37.1	≥36.8%	103.9	2.40	0.90	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	36.9	≥36.8%	333.7	2.41	1.52	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	35.7	36.7%-33.7%	1.9	1.88	0.03	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	35.4	36.7%-33.7%	449.5	1.32	0.78	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	35.3	36.7%-33.7%	257.4	1.72	0.82	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	34.9	36.7%-33.7%	78.9	2.08	0.88	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	34.5	36.7%-33.7%	184.2	2.27	1.02	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	34.5	36.7%-33.7%	65.7	1.88	0.71	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	34.3	36.7%-33.7%	92.0	2.47	0.85	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	34.3	36.7%-33.7%	14.1	2.25	0.20	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	33.9	36.7%-33.7%	3469.6	1.58	1.32	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	33.9	36.7%-33.7%	61.7	3.47	0.63	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	33.8	36.7%-33.7%	34.3	2.54	0.80	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	33.8	36.7%-33.7%	27.7	1.96	0.92	2	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30221-D8RR	33.6	33.6%-26.8%	437.4	2.03	1.40	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	33.2	33.6%-26.8%	155.7	1.83	0.63	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	33.2	33.6%-26.8%	60.4	2.12	0.49	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	33.1	33.6%-26.8%	2620.8	1.77	1.59	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	33.0	33.6%-26.8%	23.6	2.13	0.70	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.9	33.6%-26.8%	40.6	3.00	0.80	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	32.7	33.6%-26.8%	1080.9	1.68	1.16	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	32.7	33.6%-26.8%	52.7	2.77	0.72	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	32.6	33.6%-26.8%	37.4	2.25	0.32	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	32.2	33.6%-26.8%	1167.1	1.79	1.37	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.2	33.6%-26.8%	107.9	2.83	0.87	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	32.2	33.6%-26.8%	77.5	2.37	0.93	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	32.1	33.6%-26.8%	623.3	2.17	1.63	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.1	33.6%-26.8%	184.3	2.77	1.20	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	31.4	33.6%-26.8%	141.6	2.35	1.15	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	31.3	33.6%-26.8%	338.6	1.95	1.44	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	31.0	33.6%-26.8%	84.8	2.29	0.85	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.9	33.6%-26.8%	313.9	1.93	1.19	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.9	33.6%-26.8%	152.8	2.50	0.97	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.8	33.6%-26.8%	73.0	1.72	0.58	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	30.5	33.6%-26.8%	65.9	2.08	0.77	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.5	33.6%-26.8%	33.7	2.99	0.75	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	30.4	33.6%-26.8%	978.3	2.20	1.76	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	30.4	33.6%-26.8%	73.7	2.38	0.72	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	30.2	33.6%-26.8%	220.8	2.29	1.40	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.1	33.6%-26.8%	102.6	2.76	1.14	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	29.8	33.6%-26.8%	471.6	2.69	1.35	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	29.7	33.6%-26.8%	176.7	2.50	1.31	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	29.7	33.6%-26.8%	106.1	2.59	0.99	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	29.5	33.6%-26.8%	178.4	1.80	0.68	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	29.4	33.6%-26.8%	484.5	2.06	1.26	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	28.8	33.6%-26.8%	30.4	7.09	0.77	2	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30221-D8RR	28.2	33.6%-26.8%	1021.3	1.85	1.47	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	27.5	33.6%-26.8%	282.4	2.20	0.95	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	26.6	33.6%-26.8%	255.1	3.10	1.19	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	25.8	26.7%-23.7%	65.5	6.61	0.74	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	25.7	26.7%-23.7%	198.1	2.13	1.17	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	25.1	26.7%-23.7%	118.8	2.94	0.74	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	24.8	26.7%-23.7%	21.2	2.97	0.24	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	24.0	26.7%-23.7%	57.2	3.02	0.87	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	23.8	26.7%-23.7%	966.1	2.07	1.47	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	23.5	≤23.6%	150.9	2.18	0.96	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	23.4	≤23.6%	132.4	2.07	1.17	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	23.4	≤23.6%	53.3	2.08	0.68	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	23.2	≤23.6%	16.2	2.28	0.30	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	22.3	≤23.6%	107.3	3.82	0.80	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	22.2	≤23.6%	971.4	1.56	1.08	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	22.2	≤23.6%	151.0	2.54	0.84	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	21.9	≤23.6%	164.0	4.08	0.93	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	21.8	≤23.6%	130.2	2.34	1.06	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	21.6	≤23.6%	142.4	2.59	0.98	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	21.6	≤23.6%	128.4	2.70	0.75	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	21.6	≤23.6%	49.7	3.73	0.76	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	21.4	≤23.6%	706.8	1.47	1.12	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	21.4	≤23.6%	35.6	2.43	0.34	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	21.3	≤23.6%	256.5	2.73	0.98	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	21.0	≤23.6%	268.4	2.07	1.24	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	20.8	≤23.6%	47.7	2.84	0.47	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	20.0	≤23.6%	207.8	2.65	1.03	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	19.9	≤23.6%	184.5	2.84	1.18	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	19.9	≤23.6%	150.8	2.54	1.06	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	19.3	≤23.6%	80.9	2.65	0.93	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	18.5	≤23.6%	168.3	2.30	1.02	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	18.5	≤23.6%	43.4	3.51	0.64	2	1	E1e1E2E2	N	E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30408-C7RR	45.4	≥36.8%	60.9	2.60	0.94	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	44.8	≥36.8%	134.4	2.53	1.35	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	44.0	≥36.8%	53.7	1.89	0.45	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	43.6	≥36.8%	166.1	2.02	1.09	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	43.1	≥36.8%	399.1	2.42	1.52	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	42.9	≥36.8%	2062.4	2.11	1.90	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	42.6	≥36.8%	200.4	3.52	1.10	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	40.5	≥36.8%	79.4	2.07	0.58	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	40.2	≥36.8%	162.3	2.60	1.10	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	40.1	≥36.8%	115.2	3.92	1.03	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	40.0	≥36.8%	187.6	1.47	1.11	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	39.7	≥36.8%	695.1	2.26	1.63	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	39.3	≥36.8%	174.4	2.40	1.04	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	38.8	≥36.8%	80.7	2.32	0.50	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	38.1	≥36.8%	313.5	1.95	1.25	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	37.8	≥36.8%	77.9	1.88	0.84	1	1	E1E1E2E2	Y	E1/E2	-
Red River 1997 x 30408-C7RR	37.3	≥36.8%	166.5	1.61	0.62	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	37.0	≥36.8%	419.0	15.19	5.59	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	37.0	≥36.8%	69.2	2.46	1.02	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	36.9	≥36.8%	361.4	2.47	1.64	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	36.7	36.7%-33.7%	120.4	2.11	1.10	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	36.2	36.7%-33.7%	116.1	2.82	1.01	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	36.0	36.7%-33.7%	333.7	2.32	1.28	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	35.1	36.7%-33.7%	107.6	1.55	1.33	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	34.7	36.7%-33.7%	761.2	2.21	1.51	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	34.6	36.7%-33.7%	107.0	2.24	1.13	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	34.4	36.7%-33.7%	143.1	2.20	1.12	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	34.4	36.7%-33.7%	77.2	2.39	1.05	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	34.0	36.7%-33.7%	411.2	2.34	1.44	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	33.8	36.7%-33.7%	308.2	2.16	1.32	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	33.8	36.7%-33.7%	145.2	2.18	1.09	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	33.7	36.7%-33.7%	269.7	2.75	1.25	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30408-C7RR	33.6	33.6%-26.8%	879.9	2.11	1.65	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	33.4	33.6%-26.8%	205.3	2.40	1.40	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	33.4	33.6%-26.8%	93.4	2.15	0.84	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	33.3	33.6%-26.8%	912.4	2.21	1.65	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	33.2	33.6%-26.8%	110.6	2.15	0.87	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	33.0	33.6%-26.8%	113.1	2.61	1.43	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	32.8	33.6%-26.8%	131.3	2.11	-4.96	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	32.6	33.6%-26.8%	128.7	2.46	0.80	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	32.5	33.6%-26.8%	153.1	2.50	1.47	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	32.2	33.6%-26.8%	643.0	1.51	1.22	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	32.2	33.6%-26.8%	158.3	2.22	1.05	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	32.1	33.6%-26.8%	88.7	2.12	0.53	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	32.0	33.6%-26.8%	1021.3	1.81	1.49	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	32.0	33.6%-26.8%	179.0	2.44	1.10	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	31.6	33.6%-26.8%	198.6	1.39	0.64	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	31.5	33.6%-26.8%	170.4	2.42	1.18	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	31.4	33.6%-26.8%	910.9	1.83	1.44	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	31.4	33.6%-26.8%	170.9	2.40	1.15	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	31.4	33.6%-26.8%	132.3	2.41	1.22	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	31.3	33.6%-26.8%	305.3	1.93	1.33	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	31.3	33.6%-26.8%	221.1	2.07	1.32	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	31.3	33.6%-26.8%	144.8	3.50	1.10	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	31.2	33.6%-26.8%	96.8	2.55	0.97	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	30.3	33.6%-26.8%	310.7	2.33	1.37	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	30.0	33.6%-26.8%	142.9	2.63	0.93	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	29.8	33.6%-26.8%	189.9	1.34	1.33	1	0	E1E1-	-	-	E1/E2
Red River 1997 x 30408-C7RR	29.5	33.6%-26.8%	920.6	2.37	1.72	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	29.5	33.6%-26.8%	119.2	3.21	1.03	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	28.8	33.6%-26.8%	1394.0	2.11	1.85	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	28.5	33.6%-26.8%	232.0	2.25	1.26	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	28.1	33.6%-26.8%	161.3	2.99	1.04	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	28.0	33.6%-26.8%	257.0	2.38	1.36	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30408-C7RR	27.9	33.6%-26.8%	268.5	2.61	1.22	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	25.6	26.7%-23.7%	3686.7	1.75	1.71	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	25.5	26.7%-23.7%	122.6	2.21	1.35	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	24.7	26.7%-23.7%	331.3	2.30	1.47	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	24.7	26.7%-23.7%	100.0	3.23	1.03	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	24.5	26.7%-23.7%	3071.3	2.09	1.93	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	24.5	26.7%-23.7%	902.3	1.73	1.32	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	24.5	26.7%-23.7%	103.2	2.05	1.23	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	24.2	26.7%-23.7%	204.1	2.13	1.15	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	24.2	26.7%-23.7%	109.7	2.99	1.02	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	23.9	26.7%-23.7%	383.8	2.36	1.44	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	23.7	26.7%-23.7%	194.4	2.58	1.16	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	23.4	≤23.6%	215.8	2.12	1.12	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	23.4	≤23.6%	67.4	2.69	0.81	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.6	≤23.6%	102.5	3.33	0.95	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.6	≤23.6%	84.0	3.06	0.70	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.2	≤23.6%	305.6	2.32	1.52	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.2	≤23.6%	93.4	2.76	0.98	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	22.1	≤23.6%	54.5	2.98	1.07	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	21.8	≤23.6%	89.6	2.15	0.50	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	21.6	≤23.6%	189.2	3.99	0.96	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	21.2	≤23.6%	217.7	2.59	1.27	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	21.1	≤23.6%	132.7	2.96	1.06	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	20.3	≤23.6%	668.2	1.78	1.25	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	20.2	≤23.6%	175.2	2.28	1.24	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	20.1	≤23.6%	66.1	1.77	0.46	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	19.5	≤23.6%	201.6	2.70	1.06	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	18.5	≤23.6%	276.4	2.79	1.26	1	0	E1E1-	-	-	E2
Red River 1997 x 30422-C7RR	46.1	≥36.8%	585.0	1.36	1.14	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	45.0	≥36.8%	250.5	2.28	1.09	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	45.0	≥36.8%	126.8	2.33	1.19	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	44.8	≥36.8%	147.8	2.34	1.02	1	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30422-C7RR	44.3	≥36.8%	301.0	1.80	1.06	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	44.1	≥36.8%	169.6	2.59	0.93	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30422-C7RR	43.6	≥36.8%	91.9	2.34	0.78	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	43.2	≥36.8%	113.4	2.42	1.08	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	42.9	≥36.8%	505.4	2.36	1.38	1	0	E1E1-	-	-	E2
Red River 1997 x 30422-C7RR	42.8	≥36.8%	115.0	2.37	0.95	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	42.7	≥36.8%	102.4	2.73	1.18	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	42.7	≥36.8%	56.1	2.42	0.53	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	42.6	≥36.8%	70.9	2.42	0.99	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	42.5	≥36.8%	83.5	1.99	0.94	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	40.9	≥36.8%	203.8	2.29	1.23	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	40.3	≥36.8%	1358.1	2.16	1.62	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	40.3	≥36.8%	325.9	2.16	1.48	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	40.1	≥36.8%	264.6	2.31	1.18	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	39.6	≥36.8%	1016.4	2.09	1.55	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	39.6	≥36.8%	362.4	1.61	1.19	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	39.6	≥36.8%	60.8	2.45	0.63	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30422-C7RR	39.5	≥36.8%	47.0	0.83	0.54	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30422-C7RR	39.4	≥36.8%	150.0	2.25	1.23	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	38.5	≥36.8%	567.4	2.13	1.39	1	0	E1E1-	-	-	E2
Red River 1997 x 30422-C7RR	38.4	≥36.8%	40.2	1.37	0.76	2	0	E1e1-	-	-	E2
Red River 1997 x 30422-C7RR	38.3	≥36.8%	335.2	2.13	1.33	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	38.3	≥36.8%	98.3	2.04	0.91	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	38.1	≥36.8%	223.6	1.64	0.96	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	38.0	≥36.8%	468.0	2.15	1.48	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	38.0	≥36.8%	226.8	2.45	1.23	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30422-C7RR	37.6	≥36.8%	151.7	2.06	1.23	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	37.6	≥36.8%	92.7	1.42	0.71	1	0	E1E1-	-	-	E2
Red River 1997 x 30422-C7RR	37.5	≥36.8%	178.0	2.19	1.41	2	0	E1e1-	-	-	E2
Red River 1997 x 30422-C7RR	37.2	≥36.8%	196.7	2.30	1.37	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30422-C7RR	37.0	≥36.8%	221.4	2.55	0.88	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30422-C7RR	36.8	≥36.8%	222.2	2.32	1.25	1	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30422-C7RR	36.5	36.7%-33.7%	1907.8	2.03	1.81	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	36.5	36.7%-33.7%	502.5	1.48	1.20	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	36.3	36.7%-33.7%	389.6	2.24	1.28	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	36.0	36.7%-33.7%	51.2	2.80	0.87	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	35.9	36.7%-33.7%	176.4	2.13	1.09	1	0	E1E1-	-	-	E2
Red River 1997 x 30422-C7RR	35.7	36.7%-33.7%	140.9	2.12	1.00	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	35.6	36.7%-33.7%	139.0	2.39	0.93	0	1	-E2E2	-	-	E1
Red River 1997 x 30422-C7RR	35.4	36.7%-33.7%	91.9	2.97	1.09	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	35.3	36.7%-33.7%	285.2	2.66	1.14	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	34.9	36.7%-33.7%	246.1	2.44	1.24	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	34.7	36.7%-33.7%	268.8	2.01	1.08	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	34.7	36.7%-33.7%	150.9	2.32	0.92	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	34.7	36.7%-33.7%	100.9	4.01	0.95	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	34.7	36.7%-33.7%	84.2	2.63	0.85	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	34.7	36.7%-33.7%	78.4	2.06	0.87	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	34.6	36.7%-33.7%	248.5	2.21	1.45	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	34.5	36.7%-33.7%	476.4	2.19	1.33	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	34.0	36.7%-33.7%	196.8	2.40	1.01	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	33.9	36.7%-33.7%	344.3	1.57	1.13	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	33.8	36.7%-33.7%	207.4	2.28	1.32	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	33.8	36.7%-33.7%	184.6	2.27	1.43	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	33.8	36.7%-33.7%	173.5	1.52	0.97	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	33.6	33.6%-26.8%	2173.7	1.80	1.64	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	33.6	33.6%-26.8%	1410.9	2.17	1.74	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	33.5	33.6%-26.8%	205.3	2.26	1.13	1	0	E1E1-	-	-	E2
Red River 1997 x 30422-C7RR	33.1	33.6%-26.8%	152.4	2.61	1.20	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	32.9	33.6%-26.8%	133.1	1.99	1.14	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	32.5	33.6%-26.8%	2821.5	2.06	1.62	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	32.5	33.6%-26.8%	474.3	2.38	1.52	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	32.4	33.6%-26.8%	184.2	2.37	1.16	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	32.1	33.6%-26.8%	262.3	2.45	1.30	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30422-C7RR	30.3	33.6%-26.8%	65.4	2.41	1.01	2	2	E1e1E2e2	N	E1/E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30422-C7RR	30.1	33.6%-26.8%	61.9	2.32	1.00	2	0	E1e1-	-	-	E2
Red River 1997 x 30422-C7RR	29.4	33.6%-26.8%	185.7	2.17	1.35	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30422-C7RR	28.9	33.6%-26.8%	156.8	2.30	1.05	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30422-C7RR	28.4	33.6%-26.8%	197.6	2.36	0.96	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30422-C7RR	28.1	33.6%-26.8%	245.5	2.32	1.25	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30422-C7RR	26.8	33.6%-26.8%	536.9	1.94	1.32	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30422-C7RR	26.5	26.7%-23.7%	262.5	2.16	1.29	0	2	-E2e2	-	-	E1
Red River 1997 x 30422-C7RR	25.8	26.7%-23.7%	294.4	2.18	1.32	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	25.8	26.7%-23.7%	25.6	2.85	0.72	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	25.7	26.7%-23.7%	191.8	2.09	1.08	2	0	E1e1-	-	-	E2
Red River 1997 x 30422-C7RR	25.6	26.7%-23.7%	43.1	2.42	1.11	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	25.2	26.7%-23.7%	62.7	2.45	0.52	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	24.6	26.7%-23.7%	556.4	1.59	1.11	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	24.5	26.7%-23.7%	277.2	2.23	1.35	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	24.5	26.7%-23.7%	142.9	2.32	0.89	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	24.1	26.7%-23.7%	226.6	2.12	1.31	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	23.7	26.7%-23.7%	152.0	2.02	1.18	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	23.2	≤23.6%	162.6	2.37	1.11	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	22.8	≤23.6%	675.0	1.31	0.82	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	22.7	≤23.6%	182.3	1.75	1.01	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	22.2	≤23.6%	290.2	2.42	1.25	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	22.0	≤23.6%	116.7	2.68	0.92	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	22.0	≤23.6%	106.8	2.78	0.91	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	21.9	≤23.6%	224.9	2.09	1.10	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	20.8	≤23.6%	211.8	1.92	1.24	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30422-C7RR	20.6	≤23.6%	923.7	1.81	1.45	2	2	E1e1E2e2	Y	-	-

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Indicator of quality of DNA. Should be greater than 1.8 to be considered pure. Less than 1.8, indicates incomplete removal of proteins during the extraction process.

[x]Secondary measurement of purity of DNA. Should be greater than 2.0 to be considered pure. Less than 2.0 indicates the presence of contaminants.

Appendix Table A9. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes from BC1F1 progeny from Roundup Ready HEAR crosses Red River 1997 x 30216-C7RR, Red River 1997 x 30220-D8RR, Red River 1997 x 30221-D8RR, Red River 1997 x 30408-C7RR, and Red River 1997 x 30422-C7RR from the 2011 spring greenhouse cycle following regular MAS procedures for determining erucic acid genotypes

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Red River 1997 x 30216-C7RR	≥36.8%	26	22	3	0	1	0	1	88.00	12.00	0.00	3.85	0.00	3.85
Red River 1997 x 30220-D8RR	≥36.8%	30	20	8	0	2	0	2	71.43	28.57	0.00	6.67	0.00	6.67
Red River 1997 x 30221-D8RR	≥36.8%	19	11	0	0	8	0	8	100.00	0.00	0.00	42.11	0.00	42.11
Red River 1997 x 30408-C7RR	≥36.8%	20	13	3	0	4	0	4	81.25	18.75	0.00	20.00	0.00	20.00
Red River 1997 x 30422-C7RR	≥36.8%	36	17	14	0	5	0	5	54.84	45.16	0.00	13.89	0.00	13.89
Total	≥36.8%	131	83	28	0	20	0	20	395.52	104.48	0.00	86.51	0.00	86.51
Mean	≥36.8%		17	6	0	4	0	4	79.10	20.90	0.00	17.30	0.00	17.30
Minimum	≥36.8%		11	0	0	1	0	1	54.84	0.00	0.00	3.85	0.00	3.85
Maximum	≥36.8%		22	14	0	8	0	8	100.00	45.16	0.00	42.11	0.00	42.11
Red River 1997 x 30216-C7RR	36.7%-33.7%	19	18	1	0	0	0	0	94.74	5.26	0.00	0.00	0.00	0.00
Red River 1997 x 30220-D8RR	36.7%-33.7%	9	7	1	0	1	0	1	87.50	12.50	0.00	11.11	0.00	11.11
Red River 1997 x 30221-D8RR	36.7%-33.7%	12	5	1	0	6	0	6	83.33	16.67	0.00	50.00	0.00	50.00
Red River 1997 x 30408-C7RR	36.7%-33.7%	12	7	2	0	3	0	3	77.78	22.22	0.00	25.00	0.00	25.00
Red River 1997 x 30422-C7RR	36.7%-33.7%	22	20	0	1	1	0	2	100.00	0.00	4.55	4.55	0.00	9.09
Total	36.7%-33.7%	74	57	5	1	11	0	12	443.35	56.65	4.55	90.66	0.00	95.20
Mean	36.7%-33.7%		11	1	0	2	0	2	88.67	11.33	0.91	18.13	0.00	19.04
Minimum	36.7%-33.7%		5	0	0	0	0	0	77.78	0.00	0.00	0.00	0.00	0.00
Maximum	36.7%-33.7%		20	2	1	6	0	6	100.00	22.22	4.55	50.00	0.00	50.00
Red River 1997 x 30216-C7RR	33.6%-26.8%	30	26	3	0	1	0	1	89.66	10.34	0.00	3.33	0.00	3.33
Red River 1997 x 30220-D8RR	33.6%-26.8%	29	19	6	0	4	0	4	76.00	24.00	0.00	13.79	0.00	13.79
Red River 1997 x 30221-D8RR	33.6%-26.8%	35	21	1	0	13	0	13	95.45	4.55	0.00	37.14	0.00	37.14
Red River 1997 x 30408-C7RR	33.6%-26.8%	33	11	10	0	12	0	12	52.38	47.62	0.00	36.36	0.00	36.36
Red River 1997 x 30422-C7RR	33.6%-26.8%	16	8	6	0	2	0	2	57.14	42.86	0.00	12.50	0.00	12.50
Total	33.6%-26.8%	143	85	26	0	32	0	32	370.63	129.37	0.00	103.13	0.00	103.13
Mean	33.6%-26.8%		17	5	0	6	0	6	74.13	25.87	0.00	20.63	0.00	20.63
Minimum	33.6%-26.8%		8	1	0	1	0	1	52.38	4.55	0.00	3.33	0.00	3.33
Maximum	33.6%-26.8%		26	10	0	13	0	13	95.45	47.62	0.00	37.14	0.00	37.14
Red River 1997 x 30216-C7RR	26.7%-23.7%	10	9	1	0	0	0	0	90.00	10.00	0.00	0.00	0.00	0.00
Red River 1997 x 30220-D8RR	26.7%-23.7%	13	10	2	0	1	0	1	83.33	16.67	0.00	7.69	0.00	7.69
Red River 1997 x 30221-D8RR	26.7%-23.7%	6	0	3	0	3	0	3	0.00	100.00	0.00	50.00	0.00	50.00
Red River 1997 x 30408-C7RR	26.7%-23.7%	11	3	2	0	6	0	6	60.00	40.00	0.00	54.55	0.00	54.55
Red River 1997 x 30422-C7RR	26.7%-23.7%	11	9	0	1	1	0	2	100.00	0.00	9.09	9.09	0.00	18.18
Total	26.7%-23.7%	51	31	8	1	11	0	12	333.33	166.67	9.09	121.33	0.00	130.42
Mean	26.7%-23.7%		6	2	0	2	0	2	66.67	33.33	1.82	24.27	0.00	26.08

Pedigree (BC1F1)	Category[z]	Sample Number	Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Minimum	26.7%-23.7%		0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	26.7%-23.7%		10	3	1	6	0	6	100.00	100.00	9.09	54.55	0.00	54.55
Red River 1997 x 30216-C7RR	≤23.6%	11	9	1	0	1	0	1	90.00	10.00	0.00	9.09	0.00	9.09
Red River 1997 x 30220-D8RR	≤23.6%	12	11	0	0	1	0	1	100.00	0.00	0.00	8.33	0.00	8.33
Red River 1997 x 30221-D8RR	≤23.6%	16	7	2	0	7	0	7	77.78	22.22	0.00	43.75	0.00	43.75
Red River 1997 x 30408-C7RR	≤23.6%	9	9	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
Red River 1997 x 30422-C7RR	≤23.6%	23	7	7	3	4	2	9	50.00	50.00	13.04	17.39	8.70	39.13
Total	≤23.6%	71	43	10	3	13	2	18	417.78	82.22	13.04	78.57	8.70	100.30
Mean	≤23.6%		9	2	1	3	0	4	83.56	16.44	2.61	15.71	1.74	20.06
Minimum	≤23.6%		7	0	0	0	0	0	50.00	0.00	0.00	0.00	0.00	0.00
Maximum	≤23.6%		11	7	3	7	2	9	100.00	50.00	13.04	43.75	8.70	43.75
Total Plants Evaluated (%)		80.00												
Overall Accuracy (%)		79.52												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

Appendix Table A10. Phenotypic and genotypic results for erucic acid (C22:1) content for BC1F1 progeny from conventional HEAR crosses 08C344 x 30408-C7RR and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following the newly developed SNP MAS procedure for determining erucic acid genotypes

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	50.1	≥40.8%	246.4	2.2	1.22	1	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	49.5	≥40.8%	191.1	1.72	1.34	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	47.2	≥40.8%	171.4	2.23	0.91	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	46.5	≥40.8%	498.9	1.96	0.92	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	46.3	≥40.8%	69.7	2.21	1.03	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	45.6	≥40.8%	176.2	2.13	1.11	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	44.8	≥40.8%	180.2	1.35	0.76	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	44.5	≥40.8%	363.6	2.14	1.11	1	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	44.4	≥40.8%	260.2	2.14	1.11	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.8	≥40.8%	429.6	2.1	1.01	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.7	≥40.8%	109.2	2.14	0.99	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	43.6	≥40.8%	112.2	2.32	1.1	1	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	43.3	≥40.8%	102.3	2.14	0.98	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	43.3	≥40.8%	78.1	2.05	0.62	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	43.2	≥40.8%	107.3	1.93	0.31	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	42.7	≥40.8%	209.0	1.94	0.96	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	42.6	≥40.8%	18.7	1.81	0.26	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	42.0	≥40.8%	336.6	2.19	1.37	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	41.9	≥40.8%	287.1	2.11	1.05	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	41.9	≥40.8%	204.3	2.09	1.83	1	1	E1E1E2E2	Y	-	-
08C344 x 30408-C7RR	41.0	≥40.8%	56.4	2.17	1.16	1	2	E1E1E2e2	N	E2	-
08C344 x 30408-C7RR	40.4	40.7%-37.7%	11.1	2.36	1.32	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	40.2	40.7%-37.7%	582.2	2.01	1.12	2	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	40.2	40.7%-37.7%	313.3	2.26	1.01	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	39.9	40.7%-37.7%	181.8	2.12	1.15	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	39.4	40.7%-37.7%	835.9	1.99	0.7	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	39.0	40.7%-37.7%	384.5	2.13	0.92	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	38.7	40.7%-37.7%	601.7	1.48	0.8	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	38.6	40.7%-37.7%	550.1	2.18	1.11	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	38.5	40.7%-37.7%	601.0	1.5	0.92	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	38.5	40.7%-37.7%	112.2	2.14	0.6	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	38.5	40.7%-37.7%	5.1	0.86	0.59	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	38.4	40.7%-37.7%	254.2	2.45	0.74	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	38.3	40.7%-37.7%	84.4	1.56	0.86	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	38.0	40.7%-37.7%	751.1	2.19	0.86	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.9	40.7%-37.7%	234.6	2.01	1.14	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.8	40.7%-37.7%	13185.8	2.07	0.77	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	37.7	40.7%-37.7%	297.8	1.54	0.44	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.5	37.6%-31.4%	274.9	2.28	1.05	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	37.3	37.6%-31.4%	79.9	2.11	1.2	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.2	37.6%-31.4%	377.0	2.07	1.06	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	37.2	37.6%-31.4%	133.4	2.1	0.58	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	37.1	37.6%-31.4%	31.3	2.09	0.96	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	36.9	37.6%-31.4%	445.7	2.15	0.74	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	36.6	37.6%-31.4%	668.9	2.12	1.02	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	36.5	37.6%-31.4%	248.7	2.24	1.18	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	36.3	37.6%-31.4%	176.0	2.24	0.92	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	36.3	37.6%-31.4%	89.5	2.28	0.73	2	0	E1e1-	-	-	E2

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	36.2	37.6%-31.4%	72.1	2.01	1.46	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.8	37.6%-31.4%	191.3	2.07	0.92	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	35.7	37.6%-31.4%	193.3	2.29	1.38	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	35.7	37.6%-31.4%	99.9	2.12	0.5	1	0	E1E1-	-	-	E2
08C344 x 30408-C7RR	35.6	37.6%-31.4%	283.4	2.15	1.36	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	35.6	37.6%-31.4%	113.4	2.72	0.94	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.5	37.6%-31.4%	150.6	2.12	1.25	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	35.4	37.6%-31.4%	381.8	2.46	1.38	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.4	37.6%-31.4%	261.9	1.44	0.96	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	35.2	37.6%-31.4%	430.9	2.38	1.08	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.2	37.6%-31.4%	85.7	2.06	1.09	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	35.0	37.6%-31.4%	85.5	2.25	0.72	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	34.9	37.6%-31.4%	713.3	2.3	1.03	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	34.8	37.6%-31.4%	233.6	2	0.68	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	34.8	37.6%-31.4%	225.0	2.13	1.35	2	1	E1e1E2E2	Y	-	-
08C344 x 30408-C7RR	34.6	37.6%-31.4%	76.8	2.14	0.87	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	34.3	37.6%-31.4%	71.3	2.09	0.96	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	34.1	37.6%-31.4%	72.0	2.05	1.14	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	34.1	37.6%-31.4%	32.9	1.9	0.77	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	34.0	37.6%-31.4%	184.4	2.18	1	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30408-C7RR	34.0	37.6%-31.4%	161.5	2.08	1.41	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30408-C7RR	33.7	37.6%-31.4%	117.9	2.07	0.84	1	2	E1E1E2e2	Y	-	-
08C344 x 30408-C7RR	33.6	37.6%-31.4%	250.0	2.05	1.08	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	33.2	37.6%-31.4%	353.6	2.21	1.24	1	2	E1E1E2e2	N	E1	-
08C344 x 30408-C7RR	32.4	37.6%-31.4%	93.6	2.29	0.96	2	1	E1e1E2E2	N	E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30408-C7RR	32.2	37.6%-31.4%	66.1	2.18	1.25	2	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	31.8	37.6%-31.4%	142.0	1.86	0.42	2	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	31.3	31.3%-28.3%	245.8	2.08	0.89	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	31.3	31.3%-28.3%	244.6	2.29	0.51	0	2	-E2e2	-	-	E1
08C344 x 30408-C7RR	30.1	31.3%-28.3%	275.2	1.7	0.47	0	1	-E2E2	-	-	E1
08C344 x 30408-C7RR	29.9	31.3%-28.3%	90.4	2.07	1.01	2	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	29.4	31.3%-28.3%	209.5	1.83	1.79	2	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	29.2	31.3%-28.3%	138.8	2	0.75	2	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	28.0	≤28.2%	435.7	2.17	1.07	2	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	27.5	≤28.2%	73.9	2.14	0.75	2	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	27.3	≤28.2%	306.0	2.04	0.44	2	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	26.9	≤28.2%	492.4	2.05	0.83	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	26.9	≤28.2%	421.4	2.32	0.89	2	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	26.6	≤28.2%	449.9	2.14	0.79	2	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	26.6	≤28.2%	84.1	1.96	0.78	2	1	E1e1E2E2	N	E2	-
08C344 x 30408-C7RR	26.3	≤28.2%	447.9	2.27	2.29	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	26.2	≤28.2%	64.5	2.19	0.87	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	25.9	≤28.2%	331.7	1.46	0.31	0	0	--	-	-	E1/E2
08C344 x 30408-C7RR	25.5	≤28.2%	487.4	1.95	1.3	2	0	E1e1-	-	-	E2
08C344 x 30408-C7RR	24.3	≤28.2%	151.0	2.11	1.11	2	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	23.8	≤28.2%	192.5	2.15	1.13	2	2	E1e1E2e2	Y	-	-
08C344 x 30408-C7RR	23.0	≤28.2%	198.8	2.18	1.37	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	48.4	≥40.8%	2835.1	1.93	0.88	1	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	46.4	≥40.8%	127.1	1.77	0.67	1	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	46.3	≥40.8%	79.0	2.1	1.2	1	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	46.3	≥40.8%	9.6	2.12	1.53	0	1	-E2E2	-	-	E1
08C344 x 30422-C7RR	46.0	≥40.8%	243.5	2.11	1.39	0	1	-E2E2	-	-	E1
08C344 x 30422-C7RR	44.9	≥40.8%	485.0	2.08	0.99	1	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	44.6	≥40.8%	332.1	1.9	0.54	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	44.6	≥40.8%	328.7	2.04	0.87	1	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	44.3	≥40.8%	251.0	2.06	1.09	2	1	E1e1E2E2	N	E1	-
08C344 x 30422-C7RR	44.0	≥40.8%	406.2	1.73	0.68	1	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	43.5	≥40.8%	209.0	2.1	1.22	0	1	-E2E2	-	-	E1
08C344 x 30422-C7RR	43.4	≥40.8%	161.2	1.97	0.74	1	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	43.2	≥40.8%	1.8	1.28	0.3	0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	43.1	≥40.8%	285.8	1.93	0.58	1	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	42.8	≥40.8%	44.4	0.36	0.05	0	0	--	-	-	E1/E2
08C344 x 30422-C7RR	42.7	≥40.8%	14.9	1.94	1.21	2	1	E1e1E2E2	N	E1	-
08C344 x 30422-C7RR	42.6	≥40.8%	322.7	2.17	1.26	1	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	42.3	≥40.8%	114.5	1.41	0.83	1	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	41.6	≥40.8%	163.7	1.95	0.96	1	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	41.5	≥40.8%	291.9	1.67	0.83	1	1	E1E1E2E2	Y	-	-
08C344 x 30422-C7RR	41.4	≥40.8%	586.4	1.65	1.67	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	41.3	≥40.8%	1216.6	2	0.93	1	2	E1E1E2e2	N	E2	-
08C344 x 30422-C7RR	41.2	≥40.8%	425.1	2.04	1.32	2	1	E1e1E2E2	N	E1	-
08C344 x 30422-C7RR	40.7	40.7%-37.7%	69.6	1.81	1.43	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	40.5	40.7%-37.7%	120.8	2.15	0.97	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	40.0	40.7%-37.7%	292.5	1.96	1.04	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	39.8	40.7%-37.7%	181.7	1.86	0.33	2	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	39.6	40.7%-37.7%	227.8	2.16	1.32	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	39.2	40.7%-37.7%	171.9	1.88	0.66	1	1	E1E1E2E2	N	E1/E2	-
08C344 x 30422-C7RR	39.2	40.7%-37.7%	108.7	2.02	0.4	1	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	38.9	40.7%-37.7%	297.2	3.25	1.18	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	38.9	40.7%-37.7%	29.6	2.09	0.64	2	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	38.7	40.7%-37.7%	266.6	1.71	0.72	0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	38.6	40.7%-37.7%	386.1	1.88	1.24	2	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	38.1	40.7%-37.7%	316.7	1.84	0.37	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	38.1	40.7%-37.7%	1.1	2.08	1.43	2	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	38.0	40.7%-37.7%	167.0	1.96	0.53	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	37.9	40.7%-37.7%	122.1	1.87	0.78	2	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	37.8	40.7%-37.7%	440.5	1.89	0.7	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	37.5	37.6%-31.4%	158.7	1.79	1.37	2	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	37.4	37.6%-31.4%	14.5	1.95	0.89	2	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	37.0	37.6%-31.4%	248.5	2.19	1.66	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.9	37.6%-31.4%	194.8	1.58	0.38	1	0	E1E1-	-	-	E2
08C344 x 30422-C7RR	36.7	37.6%-31.4%	289.5	2.15	1.88	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.6	37.6%-31.4%	426.3	2.09	1	2	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	36.6	37.6%-31.4%	48.0	1.6	1.54	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.6	37.6%-31.4%	13.8	2.03	1.28	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.5	37.6%-31.4%	122.6	1.68	0.33	0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	36.5	37.6%-31.4%	49.2	1.87	0.64	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.4	37.6%-31.4%	157.3	1.76	0.47	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.2	37.6%-31.4%	94.3	0.85	-52.6	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	36.2	37.6%-31.4%	40.1	1.77	0.63	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.9	37.6%-31.4%	222.8	2.1	1.37	1	2	E1E1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	35.8	37.6%-31.4%	116.9	1.64	1.5	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.8	37.6%-31.4%	43.7	1.86	0.65	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.7	37.6%-31.4%	621.2	2.07	1.48	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	35.3	37.6%-31.4%	219.6	2.05	0.81	2	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	35.0	37.6%-31.4%	76.0	2.06	0.92	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	34.7	37.6%-31.4%	209.2	1.21	0.73	2	2	E1e1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	34.5	37.6%-31.4%	54.1	1.91	1	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	34.2	37.6%-31.4%	167.2	1.66	0.92	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	34.2	37.6%-31.4%	37.9	2.05	0.42	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	33.8	37.6%-31.4%	179.7	1.18	0.7	2	1	E1e1E2E2	Y	-	-
08C344 x 30422-C7RR	33.8	37.6%-31.4%	167.8	2.05	0.8	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	33.0	37.6%-31.4%	70.9	1.79	0.65	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	32.8	37.6%-31.4%	143.6	2	0.91	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	32.6	37.6%-31.4%	284.1	1.84	0.62	1	2	E1E1E2e2	Y	-	-
08C344 x 30422-C7RR	31.2	31.3%-28.3%	81.5	1.92	0.95	1	2	E1E1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	30.9	31.3%-28.3%	242.2	3.52	1.18	1	2	E1E1E2e2	N	E1/E2	-
08C344 x 30422-C7RR	30.0	31.3%-28.3%	19.3	1.93	0.54	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	29.4	31.3%-28.3%	299.3	1.72	1.63	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	29.0	31.3%-28.3%	74.2	2.04	1	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	28.5	31.3%-28.3%	357.0	2.2	1.16	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	28.0	≤28.2%	17.3	2.08	0.82	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.8	≤28.2%	224.5	1.58	0.75	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.5	≤28.2%	7819.6	1.98	0.93	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.2	≤28.2%	244.9	1.96	0.88	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	27.1	≤28.2%	206.7	-0.15	-0.19	0	2	-E2e2	-	-	E1

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
08C344 x 30422-C7RR	26.8	≤28.2%	793.7	2.23	0.93	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.8	≤28.2%	196.4	2.13	0.51	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.7	≤28.2%	54.0	2.01	0.57	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.5	≤28.2%	181.3	1.98	0.82	0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	26.4	≤28.2%	144.7	1.78	0.59	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.1	≤28.2%	171.0	2.04	1.13	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	26.0	≤28.2%	233.3	1.51	1.17	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.8	≤28.2%	142.8	2.03	1.09	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.8	≤28.2%	34.3	2.42	1.21	0	2	-E2e2	-	-	E1
08C344 x 30422-C7RR	25.6	≤28.2%	292.5	2.16	1.43	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.6	≤28.2%	270.4	1.91	0.57	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.6	≤28.2%	42.1	2.01	0.96	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.5	≤28.2%	124.7	1.93	1.12	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	25.5	≤28.2%	52.8	1.99	0.74	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	24.9	≤28.2%	6111.3	1.94	0.79	2	0	E1e1-	-	-	E2
08C344 x 30422-C7RR	24.8	≤28.2%	198.5	1.82	1.04	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	24.8	≤28.2%	28.8	2.05	0.79	2	2	E1e1E2e2	Y	-	-
08C344 x 30422-C7RR	24.4	≤28.2%	207.6	2.54	0.75	2	2	E1e1E2e2	Y	-	-

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Indicator of quality of DNA. Should be greater than 1.8 to be considered pure. Less than 1.8, indicates incomplete removal of proteins during the extraction process.

[x]Secondary measurement of purity of DNA. Should be greater than 2.0 to be considered pure. Less than 2.0 indicates the presence of contaminants.

Appendix Table A11. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes from BC1F1 progeny from conventional HEAR crosses 08C344 x 30408-C7RR and 08C344 x 30422-C7RR from the 2011 spring greenhouse cycle following the regular and newly developed SNP MAS procedures for determining erucic acid genotypes

			Regular SCAR Procedure											
			Number						Frequency (%)					
Pedigree (BC1F1)	Category[z]	Sample Number	Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 30408-C7RR	≥40.8%	21	17	1	0	3	0	3	94.44	5.56	0.00	14.29	0.00	14.29
08C344 x 30422-C7RR	≥40.8%	23	7	7	3	4	2	9	50.00	50.00	13.04	17.39	8.70	39.13
Total	≥40.8%	44	24	8	3	7	2	12	75.00	25.00	6.82	15.91	4.55	27.27
Mean	≥40.8%		12	4	2	4	1	6	72.22	27.78	6.52	15.84	4.35	26.71
Minimum	≥40.8%		7	1	0	3	0	3	50.00	5.56	0.00	14.29	0.00	14.29
Maximum	≥40.8%		17	7	3	4	2	9	94.44	50.00	13.04	17.39	8.70	39.13
08C344 x 30408-C7RR	40.7%-37.7%	17	9	1	0	7	0	7	90.00	10.00	0.00	41.18	0.00	41.18
08C344 x 30422-C7RR	40.7%-37.7%	16	5	6	1	4	0	5	45.45	54.55	6.25	25.00	0.00	31.25
Total	40.7%-37.7%	33	14	7	1	11	0	12	66.67	33.33	3.03	33.33	0.00	36.36
Mean	40.7%-37.7%		7	4	1	6	0	6	67.73	32.27	3.13	33.09	0.00	36.21
Minimum	40.7%-37.7%		5	1	0	4	0	5	45.45	10.00	0.00	25.00	0.00	31.25
Maximum	40.7%-37.7%		9	6	1	7	0	7	90.00	54.55	6.25	41.18	0.00	41.18
08C344 x 30408-C7RR	37.6%-31.4%	37	24	3	0	10	0	10	88.89	11.11	0.00	27.03	0.00	27.03
08C344 x 30422-C7RR	37.6%-31.4%	28	22	3	1	2	0	3	88.00	12.00	3.57	7.14	0.00	10.71
Total	37.6%-31.4%	65	46	6	1	12	0	13	88.46	11.54	1.54	18.46	0.00	20.00
Mean	37.6%-31.4%		23	3	1	6	0	7	88.44	11.56	1.79	17.08	0.00	18.87
Minimum	37.6%-31.4%		22	3	0	2	0	3	88.00	11.11	0.00	7.14	0.00	10.71
Maximum	37.6%-31.4%		24	3	1	10	0	10	88.89	12.00	3.57	27.03	0.00	27.03
08C344 x 30408-C7RR	31.3%-28.3%	6	1	1	2	2	0	4	50.00	50.00	33.33	33.33	0.00	66.67
08C344 x 30422-C7RR	31.3%-28.3%	6	4	2	0	0	0	0	66.67	33.33	0.00	0.00	0.00	0.00
Total	31.3%-28.3%	12	5	3	2	2	0	4	62.50	37.50	16.67	16.67	0.00	33.33
Mean	31.3%-28.3%		3	2	1	1	0	2	58.33	41.67	16.67	16.67	0.00	33.33
Minimum	31.3%-28.3%		1	1	0	0	0	0	50.00	33.33	0.00	0.00	0.00	0.00
Maximum	31.3%-28.3%		4	2	2	2	0	4	66.67	50.00	33.33	33.33	0.00	66.67
08C344 x 30408-C7RR	≤28.2%	14	3	2	0	9	0	9	60.00	40.00	0.00	64.29	0.00	64.29
08C344 x 30422-C7RR	≤28.2%	23	15	1	3	4	0	7	93.75	6.25	13.04	17.39	0.00	30.43
Total	≤28.2%	37	18	3	3	13	0	16	85.71	14.29	8.11	35.14	0.00	43.24
Mean	≤28.2%		9	2	2	7	0	8	76.88	23.13	6.52	40.84	0.00	47.36
Minimum	≤28.2%		3	1	0	4	0	7	60.00	6.25	0.00	17.39	0.00	30.43
Maximum	≤28.2%		15	2	3	9	0	9	93.75	40.00	13.04	64.29	0.00	64.29
Total Plants Evaluated (%)		87.01												
Overall Accuracy (%)		79.85												

Pedigree (BC1F1)	Category[z]	Sample Number	New SNP Procedure											
			Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
08C344 x 30408-C7RR	≥40.8%	21	9	9	0	3	0	3	50.00	50.00	0.00	14.29	0.00	14.29
08C344 x 30422-C7RR	≥40.8%	23	6	11	4	2	0	6	35.29	64.71	17.39	8.70	0.00	26.09
Total	≥40.8%	44	15	20	4	5	0	9	42.86	57.14	9.09	11.36	0.00	20.45
Mean	≥40.8%		8	10	2	3	0	5	42.65	57.35	8.70	11.49	0.00	20.19
Minimum	≥40.8%		6	9	0	2	0	3	35.29	50.00	0.00	8.70	0.00	14.29
Maximum	≥40.8%		9	11	4	3	0	6	50.00	64.71	17.39	14.29	0.00	26.09
08C344 x 30408-C7RR	40.7%-37.7%	17	9	7	0	1	0	1	56.25	43.75	0.00	5.88	0.00	5.88
08C344 x 30422-C7RR	40.7%-37.7%	16	7	5	1	3	0	4	58.33	41.67	6.25	18.75	0.00	25.00
Total	40.7%-37.7%	33	16	12	1	4	0	5	57.14	42.86	3.03	12.12	0.00	15.15
Mean	40.7%-37.7%		8	6	1	2	0	3	57.29	42.71	3.13	12.32	0.00	15.44
Minimum	40.7%-37.7%		7	5	0	1	0	1	56.25	41.67	0.00	5.88	0.00	5.88
Maximum	40.7%-37.7%		9	7	1	3	0	4	58.33	43.75	6.25	18.75	0.00	25.00
08C344 x 30408-C7RR	37.6%-31.4%	37	16	15	0	6	0	6	51.61	48.39	0.00	16.22	0.00	16.22
08C344 x 30422-C7RR	37.6%-31.4%	28	23	2	1	2	0	3	92.00	8.00	3.57	7.14	0.00	10.71
Total	37.6%-31.4%	65	39	17	1	8	0	9	69.64	30.36	1.54	12.31	0.00	13.85
Mean	37.6%-31.4%		20	9	1	4	0	5	71.81	28.19	1.79	11.68	0.00	13.47
Minimum	37.6%-31.4%		16	2	0	2	0	3	51.61	8.00	0.00	7.14	0.00	10.71
Maximum	37.6%-31.4%		23	15	1	6	0	6	92.00	48.39	3.57	16.22	0.00	16.22
08C344 x 30408-C7RR	31.3%-28.3%	6	1	2	2	1	0	3	33.33	66.67	33.33	16.67	0.00	50.00
08C344 x 30422-C7RR	31.3%-28.3%	6	4	2	0	0	0	0	66.67	33.33	0.00	0.00	0.00	0.00
Total	31.3%-28.3%	12	5	4	2	1	0	3	55.56	44.44	16.67	8.33	0.00	25.00
Mean	31.3%-28.3%		3	2	1	1	0	2	50.00	50.00	16.67	8.33	0.00	25.00
Minimum	31.3%-28.3%		1	2	0	0	0	0	33.33	33.33	0.00	0.00	0.00	0.00
Maximum	31.3%-28.3%		4	2	2	1	0	3	66.67	66.67	33.33	16.67	0.00	50.00
08C344 x 30408-C7RR	≤28.2%	14	5	1	0	4	4	8	83.33	16.67	0.00	28.57	28.57	57.14
08C344 x 30422-C7RR	≤28.2%	23	19	0	3	1	0	4	100.00	0.00	13.04	4.35	0.00	17.39
Total	≤28.2%	37	24	1	3	5	4	12	96.00	4.00	8.11	13.51	10.81	32.43
Mean	≤28.2%		12	1	2	3	2	6	91.67	8.33	6.52	16.46	14.29	37.27
Minimum	≤28.2%		5	0	0	1	0	4	83.33	0.00	0.00	4.35	0.00	17.39
Maximum	≤28.2%		19	1	3	4	4	8	100.00	16.67	13.04	28.57	28.57	57.14
Total Plants Evaluated (%)		99.35												
Overall Accuracy (%)		64.71												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

Appendix Table A12. Phenotypic and genotypic results for erucic acid (C22:1) content for BC1F1 progeny from Roundup Ready HEAR crosses 08C344 x 30221-D8RR and 08C344 x 30408-C7RR from the 2011 spring greenhouse cycle following the newly developed SNP MAS procedure for determining erucic acid genotypes

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30221-D8RR	45.9	≥36.8%	64.1	2.62	0.64	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30221-D8RR	45.5	≥36.8%	86.7	2.96	0.93	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30221-D8RR	44.9	≥36.8%	65.7	2.71	0.72	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30221-D8RR	42.3	≥36.8%	126	2.73	1.03	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	42.2	≥36.8%	93.3	2.13	0.69	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30221-D8RR	41.1	≥36.8%	39.6	2.89	0.79	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	40.9	≥36.8%	38.6	2.32	0.75	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	40.9	≥36.8%	161.8	1.92	0.91	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30221-D8RR	40.3	≥36.8%	149.6	2.55	0.97	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	40.2	≥36.8%	160.8	2.28	1.12	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	39.5	≥36.8%	889.8	1.82	1.45	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	38.9	≥36.8%	389	1.97	1.29	2	1	E1e1E2E2	N	E1	-
Red River 1997 x 30221-D8RR	38.9	≥36.8%	128.2	4.26	0.88	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30221-D8RR	37.9	≥36.8%	38.2	2.33	0.82	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30221-D8RR	37.7	≥36.8%	2519.9	1.92	1.79	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	37.6	≥36.8%	269.4	2.48	1.3	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	37.2	≥36.8%	60.8	2.52	0.62	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	37.1	≥36.8%	103.9	2.4	0.9	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	36.9	≥36.8%	333.7	2.41	1.52	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	35.7	36.7%-33.7%	1.9	1.88	0.03	1	0	E1E1-	-	-	E2
Red River 1997 x 30221-D8RR	35.4	36.7%-33.7%	449.5	1.32	0.78	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	35.3	36.7%-33.7%	257.4	1.72	0.82	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	34.9	36.7%-33.7%	78.9	2.08	0.88	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	34.5	36.7%-33.7%	184.2	2.27	1.02	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	34.5	36.7%-33.7%	65.7	1.88	0.71	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	34.3	36.7%-33.7%	92	2.47	0.85	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	34.3	36.7%-33.7%	14.1	2.25	0.2	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	33.9	36.7%-33.7%	3469.6	1.58	1.32	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	33.9	36.7%-33.7%	61.7	3.47	0.63	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	33.8	36.7%-33.7%	34.3	2.54	0.8	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	33.8	36.7%-33.7%	27.7	1.96	0.92	2	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30221-D8RR	33.6	33.6%-26.8%	437.4	2.03	1.4	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	33.2	33.6%-26.8%	155.7	1.83	0.63	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	33.2	33.6%-26.8%	60.4	2.12	0.49	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	33.1	33.6%-26.8%	2620.8	1.77	1.59	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	33.0	33.6%-26.8%	23.6	2.13	0.7	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.9	33.6%-26.8%	40.6	3	0.8	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.7	33.6%-26.8%	52.7	2.77	0.72	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	32.7	33.6%-26.8%	1080.9	1.68	1.16	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	32.6	33.6%-26.8%	37.4	2.25	0.32	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.2	33.6%-26.8%	1167.1	1.79	1.37	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.2	33.6%-26.8%	107.9	2.83	0.87	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	32.2	33.6%-26.8%	77.5	2.37	0.93	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	32.1	33.6%-26.8%	623.3	2.17	1.63	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	32.1	33.6%-26.8%	184.3	2.77	1.2	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	31.4	33.6%-26.8%	141.6	2.35	1.15	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	31.3	33.6%-26.8%	338.6	1.95	1.44	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	31.0	33.6%-26.8%	84.8	2.29	0.85	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.9	33.6%-26.8%	313.9	1.93	1.19	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.9	33.6%-26.8%	152.8	2.5	0.97	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.8	33.6%-26.8%	73	1.72	0.58	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	30.5	33.6%-26.8%	33.7	2.99	0.75	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.5	33.6%-26.8%	65.9	2.08	0.77	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	30.4	33.6%-26.8%	978.3	2.2	1.76	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	30.4	33.6%-26.8%	73.7	2.38	0.72	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30221-D8RR	30.2	33.6%-26.8%	220.8	2.29	1.4	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	30.1	33.6%-26.8%	102.6	2.76	1.14	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	29.8	33.6%-26.8%	471.6	2.69	1.35	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	29.7	33.6%-26.8%	176.7	2.5	1.31	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	29.7	33.6%-26.8%	106.1	2.59	0.99	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30221-D8RR	29.5	33.6%-26.8%	178.4	1.8	0.68	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	29.4	33.6%-26.8%	484.5	2.06	1.26	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	28.8	33.6%-26.8%	30.4	7.09	0.77	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30221-D8RR	28.2	33.6%-26.8%	1021.3	1.85	1.47	2	1	E1e1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30221-D8RR	27.5	33.6%-26.8%	282.4	2.2	0.95	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	26.6	26.7%-23.7%	255.1	3.1	1.19	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	25.8	26.7%-23.7%	65.5	6.61	0.74	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	25.7	26.7%-23.7%	198.1	2.13	1.17	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	25.1	26.7%-23.7%	118.8	2.94	0.74	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	24.8	26.7%-23.7%	21.2	2.97	0.24	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	24.0	26.7%-23.7%	57.2	3.02	0.87	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	23.8	26.7%-23.7%	966.1	2.07	1.47	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	23.5	≤23.6%	150.9	2.18	0.96	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	23.4	≤23.6%	53.3	2.08	0.68	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	23.4	≤23.6%	132.4	2.07	1.17	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	23.2	≤23.6%	16.2	2.28	0.3	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	22.3	≤23.6%	107.3	3.82	0.8	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	22.2	≤23.6%	971.4	1.56	1.08	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	22.2	≤23.6%	151	2.54	0.84	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	21.9	≤23.6%	164	4.08	0.93	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	21.8	≤23.6%	130.2	2.34	1.06	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	21.6	≤23.6%	142.4	2.59	0.98	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	21.6	≤23.6%	128.4	2.7	0.75	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	21.6	≤23.6%	49.7	3.73	0.76	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	21.4	≤23.6%	706.8	1.47	1.12	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	21.4	≤23.6%	35.6	2.43	0.34	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30221-D8RR	21.3	≤23.6%	256.5	2.73	0.98	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	21.0	≤23.6%	268.4	2.07	1.24	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	20.8	≤23.6%	47.7	2.84	0.47	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30221-D8RR	20.0	≤23.6%	207.8	2.65	1.03	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	19.9	≤23.6%	150.8	2.54	1.06	2	0	E1e1-	-	-	E2
Red River 1997 x 30221-D8RR	19.9	≤23.6%	184.5	2.84	1.18	2	1	E1e1E2E2	N	E2	-
Red River 1997 x 30221-D8RR	19.3	≤23.6%	80.9	2.65	0.93	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	18.5	≤23.6%	168.3	2.3	1.02	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30221-D8RR	18.5	≤23.6%	43.4	3.51	0.64	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	45.4	≥36.8%	60.9	2.6	0.94	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	44.8	≥36.8%	134.4	2.53	1.35	1	1	E1E1E2E2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30408-C7RR	44.0	≥36.8%	53.7	1.89	0.45	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	43.6	≥36.8%	166.1	2.02	1.09	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	43.1	≥36.8%	399.1	2.42	1.52	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	42.9	≥36.8%	2062.4	2.11	1.9	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	42.6	≥36.8%	200.4	3.52	1.1	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	40.5	≥36.8%	79.4	2.07	0.58	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	40.2	≥36.8%	162.3	2.6	1.1	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	40.1	≥36.8%	115.2	3.92	1.03	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	40.0	≥36.8%	187.6	1.47	1.11	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	39.7	≥36.8%	695.1	2.26	1.63	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	39.3	≥36.8%	174.4	2.4	1.04	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	38.8	≥36.8%	80.7	2.32	0.5	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	38.1	≥36.8%	313.5	1.95	1.25	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	37.8	≥36.8%	77.9	1.88	0.84	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	37.3	≥36.8%	166.5	1.61	0.62	2	0	E1e1-	-	-	E2
Red River 1997 x 30408-C7RR	37.0	≥36.8%	419	15.19	5.59	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	37.0	≥36.8%	69.2	2.46	1.02	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	36.9	≥36.8%	361.4	2.47	1.64	1	2	E1E1E2e2	N	E2	-
Red River 1997 x 30408-C7RR	36.7	36.7%-33.7%	120.4	2.11	1.1	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	36.2	36.7%-33.7%	116.1	2.82	1.01	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	36.0	36.7%-33.7%	333.7	2.32	1.28	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	35.1	36.7%-33.7%	107.6	1.55	1.33	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	34.7	36.7%-33.7%	761.2	2.21	1.51	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	34.6	36.7%-33.7%	107	2.24	1.13	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	34.4	36.7%-33.7%	143.1	2.2	1.12	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	34.4	36.7%-33.7%	77.2	2.39	1.05	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	34.0	36.7%-33.7%	411.2	2.34	1.44	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	33.8	36.7%-33.7%	145.2	2.18	1.09	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	33.8	36.7%-33.7%	308.2	2.16	1.32	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	33.7	36.7%-33.7%	269.7	2.75	1.25	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	33.6	33.6%-26.8%	879.9	2.11	1.65	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	33.4	33.6%-26.8%	205.3	2.4	1.4	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	33.4	33.6%-26.8%	93.4	2.15	0.84	1	1	E1E1E2E2	N	E1/E2	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30408-C7RR	33.3	33.6%-26.8%	912.4	2.21	1.65	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	33.2	33.6%-26.8%	110.6	2.15	0.87	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	33.0	33.6%-26.8%	113.1	2.61	1.43	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	32.8	33.6%-26.8%	131.3	2.11	-4.96	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	32.6	33.6%-26.8%	128.7	2.46	0.8	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30408-C7RR	32.5	33.6%-26.8%	153.1	2.5	1.47	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30408-C7RR	32.2	33.6%-26.8%	158.3	2.22	1.05	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	32.2	33.6%-26.8%	643	1.51	1.22	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	32.1	33.6%-26.8%	88.7	2.12	0.53	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	32.0	33.6%-26.8%	179	2.44	1.1	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	32.0	33.6%-26.8%	1021.3	1.81	1.49	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	31.6	33.6%-26.8%	198.6	1.39	0.64	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	31.5	33.6%-26.8%	170.4	2.42	1.18	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	31.4	33.6%-26.8%	910.9	1.83	1.44	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	31.4	33.6%-26.8%	170.9	2.4	1.15	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	31.4	33.6%-26.8%	132.3	2.41	1.22	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	31.3	33.6%-26.8%	305.3	1.93	1.33	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	31.3	33.6%-26.8%	144.8	3.5	1.1	1	0	E1E1-	-	-	E2
Red River 1997 x 30408-C7RR	31.3	33.6%-26.8%	221.1	2.07	1.32	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	31.2	33.6%-26.8%	96.8	2.55	0.97	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	30.3	33.6%-26.8%	310.7	2.33	1.37	2	2	E1e1E2e2	N	E1/E2	-
Red River 1997 x 30408-C7RR	30.0	33.6%-26.8%	142.9	2.63	0.93	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	29.8	33.6%-26.8%	189.9	1.34	1.33	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	29.5	33.6%-26.8%	920.6	2.37	1.72	1	1	E1E1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	29.5	33.6%-26.8%	119.2	3.21	1.03	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	28.8	33.6%-26.8%	1394	2.11	1.85	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	28.5	33.6%-26.8%	232	2.25	1.26	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	28.1	33.6%-26.8%	161.3	2.99	1.04	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	28.0	33.6%-26.8%	257	2.38	1.36	1	2	E1E1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	27.9	33.6%-26.8%	268.5	2.61	1.22	2	1	E1e1E2E2	Y	-	-
Red River 1997 x 30408-C7RR	25.6	26.7%-23.7%	3686.7	1.75	1.71	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	25.5	26.7%-23.7%	122.6	2.21	1.35	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	24.7	26.7%-23.7%	331.3	2.3	1.47	2	2	E1e1E2e2	Y	-	-

Pedigree (BC1F1)	C22:1 Phenotype	Category[z]	DNA Conc. (ng/µl)	260/280[y]	260/230[x]	SNP (E1e1)	SCAR (E2e2)	C22:1 Genotype	Result		
									Correct Y/N?	Error E1/E2?	Missing E1/E2?
Red River 1997 x 30408-C7RR	24.7	26.7%-23.7%	100	3.23	1.03	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	24.5	26.7%-23.7%	3071.3	2.09	1.93	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	24.5	26.7%-23.7%	902.3	1.73	1.32	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	24.5	26.7%-23.7%	103.2	2.05	1.23	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	24.2	26.7%-23.7%	109.7	2.99	1.02	1	1	E1E1E2E2	N	E1/E2	-
Red River 1997 x 30408-C7RR	24.2	26.7%-23.7%	204.1	2.13	1.15	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	23.9	26.7%-23.7%	383.8	2.36	1.44	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	23.7	26.7%-23.7%	194.4	2.58	1.16	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	23.4	≤23.6%	215.8	2.12	1.12	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	23.4	≤23.6%	67.4	2.69	0.81	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.6	≤23.6%	102.5	3.33	0.95	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.6	≤23.6%	84	3.06	0.7	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.2	≤23.6%	305.6	2.32	1.52	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	22.2	≤23.6%	93.4	2.76	0.98	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	22.1	≤23.6%	54.5	2.98	1.07	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	21.8	≤23.6%	89.6	2.15	0.5	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	21.6	≤23.6%	189.2	3.99	0.96	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	21.2	≤23.6%	217.7	2.59	1.27	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	21.1	≤23.6%	132.7	2.96	1.06	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	20.3	≤23.6%	668.2	1.78	1.25	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	20.2	≤23.6%	175.2	2.28	1.24	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	20.1	≤23.6%	66.1	1.77	0.46	1	2	E1E1E2e2	N	E1	-
Red River 1997 x 30408-C7RR	19.5	≤23.6%	201.6	2.7	1.06	2	2	E1e1E2e2	Y	-	-
Red River 1997 x 30408-C7RR	18.5	≤23.6%	276.4	2.79	1.26	1	2	E1E1E2e2	N	E1	-

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Indicator of quality of DNA. Should be greater than 1.8 to be considered pure. Less than 1.8, indicates incomplete removal of proteins during the extraction process.

[x]Secondary measurement of purity of DNA. Should be greater than 2.0 to be considered pure. Less than 2.0 indicates the presence of contaminants.

Appendix Table A13. Frequencies and statistics of correct/incorrect and missing erucic acid (C22:1) genotypes from BC1F1 progeny from Roundup Ready HEAR crosses Red River 1997 x 30221-D8RR and Red River 1997 x 30408-C7RR from the 2011 spring greenhouse cycle following the regular and newly developed SNP MAS procedure for determining erucic acid genotypes

			Regular SCAR Procedure											
			Number						Frequency (%)					
Pedigree (BC1F1)	Category[z]	Sample Number	Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Red River 1997 x 30221-D8RR	≥36.8%	19	11	0	0	8	0	8	100.00	0.00	0.00	42.11	0.00	42.11
Red River 1997 x 30408-C7RR	≥36.8%	20	13	3	0	4	0	4	81.25	18.75	0.00	20.00	0.00	20.00
Total	≥36.8%	39	24	3	0	12	0	12	88.89	11.11	0.00	30.77	0.00	30.77
Mean	≥36.8%		12	2	0	6	0	6	90.63	9.38	0.00	31.05	0.00	31.05
Minimum	≥36.8%		11	0	0	4	0	4	81.25	0.00	0.00	20.00	0.00	20.00
Maximum	≥36.8%		13	3	0	8	0	8	100.00	18.75	0.00	42.11	0.00	42.11
Red River 1997 x 30221-D8RR	36.7%-33.7%	12	5	1	0	6	0	6	83.33	16.67	0.00	50.00	0.00	50.00
Red River 1997 x 30408-C7RR	36.7%-33.7%	12	7	2	0	3	0	3	77.78	22.22	0.00	25.00	0.00	25.00
Total	36.7%-33.7%	24	12	3	0	9	0	9	80.00	20.00	0.00	37.50	0.00	37.50
Mean	36.7%-33.7%		6	2	0	5	0	5	80.56	19.44	0.00	37.50	0.00	37.50
Minimum	36.7%-33.7%		5	1	0	3	0	3	77.78	16.67	0.00	25.00	0.00	25.00
Maximum	36.7%-33.7%		7	2	0	6	0	6	83.33	22.22	0.00	50.00	0.00	50.00
Red River 1997 x 30221-D8RR	33.6%-26.8%	35	21	1	0	13	0	13	95.45	4.55	0.00	37.14	0.00	37.14
Red River 1997 x 30408-C7RR	33.6%-26.8%	33	11	10	0	12	0	12	52.38	47.62	0.00	36.36	0.00	36.36
Total	33.6%-26.8%	68	32	11	0	25	0	25	74.42	25.58	0.00	36.76	0.00	36.76
Mean	33.6%-26.8%		16	6	0	13	0	13	73.92	26.08	0.00	36.75	0.00	36.75
Minimum	33.6%-26.8%		11	1	0	12	0	12	52.38	4.55	0.00	36.36	0.00	36.36
Maximum	33.6%-26.8%		21	10	0	13	0	13	95.45	47.62	0.00	37.14	0.00	37.14
Red River 1997 x 30221-D8RR	26.7%-23.7%	6	0	3	0	3	0	3	0.00	100.00	0.00	50.00	0.00	50.00
Red River 1997 x 30408-C7RR	26.7%-23.7%	11	3	2	0	6	0	6	60.00	40.00	0.00	54.55	0.00	54.55
Total	26.7%-23.7%	17	3	5	0	9	0	9	37.50	62.50	0.00	52.94	0.00	52.94
Mean	26.7%-23.7%		2	3	0	5	0	5	30.00	70.00	0.00	52.27	0.00	52.27
Minimum	26.7%-23.7%		0	2	0	3	0	3	0.00	40.00	0.00	50.00	0.00	50.00
Maximum	26.7%-23.7%		3	3	0	6	0	6	60.00	100.00	0.00	54.55	0.00	54.55
Red River 1997 x 30221-D8RR	≤23.6%	16	7	2	0	7	0	7	77.78	22.22	0.00	43.75	0.00	43.75
Red River 1997 x 30408-C7RR	≤23.6%	9	9	0	0	0	0	0	100.00	0.00	0.00	0.00	0.00	0.00
Total	≤23.6%	25	16	2	0	7	0	7	88.89	11.11	0.00	28.00	0.00	28.00
Mean	≤23.6%		8	1	0	4	0	4	88.89	11.11	0.00	21.88	0.00	21.88
Minimum	≤23.6%		7	0	0	0	0	0	77.78	0.00	0.00	0.00	0.00	0.00
Maximum	≤23.6%		9	2	0	7	0	7	100.00	22.22	0.00	43.75	0.00	43.75
Total Plants Evaluated (%)		75.00												
Overall Accuracy (%)		78.38												

Pedigree (BC1F1)	Category[z]	Sample Number	New SNP Procedure											
			Number						Frequency (%)					
			Correct	Incorrect	Missing E1	Missing E2	Missing E1 & E2	Total Missing	Correct[y]	Incorrect[x]	Missing E1	Missing E2	Missing E1 & E2	Total Missing
Red River 1997 x 30221-D8RR	≥36.8%	19	9	5	0	2	0	2	44.44	55.56	0.00	10.00	0.00	10.00
Red River 1997 x 30408-C7RR	≥36.8%	20	9	4	0	2	0	2	55.56	44.44	0.00	18.18	0.00	18.18
Total	≥36.8%	39	18	9	0	4	0	4	66.67	33.33	0.00	10.26	0.00	10.26
Mean	≥36.8%		9	5	0	2	0	2	50.00	50.00	0.00	14.09	0.00	14.09
Minimum	≥36.8%		9	4	0	2	0	2	44.44	44.44	0.00	10.00	0.00	10.00
Maximum	≥36.8%		9	5	0	2	0	2	55.56	55.56	0.00	18.18	0.00	18.18
Red River 1997 x 30221-D8RR	36.7%-33.7%	12	6	5	0	1	0	1	37.50	62.50	0.00	0.00	0.00	0.00
Red River 1997 x 30408-C7RR	36.7%-33.7%	12	10	4	0	0	0	0	42.86	57.14	0.00	12.50	0.00	12.50
Total	36.7%-33.7%	24	16	9	0	1	0	1	64.00	36.00	0.00	4.17	0.00	4.17
Mean	36.7%-33.7%		8	5	0	1	0	1	40.18	59.82	0.00	6.25	0.00	6.25
Minimum	36.7%-33.7%		6	4	0	0	0	0	37.50	57.14	0.00	0.00	0.00	0.00
Maximum	36.7%-33.7%		10	5	0	1	0	1	42.86	62.50	0.00	12.50	0.00	12.50
Red River 1997 x 30221-D8RR	33.6%-26.8%	34	22	13	0	0	0	0	64.86	35.14	0.00	5.13	0.00	5.13
Red River 1997 x 30408-C7RR	33.6%-26.8%	33	17	14	0	2	0	2	61.11	38.89	0.00	5.26	0.00	5.26
Total	33.6%-26.8%	67	39	27	0	2	0	2	59.09	40.91	0.00	2.99	0.00	2.99
Mean	33.6%-26.8%		20	14	0	1	0	1	62.99	37.02	0.00	5.20	0.00	5.20
Minimum	33.6%-26.8%		17	13	0	0	0	0	61.11	35.14	0.00	5.13	0.00	5.13
Maximum	33.6%-26.8%		22	14	0	2	0	2	64.86	38.89	0.00	5.26	0.00	5.26
Red River 1997 x 30221-D8RR	26.7%-23.7%	7	2	4	0	1	0	1	50.00	50.00	8.33	25.00	0.00	33.33
Red River 1997 x 30408-C7RR	26.7%-23.7%	11	4	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
Total	26.7%-23.7%	18	6	4	0	1	0	1	60.00	40.00	0.00	5.56	0.00	5.56
Mean	26.7%-23.7%		3	2	0	1	0	1	25.00	25.00	4.17	12.50	0.00	16.67
Minimum	26.7%-23.7%		2	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	26.7%-23.7%		4	4	0	1	0	1	50.00	50.00	8.33	25.00	0.00	33.33
Red River 1997 x 30221-D8RR	≤23.6%	23	7	18	0	2	0	2	33.33	66.67	0.00	10.00	0.00	10.00
Red River 1997 x 30408-C7RR	≤23.6%	16	10	21	0	0	0	0	40.00	60.00	0.00	0.00	0.00	0.00
Total	≤23.6%	39	17	39	0	2	0	2	30.36	69.64	0.00	5.13	0.00	5.13
Mean	≤23.6%		9	20	0	1	0	1	36.67	63.34	0.00	5.00	0.00	5.00
Minimum	≤23.6%		7	18	0	0	0	0	33.33	60.00	0.00	0.00	0.00	0.00
Maximum	≤23.6%		10	21	0	2	0	2	40.00	66.67	0.00	10.00	0.00	10.00
Total Plants Evaluated (%)		94.65												
Overall Accuracy (%)		54.24												

[z]Based on Mendelian genetics with 1 E1E1E2E2: 2 E1E1E2e2 or E1e1E2E2: 1 E1e1E2e2 ratio

[y]Frequency correct does not include missing results

[x]Frequency incorrect does not include missing results

7.2 Appendix B

Appendix Table B1. Results from the modification testing of the traditional extraction method for determining chlorophyll concentration (chll) in ppm. Group 1 - followed regular protocol; Group 2 - filter, measure on same day; Group 3 - filter, settle overnight, measure next day; Group 4 - settle overnight, measure next day

		Absorbance				Chll	
Group	ID	665	625	705	A_{Corr}	Test[z]	GRL[y]
1	125465	0.986	1.061	0.899	0.006	1.0	4.4
2	125465	0.125	0.122	0.100	0.014	2.3	4.4
3	125465	0.110	0.104	0.084	0.016	2.6	4.4
4	125465	0.334	0.356	0.284	0.013	2.1	4.4
1	2007 - GF436	1.263	1.333	1.173	0.010	1.6	5.1
2	2007 - GF436	0.308	0.312	0.260	0.022	3.6	5.1
3	2007 - GF436	0.046	0.027	0.018	0.023	3.7	5.1
4	2007 - GF436	0.261	0.265	0.206	0.025	4.1	5.1
1	1227785	1.198	1.248	1.047	0.050	8.1	12.6
2	1227785	0.116	0.073	0.053	0.053	8.6	12.6
3	1227785	0.166	0.129	0.097	0.053	8.6	12.6
4	1227785	0.539	0.541	0.426	0.055	8.9	12.6
1	2006 - GF345	1.442	1.472	1.254	0.079	12.8	14.4
2	2006 - GF345	0.244	0.200	0.162	0.063	10.2	14.4
3	2006 - GF345	0.104	0.049	0.027	0.066	10.7	14.4
4	2006 - GF345	0.644	0.649	0.513	0.063	10.2	14.4
1	2006 - GF241	1.537	1.498	1.315	0.130	21.1	31.5
2	2006 - GF241	0.570	0.482	0.398	0.130	21.1	31.5
3	2006 - GF241	0.185	0.071	0.034	0.132	21.5	31.5
4	2006 - GF241	0.369	0.278	0.204	0.128	20.8	31.5
1	1317806	1.655	1.643	1.438	0.115	18.7	32.5
2	1317806	0.146	0.054	0.026	0.106	17.2	32.5
3	1317806	0.235	0.149	0.104	0.109	17.7	32.5
4	1317806	0.670	0.616	0.484	0.120	19.5	32.5
1	2006 - GF364	1.507	1.383	1.171	0.230	37.4	60.2
2	2006 - GF364	0.581	0.401	0.314	0.223	36.2	60.2
3	2006 - GF364	0.365	0.159	0.087	0.241	39.2	60.2
4	2006 - GF364	0.535	0.369	0.255	0.223	36.2	60.2
1	CN04CL11	1.506	1.279	1.027	0.352	57.2	66.3
2	CN04CL11	0.828	0.562	0.412	0.341	55.4	66.3
3	CN04CL11	0.522	0.228	0.122	0.347	56.4	66.3
4	CN04CL11	0.765	0.486	0.324	0.360	58.5	66.3

[z]Calculated based on corrected absorbance (A_{Corr});

[y]Determined by GRL, considered accurate.

Appendix Table B2. Absorbance values at 665 nm, 625 nm, and 705 nm used to calculate chlorophyll concentration (chll) in ppm determined following modifications to the traditional extraction method. Each sample was analyzed in triplicate

		Absorbance				
Replicate	ID	665 nm	625 nm	705 nm	A_{Corr}	chll
1	333	0.356	0.226	0.171	0.158	25.7
2	333	0.311	0.175	0.123	0.162	26.3
3	333	0.398	0.274	0.218	0.152	24.7
1	623	0.193	0.118	0.088	0.090	14.6
2	623	0.277	0.212	0.170	0.085	13.8
3	623	0.228	0.165	0.128	0.081	13.2
1	1204174	0.162	0.059	0.032	0.116	18.9
2	1204174	0.183	0.081	0.051	0.117	19.0
3	1204174	0.507	0.435	0.356	0.111	18.0
1	1224925	1.227	1.067	0.938	0.225	36.6
2	1224925	0.728	0.575	0.472	0.205	33.3
3	1224925	1.349	1.166	1.004	0.265	43.1
1	1228059	0.543	0.567	0.490	0.015	2.4
2	1228059	0.065	0.054	0.042	0.018	2.9
3	1228059	0.400	0.415	0.353	0.017	2.8
1	CN02HF35	1.499	1.094	0.879	0.511	83.0
2	CN02HF35	0.551	0.135	0.028	0.469	76.2
3	CN02HF35	0.670	0.206	0.080	0.528	85.8
1	2004 - C496	0.211	0.161	0.129	0.066	10.7
2	2004 - C496	0.067	0.023	0.012	0.050	8.1
3	2004 - C496	0.127	0.079	0.057	0.059	9.6
1	2004 - C905	0.368	0.344	0.292	0.050	8.1
2	2004 - C905	0.125	0.088	0.071	0.045	7.3
3	2004 - C905	0.306	0.269	0.224	0.060	9.8
1	2004 - GF340	0.504	0.471	0.400	0.068	11.1
2	2004 - GF340	0.128	0.055	0.029	0.086	14.0
3	2004 - GF340	0.472	0.426	0.356	0.081	13.2
1	2004 - GF516	0.049	0.039	0.031	0.014	2.3
2	2004 - GF516	0.016	0.052	0.040	0.016	2.6
3	2004 - GF516	0.517	0.525	0.469	0.020	3.3
1	2004 - OP041b	0.259	0.193	0.151	0.087	14.1
2	2004 - OP041b	0.350	0.300	0.242	0.079	12.8
3	2004 - OP041b	0.123	0.048	0.024	0.087	14.1
1	2006 - GF422	0.732	0.529	0.424	0.255	41.4
2	2006 - GF422	0.799	0.608	0.491	0.250	40.6
3	2006 - GF422	0.703	0.459	0.358	0.295	47.9
1	2006 - GF948	0.336	0.297	0.246	0.064	10.4
2	2006 - GF948	0.152	0.100	0.076	0.064	10.4
3	2006 - GF948	0.472	0.430	0.371	0.068	11.1
1	2006 - OP344	0.193	0.170	0.144	0.035	5.7
2	2006 - OP344	0.272	0.258	0.216	0.036	5.9
3	2006 - OP344	0.354	0.343	0.289	0.037	6.0

Replicate	ID	Absorbance				chll
		665 nm	625 nm	705 nm	A_{Corr}	
1	2006 - OP599	0.137	0.068	0.045	0.081	13.2
2	2006 - OP599	0.144	0.081	0.056	0.076	12.4
3	2006 - OP599	0.106	0.038	0.016	0.079	12.8
1	2007 - C762	0.756	0.712	0.610	0.095	15.4
2	2007 - C762	0.168	0.088	0.054	0.098	15.9
3	2007 - C762	0.711	0.655	0.557	0.105	17.1
1	2007 - OP346	0.124	0.092	0.073	0.042	6.8
2	2007 - OP346	0.122	0.092	0.073	0.040	6.5
3	2007 - OP346	0.096	0.051	0.037	0.052	8.5
1	2008 - OP352	0.908	0.918	0.809	0.044	7.2
2	2008 - OP352	0.233	0.209	0.178	0.040	6.5
3	2008 - OP352	0.501	0.499	0.431	0.036	5.9
1	2010 - OP491	0.303	0.275	0.237	0.048	7.8
2	2010 - OP491	0.220	0.185	0.156	0.050	8.1
3	2010 - OP491	0.091	1.115	1.004	0.031	5.0

Appendix Table B3. All samples in the calibration file with reference data (chll) in ppm and absorbance values for each wavelength starting at 650 nm to 2498 nm. For simplicity, data is shown in 8 nm increments in the 650 nm to 690 nm (chll peak) range and in approximately 100 nm increments from there on up

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
1204020	27.6	1.12	1.11	1.10	1.08	1.04	1.00	0.94	0.65	0.39	0.28	0.19	0.40	0.23	0.49	0.63	0.57	0.81	0.71	0.94	0.95	1.01	1.02	1.31	1.29	1.34
1204041	36.3	1.07	1.06	1.05	1.02	0.99	0.96	0.89	0.62	0.39	0.28	0.20	0.39	0.23	0.47	0.61	0.56	0.79	0.70	0.92	0.92	0.99	0.99	1.28	1.26	1.32
1204231	22.0	1.05	1.04	1.02	1.00	0.97	0.92	0.85	0.59	0.37	0.27	0.19	0.42	0.24	0.52	0.67	0.61	0.89	0.78	1.01	1.02	1.10	1.10	1.39	1.38	1.42
1217407	23.7	1.08	1.07	1.06	1.04	1.00	0.97	0.91	0.64	0.40	0.29	0.20	0.40	0.23	0.49	0.65	0.59	0.82	0.73	0.96	0.97	1.04	1.04	1.31	1.30	1.36
1222132	24.4	1.13	1.12	1.11	1.09	1.06	1.02	0.95	0.65	0.40	0.28	0.20	0.39	0.22	0.48	0.61	0.55	0.80	0.70	0.93	0.92	0.99	0.99	1.30	1.28	1.34
1224925	23.3	1.05	1.04	1.03	1.01	0.98	0.95	0.89	0.63	0.41	0.30	0.22	0.41	0.24	0.48	0.62	0.56	0.80	0.70	0.92	0.92	0.99	0.98	1.27	1.25	1.31
1227839	20.3	1.07	1.06	1.05	1.03	0.99	0.95	0.88	0.62	0.40	0.30	0.21	0.41	0.24	0.49	0.63	0.57	0.83	0.73	0.94	0.94	1.01	1.01	1.31	1.29	1.35
1228059	13.4	1.12	1.10	1.09	1.06	1.04	1.00	0.94	0.65	0.39	0.28	0.19	0.41	0.22	0.48	0.62	0.56	0.83	0.72	0.94	0.93	1.01	1.00	1.31	1.29	1.34
1317041	25.5	1.07	1.06	1.05	1.03	1.00	0.96	0.90	0.62	0.37	0.27	0.19	0.38	0.22	0.47	0.61	0.55	0.79	0.69	0.92	0.93	1.00	1.00	1.30	1.28	1.33
1317610	24.5	1.14	1.13	1.12	1.10	1.07	1.03	0.97	0.68	0.42	0.30	0.21	0.40	0.23	0.48	0.62	0.57	0.82	0.72	0.94	0.95	1.02	1.01	1.31	1.29	1.35
1317809	62.7	1.06	1.05	1.04	1.02	0.99	0.94	0.86	0.59	0.36	0.26	0.19	0.40	0.23	0.48	0.63	0.57	0.83	0.72	0.94	0.95	1.03	1.03	1.32	1.30	1.35
1317812	37.2	1.12	1.11	1.09	1.07	1.04	1.01	0.94	0.66	0.40	0.29	0.20	0.40	0.23	0.48	0.62	0.56	0.82	0.71	0.94	0.93	1.01	1.00	1.32	1.30	1.35
1328042	26.4	1.06	1.05	1.03	1.01	0.98	0.94	0.88	0.61	0.38	0.28	0.20	0.39	0.23	0.47	0.61	0.55	0.80	0.70	0.92	0.93	1.00	1.00	1.29	1.27	1.33
1328529	23.7	1.08	1.07	1.06	1.04	1.01	0.97	0.91	0.63	0.39	0.29	0.21	0.40	0.24	0.48	0.62	0.57	0.81	0.71	0.93	0.94	1.01	1.01	1.27	1.26	1.32
1346054	39.3	1.11	1.10	1.09	1.07	1.04	0.99	0.92	0.64	0.41	0.30	0.21	0.41	0.23	0.49	0.64	0.58	0.85	0.74	0.95	0.97	1.05	1.06	1.34	1.33	1.37
CN02HF31	39.5	1.12	1.11	1.10	1.08	1.05	1.01	0.95	0.68	0.44	0.32	0.23	0.40	0.24	0.48	0.61	0.55	0.77	0.68	0.90	0.91	0.96	0.97	1.20	1.19	1.25
CN02HF35	56.7	1.08	1.07	1.07	1.05	1.01	0.96	0.88	0.62	0.40	0.29	0.21	0.39	0.23	0.47	0.62	0.56	0.80	0.70	0.91	0.93	1.00	1.01	1.27	1.25	1.31
CN04CL08	34.4	1.18	1.18	1.17	1.15	1.12	1.07	1.00	0.71	0.43	0.31	0.21	0.39	0.23	0.46	0.59	0.54	0.79	0.69	0.89	0.89	0.97	0.98	1.27	1.25	1.30
CN04CL11	37.3	1.13	1.12	1.12	1.10	1.06	1.02	0.94	0.66	0.40	0.29	0.20	0.39	0.23	0.47	0.61	0.56	0.81	0.70	0.92	0.93	1.00	1.00	1.30	1.28	1.33
CN06CL04	9.9	1.02	1.01	1.00	0.98	0.96	0.92	0.87	0.62	0.40	0.29	0.21	0.40	0.23	0.47	0.62	0.56	0.82	0.72	0.92	0.94	1.02	1.02	1.32	1.30	1.35
CN07CL12	31.9	1.13	1.12	1.12	1.10	1.07	1.03	0.96	0.69	0.44	0.31	0.22	0.40	0.23	0.46	0.60	0.55	0.81	0.70	0.89	0.90	0.99	0.99	1.28	1.26	1.31
CN6CL017	39.2	1.06	1.06	1.05	1.04	0.99	0.94	0.86	0.59	0.37	0.27	0.19	0.41	0.23	0.50	0.64	0.58	0.86	0.75	0.96	0.97	1.05	1.06	1.36	1.35	1.39
110	23.9	1.14	1.13	1.12	1.10	1.07	1.04	0.97	0.69	0.42	0.30	0.22	0.40	0.24	0.47	0.60	0.55	0.80	0.70	0.90	0.90	0.97	0.98	1.27	1.25	1.30
121	30.3	1.08	1.07	1.06	1.04	1.01	0.97	0.90	0.63	0.38	0.28	0.19	0.38	0.22	0.46	0.60	0.54	0.78	0.68	0.89	0.90	0.97	0.97	1.26	1.24	1.29
199	25.8	1.11	1.10	1.09	1.07	1.04	1.00	0.94	0.66	0.42	0.31	0.22	0.40	0.24	0.48	0.61	0.55	0.81	0.70	0.91	0.91	0.98	0.99	1.27	1.25	1.30
234	36.8	1.10	1.09	1.08	1.06	1.03	0.99	0.92	0.65	0.41	0.30	0.22	0.40	0.24	0.48	0.62	0.56	0.80	0.70	0.91	0.92	1.00	0.99	1.27	1.25	1.31
276	18.3	1.13	1.11	1.10	1.08	1.05	1.02	0.96	0.67	0.42	0.30	0.21	0.40	0.23	0.48	0.62	0.56	0.81	0.71	0.92	0.92	1.00	1.00	1.29	1.27	1.32
333	32.7	1.10	1.10	1.09	1.07	1.03	0.99	0.92	0.64	0.39	0.29	0.20	0.40	0.23	0.48	0.62	0.56	0.81	0.71	0.92	0.93	1.01	1.01	1.29	1.27	1.33
414	31.5	1.14	1.13	1.12	1.10	1.06	1.02	0.95	0.67	0.41	0.30	0.22	0.40	0.24	0.48	0.61	0.56	0.82	0.71	0.92	0.92	0.99	1.00	1.29	1.27	1.32
457	31.1	1.11	1.10	1.09	1.07	1.04	1.00	0.93	0.65	0.41	0.31	0.23	0.41	0.25	0.48	0.62	0.56	0.80	0.71	0.91	0.91	0.99	0.99	1.26	1.24	1.30
599	27.3	0.97	0.96	0.95	0.93	0.90	0.85	0.79	0.56	0.38	0.29	0.21	0.42	0.24	0.49	0.63	0.57	0.84	0.73	0.93	0.95	1.02	1.03	1.32	1.30	1.34

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
623	20.3	1.08	1.07	1.05	1.04	1.00	0.97	0.91	0.64	0.40	0.29	0.21	0.40	0.23	0.48	0.61	0.55	0.81	0.71	0.91	0.92	0.99	1.00	1.29	1.27	1.32
714	24.4	1.14	1.13	1.12	1.10	1.07	1.03	0.97	0.67	0.41	0.29	0.21	0.39	0.23	0.47	0.60	0.54	0.79	0.69	0.90	0.90	0.98	0.98	1.27	1.25	1.31
777	26.4	1.07	1.06	1.05	1.03	1.00	0.95	0.89	0.63	0.41	0.31	0.22	0.43	0.25	0.50	0.64	0.58	0.85	0.74	0.94	0.96	1.03	1.04	1.32	1.31	1.35
802	27.5	1.09	1.08	1.07	1.05	1.02	0.98	0.91	0.65	0.42	0.32	0.23	0.42	0.26	0.49	0.63	0.57	0.83	0.73	0.92	0.94	1.01	1.01	1.30	1.28	1.33
869	20.3	1.16	1.15	1.14	1.12	1.09	1.05	0.99	0.70	0.43	0.31	0.22	0.39	0.23	0.46	0.59	0.53	0.77	0.67	0.88	0.88	0.94	0.94	1.22	1.20	1.26
931	26.1	1.10	1.09	1.08	1.06	1.02	0.98	0.92	0.64	0.40	0.29	0.21	0.41	0.24	0.49	0.63	0.57	0.82	0.72	0.93	0.94	1.01	1.01	1.30	1.28	1.33
1204027	25.7	1.02	1.01	1.00	0.97	0.94	0.90	0.84	0.57	0.35	0.26	0.18	0.38	0.22	0.47	0.62	0.56	0.80	0.70	0.92	0.93	1.01	1.01	1.31	1.29	1.34
1204088	27.6	1.06	1.05	1.04	1.02	0.98	0.95	0.88	0.62	0.39	0.29	0.21	0.40	0.24	0.48	0.63	0.57	0.82	0.72	0.93	0.94	1.03	1.02	1.31	1.29	1.35
1217125	24.9	1.03	1.02	1.01	0.99	0.95	0.91	0.85	0.59	0.37	0.27	0.20	0.41	0.24	0.50	0.66	0.60	0.87	0.76	0.99	1.01	1.09	1.10	1.37	1.35	1.40
1217446	23.6	1.10	1.09	1.08	1.06	1.02	0.98	0.92	0.64	0.39	0.28	0.20	0.38	0.22	0.46	0.60	0.55	0.78	0.68	0.90	0.90	0.98	0.98	1.27	1.25	1.31
1222928	35.4	1.07	1.06	1.05	1.03	0.99	0.94	0.87	0.60	0.37	0.27	0.19	0.38	0.22	0.46	0.61	0.55	0.79	0.70	0.90	0.91	1.00	1.00	1.29	1.27	1.33
1225465	19.4	1.07	1.06	1.05	1.03	1.00	0.96	0.90	0.65	0.43	0.33	0.24	0.42	0.25	0.48	0.61	0.55	0.81	0.71	0.89	0.90	0.98	0.98	1.27	1.25	1.30
1227851	7.4	1.10	1.08	1.07	1.05	1.02	0.99	0.93	0.65	0.39	0.28	0.20	0.40	0.22	0.47	0.59	0.54	0.80	0.70	0.89	0.89	0.97	0.97	1.28	1.26	1.31
1228084	21.3	1.06	1.05	1.03	1.01	0.98	0.94	0.88	0.61	0.38	0.27	0.19	0.40	0.23	0.47	0.62	0.57	0.82	0.72	0.92	0.93	1.02	1.01	1.31	1.29	1.34
1317044	25.0	1.09	1.08	1.07	1.05	1.02	0.98	0.92	0.63	0.37	0.27	0.19	0.38	0.22	0.46	0.60	0.54	0.79	0.69	0.91	0.91	0.99	0.98	1.29	1.27	1.33
1317806	43.6	1.11	1.11	1.10	1.08	1.05	1.00	0.94	0.66	0.40	0.29	0.21	0.41	0.23	0.49	0.63	0.57	0.84	0.73	0.95	0.95	1.03	1.03	1.32	1.30	1.35
1317810	53.1	1.08	1.07	1.06	1.04	1.00	0.96	0.89	0.61	0.37	0.27	0.19	0.39	0.23	0.47	0.62	0.56	0.80	0.70	0.91	0.93	1.01	1.01	1.29	1.27	1.33
1317813	25.6	1.06	1.05	1.03	1.01	0.98	0.95	0.89	0.62	0.39	0.28	0.20	0.39	0.22	0.47	0.60	0.55	0.80	0.70	0.91	0.91	0.99	0.99	1.30	1.28	1.33
1328130	30.3	1.08	1.07	1.06	1.04	1.00	0.96	0.90	0.62	0.37	0.27	0.20	0.39	0.23	0.47	0.61	0.55	0.80	0.70	0.91	0.92	0.99	1.00	1.29	1.27	1.33
1328660	25.9	1.09	1.08	1.07	1.05	1.02	0.98	0.91	0.64	0.40	0.30	0.22	0.41	0.25	0.50	0.65	0.59	0.84	0.74	0.95	0.97	1.05	1.05	1.31	1.30	1.35
CN00CL11	34.0	1.07	1.06	1.05	1.03	1.00	0.96	0.89	0.63	0.41	0.30	0.22	0.39	0.23	0.46	0.60	0.54	0.78	0.69	0.88	0.89	0.97	0.97	1.24	1.22	1.28
CN02HF32	62.9	1.07	1.07	1.06	1.04	1.01	0.97	0.89	0.64	0.41	0.30	0.22	0.39	0.24	0.47	0.61	0.55	0.77	0.68	0.89	0.90	0.96	0.96	1.20	1.19	1.25
CN03CL10	35.4	1.08	1.07	1.06	1.04	1.01	0.97	0.90	0.64	0.40	0.29	0.21	0.39	0.23	0.46	0.60	0.55	0.79	0.69	0.88	0.90	0.98	0.99	1.28	1.25	1.31
CN04CL09	47.3	1.14	1.14	1.13	1.12	1.08	1.02	0.94	0.66	0.41	0.29	0.21	0.39	0.23	0.46	0.60	0.55	0.80	0.69	0.89	0.90	0.98	0.98	1.28	1.26	1.31
CN05CL09	18.7	1.11	1.10	1.09	1.07	1.04	1.01	0.95	0.67	0.41	0.29	0.20	0.38	0.22	0.46	0.60	0.54	0.79	0.69	0.89	0.91	0.97	0.98	1.27	1.25	1.30
CN06CL14	10.7	0.97	0.96	0.95	0.93	0.91	0.88	0.82	0.60	0.39	0.29	0.21	0.40	0.23	0.47	0.61	0.56	0.81	0.71	0.90	0.92	1.01	1.01	1.30	1.28	1.33
CN07CL17	39.8	1.10	1.09	1.08	1.07	1.03	0.99	0.92	0.65	0.41	0.29	0.21	0.39	0.23	0.46	0.61	0.55	0.81	0.70	0.90	0.91	1.00	1.00	1.29	1.27	1.32
101	34.9	1.06	1.05	1.05	1.03	0.99	0.95	0.87	0.61	0.38	0.28	0.20	0.40	0.23	0.47	0.61	0.56	0.83	0.72	0.91	0.92	1.01	1.01	1.31	1.29	1.33
111	24.7	1.13	1.12	1.11	1.09	1.06	1.02	0.95	0.67	0.41	0.29	0.21	0.39	0.23	0.46	0.59	0.54	0.80	0.69	0.88	0.89	0.97	0.97	1.26	1.24	1.29
161	34.3	1.04	1.03	1.02	1.00	0.96	0.92	0.85	0.59	0.37	0.27	0.20	0.38	0.22	0.45	0.59	0.54	0.78	0.68	0.87	0.89	0.97	0.98	1.25	1.23	1.29
201	22.5	1.11	1.10	1.09	1.07	1.04	1.01	0.94	0.67	0.43	0.32	0.23	0.41	0.25	0.47	0.60	0.55	0.80	0.69	0.88	0.89	0.97	0.97	1.25	1.23	1.28
239	36.7	1.09	1.08	1.07	1.05	1.01	0.97	0.90	0.64	0.41	0.30	0.22	0.40	0.24	0.47	0.61	0.56	0.79	0.69	0.88	0.90	0.98	0.98	1.25	1.23	1.28
283	19.1	1.10	1.09	1.08	1.06	1.03	0.99	0.93	0.65	0.40	0.29	0.21	0.40	0.23	0.46	0.60	0.54	0.80	0.69	0.88	0.89	0.98	0.98	1.27	1.25	1.30

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
376	30.4	1.06	1.06	1.05	1.03	0.99	0.95	0.89	0.62	0.39	0.29	0.21	0.40	0.23	0.47	0.60	0.55	0.80	0.70	0.88	0.90	0.99	0.99	1.27	1.25	1.30
432	35.4	1.10	1.10	1.09	1.07	1.03	0.99	0.92	0.64	0.41	0.31	0.23	0.40	0.24	0.46	0.60	0.55	0.79	0.69	0.87	0.88	0.97	0.97	1.24	1.22	1.28
501	33.6	1.07	1.07	1.06	1.04	1.00	0.96	0.89	0.62	0.39	0.29	0.21	0.39	0.23	0.46	0.60	0.55	0.79	0.69	0.88	0.90	0.98	0.99	1.27	1.25	1.30
608	18.9	1.02	1.01	1.00	0.98	0.95	0.91	0.84	0.60	0.40	0.30	0.22	0.42	0.24	0.48	0.62	0.56	0.85	0.73	0.91	0.93	1.01	1.03	1.33	1.31	1.34
628	17.8	1.08	1.07	1.06	1.04	1.01	0.97	0.92	0.64	0.40	0.29	0.21	0.39	0.23	0.46	0.60	0.54	0.80	0.69	0.88	0.90	0.98	0.99	1.28	1.25	1.30
720	31.8	1.12	1.11	1.10	1.08	1.05	1.01	0.94	0.65	0.40	0.29	0.20	0.39	0.23	0.46	0.60	0.54	0.79	0.69	0.88	0.89	0.97	0.98	1.26	1.24	1.29
789	25.4	1.03	1.03	1.02	1.00	0.96	0.92	0.86	0.62	0.42	0.32	0.24	0.44	0.26	0.50	0.64	0.58	0.85	0.75	0.92	0.95	1.03	1.04	1.31	1.29	1.33
809	26.1	1.10	1.09	1.08	1.06	1.03	0.99	0.93	0.65	0.40	0.30	0.21	0.40	0.23	0.47	0.60	0.54	0.80	0.70	0.88	0.90	0.98	0.98	1.28	1.25	1.30
876	25.5	1.11	1.10	1.09	1.07	1.04	1.00	0.94	0.66	0.42	0.31	0.22	0.40	0.24	0.47	0.60	0.54	0.79	0.69	0.88	0.89	0.97	0.97	1.25	1.23	1.28
998	22.7	1.08	1.07	1.06	1.04	1.00	0.97	0.91	0.63	0.39	0.28	0.20	0.39	0.23	0.46	0.61	0.55	0.80	0.69	0.89	0.90	0.99	0.99	1.28	1.26	1.31
1204028	37.8	1.07	1.06	1.04	1.02	0.99	0.95	0.89	0.62	0.38	0.28	0.20	0.40	0.23	0.47	0.61	0.56	0.82	0.71	0.90	0.92	1.01	1.02	1.31	1.29	1.34
1204174	22.5	1.06	1.05	1.04	1.02	0.98	0.94	0.88	0.62	0.38	0.28	0.20	0.41	0.24	0.49	0.64	0.59	0.85	0.75	0.95	0.97	1.06	1.07	1.34	1.32	1.37
1217193	21.3	1.05	1.04	1.03	1.01	0.97	0.94	0.88	0.62	0.38	0.28	0.20	0.40	0.23	0.48	0.64	0.59	0.84	0.74	0.94	0.97	1.06	1.07	1.34	1.32	1.38
1217731	27.2	1.11	1.10	1.08	1.06	1.03	0.99	0.93	0.64	0.39	0.28	0.20	0.39	0.22	0.46	0.59	0.54	0.79	0.69	0.88	0.89	0.98	0.98	1.29	1.26	1.31
1223922	27.4	1.13	1.12	1.11	1.09	1.06	1.02	0.96	0.67	0.42	0.30	0.21	0.39	0.23	0.46	0.60	0.54	0.78	0.69	0.87	0.88	0.97	0.97	1.27	1.25	1.31
1227785	19.9	1.01	1.01	0.99	0.97	0.94	0.90	0.84	0.60	0.40	0.30	0.23	0.42	0.24	0.47	0.61	0.55	0.82	0.71	0.88	0.90	0.99	0.99	1.29	1.26	1.31
1227968	10.8	1.09	1.07	1.06	1.04	1.01	0.99	0.93	0.66	0.40	0.29	0.20	0.41	0.22	0.47	0.60	0.55	0.83	0.72	0.89	0.90	1.00	0.99	1.30	1.28	1.33
1228114	25.5	1.10	1.09	1.07	1.05	1.02	0.99	0.93	0.64	0.39	0.28	0.19	0.39	0.22	0.47	0.62	0.56	0.81	0.71	0.91	0.93	1.01	1.02	1.31	1.29	1.34
1317101	26.3	1.12	1.11	1.10	1.08	1.05	1.01	0.96	0.67	0.40	0.28	0.20	0.40	0.22	0.47	0.61	0.55	0.81	0.71	0.91	0.91	0.99	0.99	1.30	1.28	1.33
1317808	57.0	1.06	1.05	1.04	1.02	0.98	0.94	0.88	0.61	0.37	0.27	0.19	0.40	0.23	0.47	0.62	0.56	0.82	0.72	0.91	0.93	1.01	1.02	1.31	1.29	1.34
1317811	38.4	1.12	1.11	1.10	1.08	1.05	1.01	0.94	0.66	0.40	0.29	0.20	0.39	0.23	0.47	0.61	0.56	0.81	0.71	0.91	0.92	1.00	1.01	1.31	1.29	1.34
1322623	23.8	1.07	1.06	1.04	1.02	0.99	0.96	0.90	0.62	0.38	0.27	0.19	0.38	0.22	0.46	0.60	0.54	0.78	0.69	0.89	0.90	0.98	0.97	1.27	1.25	1.31
1328355	28.5	1.06	1.05	1.04	1.02	0.99	0.96	0.90	0.63	0.38	0.29	0.21	0.40	0.24	0.48	0.63	0.57	0.82	0.72	0.92	0.94	1.02	1.02	1.29	1.28	1.33
1331242	28.4	1.10	1.09	1.08	1.06	1.03	0.99	0.92	0.65	0.40	0.29	0.21	0.41	0.24	0.48	0.63	0.58	0.84	0.73	0.93	0.95	1.04	1.04	1.32	1.30	1.35
CN01CL13	38.5	1.05	1.04	1.03	1.01	0.98	0.94	0.87	0.63	0.41	0.30	0.22	0.40	0.24	0.47	0.61	0.56	0.79	0.70	0.89	0.91	0.98	0.98	1.24	1.22	1.28
CN02HF33	59.7	1.07	1.06	1.05	1.03	1.00	0.95	0.88	0.62	0.40	0.29	0.21	0.39	0.23	0.47	0.61	0.56	0.78	0.69	0.90	0.91	0.98	0.98	1.23	1.22	1.28
CN03CL12	36.6	1.11	1.11	1.10	1.09	1.05	1.01	0.93	0.66	0.41	0.30	0.21	0.40	0.23	0.47	0.61	0.56	0.81	0.71	0.90	0.92	1.00	1.01	1.30	1.28	1.33
CN04CL10	47.0	1.13	1.13	1.13	1.11	1.07	1.02	0.94	0.66	0.41	0.29	0.20	0.40	0.22	0.47	0.61	0.55	0.81	0.71	0.90	0.92	1.00	1.00	1.29	1.27	1.32
CN06CL02	12.4	1.04	1.03	1.02	1.00	0.97	0.94	0.89	0.64	0.41	0.30	0.21	0.40	0.23	0.47	0.62	0.56	0.82	0.71	0.91	0.93	1.01	1.02	1.31	1.29	1.34
CN07CL08	17.6	1.08	1.07	1.06	1.04	1.02	0.98	0.92	0.66	0.41	0.30	0.21	0.39	0.23	0.46	0.60	0.55	0.80	0.70	0.88	0.90	0.99	0.99	1.28	1.26	1.31
CN07CL18	44.2	1.06	1.06	1.05	1.04	1.00	0.94	0.86	0.60	0.38	0.28	0.20	0.41	0.23	0.48	0.63	0.58	0.85	0.74	0.92	0.94	1.04	1.04	1.34	1.32	1.37
107	30.0	1.15	1.14	1.13	1.11	1.08	1.04	0.97	0.68	0.42	0.29	0.21	0.39	0.22	0.46	0.59	0.54	0.80	0.69	0.87	0.88	0.97	0.97	1.27	1.24	1.29
112	24.6	1.10	1.10	1.09	1.07	1.03	1.00	0.93	0.66	0.41	0.30	0.21	0.40	0.23	0.46	0.60	0.54	0.80	0.69	0.87	0.88	0.97	0.98	1.26	1.24	1.29

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
171	31.5	1.06	1.05	1.04	1.02	0.99	0.95	0.88	0.62	0.39	0.28	0.20	0.39	0.23	0.46	0.60	0.54	0.80	0.69	0.87	0.89	0.98	0.99	1.28	1.25	1.30
228	29.6	1.13	1.12	1.11	1.09	1.06	1.02	0.97	0.69	0.43	0.32	0.23	0.40	0.24	0.46	0.60	0.54	0.80	0.69	0.86	0.88	0.97	0.97	1.25	1.23	1.28
255	27.6	1.11	1.10	1.09	1.07	1.04	1.00	0.93	0.66	0.42	0.32	0.23	0.41	0.25	0.47	0.61	0.55	0.81	0.70	0.87	0.89	0.98	0.99	1.26	1.24	1.29
324	28.7	1.10	1.09	1.08	1.06	1.03	0.99	0.92	0.65	0.40	0.29	0.20	0.39	0.23	0.46	0.60	0.55	0.80	0.70	0.87	0.89	0.99	0.99	1.28	1.26	1.31
393	24.4	1.12	1.11	1.10	1.08	1.05	1.01	0.95	0.67	0.43	0.32	0.23	0.41	0.24	0.46	0.60	0.54	0.80	0.69	0.86	0.88	0.97	0.98	1.25	1.23	1.28
450	37.0	1.13	1.12	1.11	1.09	1.06	1.01	0.94	0.67	0.42	0.31	0.23	0.41	0.24	0.46	0.60	0.55	0.80	0.70	0.86	0.88	0.98	0.98	1.26	1.24	1.29
525	27.3	0.97	0.97	0.96	0.94	0.91	0.86	0.80	0.57	0.38	0.29	0.21	0.41	0.24	0.47	0.61	0.56	0.83	0.72	0.88	0.91	1.01	1.02	1.31	1.28	1.33
610	18.2	1.11	1.10	1.09	1.07	1.04	1.00	0.94	0.68	0.43	0.32	0.23	0.41	0.24	0.47	0.60	0.54	0.81	0.70	0.87	0.89	0.97	0.98	1.27	1.24	1.29
700	27.9	1.10	1.09	1.08	1.06	1.03	0.99	0.92	0.64	0.39	0.28	0.20	0.39	0.23	0.46	0.60	0.54	0.80	0.70	0.87	0.89	0.99	0.99	1.29	1.27	1.32
747	24.2	1.10	1.09	1.08	1.06	1.03	0.99	0.93	0.66	0.42	0.30	0.22	0.40	0.23	0.46	0.60	0.54	0.80	0.70	0.86	0.88	0.98	0.98	1.26	1.24	1.29
801	32.7	1.04	1.04	1.03	1.01	0.97	0.92	0.85	0.61	0.42	0.32	0.24	0.44	0.26	0.50	0.64	0.58	0.86	0.75	0.91	0.94	1.03	1.04	1.32	1.29	1.34
816	16.7	1.14	1.13	1.12	1.10	1.07	1.04	0.98	0.69	0.43	0.31	0.22	0.40	0.23	0.46	0.59	0.54	0.80	0.69	0.86	0.88	0.96	0.97	1.26	1.24	1.28
912	27.2	1.09	1.09	1.08	1.06	1.03	0.99	0.93	0.66	0.42	0.31	0.22	0.41	0.24	0.47	0.60	0.55	0.81	0.70	0.87	0.89	0.98	0.98	1.26	1.24	1.29
C035	27.2	1.13	1.13	1.12	1.10	1.07	1.03	0.96	0.68	0.42	0.30	0.21	0.39	0.22	0.46	0.60	0.54	0.80	0.69	0.88	0.89	0.98	0.98	1.28	1.26	1.30
C082	20.3	1.16	1.15	1.14	1.13	1.10	1.06	1.00	0.72	0.45	0.32	0.22	0.39	0.22	0.44	0.58	0.53	0.78	0.68	0.84	0.86	0.96	0.96	1.26	1.23	1.28
C093	14.4	0.97	0.96	0.95	0.94	0.91	0.88	0.84	0.64	0.44	0.33	0.24	0.41	0.24	0.47	0.60	0.55	0.81	0.71	0.87	0.90	0.99	1.00	1.26	1.25	1.29
C165	14.4	1.18	1.17	1.16	1.14	1.11	1.08	1.03	0.73	0.43	0.30	0.21	0.38	0.22	0.45	0.58	0.52	0.78	0.67	0.86	0.87	0.95	0.95	1.25	1.23	1.27
C182	17.6	1.16	1.15	1.14	1.12	1.09	1.05	0.99	0.70	0.43	0.30	0.21	0.40	0.23	0.46	0.60	0.55	0.81	0.70	0.89	0.91	0.99	0.99	1.30	1.28	1.32
C205	14.9	1.21	1.20	1.19	1.17	1.14	1.12	1.06	0.75	0.46	0.33	0.23	0.40	0.24	0.46	0.58	0.53	0.79	0.68	0.85	0.86	0.94	0.95	1.24	1.22	1.26
C229	14.6	1.03	1.02	1.00	0.98	0.95	0.91	0.85	0.60	0.39	0.29	0.21	0.41	0.24	0.47	0.62	0.57	0.82	0.72	0.89	0.93	1.02	1.03	1.29	1.28	1.32
C306	29.3	1.20	1.19	1.18	1.16	1.13	1.09	1.02	0.72	0.45	0.32	0.23	0.41	0.24	0.47	0.61	0.55	0.81	0.70	0.88	0.90	0.99	0.99	1.28	1.26	1.31
C337	6.2	1.11	1.10	1.09	1.07	1.05	1.02	0.97	0.72	0.46	0.34	0.24	0.42	0.25	0.47	0.61	0.56	0.83	0.72	0.89	0.91	1.01	1.01	1.30	1.28	1.32
C351	19.2	0.99	0.98	0.97	0.96	0.93	0.90	0.85	0.65	0.45	0.33	0.23	0.40	0.23	0.45	0.58	0.53	0.78	0.68	0.85	0.87	0.95	0.96	1.23	1.21	1.25
C449	32.0	1.12	1.11	1.10	1.08	1.05	1.01	0.95	0.67	0.42	0.30	0.22	0.40	0.23	0.46	0.60	0.54	0.81	0.70	0.87	0.89	0.98	0.99	1.28	1.25	1.30
C528	61.7	1.13	1.13	1.13	1.11	1.06	0.99	0.89	0.62	0.40	0.29	0.21	0.40	0.23	0.47	0.61	0.56	0.83	0.72	0.88	0.91	1.01	1.02	1.30	1.28	1.32
C572	8.2	1.01	1.00	0.98	0.96	0.93	0.90	0.85	0.60	0.37	0.28	0.20	0.38	0.23	0.45	0.59	0.54	0.77	0.68	0.85	0.88	0.97	0.98	1.27	1.24	1.29
C648	8.7	1.13	1.12	1.11	1.09	1.07	1.04	0.99	0.71	0.44	0.31	0.22	0.40	0.23	0.46	0.58	0.53	0.78	0.68	0.85	0.86	0.95	0.95	1.23	1.21	1.26
C672	13.2	1.17	1.16	1.14	1.12	1.09	1.06	1.00	0.70	0.43	0.31	0.22	0.40	0.24	0.47	0.61	0.56	0.80	0.70	0.88	0.90	0.99	0.99	1.27	1.25	1.30
C673	5.7	1.19	1.18	1.17	1.15	1.13	1.11	1.06	0.77	0.47	0.33	0.23	0.41	0.24	0.48	0.61	0.55	0.82	0.71	0.90	0.91	0.99	0.99	1.29	1.27	1.32
C698	13.6	1.06	1.05	1.04	1.01	0.98	0.94	0.88	0.62	0.39	0.29	0.21	0.40	0.23	0.46	0.61	0.55	0.80	0.70	0.87	0.90	0.99	1.00	1.28	1.26	1.30
C708	10.5	1.20	1.19	1.18	1.16	1.13	1.11	1.05	0.75	0.44	0.31	0.21	0.40	0.23	0.46	0.59	0.54	0.79	0.69	0.88	0.88	0.96	0.96	1.27	1.25	1.29
C725	38.8	1.18	1.17	1.16	1.14	1.10	1.06	0.99	0.69	0.41	0.29	0.20	0.39	0.22	0.45	0.58	0.52	0.79	0.68	0.85	0.86	0.96	0.96	1.27	1.24	1.29
C735	43.1	1.17	1.16	1.16	1.14	1.10	1.05	0.97	0.70	0.45	0.33	0.23	0.40	0.24	0.46	0.60	0.54	0.81	0.70	0.86	0.88	0.98	0.99	1.28	1.26	1.30

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
C802	24.5	1.16	1.15	1.14	1.12	1.08	1.05	0.98	0.70	0.43	0.31	0.22	0.40	0.23	0.46	0.59	0.54	0.81	0.70	0.86	0.88	0.97	0.97	1.26	1.24	1.29
C846	24.5	1.15	1.14	1.13	1.11	1.08	1.03	0.97	0.68	0.43	0.31	0.22	0.41	0.24	0.47	0.61	0.55	0.83	0.71	0.88	0.90	0.99	1.00	1.29	1.27	1.31
C859	24.5	1.20	1.19	1.17	1.15	1.12	1.09	1.02	0.72	0.45	0.32	0.22	0.40	0.23	0.46	0.59	0.54	0.80	0.69	0.86	0.87	0.96	0.97	1.26	1.23	1.28
C893	15.0	0.99	0.99	0.98	0.96	0.92	0.87	0.79	0.54	0.36	0.27	0.20	0.40	0.23	0.46	0.61	0.56	0.81	0.71	0.87	0.91	1.01	1.03	1.30	1.28	1.32
C898	9.6	1.27	1.25	1.24	1.22	1.20	1.18	1.12	0.81	0.50	0.35	0.25	0.44	0.25	0.49	0.61	0.55	0.85	0.73	0.89	0.90	0.99	0.98	1.29	1.27	1.30
C899	11.0	1.15	1.14	1.12	1.11	1.08	1.05	0.99	0.71	0.44	0.31	0.22	0.39	0.23	0.45	0.58	0.53	0.77	0.67	0.85	0.86	0.95	0.95	1.25	1.23	1.28
C913	13.6	1.20	1.18	1.17	1.15	1.12	1.09	1.04	0.73	0.44	0.31	0.21	0.39	0.22	0.45	0.57	0.52	0.78	0.67	0.84	0.86	0.94	0.95	1.24	1.21	1.26
C988	8.9	1.18	1.17	1.16	1.14	1.11	1.08	1.03	0.73	0.45	0.31	0.22	0.40	0.23	0.45	0.58	0.53	0.80	0.69	0.86	0.87	0.96	0.97	1.28	1.25	1.30
GF008	10.3	1.15	1.14	1.13	1.11	1.08	1.06	1.01	0.72	0.45	0.32	0.23	0.41	0.23	0.47	0.61	0.55	0.82	0.71	0.90	0.91	0.99	0.99	1.29	1.27	1.31
GF028	25.5	1.15	1.14	1.14	1.12	1.10	1.07	1.02	0.77	0.51	0.36	0.25	0.40	0.24	0.45	0.58	0.52	0.77	0.67	0.84	0.85	0.94	0.94	1.22	1.19	1.24
GF075	7.7	1.17	1.16	1.15	1.13	1.11	1.08	1.03	0.75	0.46	0.33	0.23	0.39	0.23	0.45	0.58	0.52	0.79	0.68	0.85	0.86	0.95	0.96	1.26	1.23	1.28
GF121	4.8	0.86	0.85	0.83	0.81	0.79	0.77	0.73	0.52	0.34	0.27	0.20	0.40	0.24	0.47	0.61	0.56	0.82	0.71	0.89	0.91	1.01	1.00	1.28	1.26	1.31
GF163	11.8	1.16	1.15	1.14	1.12	1.10	1.07	1.02	0.71	0.42	0.29	0.20	0.39	0.22	0.46	0.59	0.53	0.79	0.68	0.88	0.88	0.96	0.96	1.28	1.25	1.30
GF195	13.2	1.12	1.11	1.10	1.08	1.05	1.02	0.96	0.70	0.46	0.33	0.24	0.41	0.24	0.47	0.61	0.56	0.83	0.72	0.89	0.92	1.01	1.02	1.31	1.29	1.33
GF212	8.8	0.82	0.81	0.79	0.77	0.75	0.73	0.69	0.50	0.32	0.25	0.18	0.39	0.22	0.46	0.59	0.54	0.81	0.70	0.86	0.88	0.98	0.98	1.28	1.26	1.29
GF216	27.9	1.06	1.06	1.06	1.04	1.00	0.94	0.87	0.62	0.42	0.31	0.23	0.44	0.25	0.50	0.64	0.58	0.87	0.76	0.93	0.96	1.05	1.06	1.35	1.33	1.36
GF221	10.6	1.18	1.17	1.15	1.13	1.10	1.07	1.02	0.73	0.45	0.32	0.22	0.40	0.23	0.47	0.60	0.54	0.81	0.70	0.88	0.89	0.98	0.99	1.29	1.26	1.31
GF242	5.1	0.71	0.70	0.68	0.67	0.65	0.64	0.61	0.46	0.32	0.26	0.20	0.41	0.23	0.47	0.61	0.56	0.84	0.72	0.88	0.91	1.01	1.01	1.33	1.30	1.34
GF310	14.9	1.21	1.20	1.18	1.17	1.15	1.12	1.07	0.78	0.48	0.34	0.24	0.40	0.23	0.46	0.59	0.53	0.80	0.69	0.85	0.87	0.96	0.97	1.27	1.24	1.28
GF369	31.5	1.14	1.13	1.12	1.10	1.07	1.03	0.96	0.68	0.42	0.30	0.21	0.40	0.23	0.46	0.60	0.54	0.81	0.70	0.88	0.89	0.98	0.99	1.28	1.26	1.30
GF406	8.4	0.99	0.98	0.96	0.94	0.91	0.88	0.83	0.59	0.37	0.27	0.20	0.38	0.23	0.45	0.60	0.54	0.77	0.68	0.84	0.88	0.98	0.99	1.26	1.24	1.29
GF452	12.9	0.96	0.95	0.93	0.91	0.88	0.85	0.79	0.56	0.36	0.27	0.20	0.40	0.24	0.47	0.63	0.57	0.83	0.73	0.90	0.94	1.03	1.04	1.30	1.28	1.33
GF461	14.0	1.17	1.15	1.14	1.11	1.08	1.05	0.99	0.69	0.44	0.32	0.23	0.41	0.24	0.47	0.60	0.55	0.81	0.70	0.87	0.90	0.98	1.00	1.29	1.26	1.30
GF569	13.5	1.20	1.19	1.18	1.17	1.14	1.11	1.06	0.78	0.50	0.36	0.24	0.41	0.23	0.46	0.58	0.53	0.81	0.69	0.85	0.87	0.96	0.97	1.27	1.25	1.29
GF604	11.7	1.10	1.09	1.08	1.06	1.03	0.99	0.93	0.66	0.43	0.32	0.23	0.45	0.26	0.52	0.67	0.61	0.91	0.79	0.97	1.00	1.08	1.10	1.35	1.34	1.37
GF608	27.6	1.19	1.19	1.18	1.16	1.12	1.08	1.01	0.70	0.43	0.31	0.22	0.40	0.23	0.47	0.60	0.55	0.82	0.70	0.88	0.90	0.99	1.00	1.29	1.27	1.31
GF752	12.5	0.84	0.83	0.82	0.80	0.77	0.74	0.70	0.52	0.38	0.29	0.21	0.41	0.23	0.47	0.62	0.56	0.84	0.73	0.90	0.93	1.03	1.04	1.35	1.33	1.36
GF806	26.1	0.73	0.72	0.71	0.70	0.67	0.64	0.58	0.42	0.29	0.23	0.18	0.38	0.23	0.46	0.62	0.56	0.81	0.70	0.89	0.92	1.01	1.02	1.29	1.26	1.31
GF849	12.2	1.20	1.19	1.18	1.16	1.13	1.10	1.04	0.74	0.45	0.32	0.22	0.40	0.23	0.46	0.59	0.53	0.80	0.69	0.87	0.88	0.97	0.97	1.28	1.26	1.30
GF907	24.5	1.12	1.11	1.09	1.07	1.04	1.01	0.95	0.68	0.43	0.31	0.23	0.42	0.25	0.48	0.61	0.56	0.83	0.72	0.89	0.91	1.00	1.01	1.30	1.28	1.31
GF912	12.2	1.18	1.17	1.16	1.14	1.12	1.09	1.03	0.73	0.44	0.31	0.21	0.39	0.22	0.45	0.58	0.53	0.79	0.68	0.87	0.87	0.96	0.96	1.25	1.23	1.28
GF936	10.0	1.15	1.14	1.12	1.10	1.07	1.04	0.99	0.71	0.44	0.32	0.23	0.40	0.24	0.46	0.59	0.53	0.79	0.69	0.86	0.88	0.96	0.97	1.27	1.24	1.29
OP109	11.5	1.17	1.16	1.14	1.12	1.09	1.06	1.00	0.70	0.42	0.30	0.21	0.41	0.23	0.47	0.60	0.55	0.83	0.72	0.88	0.90	1.00	1.01	1.32	1.30	1.34

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
OP117	11.9	1.20	1.18	1.17	1.15	1.12	1.08	1.03	0.72	0.45	0.33	0.23	0.41	0.24	0.47	0.60	0.55	0.81	0.70	0.87	0.89	0.98	0.99	1.28	1.26	1.30
OP126	9.5	1.20	1.18	1.17	1.15	1.13	1.10	1.05	0.75	0.46	0.33	0.23	0.42	0.23	0.47	0.60	0.54	0.84	0.72	0.89	0.90	0.99	0.99	1.31	1.29	1.32
OP171(05)	16.2	1.21	1.20	1.19	1.17	1.14	1.11	1.06	0.75	0.46	0.33	0.23	0.41	0.24	0.47	0.59	0.54	0.81	0.70	0.87	0.88	0.97	0.97	1.28	1.25	1.29
OP171(07)	34.5	1.13	1.12	1.11	1.09	1.06	1.01	0.95	0.67	0.43	0.31	0.22	0.40	0.23	0.46	0.59	0.54	0.79	0.69	0.86	0.87	0.96	0.96	1.24	1.22	1.27
OP172	11.4	1.17	1.15	1.14	1.12	1.09	1.06	1.00	0.70	0.43	0.30	0.21	0.40	0.23	0.47	0.61	0.55	0.81	0.70	0.89	0.91	1.00	1.01	1.30	1.28	1.32
OP213	9.4	1.15	1.14	1.12	1.11	1.08	1.06	1.01	0.72	0.43	0.31	0.22	0.39	0.23	0.46	0.61	0.55	0.79	0.69	0.89	0.91	0.99	1.00	1.26	1.25	1.30
OP226	11.1	1.09	1.07	1.06	1.04	1.02	0.99	0.93	0.67	0.41	0.30	0.21	0.38	0.22	0.44	0.58	0.53	0.77	0.67	0.84	0.87	0.96	0.97	1.24	1.22	1.28
OP231	10.8	1.14	1.13	1.12	1.10	1.07	1.04	0.99	0.72	0.45	0.32	0.23	0.40	0.23	0.46	0.60	0.54	0.80	0.70	0.87	0.89	0.99	1.00	1.30	1.27	1.32
OP268	13.8	1.04	1.03	1.01	0.99	0.96	0.93	0.87	0.61	0.38	0.29	0.21	0.39	0.24	0.47	0.62	0.57	0.81	0.71	0.89	0.93	1.02	1.03	1.30	1.28	1.33
OP325	10.5	1.21	1.20	1.19	1.17	1.14	1.11	1.06	0.75	0.45	0.31	0.21	0.39	0.22	0.46	0.58	0.53	0.79	0.68	0.87	0.88	0.96	0.96	1.26	1.24	1.29
OP386	8.9	1.18	1.17	1.16	1.14	1.12	1.09	1.04	0.74	0.44	0.31	0.21	0.39	0.22	0.46	0.60	0.54	0.80	0.69	0.89	0.90	0.98	0.98	1.28	1.26	1.31
OP394	14.3	1.20	1.19	1.18	1.16	1.13	1.10	1.04	0.73	0.44	0.31	0.22	0.40	0.23	0.46	0.58	0.53	0.81	0.69	0.86	0.87	0.96	0.96	1.27	1.25	1.29
OP436	12.1	1.22	1.21	1.20	1.18	1.15	1.13	1.07	0.77	0.48	0.34	0.23	0.40	0.23	0.45	0.57	0.52	0.79	0.68	0.84	0.85	0.95	0.95	1.27	1.24	1.28
OP444	10.7	1.19	1.18	1.16	1.14	1.11	1.08	1.02	0.71	0.43	0.31	0.22	0.41	0.23	0.46	0.59	0.54	0.82	0.70	0.87	0.88	0.97	0.97	1.29	1.26	1.30
OP494	8.6	1.18	1.17	1.16	1.14	1.11	1.09	1.03	0.74	0.46	0.32	0.22	0.41	0.23	0.47	0.59	0.53	0.83	0.71	0.88	0.89	0.98	0.98	1.31	1.29	1.32
OP497	10.8	1.18	1.17	1.15	1.13	1.11	1.08	1.03	0.74	0.46	0.32	0.23	0.39	0.23	0.45	0.59	0.53	0.79	0.68	0.86	0.88	0.97	0.97	1.27	1.24	1.29
OP502	11.2	1.21	1.20	1.19	1.17	1.14	1.11	1.05	0.75	0.46	0.32	0.23	0.41	0.23	0.47	0.59	0.54	0.82	0.71	0.88	0.89	0.98	0.98	1.30	1.28	1.32
OP529	15.9	1.18	1.17	1.15	1.13	1.10	1.07	1.01	0.71	0.44	0.32	0.23	0.40	0.24	0.47	0.62	0.57	0.81	0.71	0.90	0.92	1.01	1.01	1.29	1.27	1.32
OP533	8.4	1.00	0.98	0.97	0.94	0.92	0.89	0.84	0.58	0.36	0.26	0.19	0.36	0.22	0.43	0.58	0.53	0.75	0.66	0.83	0.86	0.95	0.96	1.24	1.21	1.27
OP613	12.6	1.24	1.23	1.21	1.19	1.17	1.14	1.08	0.76	0.46	0.32	0.23	0.42	0.23	0.47	0.59	0.54	0.82	0.70	0.87	0.87	0.97	0.96	1.27	1.25	1.30
OP656	24.5	1.17	1.16	1.15	1.13	1.10	1.06	1.00	0.71	0.44	0.31	0.22	0.40	0.23	0.46	0.59	0.53	0.80	0.69	0.87	0.88	0.97	0.97	1.28	1.25	1.30
OP665	12.2	1.25	1.23	1.22	1.20	1.17	1.15	1.09	0.77	0.46	0.32	0.22	0.41	0.23	0.46	0.58	0.53	0.80	0.69	0.86	0.86	0.95	0.95	1.27	1.24	1.29
OP706	13.3	1.13	1.12	1.10	1.08	1.06	1.03	0.98	0.70	0.44	0.32	0.23	0.41	0.25	0.48	0.63	0.57	0.82	0.72	0.90	0.93	1.02	1.03	1.29	1.28	1.32
OP724	10.7	1.20	1.19	1.18	1.17	1.14	1.11	1.06	0.76	0.45	0.31	0.22	0.40	0.23	0.46	0.59	0.54	0.80	0.69	0.88	0.89	0.97	0.97	1.27	1.25	1.30
OP848	8.8	1.19	1.18	1.17	1.15	1.13	1.10	1.05	0.75	0.46	0.32	0.22	0.41	0.23	0.47	0.59	0.54	0.82	0.71	0.89	0.89	0.97	0.97	1.28	1.26	1.31
OP862	15.1	1.13	1.12	1.10	1.09	1.06	1.03	0.97	0.69	0.43	0.31	0.22	0.39	0.23	0.45	0.59	0.53	0.79	0.68	0.85	0.87	0.96	0.96	1.25	1.22	1.27
C164	7.9	1.19	1.18	1.17	1.16	1.14	1.12	1.08	0.78	0.47	0.32	0.22	0.40	0.22	0.46	0.58	0.52	0.80	0.69	0.86	0.86	0.94	0.94	1.24	1.22	1.26
C190	33.2	0.99	0.98	0.97	0.95	0.92	0.88	0.82	0.59	0.38	0.28	0.20	0.40	0.22	0.46	0.60	0.54	0.80	0.70	0.86	0.89	0.97	0.98	1.26	1.24	1.28
C297	15.4	1.13	1.13	1.12	1.10	1.08	1.05	1.00	0.72	0.44	0.31	0.22	0.40	0.23	0.46	0.59	0.54	0.80	0.69	0.87	0.88	0.96	0.96	1.25	1.23	1.27
C327	29.7	1.08	1.08	1.07	1.06	1.03	0.99	0.93	0.68	0.44	0.31	0.21	0.41	0.22	0.47	0.60	0.55	0.81	0.70	0.87	0.89	0.97	0.98	1.26	1.24	1.28
C389	5.1	1.15	1.14	1.13	1.12	1.10	1.08	1.04	0.76	0.47	0.33	0.23	0.40	0.23	0.46	0.58	0.53	0.79	0.68	0.85	0.86	0.95	0.95	1.22	1.21	1.25
C502	7.3	1.18	1.17	1.16	1.15	1.13	1.10	1.05	0.77	0.46	0.32	0.22	0.40	0.23	0.46	0.58	0.53	0.79	0.68	0.86	0.86	0.95	0.94	1.23	1.21	1.26
GF342	10.2	1.11	1.10	1.09	1.08	1.05	1.02	0.97	0.70	0.44	0.31	0.22	0.42	0.23	0.48	0.61	0.55	0.85	0.73	0.90	0.91	1.00	1.00	1.30	1.28	1.32

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
GF471	13.7	1.09	1.08	1.07	1.05	1.02	0.99	0.94	0.68	0.44	0.32	0.22	0.43	0.24	0.49	0.61	0.56	0.84	0.73	0.89	0.91	0.99	1.00	1.27	1.26	1.29
GF619	10.2	1.14	1.13	1.12	1.11	1.08	1.05	1.00	0.73	0.44	0.31	0.21	0.40	0.22	0.46	0.59	0.53	0.79	0.69	0.87	0.87	0.96	0.96	1.25	1.23	1.27
GF791	12.9	1.13	1.12	1.11	1.10	1.07	1.05	1.00	0.73	0.46	0.33	0.23	0.40	0.23	0.46	0.59	0.54	0.80	0.70	0.86	0.88	0.97	0.98	1.26	1.24	1.28
OP169	7.5	1.17	1.16	1.15	1.14	1.12	1.10	1.05	0.75	0.45	0.31	0.22	0.39	0.23	0.45	0.58	0.52	0.78	0.67	0.86	0.86	0.94	0.94	1.23	1.21	1.25
OP554	9.1	1.16	1.15	1.14	1.13	1.10	1.08	1.04	0.75	0.45	0.31	0.22	0.39	0.22	0.45	0.58	0.52	0.79	0.68	0.86	0.86	0.95	0.94	1.24	1.22	1.26
OP617	12.1	1.14	1.13	1.12	1.11	1.08	1.06	1.01	0.72	0.44	0.31	0.22	0.39	0.23	0.46	0.59	0.54	0.78	0.68	0.86	0.88	0.96	0.97	1.24	1.22	1.27
OP831	9.4	1.18	1.17	1.16	1.14	1.12	1.10	1.05	0.75	0.44	0.31	0.21	0.39	0.22	0.46	0.58	0.53	0.79	0.68	0.87	0.87	0.95	0.95	1.24	1.22	1.26
OP911	8.7	1.15	1.14	1.13	1.12	1.09	1.06	1.01	0.75	0.49	0.34	0.24	0.44	0.24	0.49	0.62	0.56	0.88	0.76	0.92	0.93	1.02	1.02	1.32	1.31	1.33
OP041a	33.1	1.18	1.17	1.16	1.15	1.12	1.08	1.02	0.74	0.46	0.33	0.23	0.40	0.23	0.46	0.59	0.54	0.80	0.69	0.86	0.88	0.98	0.99	1.28	1.25	1.30

Appendix Table B4. All samples in the validation set with reference data (chll) in ppm and absorbance values for each wavelength starting at 650 nm to 2498 nm. For simplicity, data is shown in 8 nm increments in the 650 nm to 690 nm (chll peak) range and in approximately 100 nm increments from there on up

		Wavelength (nm)																								
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
2006 - OP344	5.9	1.26	1.25	1.23	1.21	1.19	1.16	1.11	0.79	0.49	0.34	0.24	0.44	0.25	0.49	0.61	0.56	0.86	0.74	0.91	0.92	1.01	1.01	1.32	1.30	1.33
2007 - OP346	7.3	1.12	1.11	1.09	1.07	1.05	1.02	0.97	0.70	0.43	0.31	0.22	0.40	0.24	0.47	0.61	0.56	0.82	0.71	0.89	0.91	1.00	1.01	1.31	1.28	1.33
2010 - OP491	7.0	1.14	1.13	1.12	1.10	1.08	1.06	1.01	0.73	0.43	0.30	0.21	0.39	0.22	0.45	0.57	0.52	0.77	0.66	0.84	0.85	0.93	0.92	1.21	1.19	1.23
2008 - OP352	6.5	1.07	1.06	1.04	1.02	1.00	0.97	0.91	0.65	0.41	0.30	0.22	0.39	0.24	0.45	0.60	0.55	0.78	0.69	0.86	0.90	0.99	1.01	1.28	1.25	1.30
2006 - GF422	43.3	1.18	1.17	1.16	1.14	1.10	1.05	0.97	0.66	0.40	0.29	0.20	0.39	0.22	0.45	0.59	0.53	0.79	0.69	0.87	0.88	0.97	0.97	1.28	1.25	1.30
2004 - C905	8.4	1.08	1.06	1.04	1.02	0.99	0.96	0.90	0.62	0.37	0.27	0.20	0.39	0.23	0.45	0.60	0.55	0.80	0.69	0.87	0.90	0.99	1.00	1.30	1.27	1.32
2004 - GF516	2.7	0.72	0.71	0.69	0.68	0.66	0.63	0.60	0.43	0.30	0.24	0.19	0.40	0.23	0.47	0.61	0.56	0.84	0.72	0.88	0.91	1.01	1.02	1.33	1.30	1.34
2006 - GF948	10.6	0.99	0.97	0.96	0.94	0.92	0.89	0.85	0.63	0.43	0.32	0.23	0.43	0.24	0.48	0.62	0.56	0.84	0.73	0.90	0.92	1.01	1.01	1.31	1.29	1.33
2006 - OP599	12.8	1.14	1.12	1.11	1.09	1.06	1.02	0.96	0.67	0.41	0.29	0.21	0.39	0.23	0.46	0.60	0.55	0.79	0.69	0.88	0.90	0.99	1.00	1.27	1.25	1.30
2004 - GF340	12.7	1.06	1.05	1.03	1.01	0.98	0.94	0.88	0.61	0.39	0.29	0.21	0.41	0.24	0.48	0.63	0.58	0.83	0.73	0.91	0.94	1.04	1.05	1.31	1.29	1.35
2007 - C762	16.1	1.15	1.14	1.13	1.11	1.09	1.05	0.99	0.71	0.44	0.32	0.22	0.40	0.23	0.45	0.58	0.53	0.79	0.68	0.85	0.87	0.95	0.96	1.25	1.23	1.27
2004 - C496	9.5	1.05	1.03	1.01	0.99	0.96	0.93	0.88	0.61	0.38	0.28	0.20	0.39	0.23	0.46	0.62	0.56	0.81	0.71	0.89	0.92	1.02	1.03	1.31	1.29	1.33
2004 - OP688	10.8	1.08	1.07	1.05	1.03	1.00	0.97	0.91	0.64	0.40	0.30	0.21	0.40	0.24	0.47	0.61	0.56	0.81	0.71	0.88	0.91	1.01	1.02	1.29	1.27	1.32
2005 - GF105	17.5	1.14	1.13	1.12	1.10	1.07	1.04	0.98	0.70	0.44	0.32	0.22	0.41	0.24	0.48	0.62	0.57	0.85	0.73	0.91	0.93	1.03	1.03	1.33	1.31	1.35
2005 - C711	39.6	1.16	1.15	1.14	1.12	1.08	1.04	0.97	0.68	0.43	0.31	0.22	0.40	0.23	0.46	0.60	0.54	0.80	0.69	0.86	0.88	0.97	0.98	1.27	1.25	1.29
2009 - C151	25.7	1.01	1.00	0.99	0.97	0.94	0.91	0.85	0.60	0.39	0.29	0.21	0.39	0.23	0.46	0.61	0.55	0.81	0.70	0.88	0.91	1.00	1.01	1.28	1.26	1.31
2007 - C619	12.8	1.10	1.08	1.07	1.05	1.02	0.99	0.93	0.66	0.41	0.29	0.21	0.39	0.23	0.46	0.61	0.55	0.80	0.70	0.88	0.91	0.99	1.01	1.28	1.26	1.31
2004 - GF377	20.5	1.04	1.03	1.02	1.00	0.96	0.92	0.86	0.60	0.39	0.29	0.22	0.40	0.24	0.47	0.61	0.56	0.80	0.70	0.87	0.90	0.99	1.00	1.24	1.22	1.27
2009 - GF481	8.1	1.14	1.13	1.11	1.09	1.07	1.04	0.99	0.70	0.44	0.31	0.22	0.41	0.23	0.48	0.62	0.56	0.84	0.73	0.93	0.94	1.03	1.03	1.34	1.32	1.36
2004 - OP041b	13.7	1.05	1.04	1.03	1.00	0.97	0.93	0.87	0.60	0.38	0.28	0.20	0.39	0.23	0.47	0.63	0.57	0.81	0.72	0.90	0.94	1.03	1.05	1.31	1.29	1.34

Appendix Table B5. All selected samples following population structuring in the calibration file used in the creation of the NIR based chlorophyll calibration equation with reference data (chll) in ppm and absorbance values for each wavelength starting at 650 nm to 2498 nm. For simplicity, data is shown in 8 nm increments in the 650 nm to 690 nm (chll peak) range and in approximately 100 nm increments from there on up

	Wavelength (nm)																									
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
1204020	27.6	1.12	1.11	1.10	1.08	1.04	1.00	0.94	0.65	0.39	0.28	0.19	0.40	0.23	0.49	0.63	0.57	0.81	0.71	0.94	0.95	1.01	1.02	1.31	1.29	1.34
1204041	36.3	1.07	1.06	1.05	1.02	0.99	0.96	0.89	0.62	0.39	0.28	0.20	0.39	0.23	0.47	0.61	0.56	0.79	0.70	0.92	0.92	0.99	0.99	1.28	1.26	1.32
1204231	22.0	1.05	1.04	1.02	1.00	0.97	0.92	0.85	0.59	0.37	0.27	0.19	0.42	0.24	0.52	0.67	0.61	0.89	0.78	1.01	1.02	1.10	1.10	1.39	1.38	1.42
1217407	23.7	1.08	1.07	1.06	1.04	1.00	0.97	0.91	0.64	0.40	0.29	0.20	0.40	0.23	0.49	0.65	0.59	0.82	0.73	0.96	0.97	1.04	1.04	1.31	1.30	1.36
1222132	24.4	1.13	1.12	1.11	1.09	1.06	1.02	0.95	0.65	0.40	0.28	0.20	0.39	0.22	0.48	0.61	0.55	0.80	0.70	0.93	0.92	0.99	0.99	1.30	1.28	1.34
1224925	23.3	1.05	1.04	1.03	1.01	0.98	0.95	0.89	0.63	0.41	0.30	0.22	0.41	0.24	0.48	0.62	0.56	0.80	0.70	0.92	0.92	0.99	0.98	1.27	1.25	1.31
1228059	13.4	1.12	1.10	1.09	1.06	1.04	1.00	0.94	0.65	0.39	0.28	0.19	0.41	0.22	0.48	0.62	0.56	0.83	0.72	0.94	0.93	1.01	1.00	1.31	1.29	1.34
1317809	62.7	1.06	1.05	1.04	1.02	0.99	0.94	0.86	0.59	0.36	0.26	0.19	0.40	0.23	0.48	0.63	0.57	0.83	0.72	0.94	0.95	1.03	1.03	1.32	1.30	1.35
1328042	26.4	1.06	1.05	1.03	1.01	0.98	0.94	0.88	0.61	0.38	0.28	0.20	0.39	0.23	0.47	0.61	0.55	0.80	0.70	0.92	0.93	1.00	1.00	1.29	1.27	1.33
1346054	39.3	1.11	1.10	1.09	1.07	1.04	0.99	0.92	0.64	0.41	0.30	0.21	0.41	0.23	0.49	0.64	0.58	0.85	0.74	0.95	0.97	1.05	1.06	1.34	1.33	1.37
CN02HF35	56.7	1.08	1.07	1.07	1.05	1.01	0.96	0.88	0.62	0.40	0.29	0.21	0.39	0.23	0.47	0.62	0.56	0.80	0.70	0.91	0.93	1.00	1.01	1.27	1.25	1.31
CN06CL04	9.9	1.02	1.01	1.00	0.98	0.96	0.92	0.87	0.62	0.40	0.29	0.21	0.40	0.23	0.47	0.62	0.56	0.82	0.72	0.92	0.94	1.02	1.02	1.32	1.30	1.35
CN07CL12	31.9	1.13	1.12	1.12	1.10	1.07	1.03	0.96	0.69	0.44	0.31	0.22	0.40	0.23	0.46	0.60	0.55	0.81	0.70	0.89	0.90	0.99	0.99	1.28	1.26	1.31
CN6CL017	39.2	1.06	1.06	1.05	1.04	0.99	0.94	0.86	0.59	0.37	0.27	0.19	0.41	0.23	0.50	0.64	0.58	0.86	0.75	0.96	0.97	1.05	1.06	1.36	1.35	1.39
333	32.7	1.10	1.10	1.09	1.07	1.03	0.99	0.92	0.64	0.39	0.29	0.20	0.40	0.23	0.48	0.62	0.56	0.81	0.71	0.92	0.93	1.01	1.01	1.29	1.27	1.33
599	27.3	0.97	0.96	0.95	0.93	0.90	0.85	0.79	0.56	0.38	0.29	0.21	0.42	0.24	0.49	0.63	0.57	0.84	0.73	0.93	0.95	1.02	1.03	1.32	1.30	1.34
869	20.3	1.16	1.15	1.14	1.12	1.09	1.05	0.99	0.70	0.43	0.31	0.22	0.39	0.23	0.46	0.59	0.53	0.77	0.67	0.88	0.88	0.94	0.94	1.22	1.20	1.26
1204027	25.7	1.02	1.01	1.00	0.97	0.94	0.90	0.84	0.57	0.35	0.26	0.18	0.38	0.22	0.47	0.62	0.56	0.80	0.70	0.92	0.93	1.01	1.01	1.31	1.29	1.34
1204088	27.6	1.06	1.05	1.04	1.02	0.98	0.95	0.88	0.62	0.39	0.29	0.21	0.40	0.24	0.48	0.63	0.57	0.82	0.72	0.93	0.94	1.03	1.02	1.31	1.29	1.35
1217125	24.9	1.03	1.02	1.01	0.99	0.95	0.91	0.85	0.59	0.37	0.27	0.20	0.41	0.24	0.50	0.66	0.60	0.87	0.76	0.99	1.01	1.09	1.10	1.37	1.35	1.40
1227851	7.4	1.10	1.08	1.07	1.05	1.02	0.99	0.93	0.65	0.39	0.28	0.20	0.40	0.22	0.47	0.59	0.54	0.80	0.70	0.89	0.89	0.97	0.97	1.28	1.26	1.31
1328660	25.9	1.09	1.08	1.07	1.05	1.02	0.98	0.91	0.64	0.40	0.30	0.22	0.41	0.25	0.50	0.65	0.59	0.84	0.74	0.95	0.97	1.05	1.05	1.31	1.30	1.35
CN00CL11	34.0	1.07	1.06	1.05	1.03	1.00	0.96	0.89	0.63	0.41	0.30	0.22	0.39	0.23	0.46	0.60	0.54	0.78	0.69	0.88	0.89	0.97	0.97	1.24	1.22	1.28
CN02HF32	62.9	1.07	1.07	1.06	1.04	1.01	0.97	0.89	0.64	0.41	0.30	0.22	0.39	0.24	0.47	0.61	0.55	0.77	0.68	0.89	0.90	0.96	0.96	1.20	1.19	1.25
101	34.9	1.06	1.05	1.05	1.03	0.99	0.95	0.87	0.61	0.38	0.28	0.20	0.40	0.23	0.47	0.61	0.56	0.83	0.72	0.91	0.92	1.01	1.01	1.31	1.29	1.33
239	36.7	1.09	1.08	1.07	1.05	1.01	0.97	0.90	0.64	0.41	0.30	0.22	0.40	0.24	0.47	0.61	0.56	0.79	0.69	0.88	0.90	0.98	0.98	1.25	1.23	1.28
283	19.1	1.10	1.09	1.08	1.06	1.03	0.99	0.93	0.65	0.40	0.29	0.21	0.40	0.23	0.46	0.60	0.54	0.80	0.69	0.88	0.89	0.98	0.98	1.27	1.25	1.30
789	25.4	1.03	1.03	1.02	1.00	0.96	0.92	0.86	0.62	0.42	0.32	0.24	0.44	0.26	0.50	0.64	0.58	0.85	0.75	0.92	0.95	1.03	1.04	1.31	1.29	1.33
1217731	27.2	1.11	1.10	1.08	1.06	1.03	0.99	0.93	0.64	0.39	0.28	0.20	0.39	0.22	0.46	0.59	0.54	0.79	0.69	0.88	0.89	0.98	0.98	1.29	1.26	1.31
1223922	27.4	1.13	1.12	1.11	1.09	1.06	1.02	0.96	0.67	0.42	0.30	0.21	0.39	0.23	0.46	0.60	0.54	0.78	0.69	0.87	0.88	0.97	0.97	1.27	1.25	1.31

	Wavelength (nm)																									
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
1227785	19.9	1.01	1.01	0.99	0.97	0.94	0.90	0.84	0.60	0.40	0.30	0.23	0.42	0.24	0.47	0.61	0.55	0.82	0.71	0.88	0.90	0.99	0.99	1.29	1.26	1.31
1227968	10.8	1.09	1.07	1.06	1.04	1.01	0.99	0.93	0.66	0.40	0.29	0.20	0.41	0.22	0.47	0.60	0.55	0.83	0.72	0.89	0.90	1.00	0.99	1.30	1.28	1.33
1317808	57.0	1.06	1.05	1.04	1.02	0.98	0.94	0.88	0.61	0.37	0.27	0.19	0.40	0.23	0.47	0.62	0.56	0.82	0.72	0.91	0.93	1.01	1.02	1.31	1.29	1.34
1317811	38.4	1.12	1.11	1.10	1.08	1.05	1.01	0.94	0.66	0.40	0.29	0.20	0.39	0.23	0.47	0.61	0.56	0.81	0.71	0.91	0.92	1.00	1.01	1.31	1.29	1.34
CN04CL10	47.0	1.13	1.13	1.13	1.11	1.07	1.02	0.94	0.66	0.41	0.29	0.20	0.40	0.22	0.47	0.61	0.55	0.81	0.71	0.90	0.92	1.00	1.00	1.29	1.27	1.32
CN06CL02	12.4	1.04	1.03	1.02	1.00	0.97	0.94	0.89	0.64	0.41	0.30	0.21	0.40	0.23	0.47	0.62	0.56	0.82	0.71	0.91	0.93	1.01	1.02	1.31	1.29	1.34
CN07CL18	44.2	1.06	1.06	1.05	1.04	1.00	0.94	0.86	0.60	0.38	0.28	0.20	0.41	0.23	0.48	0.63	0.58	0.85	0.74	0.92	0.94	1.04	1.04	1.34	1.32	1.37
525	27.3	0.97	0.97	0.96	0.94	0.91	0.86	0.80	0.57	0.38	0.29	0.21	0.41	0.24	0.47	0.61	0.56	0.83	0.72	0.88	0.91	1.01	1.02	1.31	1.28	1.33
2008 - C082	20.3	1.16	1.15	1.14	1.13	1.10	1.06	1.00	0.72	0.45	0.32	0.22	0.39	0.22	0.44	0.58	0.53	0.78	0.68	0.84	0.86	0.96	0.96	1.26	1.23	1.28
2008 - C093	14.4	0.97	0.96	0.95	0.94	0.91	0.88	0.84	0.64	0.44	0.33	0.24	0.41	0.24	0.47	0.60	0.55	0.81	0.71	0.87	0.90	0.99	1.00	1.26	1.25	1.29
2009 - C182	17.6	1.16	1.15	1.14	1.12	1.09	1.05	0.99	0.70	0.43	0.30	0.21	0.40	0.23	0.46	0.60	0.55	0.81	0.70	0.89	0.91	0.99	0.99	1.30	1.28	1.32
2004 - C229	14.6	1.03	1.02	1.00	0.98	0.95	0.91	0.85	0.60	0.39	0.29	0.21	0.41	0.24	0.47	0.62	0.57	0.82	0.72	0.89	0.93	1.02	1.03	1.29	1.28	1.32
2005 - C306	29.3	1.20	1.19	1.18	1.16	1.13	1.09	1.02	0.72	0.45	0.32	0.23	0.41	0.24	0.47	0.61	0.55	0.81	0.70	0.88	0.90	0.99	0.99	1.28	1.26	1.31
2008 - C337	6.2	1.11	1.10	1.09	1.07	1.05	1.02	0.97	0.72	0.46	0.34	0.24	0.42	0.25	0.47	0.61	0.56	0.83	0.72	0.89	0.91	1.01	1.01	1.30	1.28	1.32
2008 - C351	19.2	0.99	0.98	0.97	0.96	0.93	0.90	0.85	0.65	0.45	0.33	0.23	0.40	0.23	0.45	0.58	0.53	0.78	0.68	0.85	0.87	0.95	0.96	1.23	1.21	1.25
2007 - C449	32.0	1.12	1.11	1.10	1.08	1.05	1.01	0.95	0.67	0.42	0.30	0.22	0.40	0.23	0.46	0.60	0.54	0.81	0.70	0.87	0.89	0.98	0.99	1.28	1.25	1.30
2005 - C528	61.7	1.13	1.13	1.13	1.11	1.06	0.99	0.89	0.62	0.40	0.29	0.21	0.40	0.23	0.47	0.61	0.56	0.83	0.72	0.88	0.91	1.01	1.02	1.30	1.28	1.32
2007 - C572	8.2	1.01	1.00	0.98	0.96	0.93	0.90	0.85	0.60	0.37	0.28	0.20	0.38	0.23	0.45	0.59	0.54	0.77	0.68	0.85	0.88	0.97	0.98	1.27	1.24	1.29
2006 - C648	8.7	1.13	1.12	1.11	1.09	1.07	1.04	0.99	0.71	0.44	0.31	0.22	0.40	0.23	0.46	0.58	0.53	0.78	0.68	0.85	0.86	0.95	0.95	1.23	1.21	1.26
2006 - C672	13.2	1.17	1.16	1.14	1.12	1.09	1.06	1.00	0.70	0.43	0.31	0.22	0.40	0.24	0.47	0.61	0.56	0.80	0.70	0.88	0.90	0.99	0.99	1.27	1.25	1.30
2009 - C673	5.7	1.19	1.18	1.17	1.15	1.13	1.11	1.06	0.77	0.47	0.33	0.23	0.41	0.24	0.48	0.61	0.55	0.82	0.71	0.90	0.91	0.99	0.99	1.29	1.27	1.32
2004 - C698	13.6	1.06	1.05	1.04	1.01	0.98	0.94	0.88	0.62	0.39	0.29	0.21	0.40	0.23	0.46	0.61	0.55	0.80	0.70	0.87	0.90	0.99	1.00	1.28	1.26	1.30
2006 - C725	38.8	1.18	1.17	1.16	1.14	1.10	1.06	0.99	0.69	0.41	0.29	0.20	0.39	0.22	0.45	0.58	0.52	0.79	0.68	0.85	0.86	0.96	0.96	1.27	1.24	1.29
2008 - C735	43.1	1.17	1.16	1.16	1.14	1.10	1.05	0.97	0.70	0.45	0.33	0.23	0.40	0.24	0.46	0.60	0.54	0.81	0.70	0.86	0.88	0.98	0.99	1.28	1.26	1.30
2006 - C802	24.5	1.16	1.15	1.14	1.12	1.08	1.05	0.98	0.70	0.43	0.31	0.22	0.40	0.23	0.46	0.59	0.54	0.81	0.70	0.86	0.88	0.97	0.97	1.26	1.24	1.29
2007 - C846	24.5	1.15	1.14	1.13	1.11	1.08	1.03	0.97	0.68	0.43	0.31	0.22	0.41	0.24	0.47	0.61	0.55	0.83	0.71	0.88	0.90	0.99	1.00	1.29	1.27	1.31
2005 - C859	24.5	1.20	1.19	1.17	1.15	1.12	1.09	1.02	0.72	0.45	0.32	0.22	0.40	0.23	0.46	0.59	0.54	0.80	0.69	0.86	0.87	0.96	0.97	1.26	1.23	1.28
2004 - C893	15.0	0.99	0.99	0.98	0.96	0.92	0.87	0.79	0.54	0.36	0.27	0.20	0.40	0.23	0.46	0.61	0.56	0.81	0.71	0.87	0.91	1.01	1.03	1.30	1.28	1.32
2006 - C898	9.6	1.27	1.25	1.24	1.22	1.20	1.18	1.12	0.81	0.50	0.35	0.25	0.44	0.25	0.49	0.61	0.55	0.85	0.73	0.89	0.90	0.99	0.98	1.29	1.27	1.30
2008 - C899	11.0	1.15	1.14	1.12	1.11	1.08	1.05	0.99	0.71	0.44	0.31	0.22	0.39	0.23	0.45	0.58	0.53	0.77	0.67	0.85	0.86	0.95	0.95	1.25	1.23	1.28
2006 - C913	13.6	1.20	1.18	1.17	1.15	1.12	1.09	1.04	0.73	0.44	0.31	0.21	0.39	0.22	0.45	0.57	0.52	0.78	0.67	0.84	0.86	0.94	0.95	1.24	1.21	1.26
2007 - C988	8.9	1.18	1.17	1.16	1.14	1.11	1.08	1.03	0.73	0.45	0.31	0.22	0.40	0.23	0.45	0.58	0.53	0.80	0.69	0.86	0.87	0.96	0.97	1.28	1.25	1.30
2008 - GF028	25.5	1.15	1.14	1.14	1.12	1.10	1.07	1.02	0.77	0.51	0.36	0.25	0.40	0.24	0.45	0.58	0.52	0.77	0.67	0.84	0.85	0.94	0.94	1.22	1.19	1.24

	Wavelength (nm)																									
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
2008 - GF075	7.7	1.17	1.16	1.15	1.13	1.11	1.08	1.03	0.75	0.46	0.33	0.23	0.39	0.23	0.45	0.58	0.52	0.79	0.68	0.85	0.86	0.95	0.96	1.26	1.23	1.28
2005 - GF121	4.8	0.86	0.85	0.83	0.81	0.79	0.77	0.73	0.52	0.34	0.27	0.20	0.40	0.24	0.47	0.61	0.56	0.82	0.71	0.89	0.91	1.01	1.00	1.28	1.26	1.31
2009 - GF163	11.8	1.16	1.15	1.14	1.12	1.10	1.07	1.02	0.71	0.42	0.29	0.20	0.39	0.22	0.46	0.59	0.53	0.79	0.68	0.88	0.88	0.96	0.96	1.28	1.25	1.30
2008 - GF195	13.2	1.12	1.11	1.10	1.08	1.05	1.02	0.96	0.70	0.46	0.33	0.24	0.41	0.24	0.47	0.61	0.56	0.83	0.72	0.89	0.92	1.01	1.02	1.31	1.29	1.33
2006 - GF212	8.8	0.82	0.81	0.79	0.77	0.75	0.73	0.69	0.50	0.32	0.25	0.18	0.39	0.22	0.46	0.59	0.54	0.81	0.70	0.86	0.88	0.98	0.98	1.28	1.26	1.29
2005 - GF216	27.9	1.06	1.06	1.06	1.04	1.00	0.94	0.87	0.62	0.42	0.31	0.23	0.44	0.25	0.50	0.64	0.58	0.87	0.76	0.93	0.96	1.05	1.06	1.35	1.33	1.36
2007 - GF221	10.6	1.18	1.17	1.15	1.13	1.10	1.07	1.02	0.73	0.45	0.32	0.22	0.40	0.23	0.47	0.60	0.54	0.81	0.70	0.88	0.89	0.98	0.99	1.29	1.26	1.31
2007 - GF242	5.1	0.71	0.70	0.68	0.67	0.65	0.64	0.61	0.46	0.32	0.26	0.20	0.41	0.23	0.47	0.61	0.56	0.84	0.72	0.88	0.91	1.01	1.01	1.33	1.30	1.34
2008 - GF310	14.9	1.21	1.20	1.18	1.17	1.15	1.12	1.07	0.78	0.48	0.34	0.24	0.40	0.23	0.46	0.59	0.53	0.80	0.69	0.85	0.87	0.96	0.97	1.27	1.24	1.28
2006 - GF369	31.5	1.14	1.13	1.12	1.10	1.07	1.03	0.96	0.68	0.42	0.30	0.21	0.40	0.23	0.46	0.60	0.54	0.81	0.70	0.88	0.89	0.98	0.99	1.28	1.26	1.30
2007 - GF406	8.4	0.99	0.98	0.96	0.94	0.91	0.88	0.83	0.59	0.37	0.27	0.20	0.38	0.23	0.45	0.60	0.54	0.77	0.68	0.84	0.88	0.98	0.99	1.26	1.24	1.29
2004 - GF452	12.9	0.96	0.95	0.93	0.91	0.88	0.85	0.79	0.56	0.36	0.27	0.20	0.40	0.24	0.47	0.63	0.57	0.83	0.73	0.90	0.94	1.03	1.04	1.30	1.28	1.33
2004 - GF461	14.0	1.17	1.15	1.14	1.11	1.08	1.05	0.99	0.69	0.44	0.32	0.23	0.41	0.24	0.47	0.60	0.55	0.81	0.70	0.87	0.90	0.98	1.00	1.29	1.26	1.30
2008 - GF569	13.5	1.20	1.19	1.18	1.17	1.14	1.11	1.06	0.78	0.50	0.36	0.24	0.41	0.23	0.46	0.58	0.53	0.81	0.69	0.85	0.87	0.96	0.97	1.27	1.25	1.29
2006 - GF604	11.7	1.10	1.09	1.08	1.06	1.03	0.99	0.93	0.66	0.43	0.32	0.23	0.45	0.26	0.52	0.67	0.61	0.91	0.79	0.97	1.00	1.08	1.10	1.35	1.34	1.37
2005 - GF608	27.6	1.19	1.19	1.18	1.16	1.12	1.08	1.01	0.70	0.43	0.31	0.22	0.40	0.23	0.47	0.60	0.55	0.82	0.70	0.88	0.90	0.99	1.00	1.29	1.27	1.31
2007 - GF752	12.5	0.84	0.83	0.82	0.80	0.77	0.74	0.70	0.52	0.38	0.29	0.21	0.41	0.23	0.47	0.62	0.56	0.84	0.73	0.90	0.93	1.03	1.04	1.35	1.33	1.36
2009 - GF806	26.1	0.73	0.72	0.71	0.70	0.67	0.64	0.58	0.42	0.29	0.23	0.18	0.38	0.23	0.46	0.62	0.56	0.81	0.70	0.89	0.92	1.01	1.02	1.29	1.26	1.31
2005 - GF907	24.5	1.12	1.11	1.09	1.07	1.04	1.01	0.95	0.68	0.43	0.31	0.23	0.42	0.25	0.48	0.61	0.56	0.83	0.72	0.89	0.91	1.00	1.01	1.30	1.28	1.31
2007 - GF936	10.0	1.15	1.14	1.12	1.10	1.07	1.04	0.99	0.71	0.44	0.32	0.23	0.40	0.24	0.46	0.59	0.53	0.79	0.69	0.86	0.88	0.96	0.97	1.27	1.24	1.29
2004 - OP109	11.5	1.17	1.16	1.14	1.12	1.09	1.06	1.00	0.70	0.42	0.30	0.21	0.41	0.23	0.47	0.60	0.55	0.83	0.72	0.88	0.90	1.00	1.01	1.32	1.30	1.34
2007 - OP126	9.5	1.20	1.18	1.17	1.15	1.13	1.10	1.05	0.75	0.46	0.33	0.23	0.42	0.23	0.47	0.60	0.54	0.84	0.72	0.89	0.90	0.99	0.99	1.31	1.29	1.32
2005 - OP171a	16.2	1.21	1.20	1.19	1.17	1.14	1.11	1.06	0.75	0.46	0.33	0.23	0.41	0.24	0.47	0.59	0.54	0.81	0.70	0.87	0.88	0.97	0.97	1.28	1.25	1.29
2007 - OP171b	34.5	1.13	1.12	1.11	1.09	1.06	1.01	0.95	0.67	0.43	0.31	0.22	0.40	0.23	0.46	0.59	0.54	0.79	0.69	0.86	0.87	0.96	0.96	1.24	1.22	1.27
2006 - OP172	11.4	1.17	1.15	1.14	1.12	1.09	1.06	1.00	0.70	0.43	0.30	0.21	0.40	0.23	0.47	0.61	0.55	0.81	0.70	0.89	0.91	1.00	1.01	1.30	1.28	1.32
2009 - OP213	9.4	1.15	1.14	1.12	1.11	1.08	1.06	1.01	0.72	0.43	0.31	0.22	0.39	0.23	0.46	0.61	0.55	0.79	0.69	0.89	0.91	0.99	1.00	1.26	1.25	1.30
2008 - OP226	11.1	1.09	1.07	1.06	1.04	1.02	0.99	0.93	0.67	0.41	0.30	0.21	0.38	0.22	0.44	0.58	0.53	0.77	0.67	0.84	0.87	0.96	0.97	1.24	1.22	1.28
2008 - OP231	10.8	1.14	1.13	1.12	1.10	1.07	1.04	0.99	0.72	0.45	0.32	0.23	0.40	0.23	0.46	0.60	0.54	0.80	0.70	0.87	0.89	0.99	1.00	1.30	1.27	1.32
2004 - OP268	13.8	1.04	1.03	1.01	0.99	0.96	0.93	0.87	0.61	0.38	0.29	0.21	0.39	0.24	0.47	0.62	0.57	0.81	0.71	0.89	0.93	1.02	1.03	1.30	1.28	1.33
2009 - OP325	10.5	1.21	1.20	1.19	1.17	1.14	1.11	1.06	0.75	0.45	0.31	0.21	0.39	0.22	0.46	0.58	0.53	0.79	0.68	0.87	0.88	0.96	0.96	1.26	1.24	1.29
2005 - OP394	14.3	1.20	1.19	1.18	1.16	1.13	1.10	1.04	0.73	0.44	0.31	0.22	0.40	0.23	0.46	0.58	0.53	0.81	0.69	0.86	0.87	0.96	0.96	1.27	1.25	1.29
2004 - OP444	10.7	1.19	1.18	1.16	1.14	1.11	1.08	1.02	0.71	0.43	0.31	0.22	0.41	0.23	0.46	0.59	0.54	0.82	0.70	0.87	0.88	0.97	0.97	1.29	1.26	1.30
2009 - OP494	8.6	1.18	1.17	1.16	1.14	1.11	1.09	1.03	0.74	0.46	0.32	0.22	0.41	0.23	0.47	0.59	0.53	0.83	0.71	0.88	0.89	0.98	0.98	1.31	1.29	1.32

	Wavelength (nm)																									
ID	chll	650	658	666	674	682	690	706	802	906	1002	1106	1202	1306	1402	1506	1602	1706	1802	1906	2002	2106	2202	2306	2402	2490
2008 - OP497	10.8	1.18	1.17	1.15	1.13	1.11	1.08	1.03	0.74	0.46	0.32	0.23	0.39	0.23	0.45	0.59	0.53	0.79	0.68	0.86	0.88	0.97	0.97	1.27	1.24	1.29
2007 - OP502	11.2	1.21	1.20	1.19	1.17	1.14	1.11	1.05	0.75	0.46	0.32	0.23	0.41	0.23	0.47	0.59	0.54	0.82	0.71	0.88	0.89	0.98	0.98	1.30	1.28	1.32
2005 - OP529	15.9	1.18	1.17	1.15	1.13	1.10	1.07	1.01	0.71	0.44	0.32	0.23	0.40	0.24	0.47	0.62	0.57	0.81	0.71	0.90	0.92	1.01	1.01	1.29	1.27	1.32
2007 - OP533	8.4	1.00	0.98	0.97	0.94	0.92	0.89	0.84	0.58	0.36	0.26	0.19	0.36	0.22	0.43	0.58	0.53	0.75	0.66	0.83	0.86	0.95	0.96	1.24	1.21	1.27
2005 - OP613	12.6	1.24	1.23	1.21	1.19	1.17	1.14	1.08	0.76	0.46	0.32	0.23	0.42	0.23	0.47	0.59	0.54	0.82	0.70	0.87	0.87	0.97	0.96	1.27	1.25	1.30
2005 - OP706	13.3	1.13	1.12	1.10	1.08	1.06	1.03	0.98	0.70	0.44	0.32	0.23	0.41	0.25	0.48	0.63	0.57	0.82	0.72	0.90	0.93	1.02	1.03	1.29	1.28	1.32
2005 - OP862	15.1	1.13	1.12	1.10	1.09	1.06	1.03	0.97	0.69	0.43	0.31	0.22	0.39	0.23	0.45	0.59	0.53	0.79	0.68	0.85	0.87	0.96	0.96	1.25	1.22	1.27
2010 - C190	33.2	0.99	0.98	0.97	0.95	0.92	0.88	0.82	0.59	0.38	0.28	0.20	0.40	0.22	0.46	0.60	0.54	0.80	0.70	0.86	0.89	0.97	0.98	1.26	1.24	1.28
2010 - C327	29.7	1.08	1.08	1.07	1.06	1.03	0.99	0.93	0.68	0.44	0.31	0.21	0.41	0.22	0.47	0.60	0.55	0.81	0.70	0.87	0.89	0.97	0.98	1.26	1.24	1.28
2010 - GF342	10.2	1.11	1.10	1.09	1.08	1.05	1.02	0.97	0.70	0.44	0.31	0.22	0.42	0.23	0.48	0.61	0.55	0.85	0.73	0.90	0.91	1.00	1.00	1.30	1.28	1.32
2010 - GF471	13.7	1.09	1.08	1.07	1.05	1.02	0.99	0.94	0.68	0.44	0.32	0.22	0.43	0.24	0.49	0.61	0.56	0.84	0.73	0.89	0.91	0.99	1.00	1.27	1.26	1.29
2010 - OP554	9.1	1.16	1.15	1.14	1.13	1.10	1.08	1.04	0.75	0.45	0.31	0.22	0.39	0.22	0.45	0.58	0.52	0.79	0.68	0.86	0.86	0.95	0.94	1.24	1.22	1.26
2010 - OP911	8.7	1.15	1.14	1.13	1.12	1.09	1.06	1.01	0.75	0.49	0.34	0.24	0.44	0.24	0.49	0.62	0.56	0.88	0.76	0.92	0.93	1.02	1.02	1.32	1.31	1.33
2008 - OP041a	33.1	1.18	1.17	1.16	1.15	1.12	1.08	1.02	0.74	0.46	0.33	0.23	0.40	0.23	0.46	0.59	0.54	0.80	0.69	0.86	0.88	0.98	0.99	1.28	1.25	1.30

Appendix Table B6. Moisture content (%) in canola/rapeseed samples obtained from the Canadian Grain Commission. Each set was scanned through the NIR spectrophotometer three separate times, in the fall (grl1), winter (grl3), and spring (grl5)

	Moisture content (%)				Moisture content (%)		
ID	grl1	grl3	grl5	ID	grl1	grl3	grl5
1204020	5.4978	4.7586	3.9932	1328042	5.3720	4.9873	4.6735
1204027	5.5604	5.0122	4.1359	1328130	5.2936	5.0384	4.8358
1204028	5.3303	4.7592	3.9105	1328355	4.7252	4.5187	4.3403
1204041	5.2852	4.7136	3.8911	1328529	4.9261	4.6039	4.4836
1204088	5.0739	4.5146	3.7515	1328660	4.7794	4.4266	4.2877
1204174	5.3478	4.9592	4.1241	1331242	4.9134	4.6260	4.5168
1204231	4.7397	4.0914	3.4233	1346054	4.6089	4.5166	4.2450
1217125	5.2823	4.8246	4.3023	CN00CL11	3.9566	3.7654	3.7216
1217193	5.0151	4.5018	3.6410	CN01CL13	4.3195	4.1496	4.0391
1217407	5.6377	4.7850	4.1703	CN02HF31	5.0062	4.7496	4.2758
1217446	5.3928	4.8459	4.3134	CN02HF32	5.4126	5.2794	4.9462
1217731	5.0732	4.5760	3.6211	CN02HF33	5.1685	5.3230	4.9508
1222132	5.0998	4.5793	3.9043	CN02HF35	5.1181	4.6912	4.5603
1222928	5.0583	4.3616	3.8949	CN03CL10	4.5973	4.4253	4.3537
1223922	5.0487	4.4449	3.5634	CN03CL12	4.1975	4.2754	4.1314
1224925	5.3078	4.6935	4.0304	CN04CL08	4.3115	4.2052	4.0749
1225465	4.7062	4.0334	3.4447	CN04CL09	4.2934	4.1867	4.0111
1227785	4.5764	3.8555	3.3195	CN04CL10	4.3149	4.2822	4.0891
1227839	4.2868	3.7089	3.0691	CN04CL11	4.2960	4.1566	4.0286
1227851	4.8638	4.3473	3.8481	CN05CL09	5.4321	5.1042	4.9134
1227968	4.6885	4.1318	3.5850	CN06CL02	5.1520	4.8758	4.7204
1228059	4.3057	3.9497	3.3209	CN06CL04	4.9609	4.6833	4.5351
1228084	4.6029	3.9222	2.9532	CN06CL14	4.3378	4.0457	3.9599
1228114	4.4794	3.8440	3.2055	CN07CL08	4.2444	4.0614	3.9659
1317041	5.4222	5.0546	4.7413	CN078CL12	4.0574	3.8872	3.8063
1317044	5.7051	5.3081	5.0241	CN07CL17	4.0322	3.9270	3.7722
1317101	5.2324	4.9066	4.6723	CN07CL18	3.9270	3.9276	3.8093
1317610	5.0958	4.8401	4.3206	CN6CL17	4.3276	4.2521	3.9980
1317806	5.0383	4.7399	4.5242	101	4.6978	3.8399	2.9058
1317808	5.2821	4.9541	4.5980	107	5.0373	4.4263	3.7143
1317809	4.7452	4.2718	3.8498	110	4.4483	3.8560	3.3720
1317810	4.6567	4.3492	3.8904	111	4.9837	4.1686	3.5466
1317811	5.0931	4.6299	4.4176	112	4.3684	3.7098	3.3294
1317812	5.2097	4.8922	4.4695	121	5.1973	4.3395	3.7556
1317813	4.9817	4.5011	4.3395	161	4.6341	4.1150	3.4483
1322623	5.1527	4.5777	4.3824	171	4.4147	3.9796	3.3426
199	4.3277	3.8169	3.3153	608	3.8137	3.1489	2.6155
201	4.4424	3.9314	3.4579	610	4.3485	3.8528	2.9787
228	4.8048	3.7807	3.1620	623	4.3658	3.7481	2.9914
234	5.0892	3.9925	3.2552	628	4.4759	4.1391	3.5732
239	5.0782	4.2893	3.6474	700	4.3208	3.9378	3.4449
255	4.5386	3.6690	3.0994	714	4.5494	3.8356	3.2530
276	4.8050	4.3187	3.7122	720	5.1120	4.3938	3.7155
283	4.4362	3.9070	3.3513	747	4.5600	3.8248	3.2489
324	4.3733	4.0825	3.4862	777	4.6050	3.9289	3.3061
333	4.6766	4.1218	3.6309	789	4.5054	3.6597	3.0045
376	4.6940	4.0562	3.4599	801	3.6987	3.3091	2.6792
393	4.8380	4.2394	3.6645	802	4.3597	3.6997	3.1195
414	4.7248	3.5037	2.8334	809	4.3170	3.8439	3.2551
432	4.7349	3.6610	2.8412	816	4.3996	3.8956	3.4243
450	4.7012	3.9309	3.1910	869	4.8753	4.3260	3.5906
457	4.5318	3.8350	3.0788	876	4.4105	4.0491	3.5108
501	4.8252	4.2616	3.6357	912	4.8803	4.1313	3.4436
525	4.1536	3.7815	3.1246	931	4.8669	3.2591	2.4849
599	4.6180	4.0465	3.4830	998	4.7859	4.1306	3.5659