Microarray analysis of Methicillin-resistant *Staphylococcus aureus* in Canada

by

Sara Christianson

A Thesis submitted to the Faculty of Graduate Studies of

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Abstract

Ten epidemic strains of MRSA and various sporadic strains have been identified in Canada. Microarrays were used to study differences in gene content and gene expression, between epidemic and sporadic MRSA as well as hospital and community-associated MRSA to determine if there were specific genetic differences associated with these factors. Analysis of CGH data identified 21 highly variable regions within the MRSA genome. Comparison of the two most common nosocomial MRSA strains and the two community acquired MRSA strains identified 53 genes of potential interest. One open reading frame, SA2196, was present in the hospital-associated epidemic strains but absent in the sporadic strains. No significant correlations were observed between gene expression in epidemic and sporadic strains. This work has identified potential targets that could be contributing to the epidemic nature of some MRSA strains. These studies may lead to improved diagnostics to predict new emerging strains of MRSA and to a better understanding of the genetic basis for epidemicity.

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Table of Contents

List of Tables:

List of Figures:

List of Abbreviations

Ala	alanine
ANOVA	analysis of variation
arc	carbamate kinase
aro	shikimate dehydrogenase
ATCC	American type culture collection
ATP	adenosine triphosphate
BHI	brain heart infusion
bp	base pair
CA-MRSA	community acquired methicillin-resistant *Staphylococcus aureus*
CC	clonal complex
cDNA	complementary deoxy ribonucleic acid
CGH	comparative genomic hybridizations
CMRSA	Canadian methicillin-resistant *Staphylococcus aureus*
CNISP	Canadian nosocomial infection surveillance program
CTP	cytosine triphosphate
Cy	cyanine
DNA	deoxy ribonucleic acid
EPP	estimated probability of presence
GACK	genomotyping analysis by Charles Kim
glp	glycerol kinase
gmk	guanylate kinase
GTP	guanidine triphosphate
HAD	halo-acid dehalogenase
iGln	isoglutamine
Lys	lysine
MLST	multi-locus sequence typing
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive Staphylococcus aureus
ng	nanogram
ORF	open reading frame
PBP	penicillin binding protein
PBS	phosphate buffered saline
PCA	principle components analysis
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
pMol	pico moles
pta	phosphate acetyltransferase
PVL	Panton-Valentine leukociden
RNA	ribonucleic acid
SCC	staphylococcal cassette chromosome
SDS	sodium dodecyl sulfate

SSC	saline-sodium citrate
ST	sequence type
tpi	triosephosphate isomerase
TTP	tyrosine triphosphate
UPGMA	unweighted pair grouping method using arithmetic means
μL	micro litre
VISA	vancomycin intermediate *Staphylococcus aureus*
yqi	acetyl coenzyme A acetyltransferase

1. Introduction

1.1 Methicillin resistant *Staphylococcus aureus*

1.1.1. Development of resistance

Staphylococcus aureus is an important human pathogen which is implicated in many conditions including bacteremia, endocarditis, sepsis, metastatic infections and toxic shock syndrome (38, 66). It is a Gram-positive coccus which usually forms grape-like clusters. It is estimated that up to 50% of healthy adults are colonized by *S. aureus* at any one time, with up to 20% of those colonizations being persistent (66). Those that are colonized are far more likely to become infected or pass an infection to another person (66). *S. aureus* also produces many virulence factors which aid in its ability to cause disease. Some of these include catalase, protein A and coagulase which help evade the immune system, production of lipases, nucleases and proteases which help to invade host tissue, as well as the production of exotoxins, enterotoxins and toxic shock syndrome toxin (6). Once an infection has been established, treatment will often include antibiotics. The first class of antibiotics used to treat *S. aureus* infections were the beta-lactams.

Beta-lactam antibiotics (penicillins) were introduced in the 1940's for the treatment of bacterial infections, including *S. aureus* (65). Their activity is based on the ability to bind penicillin binding proteins (PBP) in the cell wall of organisms. Once bound, they prevent the cross linking of peptidoglycan. This

leaves the cell susceptible to osmotic pressure, which causes cell death in actively growing bacteria. This class of antibiotics is characterized by a central, four-membered, beta-lactam ring flanked by various side chains (61).

Almost immediately after the introduction of penicillin and the first generation of beta-lactam based drugs, resistance emerged in *S. aureus*. This resistance was based on enzymes which cleave the beta-lactam ring of these antibiotics and render them ineffective (beta-lactamases). To circumvent resistance to penicillins, methicillin was developed and introduced in the 1960's. Methicillin is a beta-lactam drug that is considered to be beta-lactamase resistant (86, 104). Methicillin's sidechains are larger and bulkier than those of penicillin. These sidechains hinder access of the beta-lactamase to the beta-lactam ring (65).

Methicillin resistance in *Staphylococci,* and more specifically, *S. aureus* emerged more than 40 years ago in the United Kingdom only briefly after the introduction of methicillin (9, 51). In the following decades methicillin-resistant *S. aureus* (MRSA) have been identified in many countries worldwide, and are currently the most prevalent hospital acquired infection in the United States (83) with case numbers on the rise in Canada (94). Methicillin resistance is gained through the acquisition of the staphylococcal cassette chromosome *mec* region (SCC*mec*) containing the *mec*A gene. The *mecA* gene encodes an altered PBP, called PBP2a, which is responsible for methicillin resistance. Once resistant to

methicillin, treatment options are limited. This is due to the fact that many MRSA, especially nosocomial isolates, are resistant to many other classes of antibiotics. In fact, the mobile region that carries the *mec* gene can also carry various other resistance genes to non beta-lactam drugs (50). The glycopeptide drug vancomycin, which inhibits cell wall synthesis in a different manner than beta-lactams, is one of the last lines of defense against these multi-resistant MRSA (65). There are currently three recognized types of vancomycin resistance in MRSA; intermediate resistance, hetero-resistance and high-level resistance. Isolates of MRSA which are intermediately resistant to vancomycin are referred to as VISA, or vancomycin intermediate *S. aureus*. These isolates have a minimum inhibitory concentration of 8 mg/ml. The first clinical isolate of VISA was reported in 1996 with other rare reports since. Hetero-resistance of MRSA to vancomycin was first described in 1997. These are isolates which are susceptible to vancomycin, but contain sub-populations which can grow at concentrations over 4 mg/mL (45). It has been suggested that 0-5% of all MRSA are hetero-resistant to vancomycin, with higher prevalence in various countries around the world (4). In 2002, two MRSA infections carrying the *vanA* gene, which imparts high-level vancomycin resistance, were isolated in American hospitals followed by a third strain isolated from a New York hospital in 2004 (19-21). Beyond vancomycin, newer drugs are being developed to combat multi-resistant MRSA. One of the more promising drugs is linezolid, which inhibits protein synthesis by binding to the 50S ribosomal subunit (91). After release for use in 2000, the first clinical report of a linezolid resistant MRSA strain was

published by June of 2001 (98), and one case has subsequently been observed in Canada (personal communication, M.R. Mulvey). Based on these occurrences, we are venturing closer to common bacterial infections that are untreatable by conventional antibiotic means.

1.1.2. Genetic factors involved in methicillin resistance:

1.1.2.1 Cell wall production

The peptidoglycan portion of the Gram-positive organism's cell wall is a thick layer which helps give the cell rigidity and protection against osmotic pressure. In *S. aureus* it is composed of chains of alternating sugars (glycan) attached to a pentapeptide chain linked together by five glycines (peptide). The sugar chain is alternating units of *N*-acetylmuramic acid and *N*-acetylglucosamine. The muramic acid subunits of the chain are substituted with an L-Ala-D-iGln-L-Lys-D-Ala-D-Ala pentapeptide, where Ala is alanine, Lys is lysine and iGln is isoglutamine (65). Two parallel chains are then linked by five glycine molecules. This linkage happens between the D-Ala of one strand and the L-Lys of the other. Initially, the five glycine molecules are added to the L-Lys of one strand, the terminal glycine is then linked to the fourth amino acid, D-Ala, and the terminal D-Ala is cleaved, providing energy for the linkage. This process is completed by penicillin binding proteins (PBP).

S. aureus codes for 4 PBPs that are named PBP 1-4. These PBPs are DD-peptidases that catalyze transpeptidation in the cross-linking of peptidoglycan (62). In Gram-positive bacteria, the cell wall is supported by a thick layer of covalently linked peptidoglycan. The tight cross linking in this layer gives the cell wall strength and helps to protect the cell from osmotic pressure and autolysis. Beta-lactam drugs act by binding to these PBP which cross-link the peptidoglycan thus inhibiting their function. With weakness created in the cell wall, cells become susceptible to osmotic pressure, causing lysis and therefore cell death (62).

The *mecA* gene codes for PBP2a that has very low affinity for beta-lactam drugs due to an unfavorable binding reaction at the active serine of PBP2a (54). PBPs from all organisms cluster into two classes, A and B. PBP2a is a high molecular mass, class B (subclass B1) PBP. It is typical of subclass B1 to bind methicillin poorly, and the inefficient binding confers broad spectrum resistance to beta-lactam antibiotics (44). Though PBP2a is not as efficient at transpeptidation as the other PBPs, its activity is sufficient to maintain the cell under antibiotic pressure. The PBP2a carried by various *Staphylococcal* species are very highly conserved, but their exact origin is unknown. It is closely (~88%) related to a PBP in *Staphylococcus sciuri* which does not confer resistance to methicillin and the possible method of transfer of this gene from *S. sciuri* to *S. aureus* has not yet been elucidated (22).

1.1.2.2 The SCC*mec* region

The SCC*mec* region, which carries the *mecA* gene, is a 21 to 67 kilobase (kb) region of DNA which has been inserted into the chromosome of MRSA at a position denoted as *orf*X (11). There are currently five recognized types of SCC*mec* regions, named I - V. Though contents of the types of regions vary, they all carry two cassette chromosome recombinases (*ccr)* denoted A and B which allow for transfer of the element. SCC*mec* types I – III are associated with nosocomial MRSA infections, while type IV and V are typically associated with community acquired MRSA, but are also found in nosocomial isolates (28). While it was originally thought that the SCC*mec* region was acquired only once and a single clone disseminated world-wide (57), it is now postulated that the region has actually been acquired by five different clones, on up to 20 different occasions. These five strains have now become epidemic worldwide (103). The natural method of transfer of this element is still not understood, though it is known that the *ccr* genes are sufficient to transfer the element from a high copy number plasmid into *orfX* of *S. aureus* (35).

The *mecA* gene is under the regulatory control of two genes, *mecI* and *mecRI* all of which are located on the SCC*mec* element and form the *mec* gene complex. Elements of this regulatory system are either deleted or mutated in resistant strains of *S. aureus*. The protein MecI represses transcription of *mec*A while MecRI is a sensor/transducer molecule (22). Methicillin is not an efficient inducer of MecRI, therefore, if this system is intact, cells will not be induced to

become resistant to methicillin merely by the antibiotic's presence. There are strains of *S. aureus* that are considered to be pre-methicillin resistant. These strains contain the *mec* region, but have functional regulatory genes and therefore do not possess the resistant phenotype (58).

Of the five types of SCC*mec* regions currently recognized, type I and IV SCC*mec* regions contain what is called a class B *mec* gene complex. In this case, *mecI* has been deleted and the *mecRI* gene, though present, contains a 5' deletion. This deletion is due to the insertion of a pseudoIS*1272* element. Type II and III SCC*mec* regions contain a class A *mec* gene complex. This complex contains the *mecA* gene, and intact *mecI* and *mecRI* genes (46). In order to confer resistance, this element then requires mutations in either the *mecA* promoter, *mecI* or *mecRI* genes (46). There are two other classes of *mec* gene complexes labeled C and D. Class C contains an IS*431* region associated with a deletion in the *mecRI* region and class D has a partially deleted *mecRI* region, not associated with any insertion sequence (53). Class C and D SCC*mec* regions have generally only been found in coagulase negative *Staphylococcus* species, though SCC*mec* V is a class C region.

The various types of SCC*mec* region are determined by the combination of *mec* complex and *ccr* genes they contain. Beyond these differences, there are also other differences in the gene content of the different elements. At ~20kb, the type IV and V SCC*mec* regions are the simplest elements and are most

frequently present in community acquired infections (79). It is thought that these regions represent the lowest cost to the organism's fitness and are likely the most easily transmissible of the elements (68, 78). Based on this fact, it has been hypothesized that SCC*mec* type IV is the most frequently acquired of the *mec* elements (84). In both these elements there are no other antimicrobial resistance genes that are encoded in the element. It is also noteworthy that there are multiple versions of the type IV element differing slightly in size and in the sequence of the non-coding DNA, but all have the basic structure of an IS*431* element, a partially deleted *mecRI* region, a pseudo IS*1272* element and the *ccrA2* and *B2* (84).

Type II and III elements contain a significantly greater amount of genetic content, being approximately 52kb and 67kb respectively. The uptake of other mobile genetic content by these regions has allowed for greater multi-resistance to develop. They both have class A *mec* regions. Type II codes for *ccr*A2 and B2, type III codes for *ccr*A3 and B3. They both also contain Tn*554* which encodes erythromycin resistance (*erm*A) and spectinomycin resistance (*spc*). Type II has pUB110 inserted which codes for bleomycin (*ble)* resistance as well as tobramycin resistance (*aad*D). Type III contains pT181 which carries the *tet*K gene, conferring resistance to tetracycline (49). The uptake of other mobile genetic content by these regions has allowed for greater multi-resistance to develop. Due to the extra drug resistance genes, organisms with Type II and III elements are well suited to the hospital environment.

Acquisition of the SCC*mec* region by *S. aureus* has allowed it to overcome the use of methicillin. While elements prevalent in hospitals are acquiring more resistance genes in order to best respond to environmental pressures, isolates in the community carry a region which has become easily transferable and has a low cost to organism fitness. A recent study did a retrospective analysis of 172 isolates of MRSA in Ireland and found seven novel SCC*mec* types in that sample alone, suggesting we may be underestimating the diversity of SCC*mec* regions (92).

1.1.2.3 Heteroresistance and *fem* genes

Most MRSA strains are heteroresistant to methicillin, that is, the majority of cells show low level resistance while a small sub-population of cells display high level resistance. In order to study this phenomenon, the homoresistant strain Col was mutagenized through the insertion of Tn*551* and mutants with reduced susceptibility to methicillin were selected. Genes involved in reduced susceptibility were termed *fem* genes for "factors essential for methicillin resistance" or *aux* genes for auxiliary factors. These factors are not responsible for the heteroresistant phenotype, but their deletion can create it. These genes were discovered to be housekeeping genes that aid in cell wall biosynthesis particularly in the creation of peptidoglycan precursors. There are currently seven recognized *fem* genes, with many other genes recognized to assist in high level methicillin resistance (11, 12, 29).

1.1.3. Epidemiology of MRSA

1.1.3.1. Risk factors and acquisition

It had generally been considered that MRSA is a nosocomial pathogen, that is, the infection is acquired after admission to the hospital and was not a pre-existing infection. Indeed, the common list of risk factors for MRSA infection include recent hospitalization or contact with a person who has recently been hospitalized, living in a long-term care facility, presence of a percutaneous medical device or previous antimicrobial therapy (99).

In recent years, people have started to present with cases of MRSA who have no associated risk factors. This includes an outbreak in a high school wrestling team in 1993 (63), as well as the death of four children in Minnesota in 1997 from a highly virulent strain of community acquired MRSA (CA-MRSA) (7). There has been controversy regarding the nomenclature of so called community acquired MRSA. This is because infections whose onset is in the community setting may be directly linked to the above listed risk factors in essence making them health care associated infections. Therefore a system that takes into account recent exposure to the health care system in order to more accurately distinguish community **acquired** MRSA from community **onset** MRSA has been proposed. This system differentiates community associated strains into two categories, with or with out risk factors such as health care exposure or exposure to a person recently in a health care facility. Community associated MRSA

infections with risk factors may indeed be nosocomial infections whose onset is delayed until the patient re-enters the community (88).

1.1.3.2 Emergence and differentiation of CA-MRSA

With the emergence of beta-lactamase producing strains of *S. aureus* in the 1940's, studies were undertaken to compare these organisms in the hospital and community setting. The results showed that while beta-lactamase producing strains emerged and thrived much sooner in the hospital, eventually they did begin to disseminate in the community and eventually reached nearly the same level of prevalence in the community (88). There is speculation that appearance of MRSA in the community will follow the same pattern as was recognized for beta-lactamase producing *S. aureus*. Now that MRSA rates in hospitals have hit a rate of ~50% in some cases, we may expect to see a sharp rise in the occurrence of CA-MRSA (23, 88)

Though originally thought to be strictly a nosocomial concern, MRSA has made the transition into the community and seems to be thriving. Most evidence points the independent evolution of methicillin resistance within the community and not simply a spread of hospital associated MRSA strains (79, 99). If the emergence of beta-lactam resistance in *S. aureus* in the hospital and subsequently the community is an accurate indicator, CA-MRSA is an emerging problem which will continue to worsen (23).

Studies using pulsed-field gel electrophoresis (PFGE) DNA fingerprinting have shown that true CA-MRSA are actually not related to strains generally associated with hospital infections. In fact, they are closely related to methicillin susceptible *S. aureus* (MSSA) strains generally found in the community (99). The difference between the CA-MRSA and the MSSA lies in the acquisition of a type IV or V SCC*mec* region. While type IV is typically associated with community acquired MRSA (28), all SCC*mec* types are associated with nosocomial MRSA infections.

The only other commonalities between CA-MRSA isolates from around the world is the presence of Panton-Valentine Leukociden (PVL) genes and a faster doubling time (99). Panton-valentine leukociden is a toxin found in *S. aureus* that causes leukocyte destruction and tissue necrosis (102). The genes necessary for its production are carried on a prophage (15). Due to its ability to destroy tissue, PVL is most often associated with primary skin infections and sometimes necrotizing pneumonia. It has been suggested that PVL should be used as a marker to identify CA-MRSA (99). A faster doubling time has been attributed to the overall fitness of the CA-MRSA and its ability to thrive in the community setting (7, 79). The presence of bacteriocins in a common community-associated strain, called MW2 or USA 400 have also been linked to better ability to out compete other bacteria in order to colonize a host (7).

1.1.3.3 The epidemic nature of MRSA

Currently there have been five strains of MRSA which have been identified as "pandemic" using PFGE DNA fingerprinting. These are the Iberian, New York/Japan, Brazilian, Hungarian, and pediatric clones, so named for the location which first reported them. The pediatric clone was first isolated from a children's hospital in Portugal (87). In a large-scale study of MRSA from Europe and the United States, these strains represented 68% of MRSA isolates (81). Recent studies have used multi-locus sequence typing (MLST) to study relationships between *S. aureus* strains. Multilocus sequence typing results are compared to a database of known sequence polymorphisms in seven housekeeping genes and each locus assigned a number based on this comparison. This creates what is called an allelic profile in which the combination of these seven numbers is used to generate a unique number called a sequence type (ST) (69). Studies using MLST have determined that there are at least five distinct *S. aureus* lineages which have developed methicillin resistance and are frequently isolated from patients in hospitals internationally. Closely related sequence types have been grouped with these lineages and these groups are named clonal complexes (CC). The five common clonal complexes are CC5, CC8, CC22, CC30 and CC45 (37). The five pandemic clones mentioned above are all contained in CCs 5 and 8. The Iberian, Brazilian and Hungarian strains fall within CC8, and the New York and Pediatric strains are part of CCC5. Community acquired MRSA showed an even more diverse genetic background, and in addition to the five previously mentioned CCs they also included strains from CCs 1 and 298. Most

notable of these two CCs is CC1 which includes the MW2 strain of CA-MRSA associated with four pediatric deaths in Minnesota (79), as well as the Canadian strain CMRSA 7.

Further, studies conducted in Denmark and the United Kingdom have suggested that *S. aureus* phage types that emerged as epidemic MRSA in those countries, were also the cause of epidemic infections before acquiring methicillin resistance (82). More recently, many CA-MRSA have been associated with the phage type 80/81. This phage type was responsible for pandemic penicillin-resistant infections in the 1950s (85). Other recent research has suggested that certain genetic backgrounds are more efficient at maintaining the SCC*mec* region and are therefore more "fit" to be methicillin resistant. Katayama *et al* (2005) have suggested that there are certain unknown genetic factors that may promote the acquisition of methicillin resistance, as well as factors which may predispose a strain to becoming epidemic.

Many countries around the world have implemented MRSA surveillance programs. Though a study by Cosgrove in 2005 found no increase in mortality for MRSA infections as opposed to MSSA infections, MRSA has been shown to increase length of hospital stay and hospital costs associated with the infection (27). This fact illuminates the need to track MRSA infections and help identify hospitals with serious problems in order that they may be resolved.

In 1999, the European Antimicrobial Resistance Surveillance System (EARSS) began tracking antimicrobial resistant organisms, including MRSA, in many countries across Europe. The latest report, from 2004, found an overall rate of MRSA in Europe to be 21%, but MRSA rates in various countries range from less than 1% in northern Europe, 5 to 20% in central Europe and 30-40% in southern Europe and the United Kingdom. Romania reported the highest rate of 73% (34).

In 2002, national surveillance in Belgium found that 28% of *S. aureus* bacteremias were caused by MRSA (33). A later survey of 455 MRSA isolates found that the MRSA clustered into 7 major strains, falling into the CCs 5, 8, 22, and 45, which are four of the five common nosocomial clonal clusters of MRSA (30).

In the United Kingdom, rates of MRSA among *S. aureus* infections have risen from 1-2% of bacteremias in the early nineties (96) to rates reported to range from 28% to 45% less than ten years later (32, 40). Two strains of MRSA, denoted EMRSA 15, belonging to CC22 and EMRSA 16, belonging to CC30, are the most prevalent strains in the U.K., accounting for more than 50% of the MRSA seen there (76).

Portuguese surveillance has determined a nosocomial prevalence of MRSA of 47.5%. Portuguese hospitals have observed many of the worldwide

epidemic strains of MRSA, having had high prevalence of the Portuguese clone, the Iberian clone, the Brazilian clone, EMRSA-15 and the pediatric clone. Despite a high prevalence in the nosocomial setting, Portugal has a low incidence of CA-MRSA (3).

MRSA has also been identified in Asian countries. Major hospitals in Taiwan report that 60% of *S. aureus* isolates are methicillin resistant (101). In China nosocomial incidence of MRSA has been reported to be as high as 80% (5). A 2003 study of MRSA from a hospital in Taiwan and a hospital in China found that 95% of the isolates collected were ST 239 or ST 241, which differ on only one allele (2).

In the United States of America, surveillance of MRSA from intensive care units has shown an increase from 35.9% of *S. aureus* isolates in 1992, to 64.4% of isolates being MRSA in 2003 (56). A recent study of MRSA from across the U.S.A. identified eight major pulsed-field types denoted USA100 – 800 which correspond to STs 5, 36, 8, 1, 8, 45,72 and 5 respectively. Pulsed-field types USA300 and USA400 are generally associated with CA-MRSA, the remaining types were common nosocomial strains (72).

Starting in 1995, hospitals belonging to the Canadian Nosocomial Infection Surveillance Program have submitted MRSA strains to our laboratory for the purpose of surveillance (94). These strains are then analyzed using

PFGE and macro-restriction banding patterns are compared in order to elucidate their relatedness to one another. In Canada, there are currently ten epidemic groups of MRSA, these strains have been given the annotation CMRSA 1-10, where the "C" stands for Canadian and should not be confused with community-acquired, or CA-MRSA. These strains account for approximately 85% of all MRSA identified in this study (Figure 1) (94) (M.R. Mulvey, personal communication) An epidemic strain is one which is considered to be clinically significant and is isolated from five or more hospital sites or from three or more geographical regions across Canada (93). Each CMRSA has unique traits. CMRSA-1 was initially the most prevalent strain but since 1999 has declined, CMRSA-2, the most diverse group of DNA fingerprints, has subsequently become the most prevalent strain. Canadian MRSA-3 was most likely to cause an infection, but since 1997 has virtually disappeared, being replaced by the closely related CMRSA 6. Both CMRSA 5 and 6 are associated with a single hospital site (95) while CMRSA 7 and 10 are related to strains commonly associated with community acquired MRSA outbreaks (75) and are frequently community acquired (Table 1).

Figure 1: Isolates of MRSA submitted to the CNISP MRSA surveillance study by Canadian hospitals separated by year and CMRSA type

Isolates of MRSA in Canada from 1995 to 2004

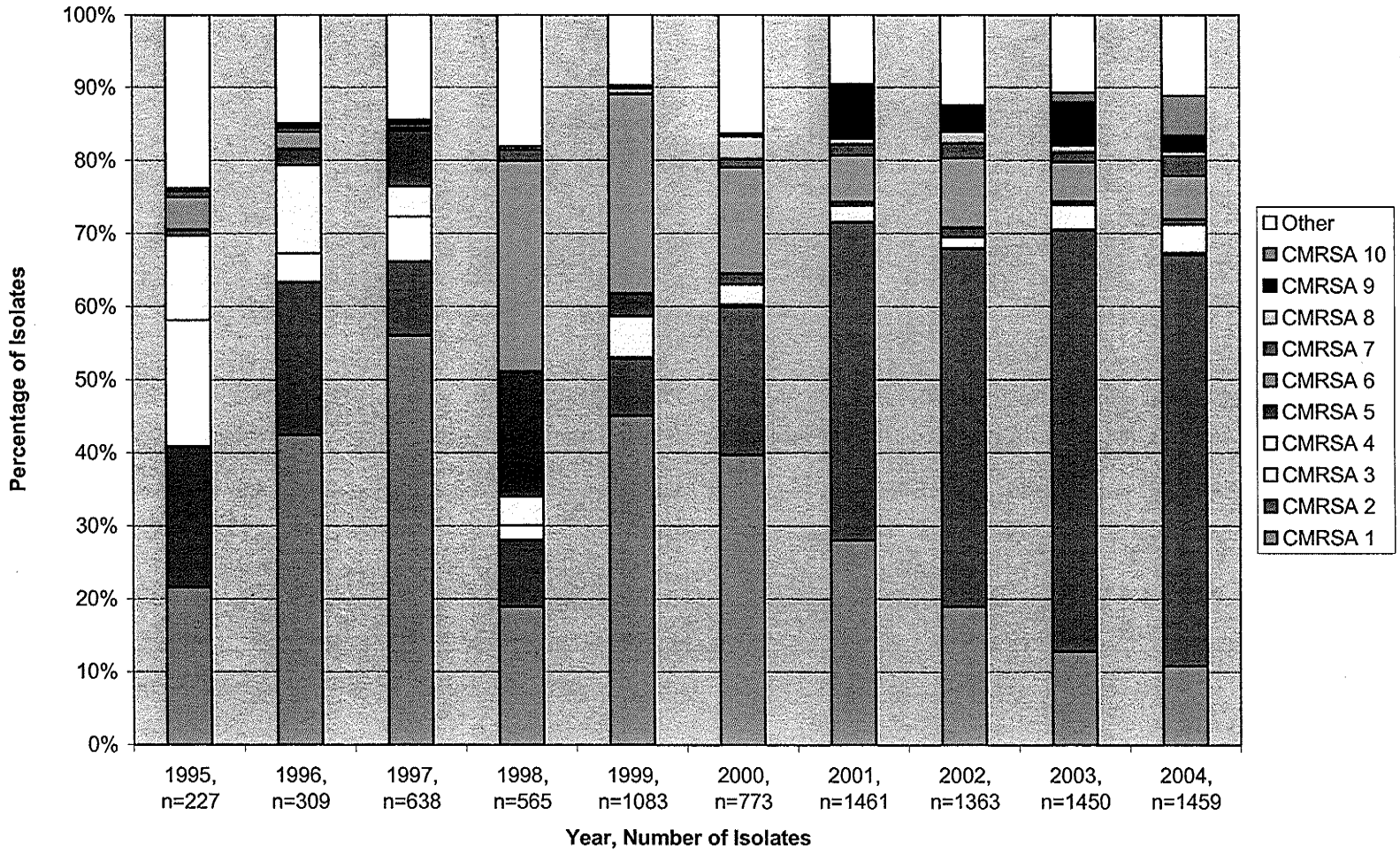


Table 1: Percentage of submitted CMRSA isolates that are associated with the community

Strain	Community Isolates	All Isolates	Percentage from Community
CMRSA1	94	2448	3.84%
CMRSA2	265	3429	7.73%
CMRSA3	4	113	3.54%
CMRSA4	20	351	5.70%
CMRSA5	11	244	4.51%
CMRSA6	20	979	2.04%
CMRSA7	34	140	24.29%
CMRSA8	8	89	8.99%
CMRSA9	10	280	3.57%
CMRSA10	48	111	43.24%

1.2. Microarrays

1.2.1. What are DNA Microarrays?:

DNA microarrays are a method for looking at the gene content and gene expression in various organisms, from humans to bacteria. They work on the same principle as Southern blots, that is, DNA can be attached to a solid substrate and probed with a single stranded DNA target. Given the appropriate hybridization conditions, this single stranded target DNA will anneal specifically to complimentary DNA attached to the solid surface. In the case of spotted microarrays like the ones used in this project, a large number of probes are covalently linked to the solid surface and the target DNA is hybridized to those probes. The microarray itself is the size of a microscope slide and is coated with various specialized substrates. This coating allows DNA to efficiently bind to the slide. Each slide can hold tens of thousands of DNA spots, each representing a specific gene or open reading frame, allowing an entire bacterial genome to be represented on one slide (42).

The first step in microarray production is deciding which open reading frames (ORFs) will be represented on the array. This can range from all of the ORFs from any one specific organism, a subset of ORFs from an organism with a larger genome or ORFs which represent genes from various sources, for example, antibiotic resistance genes. Once the ORF set has been decided, short or long oligomers which represent those ORFs must be generated. This can be done by either synthesizing 25 to 70 base pair (bp) products or by producing

larger products using polymerase chain reaction (PCR) (71). When short oligmers are used, (ie. 25 – 30 bp) each ORF is usually represented by five or more oligomers. These are sometimes referred to as oligo arrays or by the brand name, Affymetrix arrays (41).

Regardless of method of generation, DNA products can then be spotted in a grid pattern onto a solid glass substrate (47, 90). In order for DNA to covalently attach to the slide for spotted arrays, the slides are coated with various substrates, for example gamma amino propyl silane (25). This is a common, commercially available coating which is especially efficient in binding shorter oligomers. The products are bound to the slide as a highly concentrated spot of DNA to ensure it is not a limiting factor during the hybridization. The spots are printed in grid pattern to allow easy location and identification when analyzed.

The target DNA can either be genomic DNA or cDNA generated from an organisms mRNA. Genomic DNA is used as a target when researchers are simply looking at presence or absence of ORFs, ie. comparative genomics. Targets consisting of cDNA are used in expression studies, that is, studies comparing gene expression under two or more sets of conditions or between two different organisms. In either case, the target DNA is labeled using fluorescent dyes and hybridized to the arrays. Two targets are hybridized to each array. One is DNA extracted from a standard organism, or an organism grown under some standard set of conditions. The other target is the comparator strain under

study, either from a test organism or from an organism grown under a test set of conditions. Each target is labeled with a different dye (47). The two most common dyes are cyanine dyes called Cy 3, which fluoresces orange and Cy 5 which fluoresces in the far red spectrum. These two dyes are excited and read at different wavelengths and can therefore be read independently of one another. The level of fluorescence for the test DNA is compared to the level of fluorescence of the sample DNA, generating a ratio. This ratio can then be used to determine whether a specific ORF is either present or absent, or in the case of expression arrays, the level of expression of an ORF in a test organism as compared to the standard organism(55).

In the case of Affymetrix, a single color detection method is used. In this case, only the labeled sample strain is hybridized to the array. The array contains more than five spots which represent each ORF which allows for the presence or absence of ORFs to be determined based on various statistical calculations. While Affymetrix chips have the advantage of not requiring a positive control, the manufacturing process does not allow for as much flexibility in content as with the spotted arrays. Though the PCR to produce spotted arrays can be time consuming and sometimes fail, the use of synthesized 70mers can circumvent the PCR related steps.

1.2.2 Arrays for comparative genomics:

Genomic comparison microarrays allow us to compare the entire genomes of closely related organisms. This process is referred to as genomotyping (67) or, more commonly, comparative genomic hybridization (52). This technique allows the comparison of entire genomes of closely related organisms without having to sequence the whole genome (67). This process has been used to look at the relatedness of *Salmonella* serovars (24), *Escherichia coli* and *Shigella* (43). *Campylobacter jejuni* (60), *Helicobacter pylori* (13), *Mycobacterium bovis* (10) and *Staphylococcus aureus* (39) among others. It is important to note that this process only allows the determination of presence or absence/divergence of ORFs present on the array, novel ORFs or insertions in sample strains can not be identified since novel sequences are not represented on the array.

Hybridizations are accomplished by fragmenting a whole DNA preparation of both the sample and reference strains. These fragments are then labeled using a fluorescent dNTP, usually Cyanine 3 or Cyanine 5, by either chemical or enzymatic methods. Once labeled, the target is denatured and hybridized to the array. Presence or absence of an ORF in a sample organism's genome is determined by the ratio of the sample's fluorescence to that of the reference (55). If the sample target DNA anneals to the DNA on the array, the ratio of sample fluorescence to reference fluorescence is expected to be fairly high. Some researchers have used extremely high fluorescence ratios to predict the multiple copies of genes (16). If there is no complimentary sequence in the sample DNA,

or if sequence homology is low, the reference strain will have much more hybridization than the sample, therefore giving a low ratio value.

1.2.3. Arrays for expression analysis:

Expression arrays are generated in the same way as the comparative genomics arrays. The real difference in expression arrays is the preparation of the target. In this case, the organism is grown under a test set of conditions. At the appropriate time, growth is stopped and RNA is extracted. Messenger RNA is reverse transcribed generating single stranded cDNA. Reverse transcription is done with a labeled dNTP as a substrate, which allows the target to be labeled. Also extracted, reverse transcribed and labeled is the mRNA from an organism grown under a standard set of conditions, for comparison. Again, the test DNA and standard DNA are labeled with different dyes. These are then combined and hybridized to the array. The ratio of fluorescence from the test DNA to the standard DNA is used to determine weather a gene is being expressed and if it is being up or down regulated (47, 90).

1.2.4 Applications of microarrays:

Comparative genomic hybridizations can be used to study the genetic content of organisms in many ways. An example of one application of CGH is to create arrays which contain various resistance genes or toxin genes and use them as a screening tool for virulence in any one strain of bacteria. These arrays

can be used to compare common resistance genes in different bacterial species. A second application is to investigate and compare the entire genomes of organisms with different resistance profiles or different epidemiology to a standard genome.

Comparative genomic hybridizations for entire genomic studies have been used previously to study various aspects of *S. aureus*. In 2001, Fitzgerald *et al.* used CGH to investigate evolutionary relationships between thirty six *Staphylococcus aureus* strains, including eleven methicillin resistant strains. Using an array which contained ~90% of the ORFs from the methicillin resistant strain labeled Col, they were able to determine that *S. aureus* had most likely obtained the genes necessary for methicillin resistance upwards of five times. They also discovered 17 other regions of difference within the strains they studied, some of which encoded various toxins (39). In 2004, Dunman *et al* compared CGH to other molecular typing techniques showing that, though CGHs have very high discriminatory power as a typing technique, their clustering ability is similar to standard molecular typing techniques such as MLST and PFGE (31). In 2005 CGHs were used in a comparison of two hypervirulent strains of *S. aureus* to eight well characterized, less virulent strains. They found the presence of the *cna* gene and the absence of *isaB, sarT, sarU and sasG* may contribute to higher virulence (18). In 2006, Tenover *et al.* used Affymetrix arrays to characterize a common CA-MRSA found in the United States. The strain USA300-0114 has the same PFGE fingerprint pattern as the Canadian epidemic

strain and CMRSA 10. This study compared USA300 to another common CA-MRSA from the U.S., USA400, and two common American nosocomial strains of MRSA USA100 and USA500. From this comparison, a set of genes was determined which may facilitate the pathogenesis of the USA300-0114 strain (97). Another study in 2006 has used CGHs to compare the content of CA-MRSA associated with both invasive and non-invasive infections. Through this analysis, examining ten clonal clusters, they found no ORFs whose presence or absence could be associated with invasive disease (64).

Two studies using comparative genomic hybridizations to look specifically at antibiotic resistance genes in *S. aureus* were published in 2003. Volokhov *et al* crated an oligo array which can be used to identify erythromycin resistance genes, which also confer resistance to related macrolide, lincosamide and streptogramin B drugs (100). Call *et al.* developed an array which contained PCR generated probes for seventeen tetracycline resistance genes. Due to the limited number of spots for each complete array, they also developed a slide masking method which allows multiple samples to be tested on one slide, allowing for faster, more cost effective analysis (17).

Expression arrays can also be used in a number of different ways for the study of resistance. One common approach is to examine changes in the regulation of genes in response to the presence of an antibiotic. Another use is in the identification and validation of antimicrobial targets. Mongodin *et al* (2003)

used microarrays to analyze gene transcription in vancomycin resistant *Staphylococcus aureus*. Intermediately resistant strains were compared to highly resistant strains which had been created through multiple passages. Electron micrographs of the strains showed that increased resistance as associated with increased cell wall thickness. Expression analysis revealed 35 up regulated genes as well as 16 down regulated genes in the resistant strains. From these experiments, it was found that isolates resistant to vancomycin had an increase in purine biosynthesis. These results led to a hypothesis that vancomycin resistance comes at a high cost of ATP for cell energy as it is used for cell wall synthesis (73).

In 2001, Wilson *et al.* used an array containing 97% of the ORFs from *Mycobacterium tuberculosis* to examine genetic response to the drug isoniazid, commonly used to treat these infections. RNA was isolated from *M. tuberculosis* at varying time points from the initiation of drug treatment. These samples were compared to the gene expression at time zero, or just prior to the addition of isoniazid. This confirmed the involvement of various genes involved in the pathway targeted by the drug, as well as other genes involved in response to the toxic effects of isoniazid on the cell (105). A similar study was performed in yeast by Marton *et al* in 1998, which showed that microarray based expression analysis could not only be used to confirm the targets of currently available drugs, but to also identify secondary targets of experimental drugs that may be used in the future (70).

1.2.5 Limitations of microarrays:

There are many potential sources of error inherent in the microarray process. Some of these include slide printing errors, dust and particles, poor surface or hybridization, background fluorescence and multiple probe preparations (59, 77). In order to ensure statistical accuracy, each experiment must be replicated multiple times, as well as exchanging the dyes between the sample and reference DNA in order to control for unequal incorporation of dyes (1). Another drawback to arrays is the fact that only sequences present on the array can be studied, novel genetic content or highly divergent genes cannot be detected (100). As more genomes are completely sequenced arrays will become more complete and these issues will be less problematic.

1.3 Thesis Objective

Microarrays are a powerful tool allowing us to examine thousands of genes at one time. Whether used for comparative genomics or expression studies, they represent a valuable method for investigating the genetic content of bacteria. My hypothesis is that there may be some pattern of gene presence/absence or pattern of gene expression in epidemic strains not observed in sporadic isolates.

Our current aim is to investigate the gene content of the ten epidemic strains of MRSA discovered through the Canadian Nosocomial Infection Surveillance Program (CNISP), along with sporadic isolates collected during the surveillance. In order to accomplish this goal, we have designed a *S. aureus* microarray using a commercially available PCR primer set along with custom designed oligomers that can be used for both comparative genomics studies and expression studies. Using comparative genomics we hope to identify a pattern of gene presence or absence/divergence among the different sub-groups of strains in an attempt to discover what gives them a distinct epidemiologic characteristic. Specifically, we may find genes which, based on their presence or absence/divergence, can distinguish between strains that become epidemic and strains that do not or strains prevalent in communities and those found in hospitals. This data can also be compared to the genetic clustering created by PFGE and MLST analysis. Genome size can also vary by up to hundreds of kilobases (determined by PFGE), large, sequential deletions/absences can be identified through this process along with potential new DNA.

Expression studies will be used to compare the six original CMRSA strains with the sporadic isolates used in the CGH experiments. Via this analysis, we will attempt to identify gene expression patterns within the two groups of strains which affect their ability to become epidemic in our hospitals. We will also be able to analyze and compare gene expression in a number of different genetic backgrounds.

2. Materials and Methods:

2.1. Strains and growth conditions:

Strains were obtained through the ongoing surveillance of MRSA at 37 hospitals in Canada by the Canadian Nosocomial Infection Surveillance Program (CNISP). Cultures were stored at -80°C on Microbank beads (Pro-Lab Diagnostics, Ontario, Canada) according to manufacturer's directions. Experimental cultures were obtained by inoculating a single bead into 2 mL of Difco brain heart infusion broth (BHI) (Becton Dickinson, Franklin Lakes, NJ). Cultures were grown for 18-24 hours with shaking at 200 rpm at 37°C. This culture was transferred to 2 mL BHI and sub-cultured twice using the above conditions before use.

A single strain representing the most common PFGE fingerprint pattern for each of the Canadian epidemic strains was chosen arbitrarily. Four isolates whose PFGE fingerprint patterns differed by more than 7 bands, and were distant from the major CMRSA strains by principle components analysis (determined using Bionumerics software from Applied Maths, Belgium) were chosen as sporadic strains. At the time the sporadic strains were chosen, only one occurrence of each of the strains in Canada had been noted.

Standard strains, Col, N315 and Mu50 were obtained from The Institute for Genomic Research (Rockville, MD, U.S.A.), Juntendo University Department of Bacteriology (Tokyo, Japan) and the American Type Culture Collection

(Manassas, VA, U.S.A.) respectively. Table 2 contains information for strains used in this study.

Growth curves were generated using the Labsystems Bioscreen C (Thermo Electron Corporation, Waltham MA, U.S.A.). Overnight cultures were diluted 1 in 100 in 400μL fresh BHI broth and dispensed in Honeycomb 2 plates (Thermo Electron Corporation). Plates were then incubated in the Bioscreen at 37°C, with continuous shaking and optical density readings at 600nm were taken every ten minutes. Readings are recorded and graphed in a Microsoft Excel spreadsheet. Mid-log phase for all strains was determined to be between 400 and 800 ODs.

Table 2: Strains used in this study

Strain Name	CMRSA designation	Other Common Names	Source
Col	n/a		The Institute of Genomic Research (Rockville MD)
N315	n/a		Juntendo University Department of Bacteriology (Tokyo, Japan)
Mu50	n/a		ATCC
01S-0177	CMRSA 1	USA600	(93)
01S-0277	CMRSA 2	USA100/800/New York Clone	(93)
98S-0566	CMRSA 3	USA700	(93)
99S-0966	CMRSA 4	USA200/EMRSA 16	(93)
01S-0354	CMRSA 5	USA500	(94)
00S-1054	CMRSA 6	USA 700	(93)
00S-0907	CMRSA 7	USA400/MW2	(75)
00S-0331	CMRSA 8	EMRSA 15	CNISP study
01S-0965	CMRSA 9	n/a	CNISP study
04S-0073	CMRSA 10	USA300	CNISP study
98S-1344	Sporadic		CNISP study
99S-1088	Sporadic		CNISP study
00S-1635	Sporadic		CNISP study
02S-0250	Sporadic		CNISP study

2.2. Molecular Typing:

2.2.1 Pulsed-field gel electrophoresis

Isolates were typed by following the Canadian PFGE standardized method as previously described (74) with the use of *Salmonella enterica* serovar Braenderup H9812 as the molecular size standard. The *S.* Braenderup DNA plug slices were digested with *XbaI* and loaded whole into lanes 1, 5 and 10 in a 10-well gel and the MRSA strains digested with *SmaI* were melted and loaded in the remaining lanes (48).

2.2.2 Multi Locus Sequence Typing

Multi-locus sequence typing was performed using primers and PCR conditions previously described (36). *Staphylococcus aureus* MLST is based on the seven housekeeping genes carbamate kinase (arc), shikimate dehydrogenase (aro), glycerol kinase (glp), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi), and acetyl coenzyme A acetyltransferase (yqi). Primers were synthesized and sequence determined by the DNA Core Facility at the National Microbiology Laboratory (Winnipeg, MB). Sequence analysis was accomplished using the DNAstar suite of programs (DNAstar Inc., Madison, WI). Sequences for each allele were compared to the current database of alleles available at http://saureus.mlst.net website. Based on this comparison, allelic types were assigned, creating an allelic profile for each strain. Allelic types are ordered arc-aro-glp-gmk-pta-tpi-yqi. This numeric profile

is also compared to the established database of allelic profiles available at the http://saureus.mlst.net website, in order to determine sequence types.

2.2.3. SCC*mec* Typing:

Typing of SCC*mec* regions was accomplished using the primers described by Oliveira *et al* (80). These primers were separated into two mixes denoted Mec 1 and Mec 2. Primer specifics are given in Table 3. Polymerase chain reaction was carried out in a 50 µl solution containing 0.4 ng/µl template DNA, 3 mM $MgCl_2$, 0.2 mM each dATP. dCTP, dGTP and dTTP, 1x concentration of AmpliTaq Gold PCR buffer (Applied Biosystems, Foster City, CA) and 0.5 units AmpliTaq Gold (Applied Biosystems). Amplification was performed in a 96 well Gen Amp PCR System 9700 thermal cycler (Applied Biosystems) using the conditions described by Oliveira *et al* (80). Resulting products were electrophoresed on a 1.5% agarose gel in 1.5x tris borate EDTA at 10 volts/cm for 2 hours. Gels were stained with ethidium bromide for 20 minutes to allow for detection of products under ultra violet light. Expected band sizes for each SCC*mec* type are listed in Table 3.

Table 3: Primers used for SCCmec typing

Primer Pair	Forward Primer Sequence 5' to 3'	Reverse Primer Sequence 5' to 3'	Product Size (base pairs)	SCC*mec* Type Identified	Primer Mix
MEC I	ATCAAGACTTGCATTCAGGC	GCGGTTTCAATTCACTTGTC	209	II, III	Mec 1
RIF 4	GTGATTGTTCGAGATATGTGG	CGCTTTATCTGTATCTATCGC	243	III	Mec 1
RIF 5	TTCTTAAGTACACGCTGAATCG	GTCACAGTAATTCCATCAATGC	414	III	Mec 1
pT181	CAGGTCTCTTCAGATCTACG	GAAGAATGGGGAAAGCTTCAC	303	IIIa	Mec 1
MEC A	TCCAGATTACAACTTCACCAGG	CCACTTCATATCTTGTAACG	162	All (internal control)	Mec 1, Mec 2
CIF 2	TTCGAGTTGCTGATGAAGAAGG	ATTTACCACAAGGACTACCAGC	495	I	Mec 2
KDP	AATCATCTGCCATTGGTGATGC	CGAATGAAGTGAAAGAAAGTGG	284	II	Mec 2
DCS	CATCCTATGATAGCTTGGTC	CTAAATCATAGCCATGACCG	342	I, II, IV	Mec 2
pUB110	CAGGTCTCTTCAGATCTACG	GAGCCATAAACACCAATAGCC	381	Ia	Mec 2

2.3 Microarrays

2.3.1 Nucleic Acid preparation:

Genomic DNA was extracted from 600 μL of culture grown at 37°C for 18 hours. Six hundred μl 50 mg/mL lysostaphin in phosphate buffered saline (PBS, 137mM NaCl, 10 mM phosphate, 2.7mN KCl) was added to the culture and incubated at 37°C for 30 minutes in order to lyse cells. Genomic DNA was then extracted using the phenol/chloroform extraction method as described elsewhere (89). The DNA was dried and resuspended in 100μl distilled deionized H_20 (dd H_20). at 65°C for one hour. Resuspended DNA was sheared to 300-500 base pairs (bp) by sonication with the Virsonic ultrasonic cell disruptor 100 (VirTis, Gardiner, NY) for 2 minutes 30 seconds. To verify fragment sizes, sheared DNA was separated on a 1.5% agarose gel in Tris Borate EDTA by electrophoresis at 10 volts/cm for 2 hours. Gels were stained with ethidium bromide for 20 minutes to allow for detection of DNA under ultra violet light. The concentration of sheared DNA was determined at 260 nm using the Nanodrop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE) and subsequently diluted to 111ng/µL in dd H_20.

For RNA extraction, cultures grown for 18 hours at 37°C were diluted 1 in 100 in 5 mL fresh BHI broth and grown to the previously determined mid-log phase. Optical density readings were taken at 600nm using 100 µL of culture in UVette disposable cuvette (Eppendorf, Germany) in a Biophotometer

spectrophotometer (Eppendorf). RNA was stabilized at an OD_{600} of between 400 and 800 using RNA Protect Bacterial Reagent (Qiagen Inc., Valencia, CA) according to the manufacturer's directions. RNA was then extracted from the stabilized cells using the RNeasy Mini kit (Qiagen) following the manufacturer's protocol with the exception that 50 µg/mL lysostaphin (Sigma, St. Louis, MO) in PBS was used for 30 minutes at room temperature in order to lyse the cells. Residual DNA was removed from the RNA by DNase digestion, using a DNAfree kit (Ambion, Austin, TX) according the manufacturer's directions. Messenger RNA was then extracted using the MICROB*Express* Bacterial mRNA Enrichment Kit (Ambion), again in accordance with the manufacturer's protocol. Briefly, magnetic beads that are coupled with oligonucleotides are used to specifically bind ribosomal and transfer RNA in the sample. The beads are mixed with the total RNA sample where the attached oligos bind the rRNA and tRNA. These beads are removed using a magnet and purified mRNA remains in the provided buffer. The beads are washed with a wash buffer provided in the kit and again removed using a magnet. The mRNA is then precipitated by mixing one tenth of a volume of 3M sodium acetate at a pH of 5.0 and three volumes of 100% ethanol with the sample and incubating for 30 minutes at -20°C. The sample is centrifuged at 13000 x g for 30 minutes at 4°C. The supernatant is discarded and the remaining mRNA pellet washed twice with 1 mL 70% ethanol, each wash being followed by at 10 minute centrifugation at 13000 x g at 4°C with the ethanol being discarded. The pellet is then dried and resuspended in an appropriate volume of ddH_2O.

2.3.2 Array production:

Staphylococcus aureus ORFmer PCR primer pairs were purchased from Sigma Genosys (St. Louis, MO). This set of primers represents all of the ORFs from the MRSA strains that had been sequenced at the time of purchase. This includes all of the ORFs from Co,I as well as ORFs unique to the strain Mu50 which is intermediately resistant to vancomycin and N315, the MRSA progenitor strain to Mu50, in total 2,731 ORFs. Polymerase chain reaction was performed using the manufacturer's guidelines using the appropriate template DNA. Products were confirmed using agarose gel electrophoresis. Various other *S. aureus* toxins and virulence genes not included in the ORFmer set were added to the array as 70 base pair oligomers designed and synthesized by Qiagen (See Table 4). The resulting ORF set was resuspended to a concentration of 100-200 ng/μL in 50% dimethyl sulfoxide (DMSO) and 700 pico liters of each product was printed onto UltraGAPS slides (Corning Incorporated Life Sciences, Acton, MA) using TeleChem split-pins (TeleChem International Inc., Sunnyvale, CA). Printing was performed by the DNA core facility at the National Microbiology Laboratory (Winnipeg, Manitoba, Canada). The ORF set was printed in duplicate on each slide, with the replicate set being printed immediately below first. Slides were checked for quality using the Paragon Microarray Quality Control Stain Kit (Invitrogen).

Table 4: Microarray probes designed and synthesized by Qiagen

Sequence Name	Sequence 5' to 3'
cadD, N315	ATTAGTTGGTACGGTTGCAATTGTTACGATAGCAAGTTGTGGCGCCGATAATATTGGTTTATTTGTTCCG
ccrA3	GGTACCACCTATTGTTTCGGCAACGAAATATGAACATGCTCAAGCAATCCGTAATAAGAAGCAACTTCAC
ccrB3	TCGTTCATTGTCCACAATGTGGTGCACCAATGGCAGCTAGTAACACAACGAACACATTGAAAGATGGTAC
ETA	GTGTTGATTTAGCATTAATCAGATTAAAACCAGATCAAAACGGTGTTTCATTAGGCGATAAAATATCGCC
ETB	CGTTGCAAGAGAAGCAGCCAAAAACCCATCGAATATTATTTTTACACCCGCTCAAAATAGAGATGCAGAA
MW0382	AGAGGAGTGTATTATGACTATATACACACACCAATCCTTGAAATCAAGAAAGGTAAAGAAGAACCACAAA
MW0383	GCAGAAGCAAGAGTTATTAAAGCAGATCATATTGGTGAATATGATTATGACTTTTTCCCATTTAAAATAG
MW0384	ATCAATTGAAAAAATATTCTGTCGGTGGCATCACGAAGACTAATAGTAAAAAAGTTAATCACAAAGTAGA
MW0385	AAAAGTTGATCACAAAGCAGGAGTAAGAATTACTAAGGAAGATAATAAAGGTACAATCTCTCATGATGTT
MW0386	TGGCAAGCATTACCTTATCTTTGATAAACATCAAAAGTTCACTAGAATACAAATTTTTGGTAAAGATATA
MW0387	AGATGCATTTTTAGTCATAGAAGAAGAACCTGTTAAAGGAAGACAATATTCAATTGGCGGTATAAGTAAG
MW0388	GACTATCTACTGTTGGTGGTGTAACGAAGAAAAATAACCAATCTTCTGAAACTAATACACCTTTATTTAT
MW0389	TATTTAGTGTTAGTGGTGTAACAAAGAAAAATGTAAAATCAATATTTGAGTCTCTAAGAACGCCGAACTT
MW0390	TTGTATAAAGGCGCGTCAGATAAAGGTAGAATCGTTATTAATATGAAAGACGAAAAGAAATATGTAATTG
MW0391	TGTTGGTGGTGTAATACAGAATAATAAAACATCTGGAGTTGTCAGTGCACCAATATTAAATATTTCAAAA
MW0394	TAAAATGAACATTATTGATGGACCACAACTTAATGTAGTTACTTTACTTGGCACAGACAAAGAAAGGTTT
MW0397	TATAAAGAAGGCAACAGGATATTATTACACGAGCAAAGTTCATGATGAATTTAATGAAAAAGAGCATCAA
MW0398	TTGGAATGTGTATAAGCCTGTTGATTGTAATCATTTTTGTAACATCTTGCGGTGGTGGTAATAAAATCAC
MW0399	TTAAAGGTAGCTCAGTTGGATATAAGCAGATAGAGTACATGTTTTCTAAGGAGAAAGAGGATGAGACTTT
MW0400	AACGTTAGACATGTATCCTACTGAAAATCTAGAAGACTTTTATGACAAAGAGGGATATCGAGATGGAAAA
MW0401	GAATAGAAAACTTTAAATTTTTCGGACAATATGCAAACCTTAAAGAATTGAAAAATTACAACAATGGTGA
MW0758	CAAACCGATTAATACCGATAGTGTTAAAAGAATTGATTTGTCAAGAATAAATCCAAACGGAAATAGGTTT
MW0759	GTAAAACTTGTATGTATGGAGGAATAACAAAACATGAAGGAAACCACTTTGATAATGGGAACTTACAAAA
MW0760	ATATATGGTGGTGTTACTAAAGCAGAAAACAATAAATTAGATTCGCCAAGAATAATACCTATAAATTTAA
MW1937	GGCAAATCAAAATTTAGATAAACCTAGAAATATACCTATTAATCTCTGGGTCAATGGTAAGCAAAATACT
MW1938	AAGTTTCGACAAATAAAAAATTTGTTACCGCTCAAGAGATTGATATCAAATTAAGAAGGTACCTTCAAGA
rep, N315	AGTTCGAAAATCGAACGTCCAGAAGTTCGAAAATCGAACGTCCAGAAGTTCGAAAATCGAACACTAATGA
SACOL0007	GAATGGGAACGATTAAGTGGTATTCCTATTGAGGAACAGACATATGAGCGTAATCGTGAAGCAGTTGATC
SACOL0054	AAGCACAGCCTACATATCGCCCAATAAGCATACATGGAGACGTTTTGGTAGAGATGCAAACGCAAAAGAA
SACOL0057	TAGTAAATATACCAATGAAGTTTCAAAAGAACAATTCCAAGAAATTGAGAATGTAAATAATAAGGTCAAA
SACOL0072	TATTAAAGAAGAAATTGCTAATTTCAAATCGGATATTAGCCTTATCACAGACGAAAAGATTATTAATAGT
SACOL0076	ATGTTCCGGAATATCTAGTTTCTAAAGAAGCTTCTGTGCAGTCTGTTGCAGACAACGCCAATCAATATGG
SACOL0080	GAAGTGCAAAAGGATTTTACTATACTAATGAAATAAAGACGGAGAAATACGAAGTAGCTCAGGATAATCA
SACOL0093	AACATTTTCAGATTGGAAGCGTCTGAACATAAAATTCAAAAACGAACGCCTAAAGAAATTGTCTTTGCTT
SACOL0095	AAGCGGATAACAAATTCAACAAAGAACAACAAAATGCTTTCTATGAAATCTTACATTTACCTAACTTAAA
SACOL0101	ATATGGAGCGTGCAAGTGGCGTCATTATTTTGAATGATCCAGAAACGAATTATCCAATTGCAGTTATGGA
SACOL0112	ATTAACACACCCAAGATATTATAAAACATCACAAAAACACCACTATCTAATTTATCTCAATAAAAATTCA
SACOL0113	ATTTCAAAGATGTTCGATTCATTTGAGCATAACAAGCCATTTACATTTTTTGGTGACGGACTGCAAACTA
SACOL0126	GAAGCAATGACGGCTGTTGGCGCTAATAAAATAAAATGGATTGTTTTCGGTGTTGTACCACAAGCCATAT
SACOL0142	AAATCATTAATGAATGCGATAAATGATATTGCCAAAAAGTATAAAATGCCTGTGATTTATTCAACGCATC
SACOL0147	GCTGACGTATTAAAAATTGCGGATGTATGTATTGCATCGCTGATTAAAGAAGGCGTCGGTTTAGGCGTGC
SACOL0161	AAAGGACCCAATAGTATTCGAGTGTCGTTTAAAGATAATTGGGCGATTGCACATATGACAGGTGTTTTGA
SACOL0237	TACGTTGTGATGGCACATGATCGTATCGTTCCGCTGCCTAATGACATTGATTTGAGTACGATTTCATACA
SACOL0248	CCTCAAGCAGCAACTACAGCGATTGCGTTACCAGTATCAGCTGGTATCGGTGGTATAAAAGAATTAACAT
SACOL0249	GTTTTTAAGGAGATTGCAACGCCACCTAAATCTGTACAAGATGAGCTCCAATTAAATGCAGATGATACCG
SACOL0261	TTTAAATAGCGGTCTAGGCGTAACAGTGGTCTTTTTCATATATGCTCAAACACACCTTGGTTTATCAGCT

SACOL0316 ATAACTTTAGTCATCACATTTGTCGTGACTGCAATTACTGCATGGCTACCGCCAATCAGCAATGAATCAA
SACOL0318 CTATTCACAGAATCAAATGTAATGCTGGATTTGGTCACAAAGATACTACGAAGACCGCAGGTTCAAAAAG
SACOL0392 AAGTGATTGATAAAGAAGCACCTGACAATTTTATTTTAGGATTTCGAGCAACGCCAGAAGAAACCAGAGG
SACOL0393 TTTTAAAATTAAACTAATGAATGGCATTTGTAGGTCTGAAAATATGAATATGAAAAAGAAAAATAAAGGC
SACOL0394 CGCATACGTTAGGTGTCCAACCACACATTGATCATTTTCCAGAAATGTGGTTATTAAGTAGTAGCGCAAC
SACOL0409 CGGGAAAACAAGGTGTGCCAATGATGATTACAACCCTTGGTGGCCCAGCAATGAACTTTAAAGGTTCTAT
SACOL0410 GACAGCAGGTTCTAGTAAACATTATTTAATTCCTGAAATGCATTTAAAACCAATATTGAGTTACATGAAA
SACOL0414 CTCTGAACCAGTTGCAGAAGCATTTGGAGATTTAGATCCTAAAATTGATGCACGTCTTGCAGATATGAAA
SACOL0433 TGCCAGTGCTGGTGTCGTAGGCTTAGCAGTAGGTTTTGGTGCTCAAACTATAGTTAAAGACGTAATTACA
SACOL0438 TTGTTACTGAAGTTGTGTGTGATAGCGTTCAATTCCTTGAACCTAAAAATGCGCAACAAAATGGTGGCCA
SACOL0477 CGACACACTGGTATAGAGAAGTTTCTGGAATTGCAGTTGAGGGTGCAAGAAATCACGGATTATTAAATGT
SACOL0481 TTTTAATATTTGCGATAGCAGGTTGTGGCAAAGGTAATGAAACAAAAGAAGATTCAAAGGAAGAACAAAT
SACOL0482 GCCAAAGGGCAAAGTGATGAAAACAAGAGGTATGCAATTATATATTAATAGAAATACCGAAACAGCCAAA
SACOL0501 CGCATCAATCATCTCCAAGAAATGAATTTTGAAGCGGTAATATCAAATCCTTGGTTAACCGTTCTAGGGC
SACOL0511 ATGCGTGTTGGTAGAGAAATTTTAGCGACATCTGCGAACACGATTTACCTTGCCTTTTTTGGTGGCCAAC
SACOL0519 GGACAAAAATTAGGTCGTGGCTTGGTTCAAGCAGTAGAAGAGCGTGCCAAAGCACAAGAGTATAGTACGG
SACOL0527 TGCTTGGCGGTATCGGACCTTGTGGTAGGTCGTTATGTTGTTCTACATTTTTAGGGGATTTTGAACCAGT
SACOL0536 GATGGCTACGTGGTGATGATGCAAAAAGAAGTGGGCGAACGCTTAAATGCTGAAGTAGGTTCAAAAGCAT
SACOL0543 GAATATTACCTCCCTGATGTATTGTCGTTAATTTTAAATGATGGCGGCATCGTAGAAGTCTATCGTACCA
SACOL0549 ACGATATCCTGATGATTACCCTGTTCAAATTGTCACTGGTGCACGAAGCGATGGTGCGGATAACGTTGTG
SACOL0551 TGTTGTTTTATCAATCTTGCTTGTTGTCCAAAAACATCGCAATGATATTGATGCACAGGAGCGAAAAGCG
SACOL0560 CTCACAGTATTACAACTGTTGGAATGTTGTTTGAAGACAGAAGAATGTTTACACCGTATTAGAAAGGAAC
SACOL0565 TATGTTTGGTACATGCGCAGGATTAATAGTTCTAGCGCAAGATATAGTTGGTGAAGAAGGATACCTTAAC
SACOL0566 GTTTTGTCTTTGCACCATTTGCATTCTTAGTCGGTGTGCCATGGCAAGATGCAGTTCAAGCCGGTTCAGT
SACOL0582 TTTAGTGGAATTAATCATGACAGATGAATCATGGTATGTGGTAAGAAATACACCAGGCGTTACTGGTTTT
SACOL0661 CTCGTGTTTTTTGGTTTTGAGGTGTTGTTTGTATTTTATAAAATGGCTTACATATATGAAGCGTTGATTA
SACOL0667 GTTGGCGATGATGCGTTAAATGATGTAGCTCCAGCACGAGCTATGGGCATGGTTAGTGTATGGTATAAAC
SACOL0699 TACGTACATTTGCACCAACAAAGTATAAAGACCAAGAACGTACTGTAACGACTGCTAGAGACTATGCCAT
SACOL0706 TAAGCAGAGAACGTTTAATATTATTCTTTATCGCAGTGATGTTATCATCTGCTGCTGTAGCAGTAGCAGG
SACOL0723 TGCCTAACCAAACATTACAAATTCCTAATGGTGGATCAGGTGGTACAACACCAACAGCTACAACAGGTAG
SACOL0732 CCCCTTTTAATTTATTAATTAGGGGCTCTTTTGCTGTTGGTGCATTAGCAACCAACTGTATTCCTTTGTC
SACOL0739 GTTGGCGCTTGTTGCCAGAATTTTGGGGAAAAGGATTAGCAACTGAAGGCGCAAAGGCAACATTGAAGTT
SACOL0743 CAGCAGCATGGGTGTTCCGCGAACGCTTCTTAGAGATTTTACATATTGGTAAACACAAACATGTTGAAGG
SACOL0763 AATAGGTGTATGTAATTTTAATGTTCATCATCTAGAAAAATTAATGGCTCAATCAAGTATCAAACCAATG
SACOL0776 CCAGCTGGTTCTGTAGTACTTGAAGGGAAAAAATGCGGTATTGTAACTACGGATACAATTAATGATTGGT
SACOL0781 TCCAGTATACGAAATGCGTTGGGATATTGGATACGTATTGCAGCAGATTGCATTATTCCCACATATGACA
SACOL0783 AAAGTCGAACTTCCGATAGCAATGCCTGTTATAATGGCTGGTGTAAGAACGGCTATGGTATTAATTATAG
SACOL0788 TTGTTAATGTTAAAAACTTCCACGGCGGATTCTTGATTGCAGCAGTTGGTATGGCATTAGGTTTAGTATG
SACOL0794 ATGCTATTTATATTGGCAGTAGTTGGCGGGGCCCCAACACAGAAGCAGGCGGAAAGTCAGCTAACAATAT
SACOL0796 ATGTCATTTTTGTACCGCTTTTAGGAATTATGATGGGTGGGATTGTTGCAAGTTTCACAACCTTCATCTC
SACOL0800 AATGAGTTTGAAAAGCACGCCATTAAGCGTTGGTCTGAGCCTTCATTTAACGAAAAAGCAATCTTATGCG
SACOL0809 CGATCCAAAATCACAGCGTAAAGTGTTTAAAGATGCAGACGATATGGTGCATGTAGCCAAAAATCAAGGG
SACOL0814 TTGTGCCAATTCCTTCTTCGCCGGCACATGATTTATCTAGAACATTTAACCCGGTAGAAGCAGTACTAAA
SACOL0818 CAATCCGGATGATATTACAGTTGATACATTCAGAGCTTCTGGTGCAGGTGGTCAGCATATTAACAAAACT
SACOL0822 TACTATTCTGACATTAATTTTAATTGGTGCCTCAATCTATAAAGAGTATTTCCATAACGAGTGGATTATT
SACOL0829 AAACGCCTATTCGTTATCGGTGGTGGTGATTCAGCAGTAGAAGAGGGAACATTCTTAACTAAATTTGCTG
SACOL0841 AATGAGGGCTTAACTGACGAATTCGTAGTACCATTCATCGTTGAGAATCAAAATGACGGTGTTAATGATG
SACOL0879 GATGAGGACGTGCTTGCATTGTGGAATAAAGTCAATCCATCCTTCGCGCTAAAATCAATGTTTGGTGGTT
SACOL0898 TATACAAGTGGTCAACATCCCAATGTTGTGTTTTGGATTGGGTGGAATCAAGAAGCGTTTGATGTTATCG
SACOL0900 TAGAGGGGCATTATGTAGGTTATGAATTACATGACGGTAAACAGCTTTCTATTAGCGATATGATGGCCAG

SACOL0908 ATGATCCCCCAATTTCAGCTAAAGTTGGTGAAAACTATAATGGCAACAAGGGGTTAATGCTAAATCAGAG

SACOL0917 TGCATCGATGATGACACAAGCTGTTAAAGGTCATTCACTTGGAGAAGCAATGCAAATGAGCCAAGAATTT

SACOL0919 CATGATTGTGGCATTAGAGTGCTGGTCTTTATTAAATTAATTGAAAGCTACATCAAATATTCTTTAGATA
SACOL0921 ACGAAGAAGTTTATGAAACGGTTATGAATAAGCCATACACTAGATATCCAGTGTACGAGGGAGATATTGA

SACOL0923 TACCAATTTAATCAAACGAAAACATCAAGCTGAAGATCGCCGAAAGATTTTAATCAAGCAAAAACATCAA

SACOL0927 AAATTTTACCATCAGATATGGGCGGGGACTATGATGCGTTAGAAACATTAATGGCGTCAAGACCTGACAT
SACOL0930 TGTAAGGTAAAAGAGGGATACATGCACCGAGCGCAAGCAAAAAAGCCCCTAACAACTAAAGGTTGTAAGG

SACOL0954 CTTAGCAGGACATTATACACCTGGTGGCGGTTTCGTCGGCGGATTATTATTCGCAAGTTCACTTGTTATC

SACOL0972 CATTCTGCTACAAGGACTTTGCACTTAATCGAAATTATTTTTTATTCTTTTGAAAATCAAAATACTATAG

SACOL0990 GAGGGGATTAGATGGGGAAATATATTTTCAAACGATTTATTTATATGCTTATTTCTTTATTTATTATTAT

SACOL0994 GCCCGTGTATTAGCCGTTGAACCAGAATTCATTATCGCGGACGAACCAATATCGGCATTGGATGTTTCAA

SACOL1027 AAATGGATTTTGAAGAATGTGAATCAAAAGATGCGATATAGTATTAAGAAAATGTGCCTTTTATATTTAG

SACOL1037 GCATTACATTTAATGTTAAGTTTCGGTATGTTTATCGTCACTTTCATTGGTATAGTAGTAGCAATAATAA

SACOL1050 AAGAAGTGAAATGGATGGCTACGGGCGTATTAGAGGACGATATTGTCAAAGAGATATATGGCAGCTCAAA

SACOL1062 GCAAAAGATTTAACTGCACCAACTGCTGTGAAACCAACTACATCAGCTGCCAAAGATTATAACTACACTT

SACOL1076 CCACAAGTATTAGATACGCAAGGACAAACGCTTACTCGAGCTGTACATGACTTAGGTTATGCACAAGTGA

SACOL1095 CGTAGGATTCGTAGGTTTCTTTCCAGAGTCAATCAAATATTTAGGCACGGTATTGTTAATACCAGGTTCA
SACOL1097 TGAACGAAAATGCGCTTGTAACAAGCTTTTTTCAATTCTAGTCAGGGGCCCCAACATAGAGAATTTCGAA

SACOL1150 TGGTCCGTTGACTGTGTGTGCTGCTTTCGTTACCAAAGAGCATGTCCCTATTTTAAAAACTTTGGGTGTA

SACOL1156 CATTTAACGTTCATAGATTTATTAATCCCACCATTCTCAAGTCGTAAACAATATGATGACTTATCATTCA
SACOL1170 CCAACAAACAATGCAAGTTGGCGGGGGCCCCAACATAGAAGCTGGCGAAAAGTCAGCTTACAAAAATGTG

SACOL1261 TCGAAAAAAGTACCTGTTACGAAACGTCTAGAATTAAATGAAGCACTAAAAAATGAAGTTACTGCTTTTG

SACOL1262 ACTGAGATTGAAGAAGTTGCTGCGAAGACTCCTGAAAAGATCTTCAAAGAAACTATCGATCCAGTAATCG

SACOL1309 GTTCTGGCCGTCTTTCAGGATATATTAATTCTTATGGCGTTCATTCTATTCACGGACGTGCATTACCTTT

SACOL1335 TTAAGACAGGGCAAACGCGAAGTAGATAATCTAACCCTGAATACAACAGAAAAATTATATGAATATGCCA
SACOL1355 TACTTTGAAAAAGCAGTTGTGAATGATGTGGATGCATATGTTTTAAAAGAACGTTCTATAGAAGAATTGG

SACOL1358 ATATCATGATCAGTTTAATTCATCAAGAAAGTCTATCTATACTTTTACAGAAACAGTTCAACCAAATCGT

SACOL1372 TGTTATTGGCGCAATGATAATAATTGATAAAAACGCAACAACAGTTACTATGAATTTTTTAATTTACATC

SACOL1379 ATTCGCAATTGATAGAGGCTATTTTCCAGATATGGAAATGGCCTCTTTTTATAATCAAATTAATAAGAAT
SACOL1385 TTGAAAGATATTTGGCCATCAATTAAAGAAGTTTCAGATACCGTTGATAGTGTTGTAACACCTGAATTAT

SACOL1397 GACCGTGGTCCTCAATATCAAACAGCTATTTTCTACACTAATGATCATCAAAAAGAACTCGCTGAGACTT

SACOL1410 GTCATTTCACGCAAACTGTTGGCCACTATGAGTTAAAGCTTGCTGAAGGTTATGAAACACATTTAGTGGG

SACOL1412 TATTGATGATGATTGGAATTATGCTGCACAACTTGACGCTGAGAACGCGATTTTTGAGCGTTTAGATCCA
SACOL1415 TCGTAAGCATTATAAAGGTAGTAAAGAAGAGGCTTTGTCCAAAGTTGATAAGGCTTTGTCGTGGGTTAAT

SACOL1430 TTTAGAAGAAGTGAAAAAGCGCATTGATACAAATTCATTTGCATTATATAGTGGCAATGATGACAACGTC

SACOL1486 GAGATCAAATTACATCGTGAATTTATTATAGAAGTACCTTATATGAATTCAAGGAAATTTGAGCTACTGA

SACOL1512 GATATTCCAGATGTTGCCAAAGTGGCATGCCTCACAATTGACACATCAATGAGACACCTTGATGACATTA

SACOL1519 CCAAATCATGGTCCGCTAGGTGTATTGACAAAGGATCGTGTGCAATTCCATCATATGCCATATCGCCAAC

SACOL1521 CTCGAATATTGGCATATCAATTTAACTTTTTAAATAGTCATCAAAAAGATAAAACACCACAATCAACAAA
SACOL1523 TCTCATCGAAAAAGATGTGTCTGAATTTAGCGCAACTTATTTATGATTCAGGTTTTCTTACAGGTATTTA

SACOL1588 AAAAGCAGGCATGACTGAAAAAGAATTAAAGGCAATATTAGAAAGCAAAATGCTAGAATTAGGAGCAGAT

SACOL1616 AAACGTATTGCAATTATTGGTGGTTCAGGTATTGGATATGAATATCAAGCTGTCCAACAAGGCGCAGATG
SACOL1622 AAACTATAAAAACGTGCAACGTTCAATGCGTAAAAAATTACCATTTGGTATCGGTCAAATTGGTAAATCA

SACOL1634 TAGCTGTGAGCATGGAACGTTCAATTGTTAAATTAAACGAGGCAAAAGTTGCCAAAAAGATAGAGCGTTG
SACOL1635 ACTACAAGTATGTGTTTGAAGGCAATAGAAACATATGTATTGCCACAGCATTCAGTAATTGATGTTGGTA

SACOL1738 GTTGGCTTTCATATGGCCTCATTAGTATGGGCATCATTTGGATGATTATGCACATTACACGTTGTAAAGC

SACOL1780 CTTACTGCTGTTTTTTAATATTTGGATTCATTGTTGTGGTTACTTTAAAAAGTGAGCATCAATTAACGCTT
SACOL1788 CGTAAAGCTGATGAAAAAACAACTAATAATACAGCTGTTGACAAGAAATCAGGTAAACAATCTAAAAAAA

SACOL1836 GCTGTCAAAGTGCTTGTCGGTGCTGAACAATTAGACGAGTTCTTTAACAAATTTGTATTCCATGGTCGTA

SACOL1863 CACCAACTCAATTGTTGATTGGTTTTATTTATGTATGAATGAACAACTTTTTGACATCATTAAGAATATA

SACOL1893	GGAACACATTGAGATGACAGCAATGGCATATGATATTGATCGTGATGAAACGATGAATCGAGCAATGCCA
SACOL1898	AAAGTTAGAGAATTGAGTGGTCCTGTTGCGACGTCGTATACAGTCGAAGGTAACTTATTAGGACACATCT
SACOL1917	ACGGACTAGGAAGTGGAGCAGCAAACCTAGGTATAGTGGCTGCCTGTGTTACTTTCTTATTTGGCTCATT
SACOL1920	TGAGGCATTTTTACGACGTTGCCCGAATTTAAAATGGATTGCATGGTTTGCAACGGGTGTAAATACATTG
SACOL1998	GGTTATTATTTACAAGCAACTAATCATGAAAGCCAAGGTATAAAATTATTATTAGCAGCGATTATGTTTA
SACOL2002	CTGTTAAAACAGGAACTAAAGCGAAAGCAGACAGATATGTACCATACACAATTGCAGTAAATGGCACATC
SACOL2005	GGTAATACAGTGAAAGTAGTCGATAGTGCTAACGTACAAATTGTGATATTTTTACATAGTTGTTTAATCA
SACOL2016	GTTAGGTACTGCAAGTAAAGTAGAAGTAACTAAAGATAATACCACTGTTGTTGATGGTGACGGTGACGAA
SACOL2020	TTCCTAAAAGTTGGAAACTTATTGCTCAAATGCCATTTGGTGATATTCGTGAAGCTGCTGGCGAAAAAAC
SACOL2031	GATTAACAAACTCGGTGTATTAGATTTCGCTGGAGGTACGGTTGTTCATATTACATCAGGTGTTTCTGGT
SACOL2076	AAACTATTGTCATCATTAGGTGTGAATATTAAAAAAACTAAATCTCGTTTAGACATTATTAATACTTTGC
SACOL2087	TACTAAGGAACTACTAGGGGAGCCTAATGATATGGCTGAGATGAATTGTTCAGACCCTTATGACCTGATT
SACOL2116	AAAATGTGTGATTGGTTTACCGGGAGGATGTCCACTTGGGCCACGTCCAATTGATCAACATATTAAAGGA
SACOL2180	TACAGATGAAGATTATGCCATTTTAGATGCAGCCAAAGATTTAAACGACTTCTTAGGTATCAATTACTAC
SACOL2187	AAACGTAATCAAATTGAAAGCCTTTTCTCAAGATTTTCATAACCTACAAATAAATATGTCACATTTTAAG
SACOL2357	TAAAATAAATGACGTTCAAGTTGTGAGTGAAAAAGATTTAACAGGTAATATTGCGAGTTATCAAGCAGAG
SACOL2393	TGATTATTGAGGATGGAACATATGTGATGGCGAATGCACTTGGTGAAGATAACAATCCATATGCCTATGT
SACOL2414	GACTGCTCGTCGTAATTTAAATAGCCCTAACATTAAAACAAGAAAGCGTGCTTTAAAGATTATTAAGCAA
SACOL2420	TACATTAAATTTAATAATAACAACTGATTTTTATAAGAATAAAGTATCGAACCATAGTAGATACACAAAT
SACOL2479	GTCTGGCTGTTCGGATTTAGTGCAATGCGCAATTCATGATTGCCAATACTATGAAGTTCAAATGCCACAA
SACOL2527	TATTAAACGTCGTCCAAATTTCGAAATGGAAGAACGTCTTGTGCTTGAAAAGGTTAATTATGATACAAAT
SACOL2536	ACATACTTCTGAAGCGTTTAAAGAAGATACGAGATTTTATTATCATCAGTTAAAAGAAATTGACAGTCTA
SACOL2568	TCATTTAAACAACTGAGTCAAAGTCATTTAGATAGCGGATTTATTTTTTTCTTCATAGTTTATGTGTTGT
SACOL2570	GGCGGTCATGTAGCCGTGTTACCAGGGGTGACGATTGGAGAAGGCAGTGTGATTGGTGCTGGTAGTGTTG
SACOL2607	GCGACTTCTGACAACAAAGACACACAGCAACAAAATCAACCTGAAGAAGATGAACGTCAAGGTTTGAATG
SACOL2619	ATCAGGATTCTCAGCGAATTTACGCGGACTTGTGAAACCAATTGGCATCGAATTACCTGCAGCATTATCA
SACOL2649	GTTATTTTAACATTAGTTGGCGGTTTATACTTCGCATTTAAAAAATACCAAGAACGTGTTAACCAAGCAC
SACOL2657	CGGCGATGAACTTGTTTTATCTAAAGATGTCTTGGCTATAGGCGTTTCAGAACGTACATCTGCACAAGCT
SACOL2673	ATATCGGTTCATCTGAATATGAACAAGGTATTATCAATGTTATTGACGAGACATTAGAAAAGCTGAATTT
SACOL2677	TATGTCGTGGTATTTATATTTTTAAAGAAGAATCTTTTAGAATTACACATATATTACACTAGGTTTATTG
SACOL2692	ATACCGTGCTACACTATTATCCATTAAGTGAAAATACTATAATATTCGGATGGATTTTTTATTTCTTCTT
SACOLP031	CTGGAAGTCAAAGCGACGAAAGTACACAAGGTGAAGAAGCAAAAAAAGAAGACGTTCCAGCTAATCTAAA
SAP031	CTGGAAGTCAAAGCGACGAAAGTACACAAGGTGAAGAAGCAAAAAAAGAAGACGTTCCAGCTAATCTAAA
SAV1970	ACGGCATTTAGTTGTTGGATTAAAACACACAAAATTCAATGGTCGACGTTGGTACTTAGATGATTATGAA
SAV2213	AAAGTAAGACCATCAGTAAAACCTATTTGCGAAAAATGTAAAGTCATTAAACGTAAAGGTAAAGTAATGG
SAV2596	TATGATTGGTAAGGACATTGTATTGACAGCGGCTCATAATGTCTATTCTAAAGACGATAAGGGTTGGGCT
St_En_C1	AGAATGGATAATGTTAATCCGATTTTGATATAAAAAGTGAAAGTATTAGATATATTTGAAAGGTAAGTAC
St_En_D	ATATAGCATTAATTGTTATGGTGGTGAAATAGATAGGACTGCTTGTACATATGGAGGTGTCACTCCACAC
St_En_E	CAGACAGCTTTGGCGGTAAGGTGCAAAGAGGCTTGATTGTGTTTCATTCTTCTGAAGGGTCCACGGTAAG
St_En_H	ATTGGCACAGGAAAGGGTGATTGGTGCTAATGTTTGGGTAGATGGTATTCAAAAAGAAACAGAATTAATA
St_En_J	TTAACGAAAAGGGTATCTCTGAAAAGATAATGACAGAAGATCAATTTTTAGATTACACACTATTATTTAA
St_En_K	TTGATGTTTTTGGTATTAGTTATAATGGCCAGTGTAACACTAAATACATATATGGTGGAATCACAGCTAC
tetK	TGCTGCATTCCCTTCACTGATTATGGTGGTTGTAGCTAGAAATATTACAAGAAAAAAACAAGGCAAAGCC

Notes :
St_En : staphylococcal enterotoxin
SACOL : ORFs from *Staphylococcus aureus* strain Col, annotation from http://www.tigr.org Comprehensive Microbial Resource, numbers are analogous to SA numbers from the Sigma Genosys *Staphylococcus aures* ORFmer set.
MW : ORFs from *Staphylococcus aureus* strain MW2, annotation from http://www.tigr.org Comprehensive Microbial Resource
SAP : ORFs from *Staphylococcus aureus* strain N315, annotation from http://www.tigr.org Comprehensive Microbial Resource
SAV : ORFs from *Staphylococcus aureus* strain Mu50, annotation from http://www.tigr.org Comprehensive Microbial Resource

2.3.3 Comparative Genomic Hybridizations:

2.3.3.1 Target DNA preparation:

Sheared genomic DNA was labeled with deoxycytosine triphosphate (dCTP) coupled to Cy3 or Cy5 (Amersham Biosciences, Piscataway, NJ) using random primed labeling. This was accomplished with the Invitrogen Bioprime CGH kit (Invitrogen, Carlsbad, CA) following the supplied protocol. Briefly, genomic DNA was denatured and allowed to anneal with random hexamers. The Klenow fragment of DNA polymerase was then added, along with dATP, dTTP, dGTP and dCTP coupled to either Cy3 or Cy5. Labeled DNA was then purified using columns provided in the kit. After elution from the column, Cy dye incorporation was checked using the Nanodrop 2000 spectrophotometer (Nano-drop Technologies) in the Microarray setting. These measurements were used to determine the picomoles (pMol) of Cy dye incorporated per nanogram (ng) of DNA in the sample. Values over 0.020 pMol/ng were used in the experiments. Labeled DNA was then concentrated, with 100 μg of yeast transfer RNA dissolved in ddH_2O, to a volume of 5μl using Microcon YM-30 centrifugal filter devices (Millipore, Billerica, MA). Labeled sample volume was adjusted to 400 μl using distilled deionized water (ddH_20) and applied to the filter device. Filters were spun in a micro centrifuge for 10 minutes at 8000 x g. Filters were then inverted onto a new tube and the concentrated sample removed from the filter by centrifugation for 2 minutes at 8000x g. The volume of the target DNA was adjusted to 55 μl using prehybridzation buffer (3x saline-sodium citrate buffer

(SSC, Sigma) 0.1% sodium dodecyl sulfate (SDS, Sigma). The target was then boiled for 1 minute 30 seconds and applied directly to the microarray.

2.3.3.2 Array preparation:

Arrays were blocked in prehybridization buffer containing 0.1 mg/ml bovine serum albumin at 50°C for one hour. The slides were then washed by dipping in ddH_2O to rinse off any SDS and a final rinse was conducted in 100% isopropanol. Slides were then dried by centrifugation. The 55µL of labeled target from section 2.3.3.1 was applied to the array and covered with a HybriSlip coverslip (Grace Bio-Labs, Bend, OR). Arrays were incubated at 55°C overnight in ten slide hybridization chamber (Genetix, UK) containing 2 mL prehybridization buffer to keep the chamber humid.

Hybridized arrays were removed from the chamber and immediately dipped in 55°C wash buffer 1 (2x SSC, 0.1% SDS) in order to remove the coverslip. The slides were then washed in wash buffer 1 for 5 minutes at 55°C. Slides were then removed to a 10 minute, room temperature wash in wash buffer 2 (0.1x SSC, 0.1% SDS). Residual SDS was then removed by four one minute, room temperature washes in 0.1x SSC and a final rinse in 0.01x SSC. Slides were dried using centrifugation and scanned using the Virtek Chipreader (Virtek, Ontario Canada).

For each strain, a total of three hybridizations using three different biological replicates, were completed. Each hybridization consisted of the sample strain labeled with Cy3 or Cy5 and Col labeled with the opposing dye. One of the three hybridizations was a dye swap in which the dyes used to label the control and test strains were inverted.

2.3.3.3 Data Analysis:

Images generated from the Virtek Chipreader were imported to the ArrayPro Analyzer software package (Media Cybernetics, Inc., Silver Spring, MD). This software was used for spot finding and to generate fluorescence values for each spot. Data was filtered to only include spots whose fluorescence on the reference channel, the channel representing Col, was greater than two times the background fluorescence. Data that was filtered out was eliminated from any further analysis. Filtered data was then exported to Microsoft Excel (Microsoft, Seattle, WA).

Raw data from ArrayPro was edited in Excel to meet import requirements for the program Genomotyping Analysis by Charles Kim (GACK) (55). This program works on the principle that all array data has essentially the same distribution, a normal curve with a left tail. The left tail represents the spots most likely to have a negative result. By plotting the distribution of the array data and estimating the normal curve it would represent with no left tail, GACK calculates the estimated probability of presence (EPP) for each data point. This EPP value

can then be used to determine a positive/negative cutoff value for the data being analyzed. GACK requires a unique identification number for each spot, provided by ArrayPro, a gene identifier and the logarithm to the base 2 (log_2) of the ratio of the sample fluorescence to the fluorescence of Col. Data was then imported to GACK and analyzed using a bin size of 0.10, no data smoothing, normal curve peak modeling and generating a binary output with either a 0% EPP or a 100% EPP cutoff. Bin size determines how the program organizes your data in order to predict a normal curve, data smoothing can be applied to poorer data sets, but is not recommended and was not required in this case. Normal curve peak modeling means that the data is used as is to draw the normal curve, the other option, positive side peak modeling, uses only half the data to estimate the normal curve that is be represented by your data (55). The file generated contained binary data representing the absence/divergence, denoted by a 0, or presence, denoted by a 1, of each ORF in the original data set. This data was then imported to GeneMaths (Applied Maths, Belgium) using the GACK_read_data script provided to us by Applied Maths. GeneMaths was used in order to sort the data, and to insert null values wherever bad data had been filtered out. Once all three hybridizations for a strain had been imported into GeneMaths, the data was again exported to Excel. In excel all spot replicates were aligned and the following criteria applied. For any ORF, at least three of the replicates had to contain data, if more than half the spots were ones, the ORF was considered present. If more than half the spots were zeros, the ORF was considered absent/divergent. If the one and zero calls for one spot were equal,

status of that ORF was considered unknown. Once all the hybridization data had been combined, it was saved as a .txt file appropriate for import into a new GeneMaths database using the read_list script provided with the program. This database was used to draw a dendrogram , using unweighted pair grouping method using arithmetic means (UPGMA)(26), and the Euclidean distance coefficient to calculate percentage similarity between strains. These settings were recommended by the manufacturer for our specific data. The database was also used to query the data for similarities between the ORFs in each strain.

2.3.3.4 PCR validation of ORF SA2196:

In order to confirm the microarray results for the ORF SA2196, the primers for that ORF provided in the Sigma-Genosys *S.aureus* ORFmer set were used at the concentration recommended by the manufacturer. Polymerase chain reaction was carried out in a 50 µl solution containing 0.4 ng/µl template DNA, 3 mM $MgCl_2$, 0.2 mM each dATP. dCTP, dGTP and dTTp, 1x concentration of AmpliTaq Gold PCR buffer (Applied Biosystems, Foster City, CA) and 0.5 units AmpliTaq Gold (Applied Biosystems). Amplification was performed in a 96 well Gen Amp PCR System 9700 thermal cycler (Applied Biosystems) with 10 minutes at 94°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C and one minute at 72°C followed by a 5 minute extension at 72°C. Resulting products were electrophoresed on a 1.5% agarose gel in 1.5x tris borate EDTA at 10 volts/cm for 2 hours. Gels were stained with ethidium bromide for 20 minutes for viewing under UV light.

2.3.4 Expression Arrays:

2.3.4.1 Target preparation:

Messenger RNA from each strain was converted to cDNA and labeled using the Invitrogen SuperScript Indirect cDNA Labeling System (Invitrogen). This kit uses the reverse transcriptase enzyme Superscript III to convert RNA to cDNA. Amino allyl dNTPs are incorporated into the cDNA during the synthesis. Cy dyes are coupled to the amino allyl dNTPs in a chemical reaction using calcium carbonate. The manufacturer's protocol was followed with the exception of the elimination of the anchored oligo(dT) primers and the doubling of the random hexamers for the cDNA synthesis. This step was taken because prokaryotes do not have a poly adenine tail on their mRNA, so poly T primers were not able to prime the bacterial mRNA. One vial each of Cy3 and Cy5 Mono-Reactive Dye Packs (Amersham Biosciences) were resuspended in 35µL dimethyl sulfoxide provided in the kit and 5 µL was mixed with the cDNA, which was resuspended in 5 µL calcium carbonate buffer also provided in the kit. The mixture was incubated for one hour at room temperature to label the cDNA. Labeled cDNA was concentrated using the method described in the CGH section. The volume of the target was then adjusted to 55 μL using DIG easy Hyb (Roche), a commercial hybridization buffer prepared according to the manufacturer's directions.

2.3.4.2 Array preparation:

The array surface was blocked, washed and dried as previously mentioned in Section 2.3.3.2. The entire volume of labeled cDNA target was applied to the array and covered with a HybriSlip. Incubation and washes were again carried out as mentioned in section 2.3.3.2. Slides were submitted to the DNA Core Facility (National Microbiology Laboratory, Winnipeg, MB, Canada) for scanning on the Agilent G2565AA Microarray Scanner. Two experiments, each being a dye swap for the other, were completed for each strain, generating four data points for each ORF represented in the array.

2.3.4.3 Data analysis

Resulting images were imported to ArrayPro for spot finding. The fluorescence values generated were then exported to Excel (Microsoft Corporation). Spots containing calibration controls, blanks or failed PCR products were filtered out of the data. In Excel, data was was formatted appropriately for import into Partek Discovery Suite (Partek Incorporated). More specifically, the fluorescence data on each channel for array experiment completed was imported as columns of data. The first column was the labels for each of the ORFs represented, followed by all fluorescence data. In addition, the replicates contained on each array were separated into different columns. The six rows of the spreadsheet located above the fluorescence data contained the experimental data for each column. This information included chip ID (the barcode from the microarray), channel (Cy3 or Cy5), operator (technician who

performed the experiment), orientation (top or bottom set of replicates) and strain (sample strain or Col). The Partek Discovery Suite (Partek Incorporated, St. Charles, MO) program was then used for all further analysis of expression data. The data table was imported into Partek and the table inverted by the program. The inversion process in Partek is necessary to account for the finite number of columns allowed in Excel. The limited number of columns does not allow for all data to be entered in an Excel spreadsheet in the appropriate orientation for Partek. This created a table with six columns representing the above descriptive information, followed by columns representing each of the ORFs on the array. Each row represented an individual array on a single channel. All data for all slides were imported into a single table. This data was normalized by taking the $\log_2$ of the fluorescence values, after adding an offset of 0.01 to eliminate values of 0. Normalized data was scaled using the grand mean for all fluorescence values in the spreadsheet. Normalization and scaling are used to make the data from different experiments comparable between those experiments. Normalized and scaled data were then used for statistical analysis.

Partek Discovery Suite has algorithms that allow for the removal effects on the data caused by factors other than the differences between the strains. These factors include dye (channel), date of experiment, technician who performed the experiments (operator) and location of the spots on the top or bottom of the slide (orientation). These are called batch effects. Each array experiment was only performed on one date, this means that the factors of chip ID and date are

confounded, or interfere with one another and cannot be isolated from one another allowing for the removal of only one of those factors. The batch remove tool was used to remove the effects of date of experiment, channel, operator, and orientation. Analysis of variation (ANOVA) was then run on the normalized, scaled and batch removed data. This ANOVA algorithm yielded a spread sheet which contained overall p-values for each ORF on the array as well as p-values for a pairwise comparison of each sample strain to Col. These p-values represent the significance of the difference in fluorescence value between the sample and Col. The false discovery rate (FDR) tool was used to determine a cutoff for significant p-values for each pairwise comparison. Lists were created using the ORFs with significant p-values and these lists were used for comparison of the strains.

3. Results:

3.1 Strain Selection:

A strain representing the most common PFGE fingerprint pattern for each of the ten CMRSA groups was chosen. Four sporadic strains were chosen whose PFGE pattern was more than seven bands different from any strain belonging to a CMRSA group. In addition, the sporadic strains were observed only once in more than 6000 MRSA isolates collected over 7 years from hospitals across Canada. Principle components analysis (PCA), which allowed us to visually investigate the distance between strains was also used in selecting the sporadic strains. Figure 2 shows the *SmaI* restriction digest banding patterns determined by PFGE for each of the strains used in this study. Figure 3 is the PCA picture of all unique PFGE banding patterns obtained from 1995-2002, created using BioNumerics. Each ball in the diagram reflects a unique DNA fingerprint.

3.2 Growth Curves:

Growth curves were determined for each of the strains involved in the study. CMRSA 1-6, 98S-1344, 99S-1088, 00S-1635 and 02S-1635 were the strains used for the expression experiments and mid-log phase for all of these strains was determined to be between an OD_{600} of 0.4 and 0.8 (Figure 4). Though the OD_{600} at mid-log phase was the same for Col, it required about 1 hour longer to reach that level, taking approximately four hours as opposed to the

Figure 2: Dendrogram depicted of analysis of PFGE patterns from Canadian MRSA strains used in this study

Note: scale denotes percent similarity of PFGE fingerprints

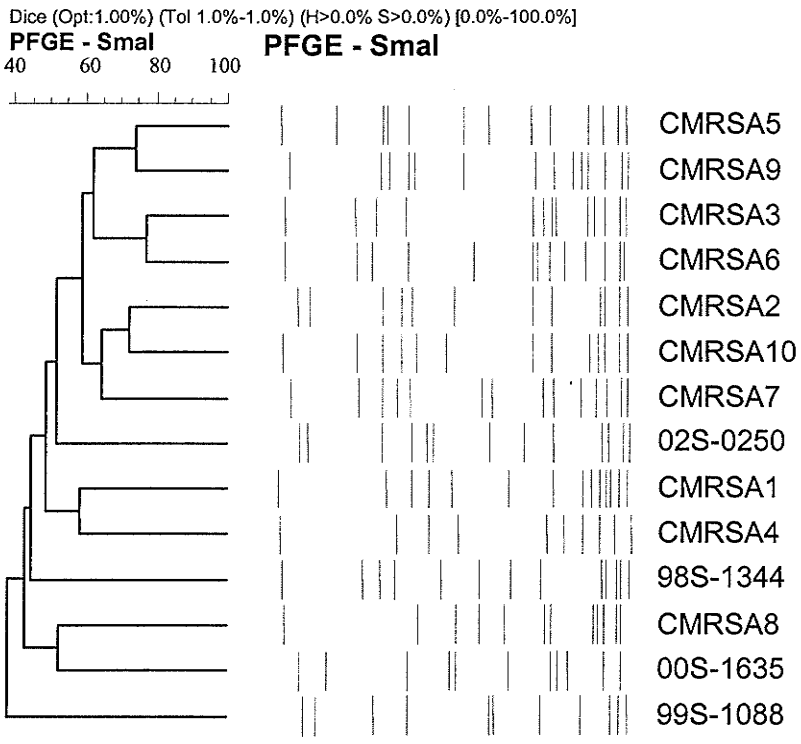

Dice (Opt:1.00%) (Tol 1.0%-1.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
PFGE - SmaI
40
60
80
100
PFGE - SmaI
CMRSA5
CMRSA9
CMRSA3
CMRSA6
CMRSA2
CMRSA10
CMRSA7
02S-0250
CMRSA1
CMRSA4
98S-1344
CMRSA8
00S-1635
99S-1088

Figure 3: Principle components analysis of strains with unique DNA fingerprints collected from 1995 - 2002

Notes: Each ball represents a unique DNA fingerprint. CMRSA strains are indicated by red balls and sporadic strains are represented by green balls. CMRSA strain not indicated on the diagram are within the clusters of white balls.
Figure generated using BioNumerics software (Applied Maths, Belgium)

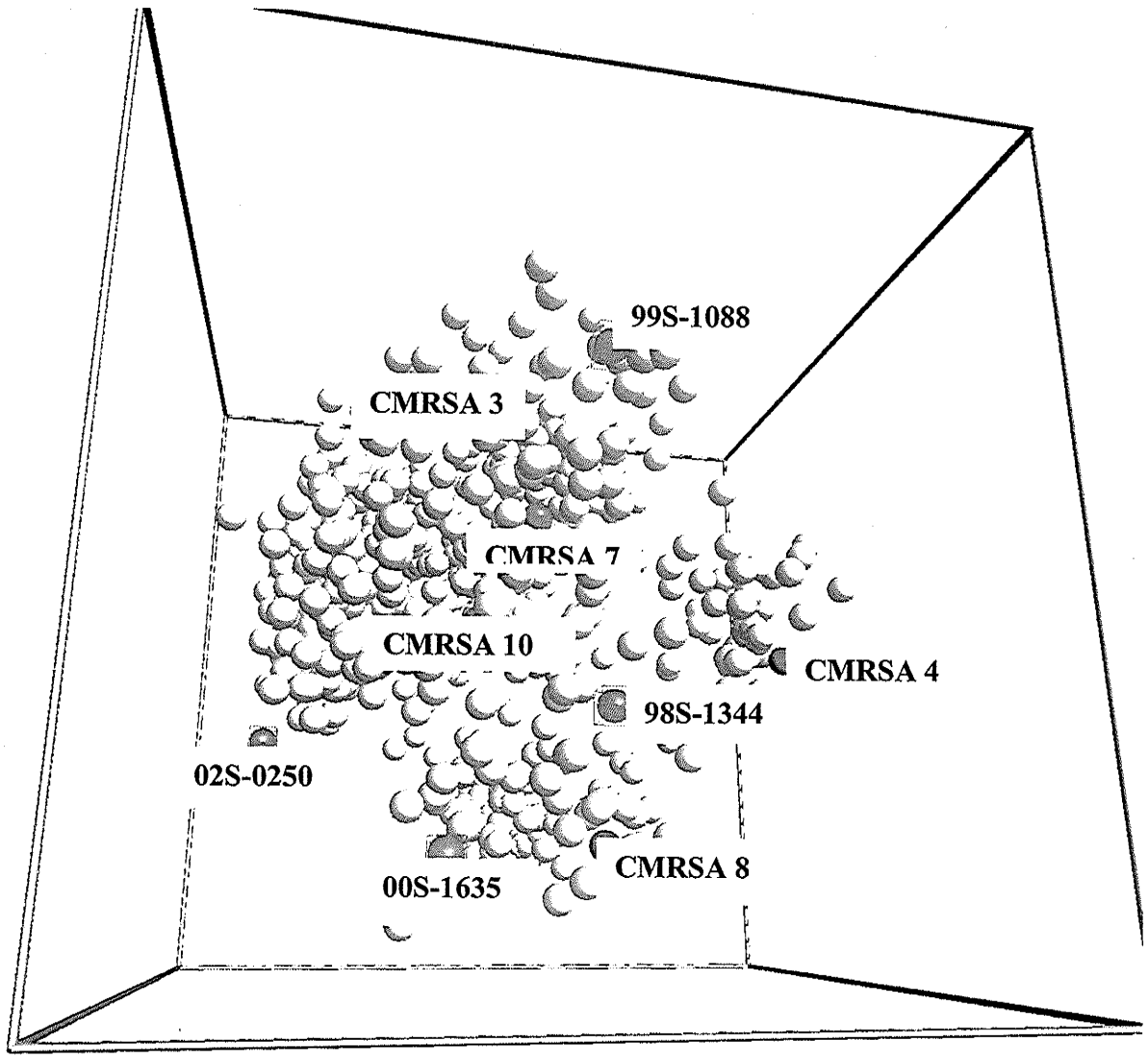
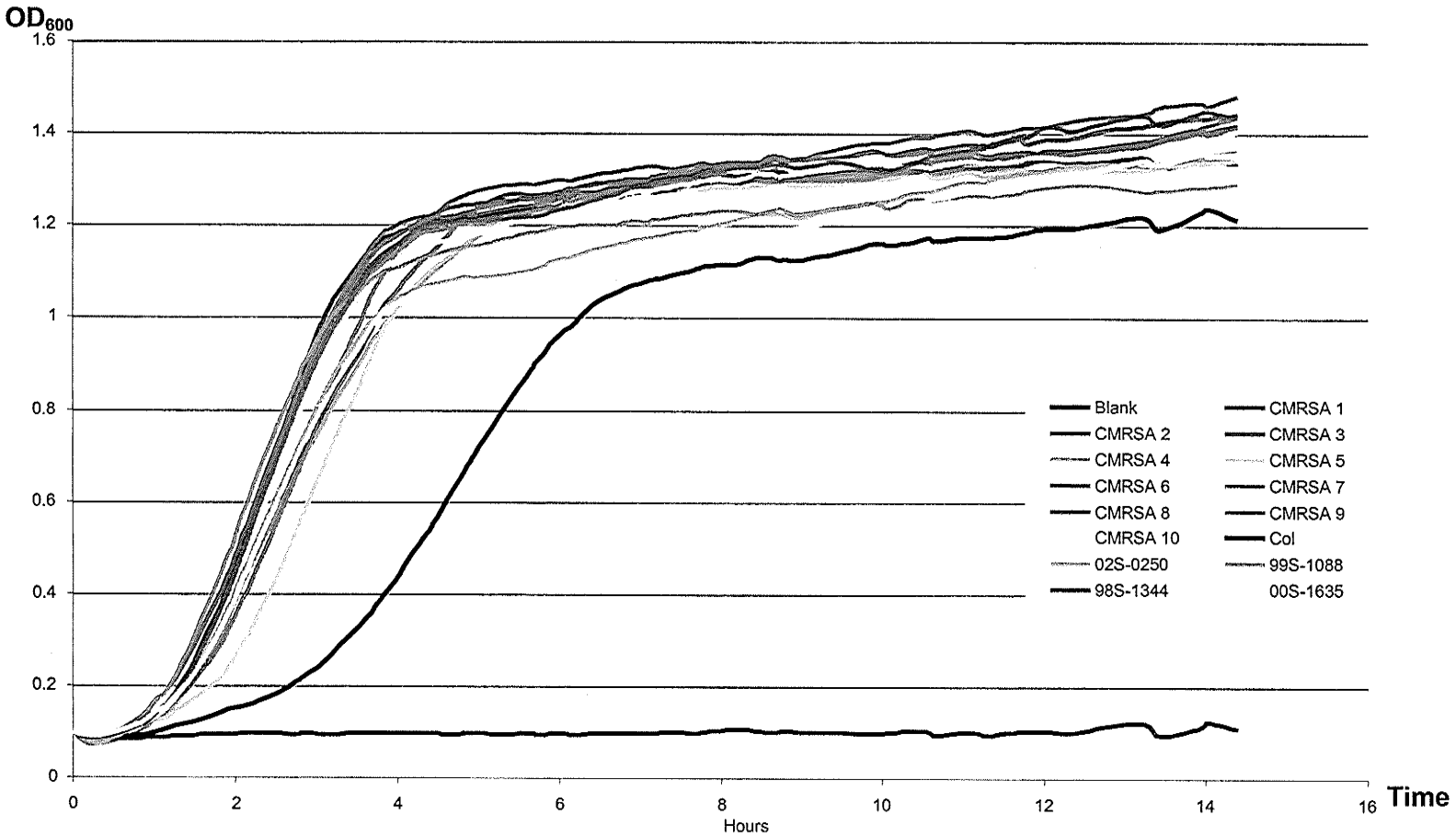

99S-1088
CMRSA 3
CMRSA 7
CMRSA 10
CMRSA 4
98S-1344
02S-0250
CMRSA 8
00S-1635

Figure 4: Growth curves for Col and Canadian MRSA strains.
Each line represents the average OD_{600} of three replicates.


OD600
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
Time
Hours
Blank
CMRSA 1
CMRSA 2
CMRSA 3
CMRSA 4
CMRSA 5
CMRSA 6
CMRSA 7
CMRSA 8
CMRSA 9
CMRSA 10
Col
02S-0250
99S-1088
98S-1344
00S-1635

three hours required for the other strains. Growth rates for the other strains were very similar, with no notable differences.

3.3 SCCmec Typing:

Figure 5 shows the results of the SCC*mec* typing performed on the 14 experimental strains. CMRSA 1, 2, 4 and 9 along with sporadic strains 00S-1635 and 02S-0250 were determined to be SCC*mec* type II, CMRSA 3 and 6 were SCC*mec* type III, and CMRSA 5, 7, 8, 10 and sporadic strains 98S-1344 and 99S-1088 were SCC*mec* type IV.

3.4 Multi-locus sequence-typing:

Table 5 contains the locus profiles, STs and clonal clusters (CC's) associated with the strains under study. Sporadic strain 02S-0250 is ST 5, the same ST as CMRSA 2 despite the fact that it is more than seven bands different from any type strain by PFGE and the PCA analysis shows it distant from the main clusters of PFGE patterns. Three strains, CMRSA 5, 9, and 10 are ST 8. Differing only at one base in the yqi allele, CMRSA 3 and 6 are ST 241 and 239 respectively. Sequence types 8, 241 and 239 all belong to CC8, whose progenitor sequence type is ST 8. CMRSA 7 belongs to ST 1 which is not generally associated with nosocomial epidemics, but with community associated strains (79). The remaining three sporadic strains have STs and CCs associated with neither nosocomial nor community epidemics. Strain 98S-1344 is ST 72, 99S-1088 is ST749. Strain 00S-1635 is ST 707, which has

Figure 5: Gel electrophoresis results from SCC*mec* typing PCR

Loading order is as follows:

Lane	Sample	SCCmec PCR mix
1	100 bp ladder	
2	SCC*mec* I	1
3	SCC*mec* I	2
4	SCC*mec* II	1
5	SCC*mec* II	2
6	SCC*mec* III	1
7	SCC*mec* III	2
8	SCC*mec* IV	1
9	SCC*mec* IV	2
10	blank	1
11	blank	2
12	100 bp ladder	
13	100 bp ladder	
14	CMRSA 1	1
15	CMRSA 1	2
16	CMRSA 2	1
17	CMRSA 2	2
18	CMRSA 3	1
19	CMRSA 3	2
20	CMRSA 4	1
21	CMRSA 4	2
22	CMRSA 5	1
23	CMRSA 5	2
24	CMRSA 6	1
25	CMRSA 6	2
26	CMRSA 7	1
27	CMRSA 7	2
28	CMRSA 8	1
29	CMRSA 8	2
30	CMRSA 9	1
31	CMRSA 9	2
32	100 bp ladder	
33	100 bp ladder	
34	CMRSA 10	1
35	CMRSA 10	2
36	98S-1344	1
37	98S-1344	2
38	99S-1088	1
39	99S-1088	2
40	00S-1635	1
41	00S-1635	2
42	02S-0250	1
43	02S-0250	2
44	100 bp ladder	

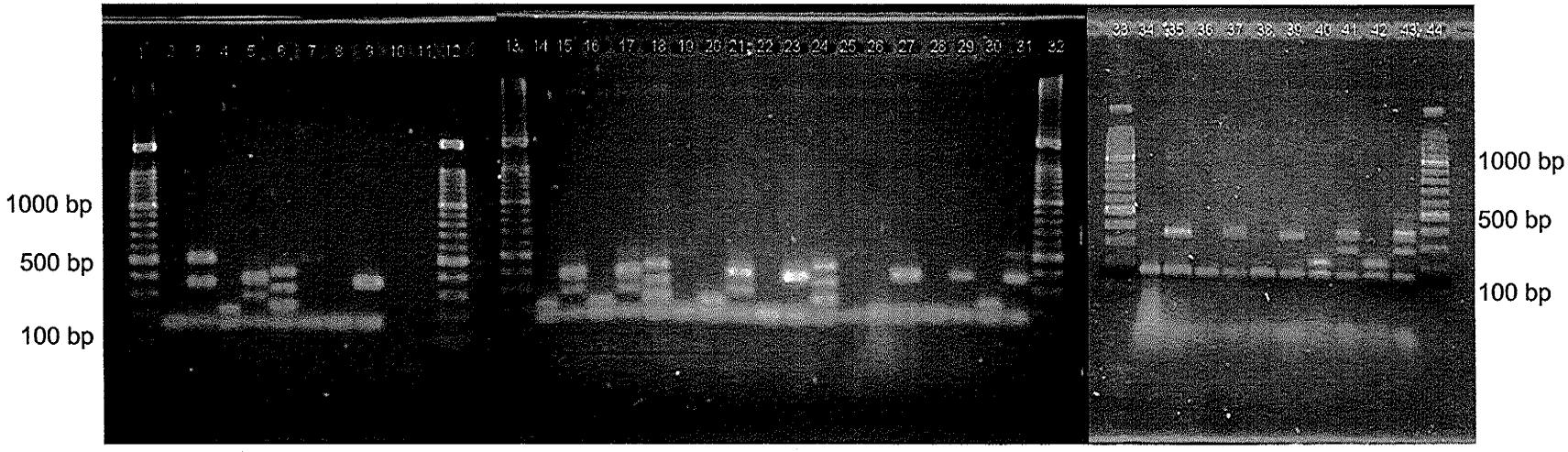

1000 bp
500 bp
100 bp
1000 bp
500 bp
100 bp

Table 5: Multi-locus Sequence Typing Data

Strain	Allelic Profile	Sequence Type	Clonal Complex
CMRSA1	10-14-8-6-10-3-2	ST45	CC45
CMRSA2	1-4-1-4-12-1-10	ST5	CC5
CMRSA3	2-3-1-1-4-4-30	ST241	CC8
CMRSA4	2-2-2-2-3-3-2	ST36	CC30
CMRSA5	3-3-1-1-4-4-3	ST8	CC8
CMRSA6	2-3-1-1-4-4-3	ST239	CC8
CMRSA7	1-1-1-1-1-1-1	ST1	CC1
CMRSA8	7-6-1-5-8-8-6	ST22	CC22
CMRSA9	3-3-1-1-4-4-3	ST8	CC8
CMRSA10	3-3-1-1-4-4-3	ST8	CC8
98S-1344	1-4-1-8-4-4-3	ST72	CC72
99S-1088	19-133-15-2-19-20-15	ST749	CC59
00S-1635	18-71-6-2-7-58-2	ST707	none
02S-0250	1-4-1-4-12-1-10	ST5	CC5

no CC associated with it when compared to the international database located on the website http://www.mlst.net database. ST749, 707 and 72 are rare, having been reported once (ST749) or twice (ST707 and ST72) in the http:www.mlst.net database. Figure 6 is the dendrogram created using the MLST allelic profile data represented in Table 5.

3.5 Comparative genomic hybridizations:

In order to test the slides and the labeling process, a hybridization using Col labeled with both Cy 3 and Cy5 was performed. The GACK program is based on the principle that, in this kind of hybridization, the $\log_2$ of fluorescence ratios will be normally distributed. Analysis of this Col/Col hybridization showed this to be true, allowing us to move forward with experimental hybridizations using GACK for data analysis (Figure 7).

Data analysis was first attempted on CMRSA 1 – 6 using a 100% EPP setting for GACK. GACK calculates an EPP value for each spot on the array. This value is an estimate of the probability a gene is present. An EPP of 100% means that GACK has estimated a 100% probability that ORF is present, anything lower than 100% is considered to be absent/divergent using the 100% EPP setting. Using these settings, it was noted that the coagulase gene (SA0209) was negative in CMRSA 1, 2 and 4. *Staphylococcus aureus* is a coagulase positive organism so these results were questioned and further validated using PCR. These PCR results indicate that the array data was in error. Lowering the EPP setting to 50% did not affect the data in question.

Figure 6: Dendrogram representing multi-locus sequence typing data for studied strains.

Distances calculated using the Dice coefficient, tree drawn using UPGMA.

Percent Similarity

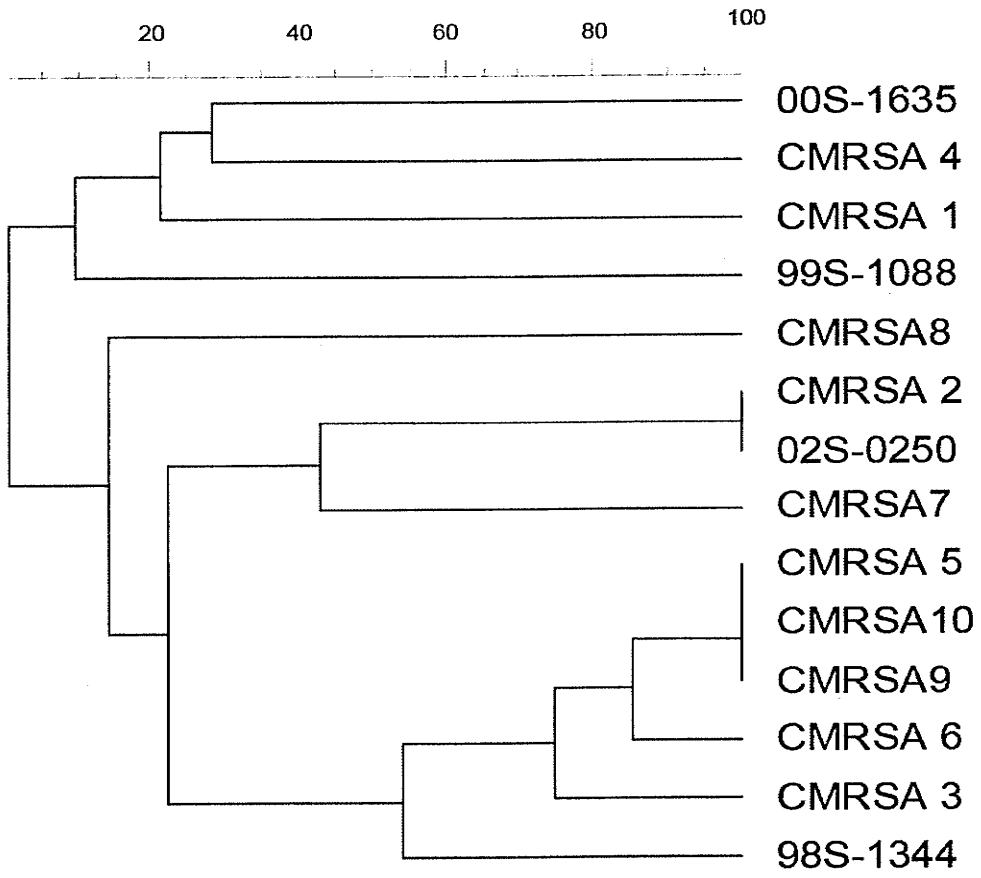
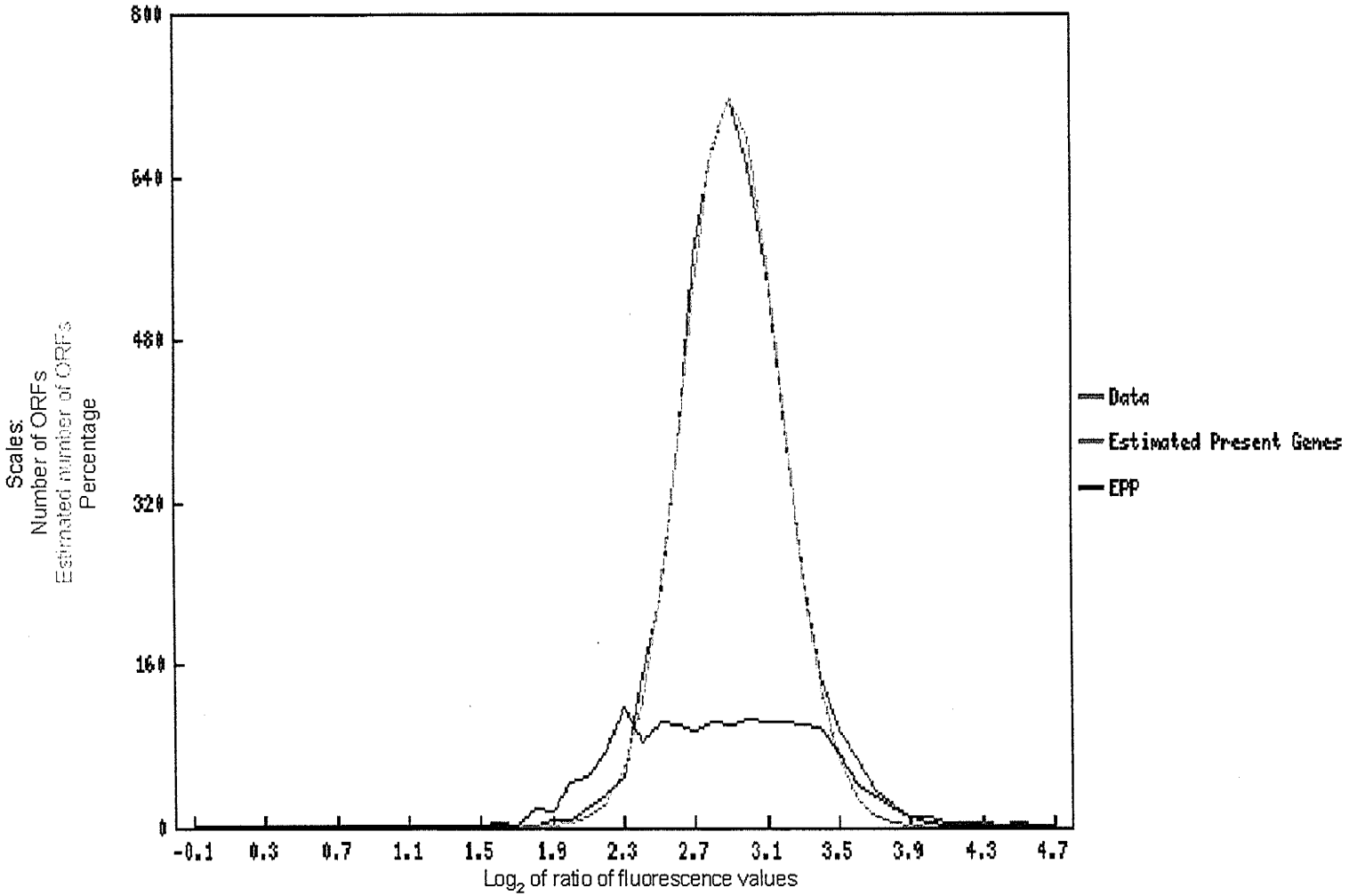


Figure 7: Frequency of the Log_2 of fluorescence ratios between ORFs in Col labeled with Cy5 and ORFs in Col labeled with Cy3, the estimated normal distribution of these values by GACK and the estimated probability of presence (EPP) of those ORFs as calculated by GACK (Graph generated using GACK software).


800
640
480
320
160
0
Scales:
Number of ORFs
Estimated number of ORFs
Percentage
-0.1
0.3
0.7
1.1
1.5
1.9
2.3
2.7
3.1
3.5
3.9
4.3
4.7
Log2 of ratio of fluorescence values
Data
Estimated Present Genes
EPP

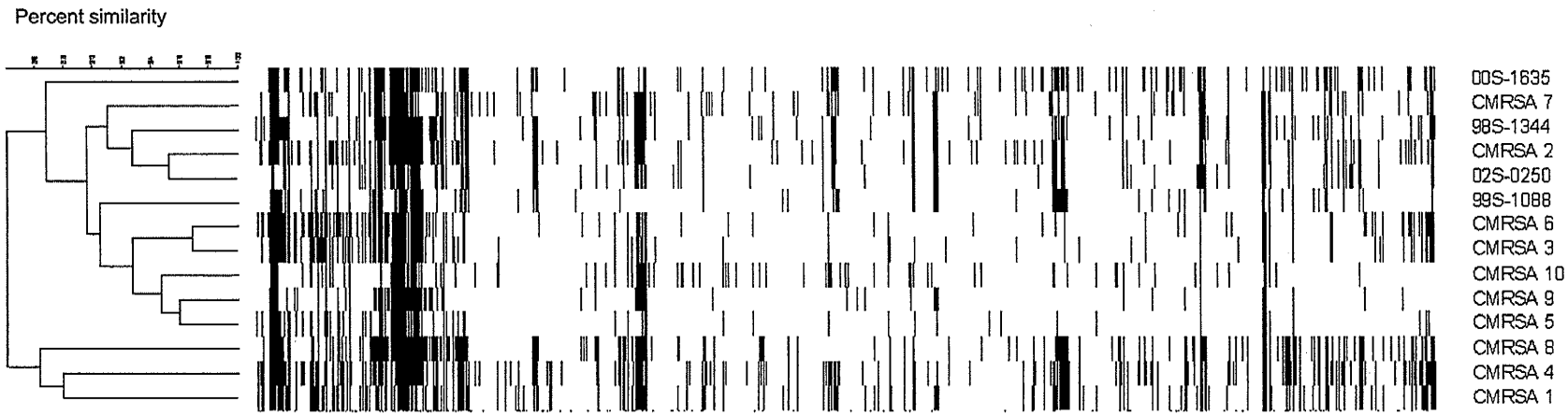
Lowering the stringency of the GACK settings to 0% generated accurate assignment of the coagulase gene.

Of the 2741 ORFs represented on the array, 37, or 1.3%, yielded no data more than 50% of the time, most likely due to either a poorly designed or poor quality PCR product or oligo printed on that spot. Eliminating these spots, we find that, with an EPP of 0%, 75.5% of the genomic content of all the strains studied is invariable when compared to Col. This means that the same set of 75.5% of the genes, or 2044 genes, are present in all strains. When the 100% EPP setting was used, this number was reduced to 49%. Based on this result, and the above coagulase PCR, future analysis was done at 0% EPP. A comparison of the two methods can be seen in Figure 8. Figure 8 also depicts the dendrogram created by microarray analysis with percent similarity calculated using the Euclidean distance coefficient and the trees drawn using UPGMA.

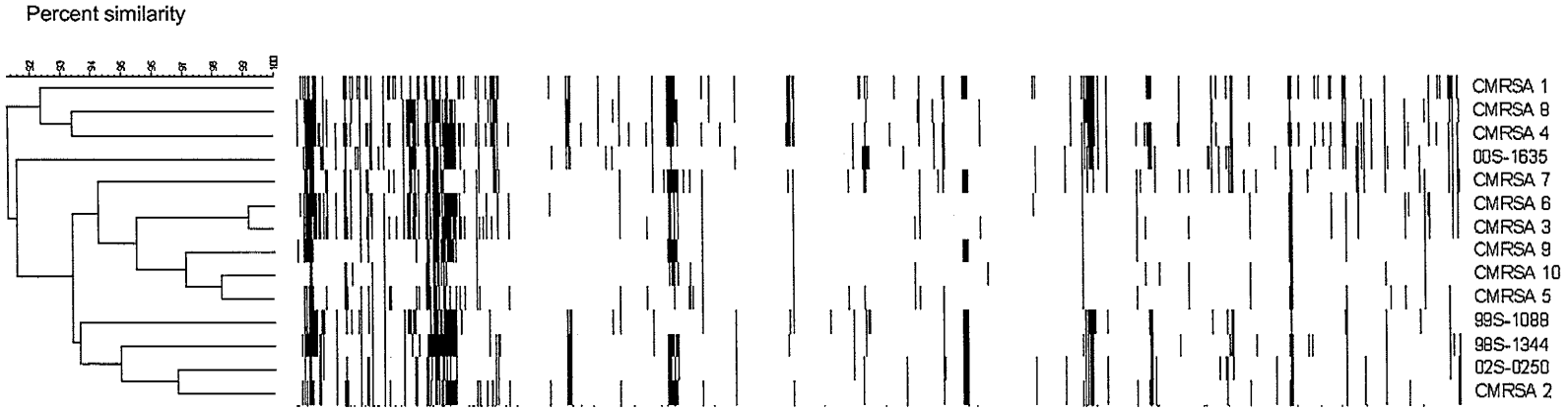
Figure 8: Comparative genomic hybridization data generated from (A) 100% estimated probability of presence (EPP) in genomotyping analysis by Charles Kim (GACK) and (B) 0% EPP in GACK.

Black regions represent the absence/divergence of an ORF. Strains tested are listed on the right hand side of the figure. Dendrogram scales represent percent similarity based on unweighted pair grouping method using arithmetic means (UPGMA)

A


Percent similarity
00S-1635
CMRSA 7
98S-1344
CMRSA 2
02S-0250
99S-1088
CMRSA 6
CMRSA 3
CMRSA 10
CMRSA 9
CMRSA 5
CMRSA 8
CMRSA 4
CMRSA 1

B


Percent similarity
CMRSA 1
CMRSA 8
CMRSA 4
00S-1635
CMRSA 7
CMRSA 6
CMRSA 3
CMRSA 9
CMRSA 10
CMRSA 5
99S-1088
98S-1344
02S-0250
CMRSA 2

In order to estimate the amount of genetic content in each strain that is not captured on the array, we compared genome size, estimated using PFGE fingerprinting band sizes, to the number of ORFs each strain shared with Col. Table 6 is a comparison of genome size, as estimated using *SmaI* macro-restriction digest band sizes, to the percentage of ORFs shared with Col, as determined using the microarray data. Using this comparison, CMRSA 1, 2 and 02S-0250 share 4-5% fewer genes with Col than CMRSA 3, 5, and 10, respectively, but this difference is not reflected in overall genome size. Also, genome size could not be associated with epidemic or sporadic strains. Epidemic strains showed an average genome size of 2463 kb, with a standard deviation of 323 kb, while sporadic strains showed an average genome size of 2502 kb with a standard deviation of 238 kb. Further looking at nosocomial strains and community-associated strains, average genome size for the nosocomial strains is 2435 kb, with a standard deviation of 298.5 kb. Average genome size for the two strains most commonly associated with the community, CMRSA 7 and 10 is 2709 kb. This value falls within two standard deviations of the average nosocomial genome size, however, a larger sample size would be required to make a statistical conclusion on the difference between these sizes.

Tables 7, 8, 9, and 10 contain CGH data for various virulence factors associated with *S. aureus*. Almost all of the adhesin genes represented on the array are present in all strains studied. The major exception in this case is

Table 6: Estimated genome sizes compared to the percentage of open reading frames shared with Col

Strain	Estimated GenomeSize[1] (kb)	Percentage ORFs Shared with Col[2]
CMRSA 1	2384	90.21%
CMRSA 2	2658	92.46%
CMRSA 3	2389	95.44%
CMRSA 4	1859	89.56%
CMRSA 5	2824	97.99%
CMRSA 6	2492	93.92%
CMRSA 7	2758	93.46%
CMRSA 8	1973	89.56%
CMRSA 9	2629	95.82%
CMRSA 10	2659	97.83%
98S-1344	2576	91.37%
99S-1088	2348	92.32%
00S-1635	2278	90.03%
02S-0250	2804	93.20%

[1] Sizing bands generated by *SmaI* digestions followed by pulsed-filed gel electrophoresis

[2] Determined using comparative genomic hybridizations

Table 7: Presence of adhesins based on comparative genomic hybridizations

NAME	GENE	DESCRIPTION	CMRSA 1	CMRSA 2	CMRSA 3	CMRSA 4	CMRSA 5	CMRSA 6	CMRSA 7	CMRSA 8	CMRSA 9	CMRSA 10	98S-1344	99S-1088	00S-1635	02S-0250
SA0095	-	Protein A precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0608	sdrC	sdrC protein	-	+	+	-	+	+	+	+	+	+	+	n/a	-	+
SA0609	sdrD	sdrD protein	n/a	+	+	-	+	+	+	+	+	+	+	+	+	+
SA0610	sdrE	sdrE protein	+	+	+	+	+	+	+	+	+	+	+	+	-	+
SA0858	ssp	secretory extracellular matrix and plasma binding protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1168	fib	fibrinogen-binding protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1169	fib	fibrinogen-binding protein precursor-related protein	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1220	-	fibronectin-binding protein A-related	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1522	ebpS	elastin binding protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2509	fnbB	fibronectin binding protein B	+	+	+	-	+	+	+	-	+	+	+	+	+	+
SA2511	fnbA	fibronectin-binding protein A	+	-	+	-	+	+	-	-	+	+	+	+	-	-
SA2652	clfB	clumping factor B	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2688	icaR	intercellular adhesion regulator	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2689	icaA	icaA protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2690	icaD	icaD protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2691	icaB	icaB protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2692	icaC	icaC protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Notes: +: Present, -: Absent/divergent, n/a: Unknown

Table 8: Presence of exoenzymes determined by comparative genomic hybridizations

NAME	GENE	DESCRIPTION	CMRSA 1	CMRSA 2	CMRSA 3	CMRSA 4	CMRSA 5	CMRSA 6	CMRSA 7	CMRSA 8	CMRSA 9	CMRSA 10	98S-1344	99S-1088	00S-1635	02S-0250
SA0024	-	5-nucleotidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0078	-	1-phosphatidylinositol phosphodiesterase precursor	+	+	+	+	+	+	+	+	+	+	n/a	n/a	n/a	n/a
SA0209	coa	coagulase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0317	geh	"lipase precursor, interruption"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0712	-	lipase/esterase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0857	-	"coagulase, putative"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0860	nuc	thermonuclease precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1028	-	serine protease	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1056	-	staphopain	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1057	-	glutamyl endopeptidase precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1357	-	staphylococcal nuclease family protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1864	-	serine protease SplF	-	+	+	+	+	+	+	-	+	+	-	-	-	+
SA1865	-	serine protease SplE	-	-	+	+	+	+	-	-	+	+	-	-	-	-
SA1866	-	serine protease SplD	-	+	+	+	+	+	-	-	+	+	-	-	-	+
SA1867	-	serine protease SplC	-	+	+	n/a	+	+	+	-	+	+	+	-	-	+
SA1868	-	serine protease SplB	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1869	-	serine protease SplA	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1970	-	staphopain	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2003	-	phospholipase C precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2194	hysA	hyaluronate lyase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2549	-	"esterase, putative"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2659	aur	aureolysin	n/a	+	+	-	+	n/a	+	+	+	+	+	+	+	+
SA2694	lip	lipase	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Notes: +: Present, -: Absent/divergent, n/a: Unknown

Table 9: Presence of toxins and superantigens based on comparative genomic hybridizations

NAME	GENE	DESCRIPTION	CMRSA 1	CMRSA 2	CMRSA 3	CMRSA 4	CMRSA 5	CMRSA 6	CMRSA 7	CMRSA 8	CMRSA 9	CMRSA 10	98S-1344	99S-1088	00S-1635	02S-0250
SA0265	-	Diarrheal toxin-like protein	n/a	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0442	-	staphylococcal enterotoxin, putative	n/a	n/a	n/a	n/a	n/a	n/a	+	-	+	+	+	+	+	n/a
SA0468	-	exotoxin 3	n/a	+	+	-	+	+	-	-	+	+	+	+	-	+
SA0469	-	exotoxin 2	-	+	n/a	n/a	n/a	+	+	-	+	+	+	+	+	+
SA0470	-	exotoxin 2	n/a	n/a	n/a	n/a	+	+	+	-	+	+	+	+	+	+
SA0472	-	exotoxin 2	-	+	+	-	+	+	+	-	+	+	+	+	+	+
SA0473	-	exotoxin 5	-	+	+	-	+	+	+	+	+	+	+	+	+	+
SA0474	-	exotoxin 4	n/a	n/a	n/a	n/a	n/a	n/a	-	-	+	+	+	n/a	-	+
SA0478	-	exotoxin 3	-	-	+	-	+	+	-	-	+	+	-	+	-	-
SA0762	-	"hemolysin, putative"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0886	ent	staphylococcal enterotoxin	-	-	+	-	+	+	+	-	-	+	-	+	+	-
SA0887	sei	staphylococcal extracellular enterotoxin type I	-	-	+	-	+	+	+	-	-	+	-	+	+	-
SA0907	seb	staphylococcal enterotoxin B	-	-	-	-	+	-	-	-	+	-	-	+	-	-
SA1178	-	"exotoxin 1, putative"	-	+	+	-	+	+	+	-	+	+	+	+	+	+
SA1179	-	"exotoxin 4, putative"	-	+	+	-	+	+	+	-	+	+	+	+	+	+
SA1180	-	"exotoxin 3, putative"	-	+	+	-	+	+	+	+	+	+	+	+	+	+
SA1657	-	enterotoxin family protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1880	lukD	leukotoxin LukD	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1881	lukS	synergohymenotropic toxin lukS	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA2004	lukF	"leukocidin precursor, F subunit, putative"	+	+	+	-	+	+	+	+	+	+	+	+	+	+
SA2006	lukM	leukotoxin LukM	-	+	+	-	+	+	+	+	+	+	+	+	+	+
SA2022	hld	delta-hemolysin	+	+	+	+	+	+	+	+	+	+	n/a	+	n/a	+
SA2160	-	"hemolysin, putative"	+	+	+	+	+	+	+	-	+	+	+	+	+	+
SA2419	-	"gamma-hemolysin, component A"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2421	-	"leukocidin precursor, S subunit"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2422	-	"leukocidin precursor, F subunit"	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Notes: +: Present, -: Absent/divergent, n/a: Unknown

Table 10: Presence of regulatory systems based in comparative genomic hybridization data

NAME	GENE	DESCRIPTION	CMRSA 1	CMRSA 2	CMRSA 3	CMRSA 4	CMRSA 5	CMRSA 6	CMRSA 7	CMRSA 8	CMRSA 9	CMRSA 10	98S-1344	99S-1088	00S-1635	02S-0250
SA0019	yycF	DNA-binding response regulator	n/a	n/a	n/a	n/a	n/a	n/a	n/a	+	+	+	+	+	+	+
SA0020	yycG	sensory box histidine kinase YycG	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0072	SA0072	"transcriptional regulator, lysR family"	+	-	-	-	+	-	+	+	+	+	-	+	+	-
SA0074	-	"transcriptional regulator, lysR family"	-	-	-	-	+	-	+	+	+	+	-	-	-	-
SA0091	-	"transcriptional regulator, GntR family"	+	+	-	-	+	-	-	-	+	+	+	+	+	+
SA0096	sarS	staphylococcal accessory regulator S	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0248	lrgB	*lrgB*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0672	sarA	staphylococcal accessory regulator A	n/a	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0757	-	"transcriptional regulator, DeoR family"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0765	saeS	sensor histidine kinase SaeS	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0766	saeR	two-component signal transduction protein SaeR	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1450	arlS	sensor histidine kinase ArlS	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1451	arlR	DNA-binding response regulator	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1534	srrB	sensor histidine kinase SrrB	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1535	srrA	DNA-binding response regulator	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1812	rot	virulence factor regulator protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1891	-	RNAIII-activating Protein TRAP	-	+	+	-	+	+	+	-	+	+	+	+	-	+
SA1905	vraR	"DNA-binding response regulator, VraR"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1906	-	"sensor histidine kinase, putative"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1942	vraR	DNA-binding response regulator VraR	+	+	+	+	+	+	+	+	+	+	+	+	+	+

SA1943	vraS	sensor histidine kinase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2023	agrB	accessory gene regulator protein B	+	-	+	+	+	+	-	+	+	+	+	+	-	-
SA2024	agrD	accessory gene regulator protein D	n/a	n/a	n/a	n/a	+	+	n/a	n/a	+	+	+	+	+	+
SA2026	agrA	accessory gene regulator protein A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2140	-	lytic regulatory protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2258	sarV	staphylococcal accessory regulator V	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2287	sarR	staphylococcal accessory regulator R	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2506	sarT	staphylococcal accessory regulator T	-	+	+	-	+	+	+	+	+	+	+	+	-	+
SA2507	sarU	staphylococcal accessory regulator U	-	+	+	-	+	+	+	+	+	+	+	+	-	+
SA2524	-	"transcriptional regulator, MarR family"	-	-	+	-	+	+	-	+	+	+	-	-	+	-

Notes: +: Present, -: Absent/divergent, n/a: Unknown

SA1169, which is involved in fibronectin binding. This ORF is deleted in CMRSA 1, 4, 8 and the sporadic strain 99S-1088 (Table 7). In the case of the exoenzymes, all represented serine proteases are absent/divergent in CMRSA 1 and 8 as well as 99S-1088. Some serine proteases are also absent/divergent in CMRSA 4 along with 98S-1344 and 00S-1635 (Table 8). With respect to the toxin and superantigen content, again we see that CMRSA 1, 4, and 8 are either lacking, or have significantly divergent versions of many of these genes (Table 9). Differences in regulatory systems are less consistent between strains (Table 10).

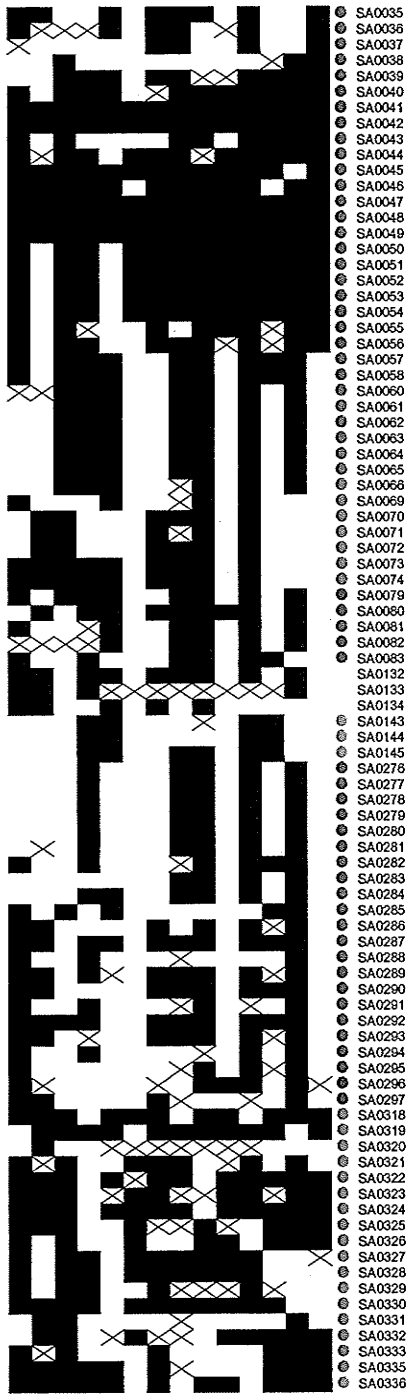
To determine areas in the genome where mutations or deletions might be more common, regions with three or more contiguous divergent/absent ORFs in three or more strains were identified using the GeneMaths software. Using this method we determined 21 regions in which mutations or deletions are most common, these regions were numbered 1-21, in order based on locus numbers (ie. SA number). These regions are listed in Table 11, along with any unique features. An illustration of the variability in presence or absence/divergence of these regions is depicted in Figure 9. At 0% EPP, nine ORFs are absent in all strains, five of which are contained in region 2, the SCCmec region, and four of which are contained in region 7, bacteriophage L54a.

In order to better focus our comparison of epidemic strains and sporadic strains, we compared 98S-1344, 99S-1088, and 00S-1635, the three sporadic

Table 11: Locations and features of variable regions in the *S. aureus* genome with frequent mutations

Variable Region	Number of ORFs	Location	Features
1	31	SA0035-SA0066	SCCmec
2	6	SA0069-SA0074	Transcritional regulator
3	5	SA0079-SA0083	all hypothetical proteins
4	3	SA0132-SA0134	degenerate replication and transposase proteins
5	3	SA0143-SA0145	cap5 H, I and J
6	22	SA0276-SA0297	Diarrheal toxin
7	72	SA0318-SA0390	Bacteriophage L54a
8	13	SA0468-SA0483	Exotoxins 2,3,4 and 5
9	9	SA0644-SA0653	all hypothetical proteins
10	3	SA0848-SA0850	all hypothetical proteins
11	27	SA0885-SA0911	pathogenicity island, Enterotoxins b and i
12	9	SA1339-SA1348	all hypothetical proteins
13	3	SA1352-SA1354	ABC transporter proteins
14	14	SA1573-SA1586	traG, FtsK like protein, integrase/recombinase
15	5	SA1857-SA1861	restriction enzyme hsdS
16	21	SA1864-SA1884	Epidermin related protiens, luk S and D, serine proteases
17	4	SA2012-SA2015	Acetyltransferase, terminase, integrase/recombinase
18	6	SA2200-SA2205	clpA related protein
19	5	SA2494-SA2498	all hypothetical proteins
20	3	SA2505-SA2507	sar T and U
21	5	SA2726-SA2730	2 integrase/recombinase related genes

Figure 9: Heat map depicting variable regions in each strain.
Each variable region is represented on the side of the diagram by a new color. White blocks in the heat map represent presence of an ORF, black blocks the absence/divergence of an ORF and an X denotes a spot which failed on the microarray.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CMRSA 6
CMRSA 5
CMRSA 4
CMRSA 3
CMRSA 2
CMRSA 10
CMRSA 1
99S-1088
98S-1344
02S-0250
00S-1635
SA0035
SA0036
SA0037
SA0038
SA0039
SA0040
SA0041
SA0042
SA0043
SA0044
SA0045
SA0046
SA0047
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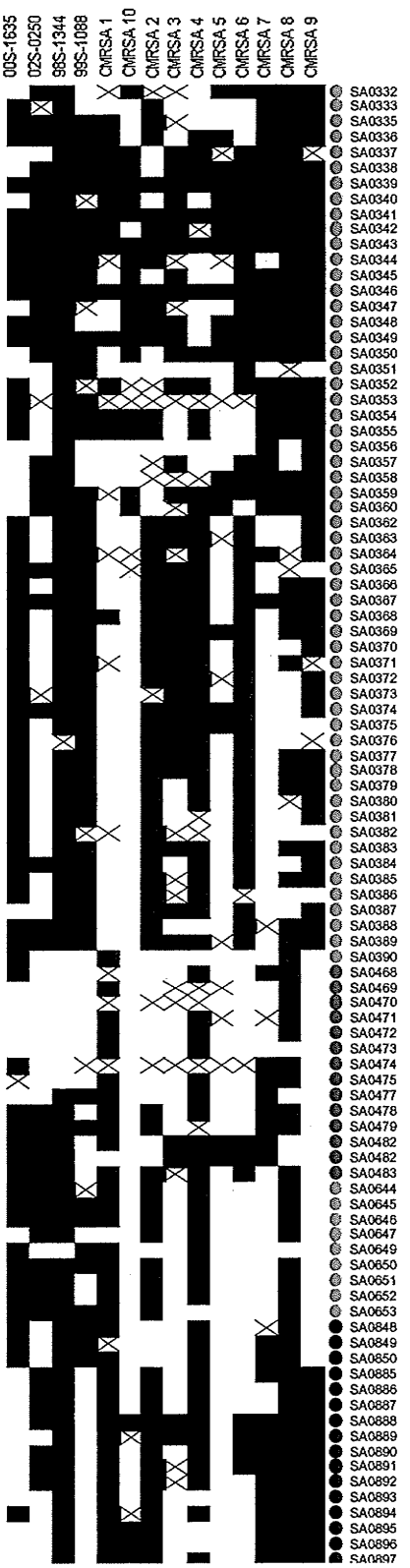
SA0336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02S-0250
98S-1344
98S-1088
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CMRSA 2
CMRSA 3
CMRSA 4
CMRSA 5
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CMRSA 7
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CMRSA 9
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SA0333
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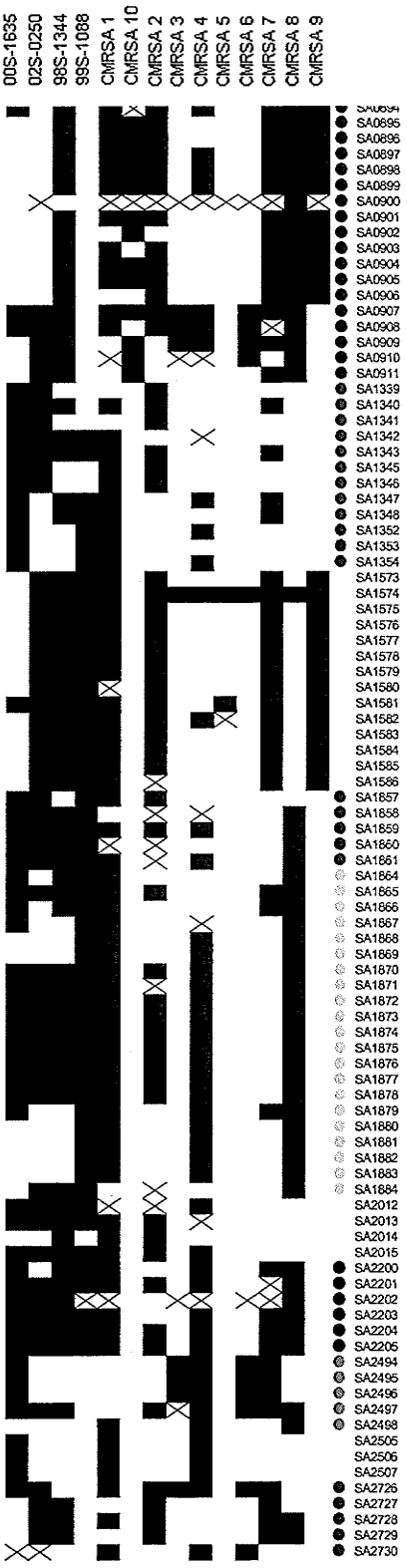
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strains unrelated to the CMRSA strains using MLST, to the eight epidemic strains not associated with the community (CMRSA 1-6, 8 and 9). Sporadic strains have been linked to CA-MRSA in the literature so may skew our comparison (5). Using this comparison, one ORF, SA2196, was found to be absent/divergent in the sporadic strains and present in the epidemic strains. This ORF is related to the haloacid dehalogenase (HAD) superfamily of enzymes. Its function in *Staphylococci* is currently unknown, but its closest match to a known enzyme is an HAD superfamily hydrolase in *Bacillus licheniformis* with 30.7% identity and 51.9% similarity based on amino acid sequence comparison. It is unknown if the presence of this gene would give an advantage to nosocomial isolates of MRSA allowing them to be maintained in a hospital setting.

Comparison of the community associated strains to the hospital associated strains revealed one ORF, SA0046, present in the community strains but absent in the hospital strains. In order to further focus our study on nosocomial and community associated MRSA, the two most common nosocomial strains, CMRSA 1 and 2, were compared to the two most common community strains, CMRSA 7 and 10. This comparison is shown in Table 12. Major differences include the SCC*mec* type IV element present in CMRSA 7 and 10 and not in CMRSA 1 and 2 which have the type II SCC*mec* region, variable region 9 in its entirety is not present in CMRSA 1 and 2, but is present in CMRSA 7 and 10. Variable region 9 is composed of 9 ORFs representing hypothetical proteins. The cluster of epidermin related genes in variable region 16 is absent

in CMRSA 1 and 2 but present in CMRSA 7 and 10. This portion of variable region 16 is also deleted in CMRSA 4 and 8 as well as the sporadic strains.

Table 12: Gene differences between the two most common nosocomial CMRSA strains, CMRSA 1 and 2, and the two recognized community acquired CMRSA strains, CMRSA 7 and 10

CMRSA 1 CMRSA 2	CMRSA 7 CMRSA 10	Locus	Gene	Variable Region	Protein Function
+	-	SA0326	n/a	7	"bacteriophage L54a, hypothetical protein"
+	-	SA0345	n/a	7	"bacteriophage L54a, hypothetical protein"
+	-	SA0350	n/a	7	"bacteriophage L54a, conserved hypothetical protein"
+	-	SA0360	n/a	7	"bacteriophage L54a, hypothetical protein"
+	-	SA0902	n/a	11	"Tn557, ORF6"
+	-	SA0909	n/a	11	hypothetical protein
+	-	SA0911	n/a	11	hypothetical protein
-	+	SA0029	n/a		HMG-CoA synthase
-	+	SA0030	n/a		hypothetical protein
-	+	SA0035	n/a	1	hypothetical protein
-	+	SA0036	n/a	1	"IS1272, transposase"
-	+	SA0046	n/a	1	metallo-beta-lactamase family protein
-	+	SA0073	n/a	2	conserved hypothetical protein
-	+	SA0074	n/a	2	"transcriptional regulator, lysR family"
-	+	SA0080	n/a	3	staphylococcus tandem lipoprotein
-	+	SA0132	n/a	4	"replication initiation protein, degenerate"
-	+	SA0134	n/a	4	"transposase, IS200 family, degenerate"
-	+	SA0368	n/a	7	"bacteriophage L54a, conserved hypothetical protein"
-	+	SA0483	n/a	8	hypothetical protein
-	+	SA0644	n/a	9	conserved hypothetical protein
-	+	SA0645	n/a	9	conserved hypothetical protein
-	+	SA0646	n/a	9	conserved hypothetical protein
-	+	SA0650	n/a	9	conserved hypothetical protein
-	+	SA0651	n/a	9	conserved hypothetical protein
-	+	SA0652	n/a	9	conserved hypothetical protein
-	+	SA0653	n/a	9	conserved hypothetical protein
-	+	SA0886	ent	11	staphylococcal enterotoxin
-	+	SA0887	sei	11	staphylococcal extracellular enterotoxin type I
-	+	SA1043	n/a		"glycosyl transferase, group 1"
-	+	SA1345	n/a	12	hypothetical protein
-	+	SA1346	n/a	12	hypothetical protein
-	+	SA1533	n/a		hypothetical protein
-	+	SA1839	n/a		"transposase, IS200 family"
-	+	SA1851	n/a		hypothetical protein
-	+	SA1854	n/a		hypothetical protein
-	+	SA1857	n/a	15	hypothetical protein
-	+	SA1859	n/a	15	hypothetical protein
-	+	SA1870	n/a	16	hypothetical protein
-	+	SA1872	epiE	16	epidermin immunity protein F
-	+	SA1873	epiF	16	epidermin immunity protein F
-	+	SA1874	epiP	16	epidermin leader peptide processing serine protease EpiP
-	+	SA1875	epiD	16	epidermin biosynthesis protein EpiD
-	+	SA1876	epiC	16	"epidermin biosynthesis protein EpiC, authentic point mutation"
-	+	SA1877	epiB	16	lantibiotic epidermin biosynthesis protein EpiB

-	+	SA1878	epiA	16	lantibiotic gallidermin precursor EpiA
-	+	SA2009	n/a		"tetracenomycin polyketide synthesis O-methyltransferase TcmP, putative"
-	+	SA2010	n/a		"iron compound ABC transporter, substrate-binding protein"
-	+	SA2013	n/a	17	hypothetical protein
-	+	SA2014	n/a	17	terminase small subunit
-	+	SA2015	n/a	17	"integrase/recombinase-related protein, authentic frameshift"
-	+	SA2190	n/a		conserved hypothetical protein
-	+	SA2481	n/a		hypothetical protein
-	+	SA2631	n/a		conserved hypothetical protein

(+: present, -: absent/divergent, n/a: not applicable)

3.6 Expression analysis

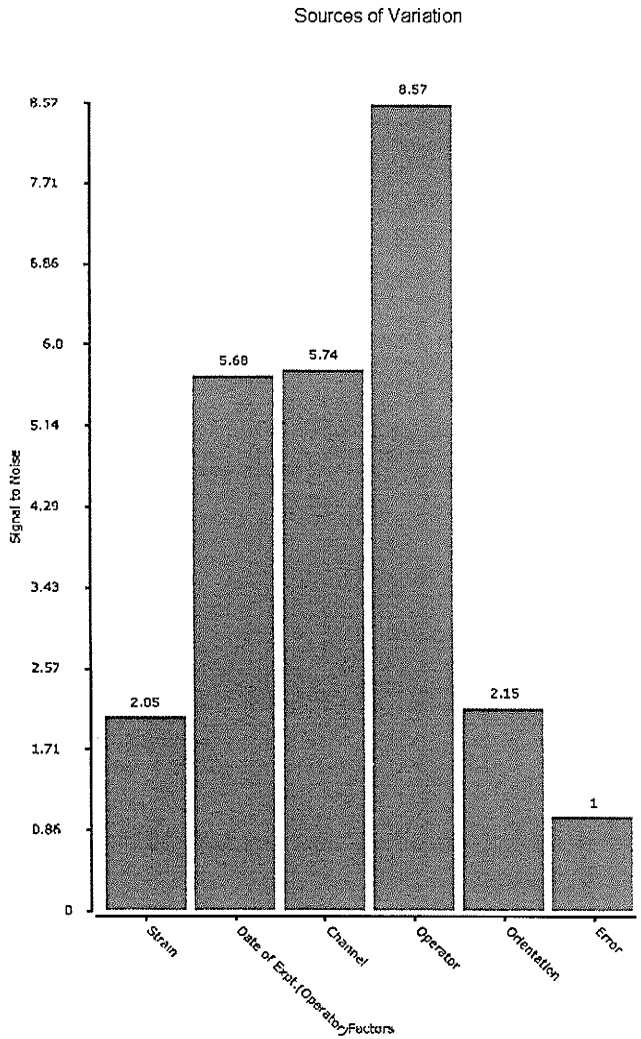
Microarray-based expression analysis was performed on CMRSA 1-6 as well as the sporadic strains. These experiments were all carried out in rich media under normal laboratory growth conditions. Gene expression in each of the experimental strains was compared to gene expression in the strain Col, grown under the same conditions.

Principle components analysis (PCA) of the expression data shows that many experimental factors, other than actual genetic (or strain) differences, affect the data. These factors include differences between the Cy dyes, date of experiment and the technician who performs the experiment. Using the batch removal tool in Partek Discovery Suite, we were able to minimize the effects of these factors on our data. Figure 10 shows the sources of variation in the data before and after batch removal. Note that the values for strain and error do not change as a result of the batch removal.

Analysis of variation was used to determine the probability that the data for each gene represented an actual change in expression, generating a p-value. Using the false discovery rate tool, cutoff p-values were determined to a significance level of 0.01. Table 13 contains the p-values determined to be the significance cutoffs as well as the number of genes in each strain which fall below that cut-off. It is important to note that p-value does not signify the level of expression, only the significance of the data. That is, a gene that is differentially

Figure 10: Sources of variation in microarray-based expression data (A) prior to batch removal and (B) after batch removal by Partek Discovery Suite.

A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Signal to Noise
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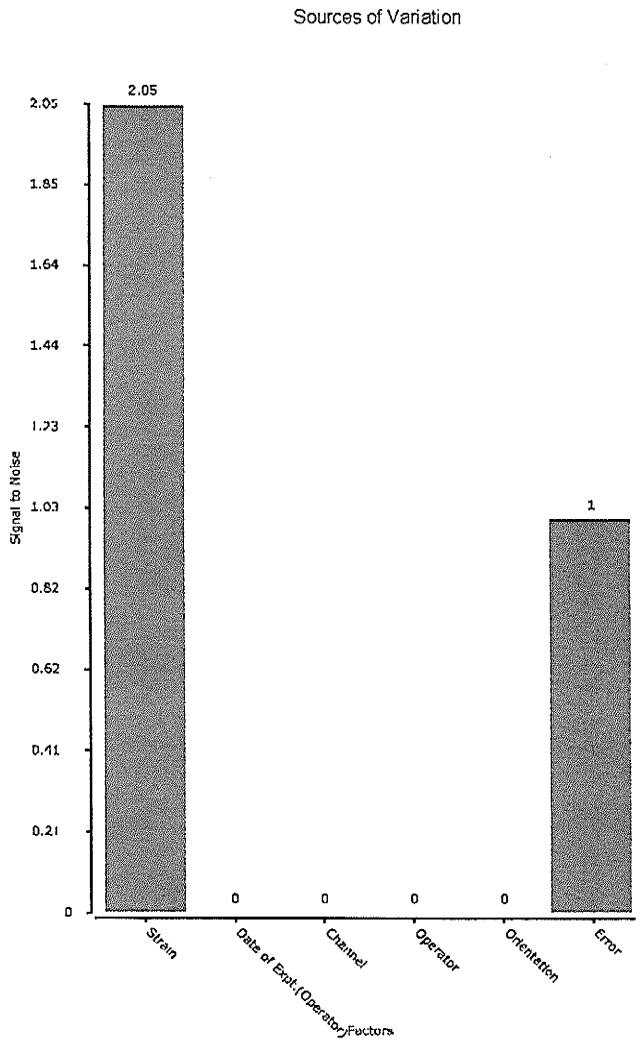
7.71
6.86
6.0
5.14
4.29
3.43
2.57
1.71
0.86
0
2.05
5.68
5.74
8.57
2.15
1
Strain
Date of Expt.(Operator)Factors
Channel
Operator
Orientation
Error

B


Sources of Variation
Signal to Noise
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1.03
0.82
0.62
0.41
0.21
0
2.05
0
0
0
0
1
Strain
Date of Expt.(Operator)Factors
Channel
Operator
Orientation
Error

Table 13: Significant p-values for comparisons of CMRSA 1-6 and four sporadic strains to Col

Comparison	P-value Cutoff	# of Significant p-values
All - Col	0.000304264	949
CMRSA 1-Col	9.17E-05	286
CMRSA 2-Col	2.24E-05	70
CMRSA 3-Col	3.88E-05	121
CMRSA 4-Col	3.82E-05	119
CMRSA 5-Col	2.98E-05	93
CMRSA 6-Col	3.40E-05	106
98S-1344 - Col	9.30E-05	290
99S-1088 - Col	7.44E-05	232
00S-1635 - Col	6.41E-07	2
02S-0250 - Col	2.50E-05	78

regulated two fold may have a higher p-value than a gene with only a 1.1 fold change in expression. Appendix 1 contains all of the differentially regulated genes, along with their fold change, as determined using the expression arrays.

Tables 14 and 15 contain the five most notably up and down regulated genes from the significantly differentially regulated genes in each strain by fold change as compared to Col. The ORF SA0204, which encodes a formate acetyl transferase involved in anaerobic energy metabolism is up-regulated 1.2 fold in the sporadic strains 98S-1344 and 99S-1088. This gene is not differentially regulated in any of the studied epidemic strains though it is present by CGH. Sporadic strain 02S-0250 shows no up-regulation of this gene, but can be excluded from the sporadic strains because it is closely related to CMRSA 2 by MLST analysis. Strain 00S-1635 has been eliminated from this analysis as the variation in the data created by a change in operators was too high and the data set could not be analyzed alongside the rest of the data. Further investigation using real-time reverse-transcriptase PCR and a greater number of sporadic strains is required to confirm this finding.

The fluorescence value for each ORF was averaged across arrays for each strain, including Col. These values were ordered from highest to lowest and the top 50% of ORFs compared among all strains. Of the 1538 ORFs included for each strain, 1140 of them were common to all strains, with 1020 of those 1140 genes being contained in the non-variable regions determined by the

CGH studies with Col. Of the 120 genes not represented by the non-variable regions, 112 of them are from the genome of Mu50. This likely means that we have overestimated the number of genes that would be expressed in *S. aureus* under the experimental growth conditions or that there is reasonable cross reaction between some genes in Col and those present on the array from Mu50.

Table 14: The five most up-regulated genes in test strains, by fold change compared to Col, as determined by microarray-based expression analysis

Strain	Locus	P-value	Fold Change
02S-0250	SAV0884	1.74E-05	1.08933
	SAP024	6.95E-08	1.08567
	SAP002	3.94E-07	1.08537
	SAV0200	1.93E-05	1.06269
	SAVP021	1.37E-05	1.0484
98S-1344	SAV1982	4.01E-24	1.36266
	SAV1985	1.64E-32	1.31717
	SA1802	4.60E-09	1.26692
	SA1477	9.39E-13	1.25721
	SA0204	8.91E-07	1.2524
99S-1088	SAP008	3.74E-08	1.27882
	SAP019	5.38E-07	1.24574
	SA0204	1.19E-05	1.22076
	SAV1964	1.09E-17	1.22003
	SAP022	3.00E-15	1.21682
CMRSA 1	SAP031	1.50E-06	1.73213
	SAP031	3.82E-09	1.73036
	cadD	9.75E-08	1.66868
	rep	9.14E-07	1.54208
	SA2619	3.47E-07	1.50226
CMRSA 2	cadD	2.10E-05	1.6127
	SAV0434	2.47E-08	1.1732
	SA0028	1.32E-08	1.17023
	SA1805	6.96E-11	1.1693
	SAV1653	4.69E-15	1.16415
CMRSA 3	SA1755	7.06E-16	1.33774
	SA1805	1.53E-12	1.18832
	SA0478	5.16E-12	1.16349
	SAV1653	6.39E-14	1.15319
	SAV2001	4.29E-10	1.14895
CMRSA 4	rep	4.24E-08	1.75392
	MW0390	7.81E-06	1.63893
	SA1261	3.30E-05	1.32029
	SA0244	2.00E-05	1.2014
	SA1805	9.34E-11	1.17112
CMRSA 5	SA0759	1.62E-09	1.1751
	SA2025	3.44E-14	1.15993
	SA0758	6.36E-07	1.15326
	SA2004	5.92E-11	1.15029
	SA0757	2.33E-06	1.14773
CMRSA 6	cadD	8.08E-06	1.56405
	SAVP027	4.58E-13	1.17414
	SA0759	1.01E-10	1.16153
	SA0758	2.61E-09	1.16089
	SA0028	1.77E-09	1.15947

Table 15: The five most down-regulated ORFs in test strains, by fold change in reference to Col, as determined using microarray based expression studies

Strain	Locus	P-value	Fold Change
02S-0250	tetK	1.07E-06	-1.9077
	SA2233	3.04E-06	-1.28476
	SA2491	2.57E-06	-1.26919
	SA0879	1.12E-05	-1.25765
	SA1377	2.02E-07	-1.24308
98S-1344	SA1508	3.79E-05	-2.96095
	SA0477	1.94E-05	-1.63926
	SA2204	1.46E-07	-1.53823
	SA0050	6.36E-14	-1.47422
	SAA0003	7.43E-10	-1.42294
99S-1088	tetK	1.77E-06	-1.92284
	SA0054	5.07E-05	-1.86902
	SA1920	6.08E-06	-1.55655
	SA0050	3.92E-12	-1.40467
	SA2120	9.08E-07	-1.3396
CMRSA 1	SA1187	8.68E-05	-1.59462
	tetK	1.50E-05	-1.50116
	SA0512	2.04E-05	-1.37562
	SA1469	5.96E-05	-1.33856
	SA1920	6.64E-05	-1.3187
CMRSA 2	SA0380	2.07E-05	-1.72905
	SA0368	3.50E-07	-1.63705
	SA0384	6.56E-07	-1.47678
	SA0050	1.13E-10	-1.32068
	SA0035	2.00E-05	-1.21553
CMRSA 3	SA0390	1.79E-05	-2.64735
	SA0054	1.43E-05	-1.87165
	SA0218	8.76E-08	-1.81634
	SA0368	2.05E-06	-1.55403
	SA0384	6.72E-06	-1.40073
CMRSA 4	tetK	3.83E-08	-2.17167
	SA2527	5.02E-06	-1.78474
	SA0994	3.37E-05	-1.65261
	SA0139	2.70E-06	-1.56679
	SA0923	5.53E-06	-1.5582
CMRSA 5	SA2024	4.39E-06	-2.00119
	SA0487	9.03E-07	-1.43595
	SA0050	1.53E-12	-1.39814
	SA0257	3.25E-06	-1.29097
	SAV2208	4.28E-07	-1.28526
CMRSA 6	SA1730	4.51E-06	-1.74692
	SA0054	7.95E-06	-1.72825
	SA0380	1.34E-05	-1.60504
	SA0384	3.20E-08	-1.47039
	SA0188	4.75E-06	-1.45445

4.0 Discussion

4.1 Growth curves

Though previous studies have found that CA-MRSA tend to have a faster growth rate than nosocomial strains (79), our growth data does not confirm this finding. The only strain in our study with a significantly slower growth rate than the other strains is Col, which represents a historic strain of MRSA.

4.2 Multilocus sequence typing

Except for CMRSA 7, all other CMRSA strains fit into the five CCs originally determined through the study of nosocomial isolates (37). CMRSA 1 belongs to CC45, CMRSA 2 to CC5, CMRSA 3, 5, 6, 9, and 10 to CC8, CMRSA 4 to CC30 and CMRSA 8 to CC22. This compliments the suggestion that a limited number of genetic backgrounds have acquired methicillin resistance and have spread throughout hospitals worldwide (82). Canadian MRSA 7 belongs to CC1, which is in agreement with its high incidence in the community as CC1 has been previously associated with community-acquired infections (75, 79).

4.3 Comparative genomic hybridizations

The CGH data were analyzed using multiple settings in GACK in order to determine the settings that would give us the most accurate output from our data. Comparing data analysis at 100% EPP and 0% EPP, the 0% EPP data better

reflects published reports that ~78% of the *S. aureus* genome is invariable, or present in all strains (39). At a setting of 100% EPP only 48% of the genome is represented as being invariable, while 75.6% is invariable at 0% EPP. A 2006 study by Lindsay *et al* using microarrays and a different analysis technique proposed that *S. aureus* has a standard set of 1954 genes which are common to all strains (64). Our data at 0% EPP is within 100 ORFs of this estimate at 2044 ORFs. The stringency of the settings at 100% EPP is more likely to detect even subtle sequence differences and is more susceptible to false negative results. A setting of 0% should be robust against minor sequence differences, and better able to pick up true deletions as well as more major sequence differences which are likely to cause a change or loss of function (55).

Analysis of genome size finds no obvious correlation with the nosocomial or epidemic nature of the strains in this study. Due to the fact that we only analyzed two strains of community-associated MRSA, further analysis would be required to make a definitive conclusion about a comparison to the genome size of nosocomial strains. When comparing genome size to percentage of ORFs shared with Col, we see a disparity in the relationship between the two. Some strains with larger genomes share a smaller portion of ORFs in common with Col than do other strains with smaller genomes. A portion of this disparity may be attributed to the genetic content in these strains that is not captured on the array. This illuminates one of the weaknesses in the spotted array system. Comparative genomics using spotted arrays have generally required that a

positive control be present for each ORF used in the analysis. This allows for the spot to be verified, as well as providing a basis for the ratio calculation used to determine positive/negative cutoff values (55). These problems have been addressed by companies such as Affymetrix which produce oligomer-based arrays that can be read on a single channel. Due to the fact that these arrays use multiple spots to verify the presence or absence/divergence of a gene, they do not require the intrinsic positive control like spotted arrays. This allows the power to include ORFs from all sequenced *S. aureus* genomes as well as various other genes of interest (31).

Virulence factors are an important aspect of how *S. aureus* causes disease (6). The CGH data allowed us to look at the various virulence factors present on the array to determine if their presence or absence could be linked with epidemic, sporadic, nosocomial or community acquired strains. The analysis of virulence factors associated with each strain determined that there is no set of virulence factors whose presence can differentiate nosocomial, community, epidemic or sporadic strains. The highest similarity, based on these virulence factors, exists between CMRSA 1, 4 and 8. The epidemiology of these strains should further be investigated to see if there is a similarity in infectivity or types and locations of infections caused by these strains of MRSA.

The 21 regions of variability elucidated through this study do not entirely correspond to a previous CGH study by Fitzgerald *et al* in 2001 which found 18

regions of difference. The two studies were performed using different array design, and the previous study performed prior to correct annotation of the Col genome. Without proper or comparable annotation of genes, comparison of the two sets of data is difficult. Though some of the regions from this study correspond directly with the previous publication, areas with many hypothetical proteins are hard to correlate. Major variable regions with similarity between the two studies include the SCC*mec* region, bacteriophage L54a, the region containing the cap5 genes, the pathogenicity island carrying enterotoxins i and b, the region which contains exotoxins 2, 3 and 4, the area containing the epidermin related proteins along with leukocidin genes and the region containing sar T, U and fibronectin binding protein A (39). The genes in these regions most likely represent two groups of genes as proposed by Lindsay *et al* in 2006. The first group is genes on mobile genetic elements such as prophages and pathogenicity islands, cassettes and transposons. The second group of genes is what Lindsay *et al* call core variable genes. These genes are generally stable but are not essential (64). They are likely gained by recombination events. By performing our array experiments using the standard annotation for the Col genome we have provided a data set which can easily be shared and compared among laboratories and researchers.

Due to the fact that the CMRSA strains are closely related by DNA fingerprinting and MLST to other strains prevalent around the world, we are able to compare our data to other published reports about similar strains. In January

of 2006, Tenover *et al* published a paper using CGH to compare four MRSA strains common in the United States. The PFGE pattern designations for these strains are USA100, USA500, USA300 and USA400 which correspond to the PFGE generated fingerprinting patterns for CMRSA 2, 5, 10 and 7 respectively (97). Though USA500, 300 and 400 have identical DNA fingerprints to the corresponding CMRSA strains, USA100 merely falls in the same pulsed-field group (less than 7 band difference) as CMRSA 2 (8, 93). Of the microarray data published by Tenover *et al.* in 2006,316 of the ORFs corresponded directly with ORFs on our microarray. This comparison finds only two differences between USA300 and CMRSA 10, 4 differences between USA500 and CMRSA 5, 37 differences between USA400 and CMRSA 7 and 53 differences between USA100 and CMRSA 2. The large number of differences between USA100 and CMRSA 2 may be expected because these are not identical strains by PFGE banding patterns, but the differences between USA400 and CMRSA 7 were unexpected due to the fact they have identical PFGE patterns. These differences are listed in Table 16. Upon further analysis of the data provided by Tenover *et al.*, 65 of the 446 ORFs presented, or 14.6%, differ in the various USA400 strains studied. CMRSA 7 differs in 11.7% of the ORFs compared between the studies. Pulsed-field gel electrophoresis is based upon macro restriction fragment analysis. It is possible

Table 16: Differences in CGH data between USA400 and CMRSA 7

Locus	Gene	COL Description	USA400	CMRSA 7
SA0060		hypothetical protein	-	+
SA0082		hypothetical protein	-	+
SA0146	cap5K	Capsular polysaccharide synthesis	-	+
SA0260		putative transposase	-	+
SA0298		hypothetical protein	-	+
SA0420		transcriptional regulator	-	+
SA0450		hypothetical protein	-	+
SA0464		transposase family protein	-	+
SA0474	set14	Exotoxin 4; N315 exotoxin 14	+	-
SA0483		hypothetical protein	-	+
SA0487		hypothetical protein	-	+
SA0500		hypothetical protein	-	+
SA0972		hypothetical protein	-	+
SA1318		hypothetical protein	-	+
SA1339		hypothetical protein	-	+
SA1342		hypothetical protein	-	+
SA1472	ebh	Pathogenicity protein	-	+
SA1521		hypothetical protein	-	+
SA1527		hypothetical protein	+	-
SA1528		hypothetical protein	-	+
SA1531		hypothetical protein	-	+
SA1533		hypothetical protein	-	+
SA1547		hypothetical protein	-	+
SA1851		hypothetical protein	-	+
SA1852		hypothetical protein	-	+
SA1866	splD	Serine protease	+	-
SA2005		hypothetical protein	-	+
SA2065		hypothetical protein	-	+
SA2194	hysA	Hyaluronate lyase	-	+
SA2336		hypothetical protein	-	+
SA2368		acetyltransferase	-	+
SA2485		hypothetical protein	-	+
SA2486		hypothetical protein	-	+
SA2498		hypothetical protein	-	+
SA2505	pls homolog	LPXTG motif cell wall anchor domain protein	-	+
SA2513		hypothetical protein	-	+
SA2588		hypothetical protein	-	+

Notes:
+: presence of an ORF
-: absence/divergence of an ORF
USA400 data obtained from supplemental material provided for reference (97)

that genetic material could be picked up or lost in small enough fragments that it is undetectable by PFGE. It is also possible that the different arrays and different analysis methods used in each of these studies may have lead to slight differences in the data sets. In fact, GACK has been noted for overestimating positive results (106), this effect would be most predominant at a setting of 0% EPP. Most of the 31 genes are represented as present in CMRSA 7 but absent in USA100, so this effect must be taken into consideration. Further investigation would be required to elucidate the true nature of these differences.

Comparison of all hospital associated CMRSA strains to the community associated CMRSA strains revealed that SA0046 is present in the community strains but absent in the hospital strains. This ORF codes for a putative protein belonging to the metallo-beta-lactamase family, the function of which is still unknown. In order to further focus on the differences between nosocomial and community-acquired MRSA in Canada, comparison of the two most common nosocomial strains, CMRSA 1 and 2 and the two CA-MRSA CMRSA strains, CMRSA 7 and 10 was completed. This analysis found some differences between these strains. The differences in SCC*mec* region are to be expected as the SCC*mec* type IV region found in CMRSA 7 and 10 has more similarities to the SCCmec region in Col (SCC*mec* type I) than does SCC*mec* type II, found in CMRSA 1 and 2. Variable region 9, as mentioned in the results, contains only hypothetical genes so the effect of deletion of these genes on phenotype is unknown. Epidermin is a lanitbiotic active against various Gram-positive

bacteria. The presence of these genes in the community-associated strains may allow for a selective advantage in a natural environment and may therefore be more important in allowing community-associated MRSA to achieve colonization despite the presence of other bacteria. This selective advantage was also suggested for bacteriocin genes present in the USA400 strain (also known as MW2) by Baba *et al* in 2002 (7). Epidermin is also produced by *Staphylococcus epidermidis*, and this region contains genes for resistance to epidermin, providing a further survival advantage in the presence *S. epidermidis*.

Again, to better focus our comparison of epidemic strains and sporadic strains, we compared the three sporadic strains unrelated to the CMRSA strains by MLST to the eight epidemic strains found in the hospital. Sporadic strains have been linked to CA-MRSA in the literature so these strains had the potential to skew our comparison. Using this comparison, one ORF, SA2196, was found to be absent/divergent in the sporadic strains and present in the epidemic strains. This ORF is related to the HAD superfamily of hydrolases. Its function in *Staphylococcus* spp. is currently unknown, so it is also not known if the presence of this gene would give an advantage to nosocomial isolates of MRSA, allowing them to be maintained in a hospital setting.

In a 2006 study using CGH, Lindsay *et al* investigated 61 invasive *S. aureus* isolates associated with community acquired infections, and 100 non-invasive *S. aureus* isolates previously collected from healthy blood donors (64).

Like the current study, they found many differences among strains, but no set of ORFs that could predict the specific characteristic of invasiveness that they were investigating. These findings, along with the findings or our current study which found no ORFs that could definitively differentiate nosocomial from community or epidemic from sporadic strains, suggest that factors like type of disease, location of acquisition or epidemicity may be linked with host factors, environmental pressures or gene expression patterns.

Dendrograms were created using PFGE banding patterns, MLST and CGHs. It is hypothesized that CGHs will provide the highest degree of discrimination because they use the highest number of factors to compare the strains. All three techniques create slightly different trees, with MLST being more similar to the CGH data than PFGE. These differences are attributable to the differences in the techniques. Pulsed field gel electrophoresis is not discriminatory based in individual genes, but rather on mutations throughout the genome at restriction sites. This may include non-coding regions where mutations are more frequent but do not affect the overall organism. It has been suggested that even though DNA fingerprinting is adequate for short term surveillance of MRSA outbreaks, it is not appropriate for long term surveillance (14). Despite this fact, because PFGE protocols are well established and data has been collected for a number of years, and due to the high discriminatory power of PFGE it is still used successfully in long term studies (97). Multi-locus sequence typing uses seven housekeeping genes as a basis for strain

comparison. These genes are essential to *S. aureus* and should mutate at a fairly slow and constant rate (36, 69). This method allows for long term surveillance and evolutionary studies of MRSA because mutations in the seven housekeeping genes should occur at a slower pace than mutations in the *SmaI* restriction sites. Comparative genomics allows us to look at thousands of ORFs and use all of these factors to elucidate relatedness between strains. Comparative genomics has previously been used successfully in a genotyping study with known strains of *S. aureus* (31). Based on the ability to include thousands of factors for comparison of strains CGH analysis has the potential to be an important tool for evolutionary studies although short term surveillance is currently served better by simpler, more standardized techniques such as PFGE while long term surveillance of MRSA has been very successful using MLST. As microarrays become more comprehensive in their overall gene content, reliable and accessible, they will certainly become a larger part of surveillance studies.

4.4 Expression analysis

Expression analysis was carried out on the six original epidemic strains and the four sporadic strains. With this analysis, as with the comparative genomic hybridization analysis, we find no set of ORFs that can definitively be used to differentiate the epidemic strains from the sporadic strains. One ORF, SA0204, does show a 1.2 fold increase in the two sporadic strains included in the analysis, but quantitative reverse transcriptase PCR testing on further sporadic strains, as well as the remaining epidemic strains, will be required to confirm this finding. When comparing all strains to Col at mid-log phase, our analysis shows only three genes whose expression difference is beyond two fold, either up or down. Not surprisingly, this suggests that, at mid-log phase, in planktonic solution, in rich media, with no antibiotic pressure, all the MRSA strains studied have very similar expression patterns.

Previous studies of bacterial gene expression using microarrays involve the application of some environmental pressure in order to investigate the genes affected by that pressure (70, 73, 105). This study instead reflects on the genes expressed in different genetic backgrounds under controlled growth conditions. We can propose that, in the absence of environmental stress, there is a core set of essential genes whose expression is required for day-to-day survival. These genes are most likely represented by the 1140 genes found to be expressed in all 10 strains studied.

Time was a limiting factor for this research, reducing the number of experimental and technical replicates that could be performed. In this case, where possible, three experimental replicates were performed, with the duplicate array on each slide being considered the technical replicate. Where not possible, two experiments were performed, with one experiment being a dye swap of the other. To increase the power of this research, further replicates should be performed.

In the case of 00S-1635, the dye swap experiment was performed by a second operator. The variability introduced by a switch in operator was very high, and indeed affected the ability of the statistical algorithms in Partek Discovery Suite to analyze the data. By separating 00S-1635 from the rest of the data, we could better find significantly differentially regulated genes in that strain, but this analysis method is not comparable to the method used for the other strains. This strain represents a weakness in our data set and should be repeated with a single technician performing the experiments.

Though this analysis under standard lab growth conditions showed very little difference in gene expression between strains, differences may exist under different conditions. Further experiments applying antibiotic pressure or under starvation conditions may reveal differences between the epidemic and sporadic strains. This study could also be expanded to include CA-MRSA, especially

those belonging to CC1 which is a genetic background more distinct to the community than the other clonal complexes.

5.0 Conclusions:

Using microarray technology, we have been able to investigate both gene content and gene expression in epidemic and sporadic strains of MRSA in Canada. Comparative genomic hybridizations were applied to epidemic and sporadic strains of MRSA. These experiments led to the identification of 21 regions within the genome which are highly variable. We did not discover a pattern of gene presence or absence that could differentiate any of the sub-groups of strains. A single ORF, SA2196 was identified in the epidemic nosocomial strains but was absent/divergent in the sporadic strains. Further testing is required to determine the significance of this finding. Focusing our attention on the most common nosocomial strains and comparing them with the CA-MRSA allowed for the identification of some targets for further investigation, including the genes related to production of and resistance to the lantibiotic epidermin.

Expression analysis allowed us to compare the first six epidemic nosocomial MRSA strains in Canada with sporadic isolates, whose PFGE patters were only seen once during the first 10 years of surveillance. Like the CGH data, expression analysis identified a single gene whose expression may differentiate these groups. A larger sample size is required to determine if this finding is significant. Indeed, expression between all strains was very similar to that of Col. This suggests that, under standard growing conditions, gene expression is very similar between all strains.

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Appendix 1:

Differentially regulated genes in CMRSA 1-6 and sporadic MRSA strains when compared to Col, as determined using microarray based expression studies

Locus	P-value	Fold Change	Strain
SA2566	2.44E-31	1.18422	CMRSA 1
SA1536	8.29E-28	-1.1108	CMRSA 1
SAP024	9.39E-25	1.18074	CMRSA 1
SA2411	3.54E-24	-1.20581	CMRSA 1
SA2412	3.45E-23	-1.14184	CMRSA 1
SA1413	7.31E-23	-1.11559	CMRSA 1
SA1885	1.56E-22	-1.09816	CMRSA 1
SAV1977	3.03E-22	1.1136	CMRSA 1
SA1974	3.51E-22	-1.20732	CMRSA 1
SAV1955	4.51E-22	1.10381	CMRSA 1
SA1743	1.60E-21	-1.12566	CMRSA 1
SA0454	1.66E-21	1.10777	CMRSA 1
SAP002	1.16E-20	1.16216	CMRSA 1
SA1805	1.78E-19	1.23035	CMRSA 1
SAV1653	2.01E-18	1.16376	CMRSA 1
SA0728	2.18E-18	-1.11594	CMRSA 1
SAV1964	4.93E-18	1.17452	CMRSA 1
SA0033	5.28E-18	-1.16887	CMRSA 1
SAV1957	5.02E-17	1.10234	CMRSA 1
SA2091	7.67E-17	-1.08815	CMRSA 1
SA2401	2.42E-16	-1.20833	CMRSA 1
SAV1985	4.48E-16	1.12132	CMRSA 1
SA1445	6.66E-16	-1.11217	CMRSA 1
SA0793	5.91E-15	-1.0791	CMRSA 1
SAV1965	1.92E-14	1.08716	CMRSA 1
SAV2001	2.32E-14	1.16544	CMRSA 1
SAP029	6.51E-14	1.12866	CMRSA 1
SAV1959	7.16E-14	1.09205	CMRSA 1
SA2300	1.53E-13	-1.14197	CMRSA 1
SA0303	1.65E-13	-1.1227	CMRSA 1
SA0157	2.51E-13	-1.11785	CMRSA 1
SA2117	4.28E-13	-1.08098	CMRSA 1
SAP022	1.12E-12	1.14538	CMRSA 1
SA2498	2.07E-12	-1.0881	CMRSA 1
SA0777	2.24E-12	-1.10911	CMRSA 1
SAV0884	4.71E-12	1.12983	CMRSA 1
SAV0050	5.37E-12	1.1151	CMRSA 1
SAV0300	6.56E-12	1.09018	CMRSA 1
SA2704	7.33E-12	1.13167	CMRSA 1

SA1861	8.31E-12	-1.08844	CMRSA 1
SAV1963	1.05E-11	1.08059	CMRSA 1
SA1939	1.06E-11	-1.09203	CMRSA 1
SA1071	1.20E-11	1.05561	CMRSA 1
SA1163	1.28E-11	-1.07419	CMRSA 1
SAP008	1.39E-11	1.28365	CMRSA 1
SA1435	2.02E-11	-1.06624	CMRSA 1
SA1755	2.31E-11	1.21661	CMRSA 1
SA2396	3.28E-11	-1.23631	CMRSA 1
SA0774	3.40E-11	-1.12606	CMRSA 1
SAV0076	1.01E-10	1.10259	CMRSA 1
SA1465	1.04E-10	-1.06595	CMRSA 1
SA1786	1.35E-10	-1.08309	CMRSA 1
SA1329	1.35E-10	-1.08838	CMRSA 1
SA2103	1.40E-10	-1.06343	CMRSA 1
SA0031	1.81E-10	1.0627	CMRSA 1
SA2272	1.89E-10	-1.07602	CMRSA 1
SA2125	2.28E-10	-1.08425	CMRSA 1
SA1940	3.61E-10	1.07452	CMRSA 1
SA1211	4.96E-10	1.06928	CMRSA 1
SA1213	5.05E-10	1.13418	CMRSA 1
SAP028	5.19E-10	1.08778	CMRSA 1
SA0690	5.23E-10	1.11787	CMRSA 1
SA2261	5.71E-10	-1.05493	CMRSA 1
SA0759	6.11E-10	1.14652	CMRSA 1
SAA0001	6.53E-10	-1.19212	CMRSA 1
SA1427	8.55E-10	-1.15799	CMRSA 1
SA0271	1.22E-09	-1.10533	CMRSA 1
SAV1445	1.22E-09	-1.04602	CMRSA 1
SA1806	1.26E-09	1.0951	CMRSA 1
SA2410	2.00E-09	-1.07842	CMRSA 1
SAV1983	2.55E-09	1.0624	CMRSA 1
SA1926	2.94E-09	-1.09642	CMRSA 1
SA0503	3.08E-09	-1.15242	CMRSA 1
SAP031	3.82E-09	1.73036	CMRSA 1
SA2567	4.48E-09	1.08068	CMRSA 1
SA1573	5.73E-09	-1.20573	CMRSA 1
SA1212	6.50E-09	1.11723	CMRSA 1
SA2092	7.54E-09	-1.10162	CMRSA 1
SA1560	7.58E-09	-1.07236	CMRSA 1
SA0692	8.74E-09	1.06949	CMRSA 1
SA1383	9.19E-09	-1.16316	CMRSA 1
SA2578	9.79E-09	-1.06903	CMRSA 1
SAV0849	1.09E-08	1.06022	CMRSA 1
SA2435	1.12E-08	1.05804	CMRSA 1
SA2293	1.20E-08	-1.04321	CMRSA 1

SA2563	1.44E-08	-1.15307	CMRSA 1
SA1799	1.52E-08	1.08381	CMRSA 1
SA1066	1.53E-08	-1.07931	CMRSA 1
SA0028	1.58E-08	1.14158	CMRSA 1
SA0698	1.59E-08	1.05025	CMRSA 1
SA1447	1.64E-08	-1.05721	CMRSA 1
SA0757	1.74E-08	1.14804	CMRSA 1
SA0022	2.09E-08	-1.06403	CMRSA 1
SA1214	2.28E-08	1.11497	CMRSA 1
SA1411	2.44E-08	-1.04929	CMRSA 1
SA2577	2.49E-08	-1.05217	CMRSA 1
SAV0049	2.52E-08	1.08057	CMRSA 1
SA2616	2.71E-08	1.05773	CMRSA 1
SA0622	3.18E-08	1.07835	CMRSA 1
SA1216	3.83E-08	1.08144	CMRSA 1
SA1759	3.86E-08	1.06983	CMRSA 1
SA1243	4.12E-08	1.08229	CMRSA 1
SA0023	5.01E-08	1.04732	CMRSA 1
SA2384	5.30E-08	-1.07794	CMRSA 1
SA0034	6.13E-08	-1.07772	CMRSA 1
SA2054	6.27E-08	-1.08563	CMRSA 1
SA1812	6.54E-08	-1.12335	CMRSA 1
SA2119	6.57E-08	-1.06252	CMRSA 1
SAV0051	7.08E-08	1.37399	CMRSA 1
SA0557	7.37E-08	-1.07431	CMRSA 1
SA2572	8.71E-08	1.12492	CMRSA 1
SA2602	9.49E-08	-1.06583	CMRSA 1
34_B5	9.75E-08	1.66868	CMRSA 1
SA0689	9.86E-08	1.08101	CMRSA 1
SA2448	1.10E-07	-1.03575	CMRSA 1
SA2573	1.11E-07	1.08406	CMRSA 1
SAV0715	1.13E-07	-1.08259	CMRSA 1
SA0688	1.25E-07	1.08223	CMRSA 1
SA1744	1.47E-07	1.05179	CMRSA 1
SA2462	1.56E-07	-1.16166	CMRSA 1
SA2660	1.58E-07	-1.18092	CMRSA 1
SA2713	1.72E-07	-1.05268	CMRSA 1
SA0006	1.85E-07	-1.06832	CMRSA 1
SA2631	1.92E-07	-1.05736	CMRSA 1
SAV0081	2.27E-07	1.05889	CMRSA 1
SA0507	2.30E-07	-1.19958	CMRSA 1
SA2525	2.40E-07	-1.07983	CMRSA 1
SA0705	2.48E-07	1.05991	CMRSA 1
SA1619	2.86E-07	-1.07104	CMRSA 1
SA1669	3.09E-07	-1.05856	CMRSA 1
SA1558	3.23E-07	1.03854	CMRSA 1

SA0845	3.26E-07	-1.07129	CMRSA 1
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SA1795	3.32E-07	1.07581	CMRSA 1
SA2619	3.47E-07	1.50226	CMRSA 1
SA0747	3.63E-07	1.04787	CMRSA 1
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SA1528	4.18E-07	-1.07093	CMRSA 1
SA0875	5.37E-07	-1.17337	CMRSA 1
SA1245	6.11E-07	1.07429	CMRSA 1
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SA0696	7.34E-07	1.06894	CMRSA 1
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SA2018	8.85E-07	-1.07725	CMRSA 1
34_C5	9.14E-07	1.54208	CMRSA 1
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SA1462	1.04E-06	-1.04391	CMRSA 1
SA1515	1.05E-06	-1.06577	CMRSA 1
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SA1537	1.14E-06	-1.03838	CMRSA 1
SA1065	1.15E-06	-1.07704	CMRSA 1
SA0446	1.24E-06	-1.0772	CMRSA 1
SA0516	1.36E-06	-1.10643	CMRSA 1
SA2701	1.37E-06	1.06694	CMRSA 1
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SA1553	1.42E-06	-1.0548	CMRSA 1
SAP023	1.49E-06	1.06305	CMRSA 1
p_34_w_A5	1.50E-06	1.73213	CMRSA 1
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SA1712	1.59E-06	-1.03288	CMRSA 1
SA1804	1.60E-06	1.0753	CMRSA 1
SA0032	1.67E-06	1.06658	CMRSA 1
SA0575	1.77E-06	1.05521	CMRSA 1
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SA0866	1.87E-06	-1.11286	CMRSA 1
SA0510	1.95E-06	-1.04236	CMRSA 1
SA1561	2.06E-06	-1.07421	CMRSA 1
SA1130	2.26E-06	1.06086	CMRSA 1
SA2461	2.32E-06	-1.07939	CMRSA 1
SA0912	2.60E-06	-1.05023	CMRSA 1
SAV0065	2.83E-06	1.04165	CMRSA 1
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SAA0003	3.02E-06	-1.19791	CMRSA 1
SAV0024	3.32E-06	1.06345	CMRSA 1
SA1281	3.50E-06	-1.0722	CMRSA 1
SA1802	3.94E-06	1.15586	CMRSA 1

SA1354	4.01E-06	-1.03826	CMRSA 1
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SA2712	4.32E-06	-1.04753	CMRSA 1
SAV1787	4.36E-06	1.0284	CMRSA 1
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SAV1954	5.34E-06	1.03389	CMRSA 1
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SA2413	5.94E-06	-1.0464	CMRSA 1
SA0916	6.07E-06	-1.04495	CMRSA 1
SA1308	6.71E-06	-1.03848	CMRSA 1
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SA1395	1.04E-05	-1.05167	CMRSA 1
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SA2501	1.35E-05	-1.04833	CMRSA 1
SA0024	1.44E-05	-1.07202	CMRSA 1
SAP003	1.45E-05	1.05147	CMRSA 1
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tetK	1.50E-05	-1.50116	CMRSA 1
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SA1444	1.74E-05	-1.09035	CMRSA 1
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SA0617	1.85E-05	1.0303	CMRSA 1
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SA0065	1.94E-05	-1.27582	CMRSA 1
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SA1847	2.14E-05	-1.06042	CMRSA 1
SAV1982	2.21E-05	1.0837	CMRSA 1

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SA0720	2.61E-05	-1.0747	CMRSA 1
SA0993	2.64E-05	-1.06112	CMRSA 1
SA1472	2.66E-05	1.03809	CMRSA 1
SAV0080	2.72E-05	1.03448	CMRSA 1
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SA2488	2.80E-05	-1.04432	CMRSA 1
SA2582	2.95E-05	1.04868	CMRSA 1
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SA1807	4.12E-05	1.05059	CMRSA 1
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SA1122	4.27E-05	-1.03038	CMRSA 1
SAV2461	4.33E-05	1.05265	CMRSA 1
SAV0906	4.39E-05	1.03514	CMRSA 1
SA1289	4.47E-05	-1.23152	CMRSA 1
SA1622	4.57E-05	-1.09968	CMRSA 1
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SA1469	5.96E-05	-1.33856	CMRSA 1
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SA0035	6.35E-05	-1.16022	CMRSA 1
SAV0901	6.43E-05	1.02457	CMRSA 1
SA0415	6.50E-05	-1.02866	CMRSA 1
SA2703	6.52E-05	1.05796	CMRSA 1

SA1733	6.59E-05	1.03422	CMRSA 1
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SA2650	6.86E-05	1.03395	CMRSA 1
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SA2120	7.28E-05	-1.18305	CMRSA 1
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SAV0869	8.11E-05	1.02408	CMRSA 1
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SA1891	8.66E-05	-1.10612	CMRSA 1
SA1187	8.68E-05	-1.59462	CMRSA 1
SA2072	8.91E-05	-1.06064	CMRSA 1
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SAV1653	4.69E-15	1.16415	CMRSA 2
SA0033	9.71E-15	-1.16991	CMRSA 2
SA0051	1.99E-13	-1.19861	CMRSA 2
SAP002	9.60E-13	1.1276	CMRSA 2
SA1761	1.05E-11	1.15474	CMRSA 2
SA0370	1.82E-11	-1.13274	CMRSA 2
SA0774	2.26E-11	-1.15648	CMRSA 2
SA1805	6.96E-11	1.1693	CMRSA 2
SA2631	1.02E-10	-1.09304	CMRSA 2
SA0050	1.13E-10	-1.32068	CMRSA 2
SAV0849	2.99E-10	1.08144	CMRSA 2
SA2577	7.27E-10	-1.07204	CMRSA 2
SAV0050	1.54E-09	1.11524	CMRSA 2
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SA0028	1.32E-08	1.17023	CMRSA 2
SAV0434	2.47E-08	1.1732	CMRSA 2
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SA1772	2.77E-08	-1.06202	CMRSA 2
SA1773	2.87E-08	-1.08082	CMRSA 2
SAV0200	3.67E-08	1.08141	CMRSA 2
SA2176	6.20E-08	-1.06908	CMRSA 2
SAV1953	6.39E-08	1.05317	CMRSA 2
SA0301	9.31E-08	-1.1574	CMRSA 2
SAV0049	1.05E-07	1.09086	CMRSA 2
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SA0281	1.35E-07	-1.10464	CMRSA 2

SAV2209	2.71E-07	1.14765	CMRSA 2
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SA0368	3.50E-07	-1.63705	CMRSA 2
SA2509	3.63E-07	1.09822	CMRSA 2
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SA0384	6.56E-07	-1.47678	CMRSA 2
SA0778	7.70E-07	-1.05233	CMRSA 2
SA1712	7.96E-07	-1.0409	CMRSA 2
SAA0001	9.05E-07	-1.1695	CMRSA 2
SA1445	9.19E-07	-1.06628	CMRSA 2
SA2625	1.17E-06	-1.05502	CMRSA 2
SAV0413	1.32E-06	1.05105	CMRSA 2
SA1002	1.56E-06	-1.05804	CMRSA 2
SA0022	1.73E-06	-1.06308	CMRSA 2
SA0444	2.00E-06	-1.12587	CMRSA 2
SA1806	2.29E-06	1.08356	CMRSA 2
SA1996	2.57E-06	-1.0531	CMRSA 2
SAV0715	2.65E-06	-1.08555	CMRSA 2
SA2178	3.12E-06	1.04072	CMRSA 2
SA0759	3.64E-06	1.12272	CMRSA 2
SA1926	4.11E-06	-1.08369	CMRSA 2
SA0461	4.15E-06	1.04983	CMRSA 2
SA1447	4.18E-06	-1.05326	CMRSA 2
SA2261	4.61E-06	-1.04491	CMRSA 2
SA2576	4.69E-06	-1.0819	CMRSA 2
SA2605	5.07E-06	-1.07061	CMRSA 2
SA0757	6.83E-06	1.13503	CMRSA 2
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SA0772	8.35E-06	1.05071	CMRSA 2
SA0446	1.12E-05	-1.0826	CMRSA 2
SAV0081	1.20E-05	1.05777	CMRSA 2
SA2614	1.25E-05	1.05592	CMRSA 2
SA1933	1.44E-05	-1.06433	CMRSA 2
SAV0435	1.55E-05	-1.05471	CMRSA 2
SA1620	1.55E-05	-1.02848	CMRSA 2
SA1755	1.59E-05	1.15099	CMRSA 2
SA0665	1.73E-05	1.07978	CMRSA 2
SA0035	2.00E-05	-1.21553	CMRSA 2
SA0380	2.07E-05	-1.72905	CMRSA 2
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34_B5	2.10E-05	1.6127	CMRSA 2
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SA2645	5.06E-19	-1.10557	CMRSA 3
SAV0413	2.83E-18	1.11316	CMRSA 3
SA0281	3.17E-16	-1.20575	CMRSA 3
SA0370	6.85E-16	-1.17931	CMRSA 3

SA1755	7.06E-16	1.33774	CMRSA 3
SA0016	1.63E-14	-1.06525	CMRSA 3
SAV1653	6.39E-14	1.15319	CMRSA 3
SA1016	1.87E-13	1.0964	CMRSA 3
SA0051	2.45E-13	-1.1963	CMRSA 3
SA1805	1.53E-12	1.18832	CMRSA 3
SA0460	3.57E-12	1.08229	CMRSA 3
SA0478	5.16E-12	1.16349	CMRSA 3
SA1264	6.24E-12	1.12257	CMRSA 3
SAV0411	1.09E-11	1.05039	CMRSA 3
SA2631	1.10E-11	-1.09966	CMRSA 3
SA2525	1.11E-11	1.12467	CMRSA 3
SAV0849	1.22E-11	1.08944	CMRSA 3
SA0033	2.11E-11	-1.13341	CMRSA 3
SA0022	3.38E-11	-1.09754	CMRSA 3
SA0026	4.17E-11	-1.15472	CMRSA 3
SA2581	4.45E-11	-1.08649	CMRSA 3
SA0461	8.35E-11	1.0759	CMRSA 3
SA0050	9.94E-11	-1.32049	CMRSA 3
SA0777	1.97E-10	-1.1141	CMRSA 3
SA2576	2.69E-10	-1.12592	CMRSA 3
SAV2001	4.29E-10	1.14895	CMRSA 3
SAV1974	5.30E-10	-1.05915	CMRSA 3
SA0918	6.27E-10	-1.07363	CMRSA 3
SA0278	6.38E-10	-1.11965	CMRSA 3
SA2678	7.51E-10	-1.14022	CMRSA 3
SA2577	8.72E-10	-1.07124	CMRSA 3
SA1806	9.57E-10	1.11412	CMRSA 3
SA2509	1.21E-09	1.12196	CMRSA 3
SA0774	1.47E-09	-1.13377	CMRSA 3
SA0276	4.21E-09	-1.10872	CMRSA 3
SA0688	4.98E-09	1.11079	CMRSA 3
SA2578	5.70E-09	-1.08496	CMRSA 3
SA0557	1.21E-08	1.08825	CMRSA 3
SA1770	1.24E-08	-1.06893	CMRSA 3
SA2596	1.27E-08	-1.07444	CMRSA 3
SA0690	1.36E-08	1.12496	CMRSA 3
SA0768	1.41E-08	1.05916	CMRSA 3
SA2665	1.47E-08	-1.06446	CMRSA 3
SA1771	1.58E-08	-1.05837	CMRSA 3
SA1957	1.69E-08	-1.05028	CMRSA 3
SA2517	1.86E-08	-1.0489	CMRSA 3
SA2716	1.89E-08	-1.10936	CMRSA 3
SAP002	2.08E-08	1.0924	CMRSA 3
SA2088	4.20E-08	-1.12398	CMRSA 3
SA0097	4.79E-08	-1.06631	CMRSA 3

SA0387	7.55E-08	-1.35644	CMRSA 3
SA0218	8.76E-08	-1.81634	CMRSA 3
SA0454	1.22E-07	1.05506	CMRSA 3
SAV0715	1.25E-07	-1.09929	CMRSA 3
SA0034	1.67E-07	-1.0896	CMRSA 3
SA2588	1.72E-07	-1.06317	CMRSA 3
SA2530	1.87E-07	-1.08091	CMRSA 3
SAV1982	2.04E-07	1.12608	CMRSA 3
SA0689	2.36E-07	1.09281	CMRSA 3
SA1445	2.38E-07	-1.07044	CMRSA 3
SA1168	3.70E-07	1.07948	CMRSA 3
SAV0050	4.07E-07	1.0923	CMRSA 3
SA2557	4.42E-07	-1.10881	CMRSA 3
SA0028	6.42E-07	1.14423	CMRSA 3
SA2712	6.54E-07	-1.06293	CMRSA 3
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SA0053	1.21E-06	-1.09856	CMRSA 3
SA1206	1.37E-06	1.05692	CMRSA 3
SA1194	1.37E-06	-1.0557	CMRSA 3
SA2660	1.55E-06	-1.19638	CMRSA 3
SA2446	1.57E-06	-1.06145	CMRSA 3
SA1772	1.66E-06	-1.05114	CMRSA 3
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SA0368	2.05E-06	-1.55403	CMRSA 3
SA1787	2.13E-06	1.10574	CMRSA 3
SA0280	2.33E-06	-1.05823	CMRSA 3
SAV1445	2.39E-06	-1.0399	CMRSA 3
SA2681	2.84E-06	-1.06936	CMRSA 3
SA0301	3.18E-06	-1.13011	CMRSA 3
SA2055	3.21E-06	-1.05911	CMRSA 3
SA0286	3.21E-06	-1.07471	CMRSA 3
SA2592	3.48E-06	-1.22607	CMRSA 3
SA1996	4.62E-06	-1.0512	CMRSA 3
SA2052	4.80E-06	-1.07658	CMRSA 3
SA1319	5.48E-06	-1.05881	CMRSA 3
SA1926	5.84E-06	-1.08163	CMRSA 3
SA1164	6.19E-06	1.14004	CMRSA 3
SA0173	6.69E-06	-1.20617	CMRSA 3
SA0384	6.72E-06	-1.40073	CMRSA 3
SA2063	7.41E-06	1.05494	CMRSA 3
SA1924	7.59E-06	-1.05843	CMRSA 3
SA1907	7.84E-06	-1.04008	CMRSA 3
SA0912	1.02E-05	-1.05582	CMRSA 3
SA2713	1.15E-05	-1.05088	CMRSA 3

SA2605	1.15E-05	-1.06697	CMRSA 3
SAV0395	1.15E-05	1.07717	CMRSA 3
SA0484	1.17E-05	1.10737	CMRSA 3
SA2718	1.18E-05	-1.04949	CMRSA 3
SA0098	1.22E-05	-1.06393	CMRSA 3
SA1094	1.30E-05	-1.07347	CMRSA 3
SA0757	1.38E-05	1.12925	CMRSA 3
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SA0054	1.43E-05	-1.87165	CMRSA 3
SA2381	1.45E-05	-1.37791	CMRSA 3
SAV1486	1.50E-05	-1.046	CMRSA 3
SA1954	1.69E-05	-1.04537	CMRSA 3
SA2463	1.74E-05	1.03486	CMRSA 3
SA0390	1.79E-05	-2.64735	CMRSA 3
SA0759	1.85E-05	1.11167	CMRSA 3
SA2689	2.04E-05	-1.04922	CMRSA 3
SA2511	2.09E-05	1.0949	CMRSA 3
SA1137	2.28E-05	-1.20644	CMRSA 3
SA0636	2.34E-05	-1.06099	CMRSA 3
SAV0049	2.45E-05	1.06899	CMRSA 3
SA2669	2.47E-05	-1.04564	CMRSA 3
SA1794	2.51E-05	1.13185	CMRSA 3
SA2625	2.51E-05	-1.04605	CMRSA 3
SA1135	2.81E-05	-1.05724	CMRSA 3
SAV0397	3.48E-05	1.0724	CMRSA 3
SA1743	3.88E-27	-1.20848	CMRSA 4
SA0292	8.45E-15	-1.46464	CMRSA 4
SA2411	1.47E-14	-1.15041	CMRSA 4
SA1744	2.47E-14	1.10319	CMRSA 4
SAV0801	3.42E-14	1.10421	CMRSA 4
SA2401	6.35E-14	-1.22491	CMRSA 4
SA0370	7.81E-14	-1.16113	CMRSA 4
SA2410	4.40E-13	-1.12888	CMRSA 4
SA1560	8.31E-13	-1.1226	CMRSA 4
SAV1653	8.49E-13	1.14662	CMRSA 4
SA0051	9.25E-13	-1.19365	CMRSA 4
SA0061	3.28E-12	-1.26082	CMRSA 4
SA2115	3.57E-12	1.12779	CMRSA 4
SA2412	8.86E-12	-1.09137	CMRSA 4
SA0281	2.04E-11	-1.15119	CMRSA 4
SA0301	9.05E-11	-1.21576	CMRSA 4
SA1805	9.34E-11	1.17112	CMRSA 4
SA0516	1.10E-10	-1.20189	CMRSA 4
SA1290	1.20E-10	-1.07587	CMRSA 4
SA2300	1.89E-10	-1.14093	CMRSA 4
SA0461	5.61E-10	1.07316	CMRSA 4

SAP024	8.36E-10	1.09897	CMRSA 4
SA0271	1.58E-09	-1.12998	CMRSA 4
SA0793	3.41E-09	-1.06488	CMRSA 4
SA2488	3.82E-09	-1.08415	CMRSA 4
SA2462	3.85E-09	-1.24332	CMRSA 4
SA0717	3.93E-09	-1.2806	CMRSA 4
SA0406	7.90E-09	-1.5422	CMRSA 4
SA1891	1.11E-08	-1.22179	CMRSA 4
SA2407	1.44E-08	1.07001	CMRSA 4
SA0050	1.72E-08	-1.26327	CMRSA 4
SA0774	1.83E-08	-1.12402	CMRSA 4
SA1638	1.91E-08	1.09635	CMRSA 4
SAP002	2.11E-08	1.09461	CMRSA 4
SA1319	2.59E-08	-1.07815	CMRSA 4
SA1806	2.86E-08	1.10338	CMRSA 4
SA0198	2.98E-08	-1.07313	CMRSA 4
tetK	3.83E-08	-2.17167	CMRSA 4
34_C5	4.24E-08	1.75392	CMRSA 4
SA2176	5.79E-08	-1.07076	CMRSA 4
SA0046	7.48E-08	-1.10095	CMRSA 4
SA2634	8.53E-08	-1.10973	CMRSA 4
SA0517	9.05E-08	-1.1104	CMRSA 4
SAP022	1.09E-07	1.121	CMRSA 4
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SA0720	1.38E-07	-1.12379	CMRSA 4
SA1639	1.54E-07	1.08702	CMRSA 4
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SA0777	2.19E-07	-1.08818	CMRSA 4
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SA2581	2.63E-07	-1.06346	CMRSA 4
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SAV1807	2.97E-07	-1.05235	CMRSA 4
SA0619	3.75E-07	-1.15145	CMRSA 4
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SA0679	5.43E-07	-1.0676	CMRSA 4
SA2616	5.55E-07	1.06192	CMRSA 4
SAV2027	6.84E-07	1.06104	CMRSA 4
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SA1328	7.34E-07	1.0627	CMRSA 4
SAV1964	8.97E-07	1.09842	CMRSA 4

SA0820	9.30E-07	1.09095	CMRSA 4
SA0507	1.01E-06	-1.23372	CMRSA 4
SA0282	1.05E-06	-1.11578	CMRSA 4
SA2461	1.28E-06	-1.10164	CMRSA 4
SA1066	1.50E-06	-1.07939	CMRSA 4
SA0383	1.50E-06	-1.40352	CMRSA 4
SA0387	1.90E-06	-1.30358	CMRSA 4
SA0028	1.92E-06	1.14018	CMRSA 4
SA0053	2.08E-06	-1.0983	CMRSA 4
SA2392	2.25E-06	-1.1366	CMRSA 4
SA0598	2.32E-06	1.14012	CMRSA 4
SA0139	2.70E-06	-1.56679	CMRSA 4
SA2660	2.75E-06	-1.19533	CMRSA 4
SA2025	2.98E-06	-1.09231	CMRSA 4
SA0799	3.28E-06	-1.06794	CMRSA 4
SA0035	3.86E-06	-1.24713	CMRSA 4
SA1168	4.42E-06	-1.07782	CMRSA 4
SA2614	4.45E-06	1.06038	CMRSA 4
SA2608	4.52E-06	1.04521	CMRSA 4
SA2527	5.02E-06	-1.78474	CMRSA 4
SA2635	5.10E-06	-1.11412	CMRSA 4
SA1358	5.16E-06	-1.44509	CMRSA 4
SA0923	5.53E-06	-1.5582	CMRSA 4
SA0096	5.96E-06	1.07012	CMRSA 4
SA2631	6.23E-06	-1.0592	CMRSA 4
SAV0050	6.81E-06	1.08228	CMRSA 4
SA2498	7.61E-06	-1.05933	CMRSA 4
MW0390	7.81E-06	1.63893	CMRSA 4
SA0421	9.20E-06	-1.25001	CMRSA 4
SA1854	9.40E-06	-1.23799	CMRSA 4
SA2489	9.66E-06	-1.07181	CMRSA 4
SA1528	1.03E-05	-1.07377	CMRSA 4
SA2582	1.09E-05	1.06286	CMRSA 4
SA1654	1.27E-05	1.04829	CMRSA 4
SA1861	1.43E-05	-1.05979	CMRSA 4
SA1755	1.60E-05	1.15396	CMRSA 4
SA1525	1.74E-05	1.04356	CMRSA 4
SA2272	1.90E-05	-1.05599	CMRSA 4
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SA0568	2.50E-05	1.0806	CMRSA 4
SA2556	2.50E-05	1.06333	CMRSA 4
SA1292	2.80E-05	1.07691	CMRSA 4
SA0023	2.82E-05	1.04228	CMRSA 4
SAP029	2.84E-05	1.07523	CMRSA 4
SA1887	2.84E-05	-1.05848	CMRSA 4
SAV0049	2.85E-05	1.07001	CMRSA 4

SAV1954	3.01E-05	1.03738	CMRSA 4
SA1163	3.08E-05	-1.04863	CMRSA 4
SAV0081	3.27E-05	1.05552	CMRSA 4
SA1261	3.30E-05	1.32029	CMRSA 4
SA0994	3.37E-05	-1.65261	CMRSA 4
SA2391	3.79E-05	-1.10502	CMRSA 4
SA2025	3.44E-14	1.15993	CMRSA 5
SA0700	1.23E-12	1.09538	CMRSA 5
SA0050	1.53E-12	-1.39814	CMRSA 5
SA2026	2.83E-11	1.13702	CMRSA 5
SA0275	3.96E-11	1.09223	CMRSA 5
SAV0413	5.25E-11	1.07733	CMRSA 5
SA0033	5.73E-11	-1.13461	CMRSA 5
SA2004	5.92E-11	1.15029	CMRSA 5
SA1328	1.10E-10	1.08851	CMRSA 5
SA2713	1.99E-10	1.07916	CMRSA 5
SA2541	4.06E-10	1.11846	CMRSA 5
SA1528	7.30E-10	-1.11658	CMRSA 5
SA0768	1.36E-09	1.06682	CMRSA 5
SA0595	1.59E-09	1.0498	CMRSA 5
SA0759	1.62E-09	1.1751	CMRSA 5
SA1807	3.06E-09	1.09686	CMRSA 5
SA2112	6.68E-09	-1.19398	CMRSA 5
SA2422	7.31E-09	1.09836	CMRSA 5
SA0051	7.84E-09	-1.14153	CMRSA 5
SA2639	8.05E-09	1.06889	CMRSA 5
SA0774	1.20E-08	-1.12825	CMRSA 5
SA1782	1.32E-08	1.08344	CMRSA 5
SA0053	1.82E-08	-1.12546	CMRSA 5
SAV1486	2.67E-08	-1.06551	CMRSA 5
SA0370	2.87E-08	-1.1045	CMRSA 5
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SA2178	1.11E-07	1.04912	CMRSA 5
SA0617	1.54E-07	1.04749	CMRSA 5
SA0597	2.00E-07	1.08742	CMRSA 5
SA0777	2.59E-07	-1.08881	CMRSA 5
SAV1953	3.05E-07	1.05148	CMRSA 5
SA0031	3.05E-07	1.05879	CMRSA 5
SA0135	3.76E-07	1.1312	CMRSA 5
SA1072	4.17E-07	1.11465	CMRSA 5
SAP002	4.27E-07	1.08466	CMRSA 5
SAV2208	4.28E-07	-1.28526	CMRSA 5
SA1329	4.65E-07	1.07403	CMRSA 5
SA0198	5.42E-07	1.06114	CMRSA 5
SA1940	5.55E-07	1.06953	CMRSA 5

SA0758	6.36E-07	1.15326	CMRSA 5
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SA2614	8.07E-07	1.06664	CMRSA 5
SA0487	9.03E-07	-1.43595	CMRSA 5
SA0690	1.33E-06	1.10686	CMRSA 5
SA0278	1.54E-06	1.08142	CMRSA 5
SA2704	1.88E-06	1.10295	CMRSA 5
SA0664	2.19E-06	-1.17352	CMRSA 5
SA0757	2.33E-06	1.14773	CMRSA 5
SAV0715	2.35E-06	-1.08931	CMRSA 5
SA0703	2.38E-06	1.04629	CMRSA 5
SA1445	2.69E-06	-1.06494	CMRSA 5
SA1812	2.69E-06	-1.12942	CMRSA 5
SA2345	2.75E-06	1.03835	CMRSA 5
SA0257	3.25E-06	-1.29097	CMRSA 5
SA0573	3.31E-06	1.06024	CMRSA 5
SA0124	3.78E-06	1.06178	CMRSA 5
SA2024	4.39E-06	-2.00119	CMRSA 5
SA2006	4.60E-06	1.10611	CMRSA 5
SA1814	5.71E-06	1.08844	CMRSA 5
SA1389	5.97E-06	1.08243	CMRSA 5
SAA0001	6.23E-06	-1.15738	CMRSA 5
SAV0938	6.90E-06	1.06416	CMRSA 5
SA1787	6.92E-06	1.10358	CMRSA 5
SA0689	7.20E-06	1.08164	CMRSA 5
SA0552	7.31E-06	1.0808	CMRSA 5
SA2505	7.97E-06	1.09318	CMRSA 5
SA1531	8.12E-06	-1.0712	CMRSA 5
SA0281	8.21E-06	1.08041	CMRSA 5
SA0526	8.30E-06	1.03367	CMRSA 5
SA1638	8.78E-06	1.07387	CMRSA 5
SA2618	8.97E-06	1.06891	CMRSA 5
SA1982	9.27E-06	1.03365	CMRSA 5
SA1924	1.16E-05	-1.05933	CMRSA 5
SA0553	1.17E-05	-1.04953	CMRSA 5
SA1162	1.27E-05	1.06738	CMRSA 5
SAV1952	1.38E-05	-1.0937	CMRSA 5
SA0317	1.49E-05	1.08686	CMRSA 5
SA0575	1.50E-05	1.06082	CMRSA 5
SA2616	1.71E-05	1.0526	CMRSA 5
SA1678	1.78E-05	-1.06129	CMRSA 5
SA1014	1.81E-05	-1.05029	CMRSA 5
SA2650	1.96E-05	1.04536	CMRSA 5
SA2296	2.08E-05	1.06653	CMRSA 5
SA1926	2.19E-05	-1.07843	CMRSA 5
SA2056	2.23E-05	-1.08231	CMRSA 5

SAV0411	2.25E-05	1.02928	CMRSA 5
SA1594	2.36E-05	1.03341	CMRSA 5
SA2293	2.49E-05	1.03577	CMRSA 5
SA2407	2.52E-05	1.04995	CMRSA 5
SA0982	2.69E-05	-1.10716	CMRSA 5
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SA1481	2.83E-05	1.08131	CMRSA 5
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SA0033	2.53E-18	-1.18026	CMRSA 6
SA0281	5.17E-18	-1.1957	CMRSA 6
SA0051	1.22E-16	-1.20882	CMRSA 6
SA0016	3.32E-15	-1.05902	CMRSA 6
SA0370	4.81E-15	-1.14558	CMRSA 6
SA0050	1.25E-14	-1.37337	CMRSA 6
SAV1653	5.43E-14	1.13465	CMRSA 6
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SAV0050	5.81E-13	1.12738	CMRSA 6
SAV0849	6.07E-13	1.08513	CMRSA 6
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SA0774	2.29E-12	-1.14408	CMRSA 6
SA0461	8.27E-12	1.07118	CMRSA 6
SA2609	2.04E-11	1.07731	CMRSA 6
SA1805	2.46E-11	1.15227	CMRSA 6
SA0026	4.83E-11	-1.1322	CMRSA 6
SA0777	4.91E-11	-1.10327	CMRSA 6
SA1445	6.07E-11	-1.08417	CMRSA 6
SA0759	1.01E-10	1.16153	CMRSA 6
SAV0411	1.94E-10	1.04042	CMRSA 6
SA0034	3.54E-10	-1.09938	CMRSA 6
SA1771	7.40E-10	-1.05687	CMRSA 6
SA2665	1.52E-09	-1.06103	CMRSA 6
SA0028	1.77E-09	1.15947	CMRSA 6
SA0758	2.61E-09	1.16089	CMRSA 6
SA1715	3.11E-09	-1.06388	CMRSA 6
SA0031	5.16E-09	1.05827	CMRSA 6
SA2631	5.35E-09	-1.0695	CMRSA 6
SA0278	7.86E-09	-1.09373	CMRSA 6
SA1773	8.35E-09	-1.07329	CMRSA 6
SA0757	9.67E-09	1.15748	CMRSA 6
SAV0715	1.09E-08	-1.09537	CMRSA 6
SA1806	1.12E-08	1.09155	CMRSA 6
SA0022	1.48E-08	-1.06775	CMRSA 6
SA0384	3.20E-08	-1.47039	CMRSA 6
SA1617	3.60E-08	-1.05582	CMRSA 6
SA2645	7.12E-08	-1.04298	CMRSA 6

SA1926	8.51E-08	-1.08758	CMRSA 6
SA0655	1.43E-07	1.09727	CMRSA 6
SA1781	1.81E-07	1.05073	CMRSA 6
SA2221	2.27E-07	-1.05731	CMRSA 6
SA0772	2.58E-07	1.05241	CMRSA 6
SA0088	3.19E-07	-1.0496	CMRSA 6
SA0046	3.47E-07	-1.0792	CMRSA 6
SA2055	4.45E-07	-1.05678	CMRSA 6
SA2596	4.61E-07	-1.05521	CMRSA 6
SA1770	4.78E-07	-1.05105	CMRSA 6
SA2178	7.16E-07	1.03814	CMRSA 6
SA1251	7.36E-07	-1.04143	CMRSA 6
SA2576	1.04E-06	-1.07659	CMRSA 6
SA1761	1.17E-06	-1.05003	CMRSA 6
SAV0395	1.24E-06	1.0759	CMRSA 6
SA2530	1.24E-06	-1.06396	CMRSA 6
SA1432	1.49E-06	1.09514	CMRSA 6
SA1698	1.56E-06	-1.04817	CMRSA 6
SA2124	1.61E-06	1.05843	CMRSA 6
SA1787	1.74E-06	1.09349	CMRSA 6
SA0387	1.93E-06	-1.24816	CMRSA 6
SAV0397	2.40E-06	1.07361	CMRSA 6
SA1772	2.63E-06	-1.04342	CMRSA 6
SA2607	2.73E-06	1.05387	CMRSA 6
SA2577	2.76E-06	-1.04375	CMRSA 6
SAV1955	2.92E-06	1.03909	CMRSA 6
SA2652	3.51E-06	-1.06966	CMRSA 6
SA0592	3.55E-06	-1.03973	CMRSA 6
SA1429	3.74E-06	1.10176	CMRSA 6
SA1899	3.77E-06	-1.04761	CMRSA 6
SA0271	4.34E-06	-1.07589	CMRSA 6
SA1730	4.51E-06	-1.74692	CMRSA 6
SA2052	4.70E-06	-1.06648	CMRSA 6
SA0188	4.75E-06	-1.45445	CMRSA 6
SAV1964	5.23E-06	1.07698	CMRSA 6
SA2376	5.30E-06	1.08003	CMRSA 6
SA0556	6.61E-06	-1.05285	CMRSA 6
SA1428	6.90E-06	1.087	CMRSA 6
SA0276	7.34E-06	-1.06607	CMRSA 6
SA1946	7.78E-06	1.02956	CMRSA 6
SA0054	7.95E-06	-1.72825	CMRSA 6
34_B5	8.08E-06	1.56405	CMRSA 6
SA2509	8.93E-06	1.07301	CMRSA 6
SA2584	9.57E-06	-1.05899	CMRSA 6
SA0035	9.79E-06	-1.19192	CMRSA 6
SA0478	1.16E-05	1.08143	CMRSA 6

SA0380	1.34E-05	-1.60504	CMRSA 6
SA2107	1.62E-05	-1.18963	CMRSA 6
SA1612	1.76E-05	-1.04754	CMRSA 6
SAV1486	1.78E-05	-1.03963	CMRSA 6
SAV0049	1.82E-05	1.06147	CMRSA 6
SA1697	1.85E-05	-1.03641	CMRSA 6
SA1712	1.89E-05	-1.02987	CMRSA 6
SA0595	2.02E-05	1.02765	CMRSA 6
SA1724	2.19E-05	-1.03206	CMRSA 6
SA1232	2.32E-05	-1.0319	CMRSA 6
SA2541	2.41E-05	1.06216	CMRSA 6
SA0369	2.61E-05	-1.37374	CMRSA 6
SA0375	2.64E-05	-1.23683	CMRSA 6
SA2660	2.65E-05	-1.14037	CMRSA 6
SA0014	2.80E-05	-1.02948	CMRSA 6
SA2616	2.95E-05	1.0429	CMRSA 6
SA2661	3.04E-05	-1.30214	CMRSA 6
SA0381	3.05E-05	-1.42312	CMRSA 6
SA1613	3.06E-05	-1.03417	CMRSA 6
SA2198	3.09E-05	1.04635	CMRSA 6
SA1363	3.33E-05	1.05936	CMRSA 6
SAV1985	1.64E-32	1.31717	98S-1344
SAV1982	4.01E-24	1.36266	98S-1344
SAV1983	1.86E-21	1.16028	98S-1344
SAV1984	3.07E-21	1.17819	98S-1344
SAV1955	4.80E-21	1.12714	98S-1344
SAV1986	4.87E-21	1.22919	98S-1344
SA1553	1.99E-18	-1.17645	98S-1344
SA0370	6.10E-17	-1.20965	98S-1344
SA0051	7.68E-17	-1.27411	98S-1344
SAV1954	1.10E-15	1.09093	98S-1344
SA1475	2.81E-15	1.17704	98S-1344
SAV1963	1.29E-14	1.12353	98S-1344
SAV2001	4.98E-14	1.20813	98S-1344
SA0050	6.36E-14	-1.47422	98S-1344
SA2014	6.37E-14	-1.12525	98S-1344
SA2672	9.31E-14	-1.09837	98S-1344
SA1861	4.87E-13	-1.12707	98S-1344
SA0774	5.62E-13	-1.19227	98S-1344
SA1477	9.39E-13	1.25721	98S-1344
SA1349	1.25E-12	-1.09413	98S-1344
SAV1980	1.55E-12	1.15532	98S-1344
SA1924	1.78E-12	-1.11765	98S-1344
SAV1967	1.98E-12	1.11894	98S-1344
SA1445	2.12E-12	-1.11793	98S-1344
SA0815	2.47E-12	1.14292	98S-1344

SA0946	3.43E-12	1.11442	98S-1344
SA2631	3.84E-12	-1.11203	98S-1344
SA0759	6.98E-12	1.2156	98S-1344
SA1926	7.06E-12	-1.15807	98S-1344
SAV1965	8.40E-12	1.09506	98S-1344
SA2530	8.93E-12	-1.1287	98S-1344
SA2505	1.02E-11	1.16325	98S-1344
SA2674	1.50E-11	-1.09118	98S-1344
SA2716	2.29E-11	-1.15357	98S-1344
SA1773	2.38E-11	-1.11367	98S-1344
SA1019	2.75E-11	1.097	98S-1344
SAV1964	3.54E-11	1.1508	98S-1344
SAV1957	5.08E-11	1.09203	98S-1344
SA1697	5.67E-11	-1.07853	98S-1344
SA1824	6.18E-11	-1.17173	98S-1344
SA2385	7.95E-11	1.14707	98S-1344
SA0777	8.96E-11	-1.12772	98S-1344
SA2376	9.52E-11	1.15221	98S-1344
SA0758	9.68E-11	1.22101	98S-1344
SAA0001	1.02E-10	-1.28102	98S-1344
SA0647	1.07E-10	-1.09837	98S-1344
SA1640	1.18E-10	-1.07831	98S-1344
SA1617	1.34E-10	-1.08607	98S-1344
SA2176	1.36E-10	-1.09436	98S-1344
SA1427	1.41E-10	-1.2278	98S-1344
SA0963	3.32E-10	1.19338	98S-1344
SA1359	4.92E-10	1.07304	98S-1344
SA0964	5.03E-10	1.16094	98S-1344
SA0688	6.13E-10	1.12848	98S-1344
SA0493	6.29E-10	1.10709	98S-1344
SA2516	6.38E-10	1.10847	98S-1344
SA1761	6.55E-10	-1.08566	98S-1344
SA1788	7.39E-10	-1.22144	98S-1344
SAA0003	7.43E-10	-1.42294	98S-1344
SA2552	8.15E-10	1.09992	98S-1344
SA0826	1.05E-09	1.07147	98S-1344
SA2195	1.09E-09	-1.12133	98S-1344
SA1501	1.85E-09	-1.06909	98S-1344
SA0028	1.88E-09	1.19611	98S-1344
SAV1968	1.93E-09	1.08241	98S-1344
SA1525	2.25E-09	-1.07319	98S-1344
SA1467	2.54E-09	1.1012	98S-1344
SA0033	2.59E-09	-1.12208	98S-1344
SA0757	2.71E-09	1.20319	98S-1344
SA0031	2.84E-09	1.07335	98S-1344
SA0837	3.32E-09	1.09784	98S-1344

SA0660	3.38E-09	1.17751	98S-1344
SA2144	3.41E-09	-1.08574	98S-1344
SA0955	3.46E-09	1.07307	98S-1344
SA1802	4.60E-09	1.26692	98S-1344
SA1531	4.63E-09	-1.10595	98S-1344
SA1535	4.64E-09	1.09575	98S-1344
SA1825	4.95E-09	-1.07582	98S-1344
SA0275	5.07E-09	-1.08892	98S-1344
SA1805	5.19E-09	1.15758	98S-1344
SA0124	5.28E-09	1.08507	98S-1344
SA1543	5.31E-09	-1.05259	98S-1344
SA0689	5.77E-09	1.11597	98S-1344
SA1505	7.81E-09	-1.05713	98S-1344
SAV0715	8.17E-09	-1.12164	98S-1344
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SA1476	1.01E-08	1.16233	98S-1344
SA2399	1.01E-08	1.16429	98S-1344
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SAV1974	1.14E-08	-1.0572	98S-1344
SA2662	1.44E-08	1.05793	98S-1344
SAV1807	1.44E-08	-1.06298	98S-1344
SA1772	1.61E-08	-1.06841	98S-1344
SA0389	1.69E-08	-1.10663	98S-1344
SA1724	1.69E-08	-1.05667	98S-1344
SA2052	2.37E-08	-1.1079	98S-1344
SA1913	2.57E-08	-1.05581	98S-1344
SA0710	2.59E-08	1.05161	98S-1344
SA2261	2.82E-08	-1.06165	98S-1344
SA1799	2.95E-08	1.10478	98S-1344
SAV1822	3.62E-08	1.09847	98S-1344
SA0053	3.67E-08	-1.12696	98S-1344
SA2644	4.59E-08	-1.04349	98S-1344
SA2738	4.78E-08	-1.09069	98S-1344
SA2474	4.92E-08	1.07229	98S-1344
SA2624	4.98E-08	1.07338	98S-1344
SA0023	6.29E-08	1.06014	98S-1344
SA1232	6.51E-08	-1.0535	98S-1344
SA0755	7.36E-08	-1.09688	98S-1344
SA1528	7.74E-08	-1.10049	98S-1344
SA1255	8.51E-08	-1.07978	98S-1344
SA0478	9.56E-08	1.12647	98S-1344
SA1669	1.00E-07	-1.08042	98S-1344
SA2602	1.11E-07	-1.08543	98S-1344
SA2577	1.17E-07	-1.06355	98S-1344
SA2204	1.46E-07	-1.53823	98S-1344
SA1804	1.51E-07	1.10755	98S-1344

SA2702	1.55E-07	-1.0469	98S-1344
SA1673	1.62E-07	-1.07981	98S-1344
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SA2446	1.84E-07	-1.074	98S-1344
SA2197	1.93E-07	-1.06593	98S-1344
SA0596	2.03E-07	1.07842	98S-1344
SA1282	2.08E-07	-1.1261	98S-1344
SA1957	2.13E-07	-1.04888	98S-1344
SA2676	2.22E-07	-1.07309	98S-1344
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SA2166	6.73E-07	-1.05461	98S-1344
SA2018	6.94E-07	-1.10255	98S-1344
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SA0377	7.24E-07	-1.16232	98S-1344
SA0690	7.43E-07	1.11407	98S-1344
SA1478	7.77E-07	1.2227	98S-1344
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SA0517	1.29E-06	1.09299	98S-1344

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SA1844	6.42E-06	-1.06626	98S-1344
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SA0840	1.26E-05	1.06318	98S-1344
SA1319	1.42E-05	1.05682	98S-1344
SA0015	1.43E-05	-1.04437	98S-1344
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SA1125	4.13E-05	1.03783	98S-1344
SA0810	4.20E-05	1.03868	98S-1344
SA1600	4.47E-05	-1.04205	98S-1344
SAV0033	4.62E-05	1.03893	98S-1344
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SA0123	5.02E-05	1.0915	98S-1344
SA1472	5.02E-05	-1.04925	98S-1344
SA0317	5.07E-05	1.08367	98S-1344
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SA1367	6.07E-05	1.07502	98S-1344
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SA0016	6.41E-05	-1.02995	98S-1344
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SA1954	8.78E-05	-1.04404	98S-1344
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SAV1955	6.35E-31	1.19133	99S-1088
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SA0033	1.87E-19	-1.24809	99S-1088
SAV1954	3.37E-19	1.10865	99S-1088
SAV1963	1.09E-18	1.15281	99S-1088
SA0281	3.31E-18	-1.25572	99S-1088
SAV1964	1.09E-17	1.22003	99S-1088
SA0897	7.49E-16	1.1581	99S-1088
SAP022	3.00E-15	1.21682	99S-1088
SAV1959	6.07E-15	1.12553	99S-1088
SAP024	1.05E-14	1.14345	99S-1088

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SA2300	5.12E-13	-1.18264	99S-1088
SA2567	5.66E-13	-1.15784	99S-1088
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SAV1985	1.02E-12	1.12827	99S-1088
SA2401	3.04E-12	-1.21124	99S-1088
SA0051	3.52E-12	-1.1971	99S-1088
SAV1957	3.89E-12	1.09922	99S-1088
SA0050	3.92E-12	-1.40467	99S-1088
SA1090	5.65E-12	-1.12419	99S-1088
SA2052	2.50E-11	-1.13984	99S-1088
SA0278	2.95E-11	-1.14545	99S-1088
SA0370	4.17E-11	-1.13998	99S-1088
SAV1958	5.62E-11	1.1503	99S-1088
SAP003	1.47E-10	1.10627	99S-1088
SAV1962	1.61E-10	1.05046	99S-1088
SA0449	1.70E-10	1.06274	99S-1088
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SA1553	4.38E-10	-1.10084	99S-1088
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SA2563	2.67E-09	-1.22103	99S-1088
SA0946	2.97E-09	1.09258	99S-1088
SAP029	3.05E-09	1.12	99S-1088
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SAV1986	5.65E-08	1.10382	99S-1088
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SA1478	2.65E-06	1.20977	99S-1088
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SA2419	4.92E-06	1.11621	99S-1088
SA0690	5.06E-06	1.10368	99S-1088
SAV1952	5.19E-06	1.09394	99S-1088
SA1051	5.39E-06	1.08544	99S-1088
SA1696	5.53E-06	-1.08519	99S-1088
SAV1982	5.74E-06	1.11588	99S-1088
SA1920	6.08E-06	-1.55655	99S-1088
SA0617	6.23E-06	1.04133	99S-1088
SA2617	6.49E-06	1.05842	99S-1088
SA0730	6.81E-06	1.09239	99S-1088
SAP021	6.92E-06	1.0597	99S-1088
SA0639	7.16E-06	1.07142	99S-1088
SA0984	7.29E-06	1.0692	99S-1088
SAV0194	7.78E-06	1.11984	99S-1088

SA0046	7.78E-06	-1.08409	99S-1088
SA1506	7.87E-06	-1.09088	99S-1088
SAP028	7.95E-06	1.07533	99S-1088
SA0339	8.31E-06	-1.2582	99S-1088
SA0286	9.29E-06	-1.07624	99S-1088
SA0845	9.74E-06	-1.07771	99S-1088
SA2535	9.86E-06	1.09834	99S-1088
SA0753	1.01E-05	1.06306	99S-1088
SA1128	1.02E-05	1.05656	99S-1088
SA0665	1.07E-05	1.0881	99S-1088
SA2155	1.10E-05	-1.05632	99S-1088
SA0916	1.11E-05	-1.05638	99S-1088
SA0517	1.17E-05	1.08287	99S-1088
SA0204	1.19E-05	1.22076	99S-1088
SA1924	1.21E-05	-1.0616	99S-1088
SA2602	1.24E-05	-1.0668	99S-1088
SA0833	1.31E-05	-1.06022	99S-1088
SA1946	1.32E-05	1.03537	99S-1088
SA1764	1.32E-05	-1.06494	99S-1088
SAV0202	1.39E-05	1.13076	99S-1088
SA0084	1.40E-05	-1.0935	99S-1088
SA1787	1.44E-05	-1.11512	99S-1088
SA1619	1.57E-05	-1.07472	99S-1088
SA0742	1.64E-05	1.04705	99S-1088
SA0778	1.66E-05	-1.04768	99S-1088
SA1663	1.70E-05	-1.18882	99S-1088
SA2004	1.87E-05	1.09285	99S-1088
SA1706	1.90E-05	-1.04575	99S-1088
SA0724	1.90E-05	1.04497	99S-1088
SA1975	1.91E-05	1.04478	99S-1088
SAV0395	1.97E-05	1.08069	99S-1088
SA0688	2.00E-05	1.082	99S-1088
SA1624	2.08E-05	-1.05364	99S-1088
SA2055	2.08E-05	-1.0574	99S-1088
SA1220	2.23E-05	-1.1901	99S-1088
SA2160	2.23E-05	-1.05955	99S-1088
SA0588	2.25E-05	-1.0906	99S-1088
SA1227	2.33E-05	-1.0558	99S-1088
SA2054	2.35E-05	-1.08191	99S-1088
SA2053	2.42E-05	-1.20392	99S-1088
SA0222	2.46E-05	1.17247	99S-1088
SA1659	2.57E-05	-1.0714	99S-1088
SA0016	2.63E-05	-1.03175	99S-1088
SA1339	2.65E-05	-1.08024	99S-1088
SA1840	2.67E-05	-1.04306	99S-1088
SA1103	2.78E-05	-1.04836	99S-1088

SA0432	2.87E-05	1.13851	99S-1088
SA1981	2.88E-05	1.06503	99S-1088
SA1204	2.90E-05	-1.04964	99S-1088
SA1125	2.91E-05	1.03867	99S-1088
SA2291	3.01E-05	-1.102	99S-1088
SA0902	3.42E-05	1.17273	99S-1088
SA0855	3.48E-05	1.07113	99S-1088
SAV0411	3.64E-05	1.02953	99S-1088
SAV0715	3.73E-05	-1.07866	99S-1088
SA0673	3.74E-05	1.09857	99S-1088
SA1613	3.77E-05	-1.04187	99S-1088
SA1982	3.78E-05	1.03215	99S-1088
SA1964	3.91E-05	1.07304	99S-1088
SA1676	3.91E-05	-1.07685	99S-1088
SA2646	3.94E-05	1.05842	99S-1088
SA1562	4.05E-05	-1.04182	99S-1088
SA1251	4.11E-05	-1.04106	99S-1088
SA0741	4.30E-05	-1.04423	99S-1088
SA2371	4.42E-05	1.04967	99S-1088
SA1505	4.54E-05	-1.03716	99S-1088
SA0556	4.54E-05	-1.05845	99S-1088
SA1977	4.77E-05	1.04789	99S-1088
SA0054	5.07E-05	-1.86902	99S-1088
SA0461	5.28E-05	1.04615	99S-1088
SA1451	5.45E-05	-1.04741	99S-1088
SA1501	5.52E-05	-1.04188	99S-1088
SAVP018	5.72E-05	1.07707	99S-1088
SA1714	6.00E-05	-1.06899	99S-1088
SAV0063	6.00E-05	1.03975	99S-1088
SA1691	6.07E-05	-1.05515	99S-1088
SA2119	6.19E-05	-1.05623	99S-1088
SA0857	6.29E-05	1.04717	99S-1088
SAV1010	6.30E-05	-1.04279	99S-1088
SA2705	6.55E-05	1.03769	99S-1088
SA1359	6.80E-05	1.04285	99S-1088
SA0925	7.08E-05	1.09974	99S-1088
SA0467	7.17E-05	-1.05328	99S-1088
SA2201	3.21E-07	-1.1496	00S-1635
SA2516	6.13E-07	1.07393	00S-1635
SA2682	1.20E-11	-1.2178	02S-0250
SA2319	1.60E-10	-1.24228	02S-0250
SA1866	6.24E-10	-1.15927	02S-0250
SA1868	6.98E-10	-1.17303	02S-0250
SA2538	8.46E-10	-1.16697	02S-0250
SA1872	1.36E-09	-1.20827	02S-0250
SA0608	1.44E-09	-1.17525	02S-0250

SA2683	1.48E-09	-1.18206	02S-0250
SA2070	1.95E-09	-1.1811	02S-0250
SA2653	3.25E-09	-1.2025	02S-0250
SA0074	3.36E-09	-1.16781	02S-0250
SA2039	4.62E-09	-1.1959	02S-0250
SA0221	5.99E-09	-1.16267	02S-0250
SAV0900	6.53E-09	-1.19563	02S-0250
SA1532	6.69E-09	-1.18566	02S-0250
SA0326	7.04E-09	-1.18793	02S-0250
SA1611	9.22E-09	-1.16937	02S-0250
SA2043	1.27E-08	-1.21233	02S-0250
SA2311	1.46E-08	-1.18576	02S-0250
SA0683	3.90E-08	-1.19038	02S-0250
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SAV1975	6.43E-08	-1.12377	02S-0250
SAP024	6.95E-08	1.08567	02S-0250
SA2488	1.16E-07	-1.0745	02S-0250
SA2287	1.44E-07	-1.15278	02S-0250
SA1881	1.46E-07	-1.22745	02S-0250
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SA0404	2.53E-07	-1.13831	02S-0250
SA0341	2.63E-07	-1.14835	02S-0250
SA0691	2.67E-07	-1.16801	02S-0250
SA1164	2.97E-07	-1.204	02S-0250
SAP002	3.94E-07	1.08537	02S-0250
SA2499	4.71E-07	-1.12402	02S-0250
SA0849	5.30E-07	-1.1513	02S-0250
SA2360	5.95E-07	-1.13726	02S-0250
SAV0893	8.38E-07	-1.11724	02S-0250
SAV0854	9.43E-07	-1.18134	02S-0250
SA1121	1.03E-06	-1.15316	02S-0250
tetK	1.07E-06	-1.9077	02S-0250
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SA2404	1.37E-06	-1.20949	02S-0250
SA1867	1.49E-06	-1.10849	02S-0250
SA1880	1.52E-06	-1.15458	02S-0250
SAV0393	1.63E-06	-1.1503	02S-0250
SA2634	1.67E-06	-1.09666	02S-0250
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SA2612	2.24E-06	-1.16651	02S-0250
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SA1860	2.51E-06	-1.13205	02S-0250
SA2491	2.57E-06	-1.26919	02S-0250
SA2233	3.04E-06	-1.28476	02S-0250
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SA2011	4.59E-06	-1.13406	02S-0250
SA2687	4.71E-06	-1.19349	02S-0250
SA2690	5.51E-06	-1.12248	02S-0250
SA2046	5.64E-06	-1.15954	02S-0250
SAV2021	9.07E-06	1.04736	02S-0250
SA1431	9.58E-06	-1.14954	02S-0250
SA2626	9.93E-06	-1.15314	02S-0250
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SA0879	1.12E-05	-1.25765	02S-0250
SA2285	1.14E-05	-1.05223	02S-0250
SA2015	1.25E-05	-1.14518	02S-0250
SAV0202	1.34E-05	-1.14519	02S-0250
SAVP021	1.37E-05	1.0484	02S-0250
SA2427	1.72E-05	-1.10111	02S-0250
SAV0884	1.74E-05	1.08933	02S-0250
SA2006	1.78E-05	-1.10972	02S-0250
SAV0200	1.93E-05	1.06269	02S-0250
SA0402	1.96E-05	-1.17249	02S-0250
SA2485	2.07E-05	-1.14527	02S-0250
SA2732	2.19E-05	-1.19874	02S-0250