Genetic Diversity Among Canadian Isolates of Penicillin-Resistant *Streptococcus pneumoniae* and Characterization of Penicillin-Binding Protein 1A, 2B and 2X Mutations

By

Kimberly Anne Nichol

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Faculty of Medicine

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Kimberly Anne Nichol

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

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Dedication

To my parents, Tom and Lois, whose unconditional love and support have made this all possible.

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LIST OF ABBREVIATIONS

AP-PCR	arbitrarily-primed polymerase chain reaction
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
bp	base pairs
$CaCl_2$	calcium chloride
CFU	colony forming units
CO_2	carbon dioxide
ddNTP	dideoxynucleotide triphosphate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
H_2O	water
HCl	hydrogen chloride
LHB	lysed horse blood
$MgCl_2$	magnesium chloride
MHB	Mueller-Hinton broth
MIC	minimum inhibitory concentration
NaCl	sodium chloride
NaOH	sodium hydroxide
NCCLS	National Committee for Clinical Laboratory Standards
PBD(s)	penicillin-binding domain(s)
PBP(s)	penicillin-binding protein(s)
PCR	polymerase chain reaction

PFGE	pulsed-field gel electrophoresis
RNase	ribonuclease
rRNA	ribosomal ribonucleic acid
TBE	aqueous solution containing Tris-Borate-EDTA
$T_{10}E_1$	aqueous solution containing 10 mM Tris and 1 mM EDTA, pH 8.0
UDG	uracil DNA glycosylase
UDP	uridine diphosphate
UPGMA	unweighted pair group method using arithmetic averages
UV	ultraviolet

ABSTRACT

Increases in the prevalence of penicillin-nonsusceptible *Streptococcus pneumoniae* can be attributed to the acquisition of altered penicillin-binding protein (PBP) genes and to the geographic spread of genetically related isolates with elevated β-lactam minimum inhibitory concentrations (MICs). The objective of this thesis was to characterize *pbp1a*, *pbp2b* and *pbp2x* mutations in Canadian isolates of penicillin-nonsusceptible *S. pneumoniae* and to evaluate the relationship between genetic diversity and penicillin susceptibility as it pertains to the dissemination of resistance.

Regions of the penicillin-binding domains of *pbp1a*, *pbp2b* and *pbp2x* previously associated with β-lactam resistance were amplified using primers specific for the unaltered genes of susceptible isolates. All isolates of penicillin-susceptible *S. pneumoniae* were found, by polymerase chain reaction (PCR), not to harbor PBP alterations. Conversely, each penicillin-resistant isolate was shown to possess alterations in all three genes. Penicillin-intermediate *S. pneumoniae* contained various combinations of PBP gene alterations. PBP profiles detected by PCR included alterations in each of the three genes (2 isolates, MICs; 0.5, 1 μg/ml), mutation of *pbp1a* and *pbp2x* (1 isolate, MIC; 0.25 μg/ml) and alteration of *pbp2b* and *pbp2x* (2 isolates, MICs; 0.25, 0.12 μg/ml). These results suggest that the rapid identification of penicillin-susceptible and -resistant genotypes among clinical isolates of *S. pneumoniae* may be possible through the application of a multiplex-PCR assay.

Both pulsed-field gel electrophoresis (PFGE) and arbitrarily-primed PCR (AP-PCR) revealed homogeneity amongst penicillin-resistant (MIC; ≥ 2 μg/ml) isolates and exclusive heterogeneity amongst penicillin-intermediate (MIC; 0.12 – 1 μg/ml) and

penicillin-susceptible (MIC; ≤ 0.06 μg/ml) isolates. Four penicillin-resistant isolates with homogenous typing profiles serotyped 19F (2 isolates), 23F (1 isolate) and 14 (1 isolate), indicating several instances of probable capsular serotype switching. Sequence analysis of the penicillin-binding domains of *pbp1a*, *pbp2b* and *pbp2x* revealed identical nucleotide and amino acid substitution patterns in all isolates with penicillin MICs ≥ 1 μg/ml. These data demonstrate the important contribution of clonal spread in the overall increase of penicillin-resistant *S. pneumoniae* in Canada.

Genetic Diversity Among Canadian Isolates of Penicillin-Resistant *Streptococcus pneumoniae* and Characterization of Penicillin-Binding Protein 1A, 2B and 2X Mutations

A. INTRODUCTION

1. *Streptococcus pneumoniae*

a. Identification and Morphology

Streptococcus pneumoniae is described as a catalase-negative, facultatively anaerobic gram-positive coccus that is spherical or ovoid in shape, replicates in pairs or short chains, and is usually less than two micrometers in diameter (1). In the routine microbiology laboratory, *S. pneumoniae* is also identified on the basis of its solubility in bile salts and/or its susceptibility to ethyl hydrocupreine (optochin) (1, 2, 3). *S. pneumoniae* are nutritionally fastidious bacteria with variable nutritional requirements and growth on complex media is enhanced by the addition of blood or serum (1). Since *S. pneumoniae* requires elevated CO_2 concentrations for optimal growth, recovery of such isolates is often facilitated by incubation in an atmosphere containing 5% CO_2 (1, 2, 4, 5, 6). During growth on blood agar, pneumococcal colonies are surrounded by a greenish discoloration of the agar due to the streptococcal action of α-hemolysin on hemoglobin in the medium (2). Alpha-hemolytic colonies with depressions in their centers are characteristic of *S. pneumoniae*. Pneumococcal colonies vary in color from gray to whitish and usually glisten, although dry colonies are sometimes observed (2). *S. pneumoniae* may also produce various amounts of capsular polysaccharide, contributing to a mucoid colonial appearance.

b. Pathogenesis

Although *S. pneumoniae* is an important cause of disease, its normal ecological niche is the nasopharynx of healthy individuals. In fact, virtually all humans are colonized by this organism at one time or another and it has been well documented that nasopharyngeal carriage in healthy adults may approach 40% (7, 8). The mechanisms by which *S. pneumoniae* translocates from the nasopharynx to the lung or migrates directly into the blood, thereby giving rise to disease, are poorly understood (7). Most infections, however, do not occur after prolonged carriage but follow the recent acquisition of a given strain (9). This suggests that the immune status of the host at the moment colonization, as well as the virulence of the particular strain, determines whether pneumococci will remain confined to the nasopharynx or become invasive (9).

For many years, the virulence of *S. pneumoniae* has largely been attributed to its antiphagocytic polysaccharide capsule. More than 90 distinct capsular serotypes have been identified to date, and pneumococci belonging to these different serotypes appear to vary both in their capacity to resist phagocytosis and in their ability to elicit a humoral immune response. This ability to survive in the bloodstream and to possibly cause invasive disease appears to be a function of the chemical structure and biological properties of the capsular polysaccharide itself and is not merely related to the thickness of the capsule (9, 10, 11).

Notwithstanding the importance of the capsule in evading host defenses, host inflammatory responses to pneumococcal components such as the cell wall are also likely to contribute to tissue injury during infection (8, 12). In addition, certain pneumococcal proteins are known to play an important role in the pathogenesis of disease, either as

mediators of inflammation or by directly attacking host tissue (12). To this end, pneumococcal hydrolytic enzymes such as neuraminadase and hyaluronidase have been hypothesized to contribute to the colonization and/or invasion of the host (12). Thereafter, inhibition of epithelial ciliary movement by pneumolysin and concurrent disruption of mucosal defense mechanisms by pneumococcal IgA1 protease may facilitate initial access of *S. pneumoniae* to the bronchi and the lungs. Further damage of the epithelial monolayer by hydrogen peroxide (produced by *S. pneumoniae*) and pneumolysin may then allow direct access to the blood. Factors such as fatigue, stress, and malnutrition as well as previous viral infection, chronic disease or hospitalization, and alcohol or drug abuse can also compromise defenses of the lower respiratory tract and predispose to pneumococcal infection (2).

Proliferation of *S. pneumoniae* at the site of infection culminates in pneumococcal cell lysis with subsequent release of cell wall products and pneumolysin. Activation of autolysin by human lysozyme may also contribute to bacterial lysis, an event that in turn triggers the inflammatory process by attracting and activating phagocytes and through indirect initiation of the complement cascade. Increasing evidence supports the hypothesis that such inflammation may be responsible for the morbidity and mortality commonly associated with pneumococcal infection (7, 13).

c. Epidemiology

S. pneumoniae is an important pathogen that causes life-threatening, invasive diseases with high morbidity and mortality throughout the world (7). Serious pneumococcal infection is most prevalent in the extreme ages of life (i.e., young children and the elderly are particularly susceptible) and in individuals with underlying debilitating

conditions. *S. pneumoniae* is an important cause of septicemia, a frequent agent of bacteremia and one of the three most common pathogens associated with bacterial meningitis. It is also the leading cause of otitis media, bronchitis and sinusitis (12). These are less serious infections, but they are highly prevalent and have a significant impact on health-care costs in developed countries. Most significantly, however, *S. pneumoniae* is responsible for the majority of cases of community-acquired pneumonia. In developing countries, an estimated five million children under the age of five die each year from pneumonia, with *S. pneumoniae* being the single most common causative agent (12). In Canada there are approximately twelve cases of pneumonia per 1000 population per annum, suggesting that there are over 350000 cases in this country each year (14). Pneumonia is also an important cause for hospitalizations, particularly among elderly patients, and, despite antibiotic therapy, over 7000 mortalities each year are attributed to this infection. The most common cause of infective deaths, pneumonia is also the sixth most common cause of deaths overall.

d. Antibiotic Therapy

Before penicillin became widely available for clinical use, morbidity and mortality estimates for pneumococcal disease were extremely high. In the early part of the 20th century, optochin (ethyl hydrocupreine) was commonly used for the treatment of *S. pneumoniae* infections. As early as 1912, however, optochin-resistant pneumococci were isolated from experimentally infected mice and acquired pneumococcal resistance during therapy of patients was subsequently documented in 1917 (15). Thereafter, sulfonamides became the only antibiotics that could be used for the treatment of such infections, but sulfonamide-resistant strains were reported as early as 1943 (15). Once methods for its

commercial production were perfected and penicillin became widely available, death rates resulting from pneumococcal disease decreased dramatically. During the early 1940's, clinical isolates of *S. pneumoniae* exhibited high degrees of susceptibility to penicillin. Consequently, benzylpenicillin (also known as penicillin G) soon became the drug of choice for treating pneumococcal disease and the alternative drugs that soon followed were largely limited for use in patients known to be allergic to penicillin (16).

2. β-lactam Antibiotics

a. Types of β-lactam Compounds

β-lactam antibiotics are a highly diverse and widely used group of antimicrobial agents that share both a common chemical structure and mechanism of action (17). There are two classical β-lactam families which include the penicillins and cephalosporins, as well as various nonclassical β-lactams such as the monobactams (also known as monocyclics) and carbapenems (18). The chemical structures that distinguish these agents are shown in Figure 1.

The penicillin class of antibiotics comprises a large group of natural and semisynthetic compounds containing the chemical nucleus 6-aminopenicillanic acid. These agents constitute one of the most important groups of antibiotics and, although numerous other antibotics have been produced since the first penicillin became available, members of this group remain the drugs of choice for a large number of infectious diseases (17).

Figure 1. Chemical structures of β-lactam antibiotics[a].

a) Penicillins

β-lactam ring Thiazolidine ring

6-aminopenicillanic acid

b) Cephalosporins

β-lactam ring dihydrothiazine ring

7-aminocephalosporanic acid

c) Monobactams

β-lactam ring

d) Carbapenems

β-lactam ring

[a] Reproduced from reference 19.

Cephalosporins are derived from the fermentation products of *Cephalosporium acremonium*, an organism initially isolated in 1948 from a sewer outlet off the Sardinian coast (19). They contain a 7-aminocephalosporanic acid nucleus, which consists of a β-lactam ring fused to a six-membered dihydrothiazine ring (Figure 1b). Although the nucleus itself has little antibacterial action, various substitutions at positions three and seven alter the activity, pharmacokinetic properties and spectra of these molecules (20). The explosive growth of the cephalosporins during the past decade (more than 100 semisynthetic compounds are now available, all with similar names and often similar

properties) clearly necessitates the development of a system of classification (17). Although cephalosporins may be distinguished by their chemical structure, clinical pharmacology, resistance to β-lactamase or antimicrobial spectrum (17), the well-accepted system of classification is based on general features of antibacterial activity (19). For example, the first-generation (narrow spectrum) cephalosporins have good activity against gram-positive bacteria and relatively modest activity against gram-negative microorganisms (17, 19). In comparison, second-generation (expanded spectrum) and third-generation (broad spectrum) cephalosporins have greater activity against gram-negative organisms but are generally less active than first-generation agents against gram-positive cocci (especially *Staphylococcus aureus*). Finally, fourth-generation (extended spectrum) cephalosporins have increased stability from hydrolysis by the plasmid- and chromosomally-mediated β-lactamases of some gram-negative bacteria and as such may prove to have particular therapeutic usefulness (17).

The monobactams are nonclassical β-lactam antibiotics with various side chains affixed to a monocyclic nucleus (Figure 1c) and a gram-negative spectrum of activity resembling that of the aminoglycosides. Carbapenems are a unique class of β-lactam agents with the widest spectrum of antibacterial activity of the currently available antibiotics (19). Structurally they differ from other β-lactams in having a hydroxyethyl side chain in a *trans* configuration at position six and lack a sulfur or oxygen in the bicyclic nucleus (Figure 1d), a feature which confers stability against β-lactamases and provides for their potent activity against many gram-negative organisms (19).

b. Penicillin

i. History

In 1928, while studying *Staphylococcus* variants in a London laboratory, Scottish physician Alexander Fleming observed that a mold contaminating one of his cultures caused the bacteria in its vicinity to undergo lysis (17). Broth in which the fungus was grown was subsequently found to be markedly inhibitory for many microorganisms. Because this mold belonged to the genus *Penicillium*, Fleming named the antibacterial substance penicillin. A decade later, penicillin was developed as a systemic therapeutic agent by the concerted research of a group of investigators headed by Florey, Chain and Abraham (17). Preliminary results indicated that the crude material available at that time was able to produce dramatic therapeutic effects when administered to mice with experimentally induced streptococcal infection. Despite great obstacles to its laboratory production, enough penicillin was accumulated by 1941 to conduct therapeutic trials in several desperately ill patients. In 1942, the first clinical trials were conducted at Yale University and the Mayo Clinic (17). By spring of 1943, 200 patients had been treated with the drug and the results were so impressive that the Surgeon General of the US Army authorized trial of the antibiotic in a military hospital. Soon thereafter, penicillin was adopted throughout the medical services of the US Armed Forces.

ii. Chemistry

The basic structure of the penicillins, as shown in Figure 1a, consists of a fused β-lactam thiazolidine ring system (21). The integrity of the β-lactam ring is essential for biological activity in that metabolic transformation or chemical alteration of this portion of the molecule causes loss of all significant antibacterial activity (17). The penicillins

differ from one another in substitutions at position six, where changes in the acyl side-chain may modify the pharmacological characteristics, antibacterial properties and spectrum of the drug (17, 19, 20).

During early studies into the chemical nature of penicillin, it quickly became apparent that the product derived from industrial fermentations of *Penicillium chrysogenum* was, in fact, a family of closely related compounds differing only in the nature of the acyl side-chain. These natural penicillins consisted of penicillins F (pentenylpenicillin), G (benzylpenicillin), K (heptylpenicillin) and X (p-hydroxybenzylpenicillin). From this family, benzylpenicillin (penicillin G) was selected as the penicillin of choice on the basis of its biological properties (it had the greatest antimicrobial activity) and ease of commercial production (21).

The limitations of benzylpenicillin as an antibacterial agent soon led to efforts to produce novel penicillins with superior properties to the naturally occurring substance. Most of these early attempts, however, failed in their original objective to produce semisynthetic penicillins of clinical utility. Nevertheless, such work was instrumental in leading to the 1957 identification of the penicillin nucleus in fermentations carried out in the absence of side-chain precursors. This discovery of 6-aminopenicillanic acid meant that the number of semisynthetic penicillins that could potentially be prepared by the addition of acyl side-chain structures in the 6-amino group of the molecule was now almost unlimited. Since 1959, thousands of novel structures have been reported in the literature (21).

iii. Antimicrobial Activity

Penicillins can be conveniently classified according to their spectrum of antimicrobial activity into three distinct categories including the narrow-spectrum penicillins, narrow-spectrum penicillins resistant to staphylococcal penicillinase and broad or extended spectrum penicillins (20). Examples of commonly used penicillins, their side chains and their useful antimicrobial spectra are shown in Table 1.

The narrow-spectrum penicillins are active against almost all gram-positive bacteria as well as against many gram-negative and anaerobic microorganisms (20). For example, penicillin G is very effective against penicillin-susceptible *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, viridans streptococci, *Streptococcus bovis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasturella multocida*, anaerobic cocci, *Clostridium* spp., *Fusobacterium* spp. and many non-*fragilis* *Bacteroides* spp. (20).

Members belonging to the group of penicillinase-resistant penicillins, such as methicillin, nafcillin, oxacillin, cloxacillin and dicloxacillin, are intrinsically less active than benzylpenicillin but are stable to staphylococcal β-lactamase and consequently display significant activity against penicillin-resistant strains of *S. aureus* (21). They appear to owe this nonsusceptibility to β-lactamase to steric hindrance resulting from the configuration of their side chain (19). In addition to staphylococci, these agents are also active against streptococci, gonococci and meningococci but have no useful activity against enterococci, *Haemophilus influenzae* or enterobacteria (21).

Table 1. Classification and major properties of representative penicillins[a].

Class	Compound	Side Chain	Major Properties	
			Resistance to Penicillinase	Useful Antimicrobial Spectrum
Narrow spectrum	Penicillin G	$-CH_2-$	No	*Streptococcus* species, *Neisseria meningitidis*, many anaerobes, spirochetes, others
	Penicillin V	$-OCH_2-$	No	
Penicillinase resistant	Methicillin	OCH_3, OCH_3	Yes	*Staphylococcus aureus*, *Streptococcus* species
	Oxacillin ($R_1 = R_2 = H$) Cloxacillin ($R_1 = Cl$; $R_2 = H$) Dicloxacillin ($R_1 = R_2 = Cl$)	R_1, R_2, C—C—, N, O, C, CH_3	Yes	
	Nafcillin	OC_2H_5	Yes	
Extended (broad) spectrum	Ampicillin ($R = H$) Amoxicillin ($R = OH$)	R—, CH—, NH_2	No	*Streptococcus* species, *Listeria monocytogenes*, *Proteus mirabilis*, *Escherichia coli*
	Carbenicillin ($R = H$)	CH—, COOR	No	Above plus, *Pseudomonas* species, *Enterobacter* species, and *Proteus*
	Piperacillin	CH—, NHCO, N, O, O, N, C_2H_5	No	*Pseudomonas* species, *Enterobacter* species, *Bacteroides fragilis*, many *Klebsiella*

[a] Table adapted from reference 17.

The extended-spectrum penicillins are a group of drugs that owe their expanded activity to the ability to traverse the outer membrane of some gram-negative cell walls (20). Ampicillin and amoxicillin retain the activity of benzylpenicillin against gram-positive cocci but exhibit increased activity against *H. influenzae* and certain gram-negative bacilli, notably *E. coli*, *Salmonella* and *Shigella* spp., and *Proteus mirabilis* (21). Others such as carbenicillin and its derivatives (ticarcillin, azlocillin, mezlocillin and piperacillin) have even greater activity against gram-negative bacteria and, although are susceptible to staphylococcal penicillinase, are more stable against hydrolysis by the β-lactamases of *Enterobacteriaceae*, *Bacteroides fragilis* and *Pseudomonas aeruginosa* (19).

iv. Mode of Action

The major antibacterial action of penicillin is derived from its ability to bind to and inhibit a number of key bacterial enzymes anchored to the cytoplasmic membrane that are essential for peptidoglycan biosynthesis (19). Peptidoglycan, also known as murein or mucopeptide, is a covalently closed, net-like polymer composed of glycan strands that are cross-linked by peptide chains (17). This structure is essential for bacterial survival in most environments by providing structural integrity and rigidity to the cell wall (22). The peptidoglycan layer of all bacterial cells is basically similar, although important differences exist between gram-positive and gram-negative microorganisms. In both types of organisms, the basic macromolecular chain consists of N-acetylglucosamine alternating with its lactyl ether, N-acetylmuramic acid (21). Each muramic acid unit carries a pentapeptide, the third amino acid of which is L-lysine in most gram-positive cocci and meso-diaminopimelic acid in gram-negative bacilli (21).

The cell wall is given its rigidity by cross-links between this amino acid and D-alanine of adjacent chains, with loss of the terminal amino acid (also D-alanine) (21). Gram-negative bacilli have a very thin peptidoglycan layer which is loosely but directly cross-linked, while gram-positive cocci possess a thick layer of peptidoglycan which is tightly cross-linked through interpeptide bridges (17, 21, 22).

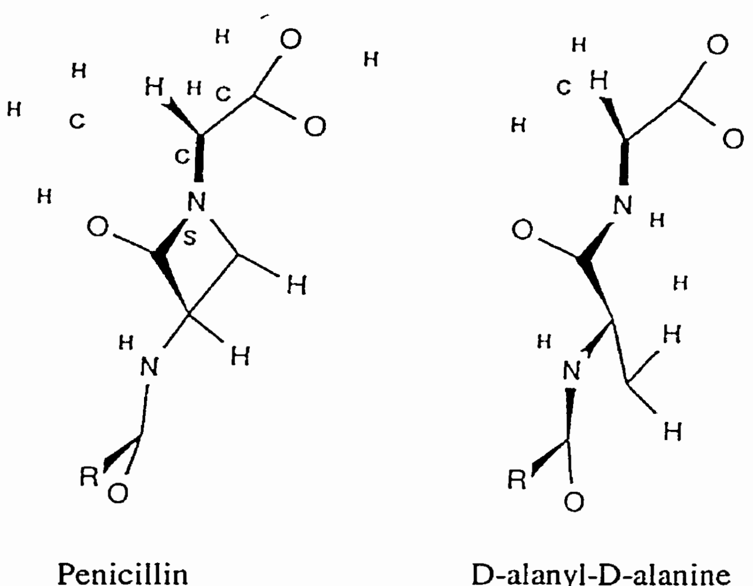
To synthesize peptidoglycan, bacteria must first assemble precursor molecules of uridine diphosphate (UDP) – linked N-acetylmuramic acid pentapeptides (22). These precursors, which are produced in the cytoplasm, are then transferred from UDP to a lipid intermediate (an isoprenoid carrier) located in the cytoplasmic membrane (22). An N-acetylglucosamine residue is then added to yield a disaccharide pentapeptide which is transported across the membrane (22). The final steps of peptidoglycan biosynthesis occur outside the cytoplasmic membrane and involve insertion of the disaccharide pentapeptide into the existing sacculus by transglycosylation and transpeptidation (22). The process of transglycosylation extends sugar chains by attaching the muramyl residue of a new precursor to a free N-acetylglucosamine residue on the existing peptidoglycan (22). Transpeptidation, which is essential for the formation of a biologically effective cell wall (18), cross-links adjacent sugar chains via their pentapeptides and represents the crucial penicillin-sensitive reaction (Figure 2) (22). These latter two stages are both catalyzed by membrane-bound enzymes known as penicillin-binding proteins (PBPs) or active-site serine transferases, which also play essential roles in cell division and morphology (22).

Figure 2. Transpeptidation: the cross-linking of murein in gram-positive bacteria[a].

[a] Adapted from reference 23.

Penicillin (and other β-lactam antibiotics) act by binding to and thereby blocking the transpeptidase activity of PBPs during cell wall synthesis (20). The ability of penicillin to inhibit these enzymes depends on conformational similarity between the amide bond of the β-lactam ring and the peptide link of the D-alanyl-D-alanine dipeptide residues in peptidoglycan precursors (Figure 3) (21, 22). Thus, in the absence of antibiotic, transpeptidation would occur by the formation of an acyl-D-alanyl-enzyme intermediate with the elimination of the terminal D-alanine residue (21). Subsequently, the acylated enzyme would interact with a free amino group of a second peptide chain or cross-bridge peptide to form a cross-link (21). This would complete the transpeptidation reaction and release the enzyme (21).

Figure 3. Stereomodels of penicillin and D-alanyl-D-alanine[a].



[a] Adapted from reference 23.

Conversely, in the presence of penicillin, PBPs undergo sequential acylation and deacylation reactions during which the amide bond of the β-lactam ring is opened by nucleophilic attack from the hydroxyl group of the PBP's active site serine (Figure 4). This generates an acylenzyme intermediate characterized by an ester bond between the enzyme and the penicilloyl moiety. Given the endocyclic nature of this β-lactam amide bond, the acylenzyme intermediate is resistant to nucleophilic attack by water and the β-lactam remains covalently bound to the PBP where it interferes with the normal cross-linking activity of these enzymes. This ability to inhibit PBPs theoretically confers on penicillin the ability to cause bacteriolysis of gram-positive bacteria such as *S. pneumoniae* by yielding a cell wall that cannot withstand osmotic forces (22). In practice, however, the actual bactericidal activity of penicillin is ultimately dependent upon its

ability to trigger membrane-associated autolytic enzymes (known as autolysins or murein hydrolases) that accelerate lysis by destroying the weakened cell wall (18, 22).

Figure 4. Acylation of the active-site serine of a PBP by penicillin[a].

[a] Reproduced from reference 22.

3. Penicillin-Binding Proteins

As mentioned previously, penicillin-binding proteins are components of the bacterial cytoplasmic membrane that catalyze the final steps of peptidoglycan biosynthesis. All pathogenic bacteria, with the exception of the mycoplasmas, possess an assortment of these enzymes; most species contain four to eight PBPs (24) with widely varied affinities for penicillin and other β-lactam antibiotics.

PBPs are multidomain proteins which, according to their domain structure, function and relatedness in peptide sequence, can be classified into two groups (25, 26, 27, 28, 29). The first group consists of monofunctional low-molecular-weight PBPs that range in size from 30 - 40 kilodaltons. By hydrolyzing the carboxy-terminal D-alanyl-D-alanine peptide bond of peptidoglycan precursors, these enzymes function as D-D-carboxypeptidases and help control the extent of peptidoglycan cross-linking by limiting the number of pentapeptide units available for transpeptidation (30). In addition, monofunctional PBPs can also hydrolyze existing peptidoglycan interpeptide bonds and therefore presumably play various roles in mediating cell division, allowing for the insertion of new peptidoglycan material, and in the recycling of old peptidoglycan (28). The second group of PBPs consists of the multimodular high-molecular-weight PBPs that are typically 50 - 100 kilodaltons in size.

S. pneumoniae contains six PBPs. These consist of five high-molecular-weight PBPs (designated PBP 1A, 1B, 2A, 2X and 2B) and the low-molecular-weight PBP 3 (31). Although β-lactam antibiotics such as penicillin do inhibit the D-alanyl-D-alanine carboxypeptidase activity of PBP 3, this is a regulatory enzyme whose inactivation can be tolerated, albeit not without some compromise in cell division and morphology. Similarly, neither PBP 1A, PBP 1B nor PBP 2A is required for growth when deleted individually, however the presence of at least PBP 1A or PBP 2A is essential for cell viability (31). On the other hand, individual deletion of either PBP 2X or PBP 2B is lethal for *S. pneumoniae* (29, 32).

The high-molecular-weight group of PBPs can be further divided into bifunctional enzymes with transpeptidase and glycosyltransferase activities (class A) and

monofunctional class B enzymes with only one well-defined function (transpeptidation) (25, 27, 28, 29, 30, 31). Of the five high-molecular-weight PBPs identified in *S. pneumoniae*, three belong to class A (PBPs 1A, 1B and 2A) while two (PBPs 2X and 2B) are class B proteins. The class A bifunctional PBPs combine in a single polypeptide chain both the transglycosylase and D,D-transpeptidase activities. Essentially, a noncleavable signal peptide which functions as a transmembrane anchor is fused to the amino end of a transglycosylase non-penicillin-binding module, which itself is fused to the amino end of a serine transferase (D,D-transpeptidase) penicillin-binding module (25, 26, 28). The two catalytic modules, which operate in a concerted manner, form a single polypeptide chain that folds on the exterior of the plasma membrane (26, 28).

To allow the bacterial cell to grow and divide, morphogenetic networks channel peptidoglycan assembly into cell wall expansion and septum formation in a cell-cycle-dependent fashion. Central to these networks are the class B PBPs which are similar in their modular design to the bienzymatic class A proteins with the single exception that the non-penicillin-binding module of these enzymes is not a transglycosylase (28). To this day, the precise function of the N-terminal domain of these class B PBPs remains unknown, although a possible role in cell shape maintenance and cell division seems most likely. Therefore, the class B PBPs combine in a single polypeptide chain a morphogenetic determinant non-penicillin-binding module and a serine transferase penicillin-binding module that is thought to prescribe species-specific traits related to peptidoglycan cross-linking (28).

In all PBPs, the catalytic centers that perform the transpeptidation reaction are defined by three conserved amino acid groupings, referred to as motifs, which constitute

essential components of the active-site cavity. In *S. pneumoniae*, these include the serine-X-X-lysine (S*XXK) motif where X represents a variable amino acid and S* is the essential active-site serine residue, a serine-X-asparagine (SXN) motif, and the lysine-X-glycine (KXG) triad (25). These motifs occur in the same order and with roughly the same spacing along the polypeptide chain of each PBP (Table 2), defining a common amino acid sequence signature within a region of the protein known as the penicillin-binding domain (PBD) (28). The PBD of high-molecular-weight PBPs is believed to start approximately 60 amino acid residues upstream of the SXXK motif and to terminate approximately 60 amino acid residues downstream from the conserved KXG motif (26). Polypeptide folding brings the three motifs close to one another, generating an active site cavity where motifs two and three each define one side of the catalytic center and where the serine of the S*XXK motif occupies a central position (28).

Table 2. Position of conserved motifs within the essential PBPs of *S. pneumoniae*.

Penicillin-Binding Protein	Protein Size (Number of Amino Acids)	Motif		
		S*XXK	SXN	KXG
1A	719	S_{370}TMK	S_{428}RN	K_{557}TG
2B	680	S_{385}VVK	S_{442}SN	K_{614}TG
2X	750	S_{337}TMK	S_{395}SN	K_{547}SG

[a] S*, active-site serine.

4. Mechanisms of Resistance

a. Molecular Mechanisms of Bacterial Resistance to β-lactam Antibiotics

β-lactam antibiotics are among the most frequently prescribed antibiotics worldwide (33). Because of the popularity of these drugs, it is not surprising that resistance to these agents has become a major therapeutic problem. Bacteria may exhibit resistance to penicillins and other β-lactam antibiotics by one or more mechanisms. In most cases, the resistance of clinical isolates is largely due to the production of bacterial enzymes known as β-lactamases, which open the β-lactam ring and cause inactivation of the antibiotic (34). Another resistance mechanism of increasing importance is the production of modified target sites (PBPs) with reduced affinities for β-lactam antibiotics (34). A third means by which gram-negative bacteria display resistance involves modification of the cell envelope, thereby creating a permeability barrier to the passage of β-lactams (34). Although somewhat less common, active efflux can also confer resistance by preventing these compounds from reaching their target (35).

β-lactamases constitute a superfamily of evolutionarily related active-site serine peptidases that catalytically destroy penicillins and cephalosporins through a serine ester mechanism of hydrolysis similar to that of the PBPs (22, 34, 35). The clinical significance of β-lactamases became apparent soon after the discovery of penicillin, with the isolation of β-lactamase-producing isolates of *S. aureus* resistant to penicillin (36). Following the advent of broad-spectrum β-lactam antibiotics, most gram-negative bacilli were discovered to produce chromosomally-mediated β-lactamases characteristic of each species, which accounted for the intrinsic resistance of organisms such as *Bacteroides*

fragilis, *Klebsiella pneumoniae*, *Enterobacteriaceae* species and *Serratia marcescens*. The discovery that β-lactamases could also be encoded by plasmids and readily transferred by conjugation meant that widespread dissemination among gram-negative bacteria, including species not previously known to possess the enzyme, was almost inevitable. Consequently, β-lactamase-producing strains of *Haemophilus influenzae* and *Neisseria gonorrhoeae* are now common causes of infection. More recently, β-lactamase-producing strains of *Enterococcus faecalis* and *Neisseria meningitidis* have also been described, but are as yet comparatively uncommon (34).

Resistance to β-lactam antibiotics due to PBP modification occurs either through chromosomal mutations in the genes encoding essential PBPs or through the acquisition of supplementary foreign genes encoding new resistant PBPs (35). The most important clinical examples arising from modified PBPs include methicillin-resistant staphylococci, penicillin-resistant pneumococci and ampicillin-resistant enterococci. However, this mechanism is also responsible for low-level penicillin resistance in *H. influenzae*, *N. gonorrhoeae* and viridans streptococci (34).

Other instances of bacterial resistance to β-lactam antibiotics are caused by the inability of the agent to penetrate to its site of action (17). In gram-negative bacteria, PBPs are protected by the outer membrane of the cell wall (34). The passage of β-lactam antibiotics across the outer membrane is consequently facilitated by porin proteins, which act as pores by allowing the diffusion of small hydrophobic molecules such as the penicillins (34). The resistance of these bacteria to β-lactam antibiotics may therefore be increased by alterations in porin structure leading to a decreased permeability (34).

Gram-positive bacteria such as *S. pneumoniae*, on the other hand, lack an outer membrane and therefore are unable to utilize this mechanism of resistance (34).

b. Penicillin Resistance in *S. pneumoniae*

Penicillin resistance in *S. pneumoniae* is due entirely to molecular changes in the high-molecular-weight PBPs that affect their interaction with the antibiotic in such a way that much higher antibiotic concentrations are required for PBP inhibition and therefore for biological activity of the drug (37, 38, 39, 40, 41, 42, 43, 44). Since penicillin and the D-alanyl-D-alanine muropeptide substrate both interact with the same active-site serine within the PBP, mutations responsible for low affinity PBP variants must be carefully positioned within the protein in order to still allow for its actual *in vivo* function (40, 45). In other words, the active site must be remodeled in such a way that the interaction with the antibiotic is severely affected whereas interaction with the peptidoglycan precursor substrate occurs virtually unhindered.

This mechanism of resistance was first recognized in 1989 by Markiewicz and Tomasz (46) and by Jabes *et al.* (47), who compared the PBPs of penicillin-sensitive and penicillin-resistant strains of *S. pneumoniae*. The results of these investigations revealed that penicillin-resistant pneumococci could be clearly distinguished from susceptible strains on the basis of alterations in their PBP profiles as well as by decreases in the affinity of these PBPs for penicillin. The mechanism by which these new 'resistant' PBPs were arising was described that same year by Dowson *et al.* (48). A comparison of the DNA sequences encoding the transpeptidase region of the PBPs of penicillin-resistant and penicillin-sensitive pneumococci revealed a mosaic pattern of nucleotide substitutions in the resistant strains. This mosaic pattern was characterized by regions of

extensive sequence alteration separated by blocks of DNA identical to the penicillin-sensitive controls. In contrast, penicillin-sensitive strains showed relatively few (i.e., approximately 12) substitutions throughout the one kilobase penicillin-binding domain. Of further interest was the observation that certain DNA alterations, as well as the resultant amino acid changes, were virtually identical among resistant isolates. Since the majority of these amino acid changes were positioned within or adjacent to the three signature motifs located within the active centers of the PBPs, these regions have been hypothesized to be important in the development of resistance (49).

c. Clinical Definition of Penicillin Resistance in Pneumococci

The most basic laboratory measurement of the activity of an antibiotic against an organism is the MIC, or minimum inhibitory concentration (19). It is defined as the lowest (antibiotic) concentration that will inhibit the growth of a test organism over a defined interval related to the organism's growth rate (19, 50). A second term that is closely associated with the MIC and is fundamental to the interpretation of these values is the 'breakpoint'. Breakpoints are the MIC values that determine the categories of susceptible, intermediate and resistant organisms and as such provide us with the power to predict the *in vivo* efficacy of a given antibiotic (19, 50).

Penicillin susceptibility implies that an infection due to *S. pneumoniae* may be appropriately treated with a penicillin concentration less than or equal to 0.06 μg/ml. The intermediate category, by comparison, includes isolates that are readily treatable with moderately increased doses of penicillin (0.1 – 1.0 μg/ml), although response rates are frequently lower than those observed for susceptible isolates. Pneumococcal isolates with MICs ≥ 2 μg/ml are routinely regarded as highly resistant to penicillin. Such isolates are

not inhibited by the usually achievable systemic concentrations of penicillin under normal dosing regimens.

5. The Evolution and Spread of Penicillin Resistance in *S. pneumoniae*

a. Origins of Altered PBPs

The altered PBPs 1A, 2B and 2X of penicillin-resistant *S. pneumoniae* appear to have arisen through multiple interspecies recombinational events, presumably mediated by genetic transformation, that have replaced parts of the pneumococcal PBP genes with the corresponding regions from the homologous PBP genes of closely related species (44, 48, 51, 52, 53, 54, 55, 56, 57). Consequently, the PBP genes of penicillin-resistant pneumococci have a mosaic structure consisting of regions that are very similar to the corresponding regions in the genes from penicillin-susceptible pneumococci and regions that differ by as much as 14 to 23% in nucleotide sequence (55, 58, 59). Sequences closely related to the mosaic blocks in the resistant PBP genes of *S. pneumoniae* are also distributed among β-lactam-resistant strains of related commensal streptococcal species (58, 60, 61, 62), documenting that a gene pool of allelic variants exists that is shared by a variety of related streptococci (44). In several studies, DNA sequences identical or closely related to the mosaic genes of resistant *S. pneumoniae* have been identified in *Streptococcus sanguis*, *S. oralis*, and *S. mitis* for *pbp2b* and *pbp2x* (54, 58, 60, 62, 63, 64) and in *S. mitis* for *pbp1a* (42, 58). It is assumed, therefore, that resistance to β-lactam antibiotics originated in commensal species, unnoticed by microbiologists mainly because these bacteria rarely cause disease (44). Thus, the appearance of penicillin-resistant pneumococci may well be a secondary event, with the genetic potential for penicillin

resistance first evolving in commensal bacteria through the introduction of point mutations (Figure 5). Once the PBPs of commensal streptococci had evolved into resistance determinants, they could then be transferred to and selected for in the pathogen *S. pneumoniae*. Finally, mutations that improve resistance, or that are required for better *in vivo* function of the protein in another genetic background, could then be introduced.

Figure 5. Model for the evolution of mosaic PBP genes as resistance determinants[a].

[a] Adapted from reference 44.

b. Epidemiology of PBP-Mediated Resistance: Horizontal *vs.* Clonal Spread

PBP-mediated resistance to penicillin can spread either through the multiplication and dissemination of resistant isolates (clonal spread) or by genetic exchange and

dissemination of mosaic PBP genes (horizontal spread) (55). In the absence of frequent horizontal genetic exchange, binary fission imposes a clonal structure on bacterial populations. Chromosomally encoded genes pass only from mother cell to daughter cell, and new alleles generated by mutation remain in the lineage in which they arose. As distinct mutations accumulate at various loci, each lineage acquires a characteristic non-random combination of alleles. Fluctuations in population size or uneven spread causes the loss of many lineages, reducing the overall diversity of the population. In a clonal population, a novel allele conferring antibiotic resistance will remain in the lineage in which it arose and will invariably be associated with other unlinked characteristics such as serotype antigens. Frequent horizontal genetic exchange, on the other hand, can disrupt this clonal structure by reassorting alleles among lineages. Consequently, the stability of a clonal population structure depends on the rate at which horizontal genetic exchange occurs relative to the rate of spread by clonal descent. Depending on the relationship of these processes, a given population of bacteria has one of a number of possible population structures, ranging from strictly clonal to nonclonal. In nonclonal or weakly clonal populations, the association of particular markers, such as antibiotic resistance or serological characteristics, may be lost (59).

To evaluate the relative importance of clonal and horizontal spread of resistance in clinical isolates of *S. pneumoniae*, epidemiological methods that are able to distinguish one mechanism from the other are required. This can be achieved through the combination of a method that indexes the overall genetic relatedness between isolates and one that can assess the relatedness of their PBPs (55, 56, 65). Resistant pneumococci that are not closely related genetically but that contain identical altered PBP genes can then be

proposed to have arisen by horizontal spread. Typically, but not invariably, they will also share a common antibiotic resistance profile and serotype (55). In contrast, isolates that are indistinguishable in terms of both their overall relatedness and the relatedness of their altered PBP genes are clearly the result of clonal spread (56, 65).

Numerous phenotypic and genotypic methods have been developed to assist in epidemiological investigations. These methods, in addition to various other techniques, include serotyping, DNA sequencing, pulsed-field gel electrophoresis (PFGE) and arbitrarily-primed PCR (AP-PCR) (66). As mentioned previously, the capsular polysaccharide is an essential virulence determinant for *S. pneumoniae* in providing protection from phagocytosis. Serotyping, based on capsular polysaccharide antigens expressed by *S. pneumoniae*, has been traditionally used for typing purposes, although it is much less discriminatory than the more recently applied molecular techniques (67). DNA sequencing, by comparison, provides an accurate means of examining the PBP genes of penicillin-resistant pneumococci, while PFGE of large DNA restriction fragments and PCR-based fingerprinting techniques such as AP-PCR facilitate the assessment of overall relatedness between isolates. By these approaches, the identification of penicillin-resistant pneumococcal clones as well as the significance of horizontal spread of resistance genes and putative serotype changes in the dissemination of penicillin resistance have been demonstrated.

c. History and Prevalence of Pneumococcal Resistance to Antibiotics

Although penicillin resistant laboratory mutants of *S. pneumoniae* were selected soon after this drug was introduced, clinical resistance to penicillin was not reported until 20 years later when investigators in Boston noted penicillin MICs in the intermediate

resistance range (0.1 – 0.2 μg/ml) for two strains but failed to recognize the significance of that resistance (68). Hansman and Bullen (69) were the first to both report and to realize the significance of penicillin resistance in *S. pneumoniae*. Their first resistant strain with a penicillin MIC of 0.6 μg/ml was isolated in Australia in 1967. Resistant strains were subsequently identified in New Guinea and Australia, where the proportion of nonsusceptible strains (MIC; ≥ 0.1 μg/ml) rose from 12% in 1970 to 33% in 1980 (68). After the initial reports by Hansman and Bullen, numerous descriptions of infections due to penicillin-resistant pneumococci began to appear in the literature; most strains showed intermediate resistance. In 1977, pneumococci resistant to penicillin began to appear in Durban, South Africa. All strains were highly resistant to penicillin (with MICs between four and eight μg/ml) and exhibited differing degrees of resistance to other antibiotics as well (68). Soon after, penicillin-resistant and multiple drug-resistant pneumococci were reported worldwide (70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82).

In terms of the geography of penicillin-resistant *S. pneumoniae*, the major foci of resistant organisms include southwest Europe (Spain, France, Portugal), central eastern regions of Europe (Hungary, Romania, Bulgaria, Turkey) and Israel, northwest Russia, South Africa, Japan and South Korea, Papua-New Guinea, Alaska, southeast and southwest North America and the South Cone of South America (83). For more than a decade, Spain and Hungary have represented the primary 'hot spots' of European pneumococcal resistance, with rates of penicillin-nonsusceptibility as high as 57% (84, 85). Recent surveys have likewise indicated that *S. pneumoniae* with reduced susceptibility to penicillin constitute a high proportion of invasive and colonizing isolates in France, Portugal and Bulgaria (85, 86). Central and northeastern European countries,

by comparison, appear to have relatively low rates of penicillin resistance. The detected rates remain less than 7% in Germany and are also low in Austria and the Czech Republic (83).

Despite their geographic proximity to southern European countries heavily colonized with resistant pneumococci, most northern and western African countries apparently maintain low rates (below 5%) of pneumococcal resistance (83). In Kenya and South Africa (where resistant pneumococci were first detected), the actual rates of resistance are thought to surpass 40% (83). Among Latin American and Caribbean countries, the lowest resistance rates are observed in Brazil (28.1%), Argentina (23.3%) and the West Indies (7.1%), while the highest rate of penicillin resistance (66.7%) is found in Mexico (87). Although data from the Middle East is somewhat scarce, recent figures suggest that the current incidence of penicillin resistance in countries such as Israel, Saudi Arabia and Lebanon may already exceed 56% (88, 89). In the Western Pacific region, studies have revealed rapidly rising rates of resistance in Korea, Singapore, Taiwan, Hong Kong, Japan and mainland China (90, 91, 92). Korea, moreover, has been considered to have the highest prevalence of penicillin and multidrug resistance (defined as resistance to antibiotics of at least three different classes) (15, 68) in the world, with nonsusceptibility to penicillin (MIC; ≥ 0.1 μg/ml) now estimated at 80% (91, 92).

In the United States, the first infection due to penicillin-nonsusceptible pneumococci (MIC; 0.25 μg/ml) was reported in 1974 (93). Between 1976 and 1988, approximately four percent of recovered isolates were found to be intermediately resistant to penicillin (16). At that point, strains with high-level resistance were extremely

uncommon (~ 0.2%). Two years later, numerous medical centers began to report a dramatic increase in penicillin nonsusceptibility among pneumococci. In 1992, new studies cited the prevalence of penicillin resistance among *S. pneumoniae* at nine percent, with two percent of isolates exhibiting high-level resistance (16). Since then, the prevalence of penicillin-resistant pneumococci in the United States has increased significantly. Current susceptibility surveys now suggest that approximately 17% of all *S. pneumoniae* isolates are intermediately resistant, while as many as 19% are highly resistant (94).

In Canada, penicillin resistance has not increased as rapidly as it has in the United States. Prior to the mid-1990s, in fact, penicillin-resistant and multidrug-resistant pneumococci were rarely isolated (95). Studies performed during the 1970s and 1980s reported *S. pneumoniae* penicillin resistance rates of 1.3 to 2.4% (79, 96); all isolates were found to be intermediately resistant. Between 1993 and 1996, various Canadian national surveys began to document an increase in the isolation of penicillin-nonsusceptible pneumococci (95, 97), with the prevalence of penicillin-intermediate and penicillin-resistant *S. pneumoniae* rising from 6.4 to 8.9% and from 2.1 to 4.4%, respectively. Thereafter, the proportion of both penicillin-intermediate and -resistant isolates in this country approximately doubled. Most recently, data from a 1997 to 1998 national surveillance study has demonstrated that *S. pneumoniae* with reduced susceptibility to penicillin constitute 21.2% of respiratory tract isolates (98). Of these, 14.8% were penicillin-intermediate and 6.4% were penicillin-resistant. In addition, penicillin nonsusceptibility was also found to be an important marker for the presence of

a multidrug-resistant phenotype, which was present in 17.1 and 36.8% of penicillin-intermediate and -resistant isolates, respectively (98).

d. Clinical Significance of Penicillin Resistance in *S. pneumoniae*

Pneumococcal resistance to penicillin has important clinical implications. Because penicillins are often inexpensive drugs, penicillin resistance means the loss of a cost-effective therapy that is often well tolerated. Furthermore, infections caused by resistant pathogens have higher rates of morbidity and mortality associated with them than do infections caused by susceptible pathogens (99). Costs incurred by prolonged hospital stays are but only a small portion of additional expenses associated with such pathogens. It has been estimated that, at least in the United States, microbial resistance could add between 100 million and 30 billion dollars annually to health care costs (33) if one considers all the unrecognized expenses that can occur and the fact that resistance, once generated, does not disappear quickly. Secondly, multidrug resistance is a frequent occurrence among penicillin-resistant pneumococci. In highly resistant isolates, significant proportions are also resistant to antibiotics such as chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole and macrolides such as erythromycin, clarithromycin and azithromycin (100). Likewise, because pneumococci become resistant to penicillin through altered PBPs, as penicillin MICs increase a parallel increase in the MICs of most, if not all, β-lactam antibiotics is also observed. Penicillin resistance may therefore prompt clinicians to use other drugs, thus increasing the possibility of developing resistance to those agents as well.

e. The Potential of Molecular Diagnostics

Effective treatment of infections caused by *S. pneumoniae* requires rapid detection of both the organism and, more importantly, its antibiotic susceptibility pattern (101). Currently, the most sensitive method of diagnosis is based on the successful culture and identification of bacteria from various specimens including blood, sputum, middle ear fluid and cerebrospinal fluid (102). By standard culture methods, presumptive identification of *S. pneumoniae* takes 12 to 24 hours, followed by biochemical tests for confirmation (103). Conventional culture-based susceptibility testing (which can be difficult to perform) requires an additional 24 hours, which means that a result is rarely available within less than 48 hours. Empiric therapy must therefore include the use of broad-spectrum antibiotics such as cephalosporins and vancomycin to ensure coverage against penicillin-resistant pneumococci. Although combinational therapy is often the only choice available to many physicians, the extensive and sometimes unnecessary use of such drugs encourages the development of further resistance.

While no method has yet replaced conventional susceptibility testing procedures, molecular diagnostics is playing an increasingly important role in the determination of antibiotic resistance profiles. For example, the recent advent of nucleic acid amplification techniques such as the polymerase chain reaction (PCR) has provided for a more rapid diagnosis, combined with high sensitivity and specificity. Given the advantages of early diagnosis of infection and appropriate antibiotic administration, the application of a PCR-based strategy for pneumococcal identification and characterization of species-specific antibiotic resistance genes could be remarkably valuable for the treatment of infectious diseases caused by *S. pneumoniae*.

6. Hypotheses and Thesis Objectives

Penicillin resistance in *S. pneumoniae* is mediated by changes in the affinities of high-molecular-weight PBPs 1A, 2A, 2B and 2X (38, 39, 43, 51). Genetic analysis, however, has shown that high-level resistance to penicillin can result from sequential alterations in only PBPs 1A, 2B and 2X (43, 104). We therefore hypothesize that if penicillin resistance in the pneumococcus is entirely due to the stepwise production of altered PBPs, then the number of PBP gene mutations contributing to such resistance will increase with progressively elevated MICs. Since alterations responsible for these low-affinity PBP variants must be carefully positioned in order to still allow for *in vivo* function of the protein, the array of substitution patterns conferring high-level resistance is likely limited. We hypothesize, moreover, that if differential nucleotide sequences can indeed act as markers for penicillin susceptibility, then rapid identification of penicillin resistance may be possible through PCR detection of mosaic PBP gene profiles.

Once acquired, PBP-mediated resistance to penicillin can spread through either the horizontal transfer of altered PBPs with diminished affinities for β-lactam antibiotics (horizontal spread) or through the multiplication and dissemination of resistant isolates (clonal spread) (44, 48, 53, 55, 57). Notwithstanding the importance of horizontal genetic exchange, we propose that clonal dissemination is a significant driving force in the increasing incidence of penicillin-resistant *S. pneumoniae* in Canada. We therefore hypothesize that a limited variety of genetic backgrounds are present among clinical isolates of penicillin-resistant pneumococci and that these clones represent a population of *S. pneumoniae* isolates with greater versatility or fitness and therefore the selective advantage to spread in an environment in which antibiotics are often misused.

The objective of this thesis was to characterize PBP 1A, 2B and 2X mutations in Canadian isolates of penicillin-nonsusceptible *S. pneumoniae* and to evaluate the relationship between pneumococcal genotypic variance and penicillin susceptibility as it pertains to the dissemination of resistance. To achieve this goal, specific objectives were devised as follows:

- To determine the degree of overall relatedness among clinical isolates of *S. pneumoniae* using a combination of AP-PCR, PFGE and serotyping.
- To investigate the utility of PCR for rapid differentiation between penicillin-resistant, -intermediate and -susceptible genotypes of *S. pneumoniae*.
- To describe the relationship between the results of *pbp1a*, *pbp2b* and *pbp2x* gene amplifications by PCR and the penicillin susceptibility (MICs) of *S. pneumoniae* isolates.
- To sequence the PBD of PBPs 1A, 2B and 2X from penicillin-resistant, -intermediate and -susceptible isolates of *S. pneumoniae* in order to identify nucleotide and/or amino acid alterations which appear to be essential for the development of penicillin resistance.
- To describe the relationship between patterns of nucleotide and/or amino acid alterations and the penicillin susceptibility (MICs) of *S. pneumoniae* isolates.

B. MATERIALS AND METHODS

1. Bacterial Isolates

a. Isolate Selection

Fifteen clinical isolates of *S. pneumoniae*, selected from more than 3600 isolates obtained as part of an ongoing Canadian Respiratory Organism Susceptibility Study (98), were tested. Selection of isolates was based upon (i) penicillin MIC (as determined by the National Committee for Clinical Laboratory Standards [NCCLS] – recommended broth microdilution method [105]) , (ii) geographic origin, (iii) date of isolation and (iv) clinical source. Specifically, isolates were randomly chosen to represent a range of susceptibilities to penicillin. The fifteen isolates consisted of five penicillin-susceptible isolates (MIC; ≤ 0.06 μg/ml), five penicillin-intermediate isolates (MIC; 0.12 - 1 μg/ml) and five penicillin-resistant isolates (MIC; ≥ 2 μg/ml). All organisms had been submitted to or isolated by the Department of Clinical Microbiology at the Health Sciences Centre in Winnipeg, Canada between August 28, 1997 and June 9, 1999. Study isolates were obtained from eleven different centres widely distributed throughout the Canadian provinces of British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec and Prince Edward Island. Sources of the isolates consisted mostly of sputum samples (53%) as well as eye, endotracheal tube, lung aspirate and trachea specimens. The clinical and demographic parameters of the *S. pneumoniae* isolates examined in this study are listed in Table 3. In addition, a penicillin-intermediate (MIC; 0.25 - 1 μg/ml) reference strain of *S. pneumoniae* (ATCC 49619) was included as a control for antibiotic susceptibility testing as well as for preliminary evaluation of PCR amplification methods and PFGE/AP-PCR techniques.

Table 3. Penicillin susceptibility and demographics of *S. pneumoniae* isolates recovered in Canada.

Isolate	Penicillin MIC (μg/ml)	Date Isolated (m/d/y)	Geographic Origin[a]	Source
6190	4	3/6/98	RUH	Sputum
8111	4	3/5/98	Van HSC	Sputum
742	2	1/17/98	VGH	Sputum
2848	2	10/1/97	HSC	Sputum
6363	2	5/14/98	UA	Trachea
3455	1	12/12/97	HSC	Endotracheal tube
14126	0.5	6/9/99	LHSC	Eye
3996	0.25	8/28/97	CLS	Sputum
11413	0.25	1/4/99	LHSC	Trachea
12276	0.12	1/3/99	MR	Sputum
3203	0.06	11/5/97	QEH	Sputum
11184	0.06	11/5/98	USL	Sputum
12244	0.06	2/11/99	CLS	Endotracheal tube
14016	0.06	1/7/99	SJH	Eye
8099	$\leq$ 0.03	3/3/98	Van HSC	Lung aspirate

[a] RUH, Royal University Hospital (Saskatoon, Saskatchewan); Van HSC, Vancouver Health Sciences Centre (Vancouver, British Columbia); VGH, Victoria General Hospital (Victoria, British Columbia); HSC, Health Sciences Centre (Winnipeg, Manitoba); UA, University of Alberta Hospitals (Edmonton, Alberta); LHSC, London Health Sciences Centre (London, Ontario); CLS, Calgary Lab Services (Calgary, Alberta); MR, Mais-Sonneve Rosemont (Montreal, Quebec); QEH, Queen Elizabeth Hospital (Prince Edward Island); USL, University de Sante de l'Estrie (Sherbrooke, Quebec); SJH, St. Joseph's Hospital (Hamilton, Ontario).

Biochemical identification of all isolates was performed by conventional laboratory methods as suggested in the Manual of Clinical Microbiology published by the American Society for Microbiology (1). Following identification, *S. pneumoniae* isolates were inoculated into skim milk and maintained at -80°C. Organisms were routinely cultured on Trypticase soy agar supplemented with 5% sheep blood and incubated for 18-24 hours at 35°C in an atmosphere containing 5% CO_2.

b. Species Confirmation

As part of the routine identification protocol, Gram-stains were performed on all isolates for the direct detection of streptococci. Previous species identification of the *S. pneumoniae* isolates was confirmed by optochin susceptibility and bile solubility testing.

i. Optochin Susceptibility Test

α. Inoculum Preparation and Antibiotic Application

Colonies were selected from an 18-20 hour subculture, inoculated into sterile saline (0.85% NaCl) and adjusted to the equivalency of a 0.5 McFarland turbidity standard (1×10^8 CFU/ml). The properly adjusted inoculum was then used to swab the entire surface of a Mueller Hinton-5% sheep blood agar plate. An optochin disk (Becton-Dickinson Microbiology Systems, Cockeysville, MD) was aseptically applied to the inoculated agar surface and plates were incubated for 20-24 hours at 35°C in 5% CO_2.

β. Interpretation of Results

Following the appropriate period of incubation, plates were examined and the zone of inhibition surrounding the disk was measured. Zone diameters of fifteen millimeters or greater were indicative of optochin sensitivity and identified the isolate as *S. pneumoniae*.

χ. Colony Counts

Colony counts were regularly performed to ensure an initial inoculum of approximately 1×10^8 CFU/ml. Viable cell counts were produced by preparing ten-fold serial dilutions of the initial inoculum suspension, plating 100 μl aliquots of each dilution on Trypticase soy-5% sheep blood agar and determining the number of viable colonies following 18-24 hours of incubation at 35°C in 5% CO_2.

ii. Bile Solubility Test

For evaluation of bile solubility (1), colonies from an 18-24 hour subculture were emulsified in 1 ml of sterile saline (0.85% NaCl) at a density equivalent to a 1.0 McFarland standard (3 x 10^8 CFU/ml). Aliquots containing 0.5 ml of the bacterial suspension were then transferred to each of two test tubes and five drops of 10% sodium deoxycholate were added to one tube. The second tube was left as is to serve as a (saline) control and all tubes were incubated for one to two hours at 35°C. Clearing of turbidity in the presence of deoxycholate when compared to the saline control indicated a positive bile solubility test and was thus indicative of *S. pneumoniae*. *S. pneumoniae* ATCC 49619 and viridans streptococci were included as positive and negative controls, respectively.

2. Antibiotic Susceptibility Testing

Penicillin susceptibilities of the 15 *S. pneumoniae* isolates were assessed by oxacillin disk diffusion and E-test methods as well as by broth macrodilution and broth microdilution procedures.

a. Oxacillin Disk Diffusion

In accordance with 2000 NCCLS performance standards (106), isolates were initially screened for penicillin resistance with a 1-µg oxacillin disk (Becton-Dickinson Microbiology Systems, Cockeysville, MD) by the Kirby-Bauer disk diffusion method. Preparation of *S. pneumoniae* inoculum suspensions and Mueller-Hinton blood agar inoculation were performed as described in section I. b. i. α. Oxacillin disks were then applied using aseptic precautions. Following a 20–24 hour incubation at 35°C in an

atmosphere enriched with 5% CO_2, plates were examined and the zones of inhibition surrounding the disks were measured. Isolates with a zone of inhibition equal to or greater than 20 mm were considered susceptible to penicillin, while an oxacillin zone size less than or equal to 19 mm identified an isolate as either non-susceptible or potentially resistant. Zone diameter interpretive standards were defined according to 2000 NCCLS guidelines (107). Colony counts on inoculum suspensions were routinely performed as described in section 1. b. i. χ. to ensure that the final inoculum concentration closely approximated 1 x 10^8 CFU/ml.

b. Broth Macrodilution

Isolates were tested for their susceptibility to penicillin by the broth macrodilution method according to the 2000 recommendations of the National Committee for Clinical Laboratory Standards (108).

i. Antibiotic Preparation

Benzylpenicillin (Sigma, St. Louis, MO) powder was used to prepare a concentrated antibiotic stock solution containing 1024 μg/ml of the antimicrobial agent. The sterilized penicillin stock solution was then dispensed in 1 ml aliquots and stored at -80°C. Vials containing the stock solution were thawed as needed and used the same day.

ii. Medium

Mueller-Hinton broth (MHB) consisting of 3 g/L of beef extract, 17.5 g/L of acid hydrolysate of casein and 1.5 g/L of starch was used as the medium of choice for all macrodilution susceptibility testing and was prepared as directed by the manufacturer (Becton Dickinson, Cockeysville, MD). Cation adjustment of this medium involved the addition of 25 μg/ml of $CaCl_2$ and 12.5 μg/ml of $MgCl_2$. To support the growth of

fastidious bacteria such as *S. pneumoniae*, Mueller-Hinton medium was further supplemented with 5% lysed horse blood (LHB).

iii. MIC Determination

For each isolate to be tested, 1 ml of MHB was added to a series of ten test tubes, omitting the first tube. Three additional tubes (broth only, broth plus organism and broth plus antibiotic), also containing 1 ml of MHB, were included as controls. Next, the concentrated penicillin stock solution was diluted in MHB to achieve a final concentration equal to twice that desired in the first test tube. One milliliter of the diluted antibiotic was then added to the first two tubes of the series as well as to the appropriate control tube, and finally to the remainder of the tubes by 1 ml two-fold serial dilutions. A standardized inoculum was prepared by suspending isolated colonies from an 18-20 hour subculture in sterile saline at a density equivalent to a 0.5 McFarland standard. The adjusted inoculum suspension was subsequently diluted 1:100 and 1 ml of this preparation was added to each tube in the dilution series and to a positive growth control tube, resulting in an initial inoculum of 5×10^5 CFU/ml.

The penicillin MIC, defined as the lowest antibiotic concentration that completely inhibited visible growth (50), was determined after incubation of the tubes for 20-24 hours at 35°C in ambient air. Penicillin-susceptible isolates were defined as having an MIC $\leq$ 0.06 μg/ml, penicillin-intermediate isolates were defined as having an MIC between 0.1 and 1 μg/ml, inclusively, and resistant isolates were defined as having an MIC $\geq$ 2 μg/ml. MIC breakpoints for defining susceptibility and resistance were in accordance with the 2000 guidelines of the NCCLS (109).

Lastly, colony counts from ten-fold serial dilutions of the original diluted inoculum suspension were performed to determine the exact initial inoculum. To this end, 100 µl aliquots of each dilution were plated on Trypticase soy-5% sheep blood agar and the number of viable colonies were noted following 18-24 hours of incubation at 35°C in 5% CO_2.

c. Broth Microdilution

The susceptibility of the *S. pneumoniae* isolates to a variety of antibiotics was assessed by a broth microdilution assay, as detailed in guidelines from the 2000 National Committee for Clinical Laboratory Standards (108). Bacterial suspensions from 24-hour agar cultures were prepared in 3 ml of inoculum water (Dade Behring, West Sacramento, CA) and adjusted to a McFarland turbidity standard of 0.5. One hundred microliters of the standardized suspension was then combined with 25 ml of cation-adjusted Mueller Hinton broth containing 3% lysed horse blood (Dade Behring, West Sacramento, CA). Dade MicroScan® (West Sacramento, CA) panels were custom made with dehydrated dilutions of the following antibiotics: amoxicillin/clavulanate, cefaclor, cefotaxime, cefuroxime, ciprofloxacin, clindamycin, erythromycin, grepafloxacin, levofloxacin, penicillin, telithromycin (HMR 3647), tetracycline, trimethoprim/sulfamethoxazole and vancomycin. Rehydration and inoculation of these panels was performed using Renok® (Dade Behring, West Sacramento, CA) inoculator sets. Panels were then stacked in groups of three to five, covered to prevent evaporation and incubated for 20-24 hours at 35°C. Colony counts were performed to verify that a final well concentration of 4-7 x 10^5 CFU/ml had been achieved. MICs, defined as the lowest concentration of a given antibiotic that completely inhibited growth of the organism (Woods and Washington,

1995), were determined after the appropriate period of incubation. Isolates were classified as susceptible, intermediate or resistant in accordance with 2000 NCCLS guidelines (109).

d. E-Test

Penicillin MICs were confirmed by the E-test method following a procedure recommended by the manufacturer (AB Biodisk, Solna, Sweden). Preparation of *S. pneumoniae* inoculum suspensions and Mueller-Hinton blood agar inoculation were performed as described in section 1. b. i. α. Etest® strips were aseptically applied to the inoculated agar surface and plates were incubated for 18-24 hours at 35°C in 5% CO_2. After the required period of incubation, MIC values were read at the point of intersection between the inhibition ellipse edge and the Etest® strip. Penicillin MIC interpretive standards were defined according to the 2000 NCCLS breakpoints (109).

3. Pulsed-Field Gel Electrophoresis

PFGE was performed for the purpose of determining the degree of overall genetic variation among isolates of *S. pneumoniae*. Preparation of total genomic DNA, restriction enzyme digestion and the actual PFGE procedures were adapted from methods described previously (110).

a. Isolation of Chromosomal DNA

S. pneumoniae cultures were grown overnight on Trypticase soy agar with 5% sheep blood and suspended in 2 ml of sterile saline (0.85% NaCl) to an optical density of 2.6 to 2.8 at 560 nanometers. One milliliter of this suspension was centrifuged at 13000 rpm for ten minutes and the resulting bacterial pellet was resuspended in 0.25 ml of PIV

solution (10 mM Tris-HCl [pH 7.6], 1 M NaCl) on ice. One hundred and fifty microliters of the PIV-bacterial cell suspension was then combined with an equal volume of 1.6% low-melting-point agarose (InCert® agarose; FMC BioProducts, Rockland, ME), dispensed in chilled plug molds (Bio-Rad Laboratories, Hercules, CA) and allowed to solidify at 4°C for 30 minutes. Cells were lysed by incubation of the DNA-embedded agarose plugs in 10 ml of fresh lysis solution (6 mM Tris-HCl [pH 7.6], 1 M NaCl, 100 mM EDTA [pH 7.5], 0.5% Brij-58, 0.2% deoxycholate, 0.5% sarcosyl, 1 mg/ml of lysozyme and 20 μg/ml of RNase) for four hours at 37°C with gentle shaking. After lysis, the plugs were transferred into 10 ml of ESP solution (0.5 M EDTA [pH 9.5], 1% sarcosyl and 50 μg/ml proteinase K) and incubated overnight at 50°C with gentle shaking. The plugs were then washed four times (for 90 minutes each time) in 45 ml of TE buffer (10 mM Tris-HCl [pH 7.5], 0.1 mM EDTA [pH 7.5]) at 37°C with gentle agitation. The DNA was then considered purified and was stored at 4°C in approximately 10 ml of TE buffer.

b. Restriction Endonuclease Digestion and PFGE of Macrorestriction Fragments

For digestion of DNA, two to three millimeter slices of the agarose plugs were incubated with 20U of *Sma*I (New England Biolabs, Mississauga, ON) for five hours in 180 μl of sterile distilled water and 20 μl of NEBuffer 4. *Sma*I cleaves within the rare restriction site 5´-GGGCCC-3´. The digested DNA plugs were then melted at 65°C for 5 to 10 minutes, loaded into the wells of a 1% Seakem® Gold (FMC BioProducts, Rockland, ME) agarose gel prepared in 0.5X TBE buffer and sealed with 1% agarose at 65°C. Restriction fragments were resolved in a contour-clamped homogeneous electric

field apparatus (CHEF DRIII; Bio-Rad Laboratories, Hercules, CA) under the following electrophoresis conditions: 6 V/cm at 95°C for 18.5 hours in 0.5X TBE buffer with switching times ramped from 2 to 30 seconds and an included angle of 120 degrees. A lambda DNA ladder PFG marker (New England Biolabs, Mississauga, ON) was run in parallel with all *S. pneumoniae* samples for use as a molecular size standard. The *S. pneumoniae* reference strain ATCC 49619 was also included in each gel to act as a procedural control. Following electrophoresis, gels were stained for 50 minutes with 50 µl of Sybr Green (Molecular Probes, Eugene, OR) in 200 ml of $T_{10}E_1$ buffer (10 mM Tris-HCl, 1 mM EDTA), destained in distilled water for 4 hours and the DNA banding patterns visualized under UV transillumination.

c. Pattern Analysis

i. Visual Inspection and Comparison

Analysis of the chromosomal DNA restriction patterns produced by PFGE was initially performed by visual inspection of Sybr Green – stained gels under UV transillumination. The total number of visible bands was counted for each isolate and patterns were compared. To interpret PFGE profiles, the following criteria were established. Briefly, isolates with identical banding patterns were considered to be genetically indistinguishable and were assigned to the same type designation (eg. type A). Isolates whose banding patterns differed by changes consistent with two genetic events (band differences of four or more) were classified as genetically different or unrelated and were designated B, C, D, etc. Finally, isolates were defined as genetically closely related or possibly genetically related if their banding patterns differed by changes consistent

with a single genetic event (resulting in a one to three band difference) and were categorized as subtypes, designated A_1 through A_n, of one another.

ii. Computer-Assisted Analysis

PFGE profiles were scanned and digitized by the Gel Doc 1000 System (Bio-Rad Laboratories, Hercules, CA) for analysis using Molecular Analyst® (Fingerprinting Plus version 1.12) software. After conversion, DNA fragments were normalized by use of the molecular size standards included on each gel (to allow for comparison between different gels). A tolerance of 1.5% in band position as compared with molecular size standards was applied during comparison of the DNA patterns. Cluster analysis was performed by the unweighted pair group method using arithmetic averages (UPGMA) and the degree of genetic relatedness among isolates was determined on the basis of Dice coefficients (i.e., number of shared bands x 2 x 100 / total number of bands in the two samples). The values obtained from the aforementioned calculations were then used to generate a dendrogram showing the hierarchic representation of linkage levels between isolates. Computer-assisted analysis and the methods and algorithms used in this study were carried out in accordance with the instructions of the manufacturer of Molecular Analyst.

d. Discriminatory Analysis

The discriminatory power (i.e., the ability to distinguish between unrelated isolates) of PFGE as an epidemiological typing method was determined on the basis of the probability that two unrelated isolates sampled from the test population would be placed into different typing groups. This numerical index of discrimination (*D*), was calculated according to the following equation (111):

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1)$$

where N is the total number of isolates in the sample population, s is the total number of PFGE patterns described, and n_j is the number of isolates belonging to the jth type.

4. Arbitrarily-Primed Polymerase Chain Reaction

For the detection of genetic diversity among clinical isolates of *S. pneumoniae*, isolates were analyzed by arbitrarily-primed PCR. This analysis was performed as described by Louie *et al.* (67) with some modifications.

a. DNA Preparation

S. pneumoniae cultures were grown overnight on Trypticase soy agar with 5% sheep blood and suspended in 2 ml of sterile saline (0.85% NaCl) to an optical density of 2.6 to 2.8 at 560 nanometers. One milliliter of this suspension was centrifuged at 13000 rpm for ten minutes and the resulting bacterial pellet was resuspended in 0.25 ml of PIV solution (10 mM Tris-HCl [pH 7.6], 1 M NaCl) on ice. One hundred and fifty microliters of the PIV-bacterial cell suspension was then combined with an equal volume of 1.6% low-melting-point agarose (InCert® agarose; FMC BioProducts, Rockland, ME), dispensed in chilled plug molds (Bio-Rad Laboratories, Hercules, CA) and allowed to solidify at 4°C for 30 minutes. Cells were lysed by incubation of the DNA-embedded agarose plugs in 10 ml of fresh lysis solution (6 mM Tris-HCl [pH 7.6], 1 M NaCl, 100 mM EDTA [pH 7.5], 0.5% Brij-58, 0.2% deoxycholate, 0.5% sarcosyl, 1 mg/ml of lysozyme and 20 µg/ml of RNase) for four hours at 37°C with gentle shaking. After lysis,

the plugs were transferred into 10 ml of ESP solution (0.5 M EDTA [pH 9.5], 1% sarcosyl and 50 μg/ml proteinase K) and incubated overnight at 50°C with gentle shaking. The plugs were then washed four times (for 90 minutes each time) in 45 ml of TE buffer (10 mM Tris-HCl [pH 7.5], 0.1 mM EDTA [pH 7.5]) at 37°C with gentle agitation. The DNA was then considered purified and was stored at 4°C in approximately 10 ml of TE buffer. Prior to AP-PCR, the DNA-embedded agarose plugs were melted at 65°C and suspended in 300 μl of sterile distilled water. An aliquot of this suspension was used as a template for PCR amplification.

b. PCR Protocol

DNA was amplified in a total volume of 50 μl, containing 10 μl of DNA template, 5 μl of 25 mM $MgCl_2$-10X PCR buffer, 1.25 mM each of dCTP, dGTP, dATP and dTTP, 100 mM of the single 10-mer primer: 5′-GGGCAATGAT-3′, 2.5U of Taq DNA polymerase (Pharmacia Biotech, Baie d'Urfe, QC) and 26 μl of sterile distilled water. The PCR reaction was performed using a Perkin-Elmer GeneAmp® PCR System 9700 and consisted of initial denaturation at 94°C for 2 minutes followed by 40 cycles at 94°C for 30 seconds, 35°C for 30 seconds and 72°C for 30 seconds. One negative control (comprised of the identical reaction mixture with sterile distilled water in place of template DNA) and one positive control (consisting of the identical reaction mixture with template DNA from *S. pneumoniae* ATCC 49619) were included with each run. To verify the reproducibility of AP-PCR typing of *S. pneumoniae*, isolates were tested under the same conditions on at least three separate occasions.

c. PCR Product Detection

The amplified products were electrophoretically separated on 1.5% Synergel/agarose (Diversified Biotech, Boston, MA) gels prepared with 0.5X Tris-Borate-EDTA (TBE) buffer. Agarose gels were routinely supplied with ethidium bromide for product visualization under UV transillumination. Electrophoresis was carried out for 90 minutes in 0.5X TBE buffer at an applied voltage of 100. A 123-bp DNA ladder (Life Technologies, Burlington, ON) was included in each run as a molecular size standard.

d. Pattern Analysis

i. Visual Inspection and Comparison

Analysis of the DNA profiles generated by AP-PCR was initially performed by visual inspection of ethidium bromide-stained gels under UV transillumination. The total number of visible bands was counted for each isolate and patterns were compared. For the purpose of this study, isolates with identical banding patterns were considered to be genetically related and were assigned to the same type designation (eg. type A). On the other hand, isolates whose banding patterns differed by more than one band were classified as genetically unrelated and were designated B, C, D, etc. Isolates were defined as genetically closely related if their banding patterns differed by only one band and were categorized as subtypes, designated A_1 through A_n, of one another.

ii. Computer-Assisted Analysis

AP-PCR profiles were scanned and digitized by the Gel Doc 1000 System (Bio-Rad Laboratories, Hercules, CA) for analysis using Molecular Analyst® (Fingerprinting Plus version 1.12) software. After conversion, DNA fragments were normalized by use

of the molecular size standards included on each gel (to allow for comparison between different gels). A tolerance of 1.5% in band position as compared with molecular size standards was applied during comparison of the DNA patterns. Cluster analysis was performed by the unweighted pair group method using arithmetic averages (UPGMA) and the degree of genetic relatedness among isolates was determined on the basis of Dice coefficients (i.e., number of shared bands x 2 x 100 / total number of bands in the two samples). The values obtained from the aforementioned calculations were then used to generate a dendrogram showing the hierarchic representation of linkage levels between isolates. Computer-assisted analysis and the methods and algorithms used in this study were carried out in accordance with the instructions of the manufacturer of Molecular Analyst®.

e. Discriminatory Analysis

The discriminatory power (i.e., the ability to distinguish between unrelated isolates) of AP-PCR as an epidemiological typing method was determined on the basis of the probability that two unrelated isolates sampled from the test population would be placed into different typing groups. This numerical index of discrimination (*D*), was calculated according to the following equation (111):

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j (n_j - 1)$$

where N is the total number of isolates in the sample population, s is the total number of AP-PCR patterns described, and n_j is the number of isolates belonging to the jth type.

5. Serotyping

All isolates were serotyped by the National Centre for Streptococcus (Edmonton, Alberta) on the basis of capsular polysaccharide antigens by the Quellung reaction. Type-specific antisera were obtained from the Statens Seruminstitut (Copenhagen, Denmark).

6. PCR Detection of PBP Gene Mutations

a. Extraction of Bacterial DNA

S. pneumoniae cultures were grown overnight on Trypticase soy agar-5% sheep blood and a small loopful of bacterial cells was emulsified in 30 µl of lysis solution. The composition of this lysis solution was previously reported (112) and consisted of 3 µl of 1 M Tris-HCl (pH 9.0), 6 µg of proteinase K, 0.225% Tween 20, 0.225% Nonidet P-40, 3 µl of 10X PCR buffer (15 mM $MgCl_2$) and sterile distilled water to a final volume of 30 µl. Bacterial cell lysis was accomplished by incubation of the cell suspensions at 60°C for 10 minutes and then at 94°C for 5 minutes using a Perkin-Elmer GeneAmp® PCR System 9700. An aliquot of this bacterial lysate was used as the template for PBP gene amplifications.

b. PCR Protocol

A multiplex-PCR strategy was used for amplification of *S. pneumoniae* PBP genes (113). Each assay required two reactions containing primers LytA (f), LytA (r), PBP 1A (f) and PBP 1A (r) and primers PBP 2B (f), PBP 2B (r), PBP 2X (f) and PBP 2X (r), respectively. The sequences of these oligonucleotide primers (synthesized by Gibco BRL Custom Primers; Life Technologies, Burlington, ON) are shown in Table 4. The 50 µl reaction mixture contained 2.5 µl of the bacterial lysate, 5 µl of 15mM $MgCl_2$-10X

PCR buffer, 1.25 mM each of dCTP, dGTP, dATP and dTTP, 100 mM of each of the appropriate primers, 2.5U of Taq DNA polymerase (Pharmacia Biotech, Baie d'Urfe, QC) and 32 µl of sterile distilled water. Amplification was performed using a Perkin-Elmer GeneAmp® PCR System 9700. The PCR cycling conditions consisted of an initial incubation at 94°C for 5 minutes followed by 25 cycles at 94°C for 20 seconds, 57°C for 20 seconds and 72°C for 15 seconds and a final extension at 72°C for 7 minutes.

Table 4. Primers used for PCR detection of PBP gene mutations.

Primer	Sequence[a] (5' - 3')	Position	Product Length (bp)
PBP 1A (f)[b]	AAACAAGGTCGGACTCAACC	2256-2275	430
PBP 1A (r)[c]	AGGTGCTACAAATTGAGAGG	2685-2666	
PBP 2B (f)	CAATCTAGAGTCTGCTATGGA	1636-1656	77
PBP 2B (r)	GGTCAATTCCTGTCGCAGTA	1712-1693	
PBP 2X (f)	CCAGGTTCCACTATGAAAGTG	1003-1023	292
PBP 2X (r)	CATCCGTCAAACCGAAACGG	1294-1275	
LytA (f)	TGAAGCGGATTATCACTGGC	694-713	273
LytA (r)	GCTAAACTCCCTGTATCAAGCG	966-945	

[a] According to data published in reference 113.
[b] (f); Primer annealing resulted in DNA extension in the forward (5' - 3') direction.
[c] (r); Primer annealing resulted in DNA extension of the complementary strand, reverse (3' - 5') direction.

c. Agarose Gel Electrophoresis

Amplified DNA fragments were analyzed by electrophoresis through 2% agarose gels containing ethidium bromide and were visualized under UV transillumination.

Electrophoresis was carried out for 75 minutes in 0.5X TBE buffer at an applied voltage of 100. A 123-bp DNA ladder (Life Technologies, Burlington, ON) was included in each run as a molecular size standard.

d. Primer Specificity

The specificity of the *S. pneumoniae* PBP gene primers was determined by PCR amplification, under identical conditions to those described above, with eight nonpneumococcal organisms. These included *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus milleri*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus oralis* and *Streptococcus sanguis*. A penicillin-susceptible *S. pneumoniae* isolate was used as a positive control.

These nonpneumococcal organisms were further tested with universal 16S rRNA primers to ensure that there were no false-negative results. To this end, PCR amplification of the 16S rRNA gene was performed using the 8FPL (5′-AGT TTG ATC CTG GCT CAG-3′) / 806R (5′-GGA CTA CCA GGG TAT CAT AT-3′) primer pair. The 50 µl reaction mixture contained 5 µl of DNA template, 5 µl of 25 mM $MgCl_2$-10X PCR buffer, 1.25 mM each of dCTP, dGTP, dATP and dUTP:dTTP in an 8:1 ratio, 100 mM of both primers, 0.5U of uracil DNA glycosylase (UDG) (Life Technologies, Burlington, ON), 2.5U of Taq DNA polymerase (Pharmacia Biotech, Baie d'Urfe, QC) and 30 µl of sterile distilled water. The PCR reaction was performed using a Perkin-Elmer GeneAmp® PCR System 9700 under the following conditions: 37°C for 10 minutes, 94°C for 10 minutes, 30 cycles at 94°C, 55°C and 72°C for 1 minute each and 72°C for 10 minutes. Agarose gel electrophoresis and product visualization was performed as described above.

e. Statistical Analysis

Multiple regression analysis (performed using Microsoft® Excel Multivariate Analysis Software, 1997) was applied to determine if, and to what degree, the presence of *pbp1a*, *pbp2b* and *pbp2x* gene mutations (as resolved by PCR) would influence the MIC of penicillin. For each *S. pneumoniae* isolate, penicillin MIC values changed to logarithm based ten and the presence (represented by the number one) or absence (indicated by the number zero) of mutation in *pbp1a*, *pbp2b* or *pbp2x* were used as the criterion and explanatory variables, respectively.

7. Sequencing

a. Preparation of Bacterial Lysates

S. pneumoniae cultures were grown overnight on Trypticase soy agar with 5% sheep blood and colonies (approximately one loopful) were emulsified in 1 ml of saline. Following centrifugation at 13000 rpm for ten minutes, supernatants were removed and the resulting bacterial pellet was resuspended in 300 µl of lysis solution containing 0.1 M NaOH, 2 M NaCl and 0.5% sodium dodecyl sulfate. Cell suspensions were then boiled for fifteen minutes, allowed to cool and 200 µl of 0.1 M Tris-HCl (pH 8.0) was added. For extraction of genomic DNA, 500 µl of phenol-chloroform-isoamyl alcohol (25:24:1) was added and the mixture was centrifuged at 13000 rpm for ten minutes. After removal of the aqueous (top) layer into a separate 1.5 ml eppendorf tube, 1 ml of cold 100% ethanol was added and DNA was precipitated at –80°C for a minimum of 30 minutes. Tubes were then centrifuged at 4°C for fifteen minutes at 13000 rpm, the supernatants discarded and the pellets allowed to air-dry for no less than half an hour. Lastly, pellets

containing the purified DNA were hydrated in 30 µl of sterile distilled water for at least one hour and the lysates either used immediately as a template for PCR amplification or stored at -20°C for future use. All DNA extraction procedures, as well as PCR preparations, were performed using positive-displacement pipettes to minimize sample to sample contamination.

b. PCR Protocol

An 800-bp region of the 16S rRNA gene and the 1.1, 1.3 and 1.1 kb gene fragments encoding the PBDs of PBPs 1A, 2B and 2X, respectively, were amplified from the chromosomal DNA of each isolate via the polymerase chain reaction. The sequences of primers used in the amplification of these genes are shown in Table 5. PCR products were subsequently purified with Microcon microconcentrators (Millipore, Bedford, MA) in accordance with the manufacturer's instructions.

i. Amplification of the 16S rRNA Gene

The 50 µl reaction mixture contained 10 µl of DNA template, 5 µl of 25 mM $MgCl_2$-10X PCR buffer, 1.25 mM each of dCTP, dGTP, dATP and dUTP:dTTP in an 8:1 ratio, 100 mM each of primers 8FPL and 806R, 0.5U of UDG (Life Technologies, Burlington, ON), 2.5U of Taq DNA polymerase (Pharmacia Biotech, Baie d'Urfe, QC) and 25 µl of sterile distilled water. Amplification was performed using a Perkin-Elmer GeneAmp® PCR System 9700 under the following conditions: 37°C for 10 minutes, 94°C for 10 minutes, 30 cycles at 94°C, 55°C and 72°C for 1 minute each and a final extension at 72°C for 10 minutes.

Table 5. Primers used for amplification of *S. pneumoniae* PBP and 16S rRNA genes.

Primer	Sequence (5' - 3')	Position	Product Length (bp)
PBP 1A (f)[a]	TGGGATGGATGTTTACACAAATG	1827-1849	1197
PBP 1A (r)[b]	GTCGTACTATTATTTGTGCTTGG	3023-3001	
PBP 2B (f)	GGCTATTCTCTAAATGACCGT	995-1015	1317
PBP 2B (r)	AGCTTAGCAATAGGTGTTGG	2311-2292	
PBP 2X (f)	TATGAAAA(G/A)GA(T/C)CGT(C/G)T(G/A)GG	958-977	1148
PBP 2X (r)	AGAGAGTCTTTCATAGCTGAAGC	2105-2083	
8FPL (f)	AGTTTGATCCTGGCTCAG	1-18	800
806R (r)	GGACTACCAGGGTATCTAAT	800-781	

[a] (f); Primer annealing resulted in DNA extension in the forward (5' - 3') direction.
[b] (r); Primer annealing resulted in DNA extension of the complementary strand, reverse (3' - 5') direction.

ii. Amplification of the Transpeptidase Domain of PBPs 1A, 2B and 2X

DNA was amplified in a total volume of 50 µl containing 10 µl of template, 5 µl of 15 mM $MgCl_2$-10X PCR buffer, 1.25 mM each of dCTP, dGTP, dATP and dTTP, 100 mM of each primer, 2.5U of Taq DNA polymerase (Pharmacia Biotech, Baie d'Urfe, QC) and 25.5 µl of sterile distilled water. The PCR reaction was performed using a Perkin-Elmer GeneAmp® PCR System 9700 under the following conditions: 94°C for 5 minutes, 30 cycles at 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 1 minute and a final extension at 72°C for 7 minutes.

c. Sequencing Reaction

Double-stranded DNA products generated by PCR were sequenced by the Sanger dideoxynucleotide (ddNTP) method of DNA sequencing (114). This technique is based on the use of ddNTP terminators where each C, A, T or G is labeled with blue, green, red or black dye, respectively. The nucleotide sequences of the PBDs of PBPs 1A, 2B and 2X were determined by sequencing with a series of oligonucleotides that were primed at intervals of ± 275 nucleotides along each gene. The sequences of these primers are shown in Table 6. The sequence of the 16S rRNA gene was determined using the universal primers 8FPL and 806R. All primers used in their respective sequencing reactions were diluted 1:100 in sterile distilled water. Sequencing reactions were performed with the ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA) and contained 4 µl of Reaction Mix, 1.6 µl of a single primer, 115 nanograms of purified PCR product and sterile distilled water to a final volume of 10 µl. Cycle sequencing was performed on the Perkin-Elmer GeneAmp® PCR System 9700 and consisted of 25 cycles at 96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes. Sequencing extension products were subsequently purified by ethanol/sodium acetate precipitation.

Table 6. Primers used for sequencing of PBP genes.

Primer	Sequence[a] (5' - 3')	Position
PBP 1A (f)	TGGGATGGATGTTTACACAAATG	1827-1849
PBP 1A – 2	CTGGGG(T/A)TC(T/A)(G/A)CTATGAAACC	2046-2067
PBP 1A – 3	AGTAGTGAAAAAATGGCTGCTG	2380-2400
PBP 1A – 4	GTAGC(T/A)CC(A/T)GATGAA(A/C)T(G/A)TTTG	2677-2699
PBP 2B (f)	GGCTATTCTCTAAATGACCGT	995-1015
PBP 2B – 2	ATTCCTTGGGAACGGTAACC	1347-1366
PBP 2B – 3	ATGGGGCAGACCTATCAACC or	1595-1614
	CTACCAGATGAATCTACTGG	1712-1731
PBP 2B – 4	CGTATTGTTGAAGGCATTTATGG	1862-1884
PBP 2X (f)	TATGAAAA(G/A)GA(T/C)CGT(C/G)T(G/A)GG	958-977
PBP 2X – 2	GACTTTGTTTGGCGTGATAT	1213-1232
PBP 2X – 3	CG(C/T)TTTAAATTTGG(G/)GTTCC	1504-1523
PBP 2X – 4	GGAAATCCTGTTTC(C/T)AAAGA	1750-1769

[a] Oligonucleotide primers permit sequencing in the forward (5' - 3') direction only.

d. Ethanol/Sodium Acetate Precipitation Protocol

For the removal of unincorporated Dye Terminators, 10 µl of each sequencing extension reaction was combined with 1 µl of 3 M sodium acetate (pH 4.6) and 25 µl of 95% ethanol. Following precipitation at room temperature for a minimum of 15 minutes, mixtures were centrifuged for 20 minutes at 13000 rpm and the supernatants were removed. Pellets were then washed with 125 µl of 70% ethanol, centrifuged at 13000 rpm for 5 minutes and the supernatants were discarded. Thereafter, pellets were dried by placing open tubes on a 90°C heating block for 1 minute. Purified sequencing products were then reconstituted in 15 µl of Template Suppression Reagent (PE Applied

Biosystems, Foster City, CA) and either analyzed immediately or stored at -20°C for no longer than one week.

e. Sequence Analysis and Manipulation

Sequence analysis of single-stranded DNA was performed with the ABI PRISM™ 310 Genetic Analyzer and Sequence Analysis Software in accordance with the instructions of the manufacturer.

i. Basic Local Alignment Search Tool (BLAST)

Each 16S rRNA gene sequence obtained from the 310 Sequence Analysis Software was entered into an internet program called BLAST Search and matched with submitted sequences from GenBank, EMBL, DDBJ and PDB databases. This program can be accessed from the NCBI homepage at http://www.ncbi.nlm.nih.gov. Results for each entry are presented as sequence alignment comparisons and most probable organism identification.

ii. Lasergene Sequence Analysis Software

Utilizing Lasergene's (DNAStar Inc., Madison, WI) Seqman II module, individual sequence fragments (four each) of *pbp1a*, *pbp2b* and *pbp2x* were assembled into a contig. The alignment of the PBD sequences of *pbp1a*, *pbp2b* or *pbp2x* from each *S. pneumoniae* isolate and comparison with the published sequence of R6, a penicillin-susceptible reference strain, was performed using Lasergene's Megalign module.

C. RESULTS

To determine the relationship between PBP alterations in *S. pneumoniae* and penicillin susceptibility, and to evaluate the mechanism(s) involved in the spread of penicillin resistance, 15 clinical isolates of *S. pneumoniae* obtained between 1997 and 1999 from across Canada were studied. The antibiotic susceptibility profiles of all isolates were initially determined by broth microdilution. Penicillin MICs were also confirmed by broth macrodilution and E-test procedures. For molecular analysis of the genetic variation among these isolates, DNA fingerprinting was performed by PFGE and AP-PCR. In addition, serotyping was utilized as a phenotypic scheme to assist in this epidemiological investigation. To assess the ability of PCR to rapidly and reliably differentiate between penicillin-resistant, -intermediate and -susceptible genotypes of *S. pneumoniae*, regions of the penicillin-binding domains of *pbp1a*, *pbp2b* and *pbp2x* previously associated with β-lactam resistance were amplified by PCR using primers specific for the unaltered genes of susceptible isolates. Finally, to identify nucleotide and/or amino acid substitutions which may be essential to the development of resistance, nucleotide sequences of the penicillin-binding domains of *pbp1a*, *pbp2b* and *pbp2x* in each isolate were determined with the ABI PRISM™ 310 Genetic Analyzer, an automated capillary electrophoresis system.

PART I. Antibiotic Susceptibility Testing of *S. pneumoniae*

1. Determination of Antibiotic Susceptibility Profiles by Broth Microdilution

Susceptibility testing of the five penicillin-susceptible (MIC; ≤ 0.06 μg/ml), five penicillin-intermediate (MIC; 0.12 – 1 μg/ml) and five penicillin-resistant (MIC; ≥ 2 μg/ml) *S. pneumoniae* isolates was performed by broth microdilution. Extended antibiograms of the studied isolates are presented in Table 7. Penicillin-susceptible *S. pneumoniae* isolates were highly susceptible to all antibiotics tested with the exception of one isolate (12244) which was highly resistant to trimethoprim/sulfamethoxazole (MIC; 8/152 μg/ml). Likewise, two of five penicillin-intermediate (MIC; 0.12 μg/ml) isolates (3996 and 12276) showed uniform susceptibility to all other antibiotics. Conversely, isolates with penicillin MICs ≥ 0.25 μg/ml tended to be more frequently resistant to at least two additional drugs. In the penicillin-resistant group, for example, all isolates were intermediately or fully resistant to trimethoprim/sulfamethoxazole, cefotaxime, cefaclor and cefuroxime. Of these, four were also resistant to tetracycline and erythromycin, with isolates 6190 and 8111 also showing reduced susceptibility to amoxicillin/clavulanic acid or clindamycin and amoxicillin/clavulanic acid, respectively. Of the five penicillin-intermediate isolates, three (11413, 14126 and 3455) were multiply resistant with various combinations of cross-resistance to ciprofloxacin, trimethoprim/sulfamethoxazole, tetracycline, cefaclor, cefuroxime and erythromycin. None of the isolates were resistant to grepafloxacin, levofloxacin, vancomycin or telithromycin.

Isolate	MIC (µg/ml) of [a]:													
	Pen	A/C	Cip	Grx	Lvx	T/S	Cft	Te	Cfr	Cd	Crm	Va	Tel	E
6190	4	4/2	1	0.25	1	4/76	1	>16	>64	≤0.12	>4	0.5	≤0.25	2
8111	4	4/2	2	0.25	1	4/76	2	>16	>64	>4	>4	≤0.25	≤0.25	>8
6363	2	2/1	0.5	0.12	1	4/76	1	>16	>64	≤0.12	>4	≤0.25	≤0.25	2
742	2	2/1	2	0.12	1	4/76	1	16	64	≤0.12	4	≤0.25	≤0.25	1
2848	2	1/0.5	2	0.25	1	4/76	1	0.5	64	≤0.12	4	≤0.25	≤0.25	≤0.25
3455	1	0.5/0.25	>4	0.5	2	4/76	0.5	0.5	32	≤0.12	2	≤0.25	≤0.25	≤0.25
14126	0.5	0.25/0.12	0.5	0.12	1	0.5/9.5	0.25	16	2	≤0.12	2	≤0.25	≤0.25	1
11413	0.25	0.25/0.12	1	0.25	1	4/76	0.12	16	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25
3996	0.12	0.12/0.06	1	0.12	1	≤0.12/2.38	≤0.06	≤0.25	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25
12276	0.12	0.06/0.03	2	0.12	1	≤0.12/2.38	≤0.06	≤0.25	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25
3203	0.06	≤0.03/0.015	1	0.12	1	0.5/9.5	≤0.06	0.5	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25
11184	0.06	≤0.03/0.015	2	0.25	1	≤0.12/2.38	≤0.06	≤0.25	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25
12244	0.06	≤0.03/0.015	1	0.25	1	8/152	≤0.06	≤0.25	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25
14016	0.06	≤0.03/0.015	1	0.06	0.5	0.5/9.5	≤0.06	≤0.25	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25
8099	≤0.03	≤0.03/0.015	0.5	0.12	0.5	0.5/9.5	≤0.06	≤0.25	≤1	≤0.12	≤0.25	≤0.25	≤0.25	≤0.25

[a] Pen, penicillin; A/C, amoxicillin/clavulanic acid; Cip, ciprofloxacin; Grx, grepafloxacin; Lvx, levofloxacin; T/S, trimethoprim/sulfamethoxazole; Cft, cefotaxime; Te, tetracycline; Cfr, cefaclor; Cd, clindamycin; Crm, cefuroxime; Va, vancomycin; Tel, telithromycin; E, erythromycin. Breakpoints (in µg/ml) per NCCLS guidelines, unless otherwise noted, are as follows. Penicillin: susceptible, ≤0.06; intermediate, 0.12 - 1; resistant, ≥2; Amoxicillin/clavulanic acid: susceptible, ≤2/1; intermediate, 4/2; resistant, ≥8/4; Grepafloxacin: susceptible, ≤0.5; intermediate, 1; resistant, ≥2; Levofloxacin: susceptible, ≤2; intermediate, 4; resistant, ≥8; Trimethoprim/sulfamethoxazole: susceptible, ≤0.5/9.5; intermediate 1/19-2/38; resistant, ≥4/76; Cefotaxime: susceptible, ≤0.5; intermediate, 1; resistant, ≥2; Tetracycline: susceptible, ≤2; intermediate, 4; resistant, ≥8; Cefaclor: susceptible, ≤1; intermediate, 2; resistant, ≥4; Clindamycin: susceptible, ≤0.25; intermediate, 0.5; resistant, ≥1; Cefuroxime: susceptible, ≤0.5; intermediate, 1; resistant, ≥2; Vancomycin: susceptible, ≤1; Erythromycin: susceptible, ≤0.25; intermediate 0.5; resistant, ≥1. Ciprofloxacin and telithromycin do not have established breakpoints.

Table 7. Antibiogram of 15 Canadian *S. pneumoniae* isolates.

2. E-test and Broth Macrodilution Confirmation of Penicillin MICs

Prior to susceptibility testing, isolates were screened for potential penicillin resistance by the oxacillin disk diffusion method. In accordance with NCCLS zone diameter interpretive standards, 10 of the 15 isolates were identified as penicillin-nonsusceptible with zone diameters for these isolates ranging in size from six to 15 mm (see Table 8). Following broth microdilution assessment of antibiotic resistance profiles, penicillin MIC data were confirmed by broth macrodilution and E-test procedures. Analysis of MIC data showed 100% agreement within ± one dilution between E-test and the microdilution reference method. A slight trend toward elevated broth macrodilution MICs was noted, with results equivalent to or one doubling dilution greater than microdilution test values for 13 of the 15 *S. pneumoniae* isolates. A four-fold MIC difference between these techniques was observed for two isolates. The penicillin MIC for *S. pneumoniae* ATCC 49619 by all methods was consistently within the proposed quality control range of 0.25 – 1 µg/ml.

Isolate	Microdilution MIC (μg/ml) [a]				Penicillin MIC (μg/ml)		Oxacillin Zone Size (mm) [b]	β-lactam Susceptibility Profile [c]			
	Pen	A/C	Cft	Crm	Macrodilution	E-Test		Pen [d]	A/C	Cft	Crm
6190	4	4/2	1	>4	16	4	6	R	I	I	R
8111	4	4/2	2	>4	8	4	6	R	I	R	R
6363	2	2/1	1	>4	8	2	6	R	S	I	R
742	2	2/1	1	4	8	2	6	R	S	I	R
2848	2	1/0.5	1	4	4	2	6	R	S	I	R
3455	1	0.5/0.25	0.5	2	2	2	6	I	S	S	R
14126	0.5	0.25/0.12	0.25	2	1	0.5	6	I	S	S	R
11413	0.25	0.25/0.12	0.12	≤0.25	0.5	0.25	10	I	S	S	S
3996	0.12	0.12/0.06	≤0.06	≤0.25	0.25	0.12	12	I	S	S	S
12276	0.12	0.06/0.03	≤0.06	≤0.25	0.25	0.12	15	I	S	S	S
3203	0.06	≤0.03/0.015	≤0.06	≤0.25	0.12	0.03	25	S	S	S	S
11184	0.06	≤0.03/0.015	≤0.06	≤0.25	0.06	0.03	27	S	S	S	S
12244	0.06	≤0.03/0.015	≤0.06	≤0.25	0.12	0.06	20	S	S	S	S
14016	0.06	≤0.03/0.015	≤0.06	≤0.25	0.06	0.03	22	S	S	S	S
8099	≤0.03	≤0.03/0.015	≤0.06	≤0.25	0.06	0.03	27	S	S	S	S

[a] Pen, penicillin; A/C, amoxicillin/clavulanic acid; Cft, cefotaxime; Crm, cefuroxime.

[b] Oxacillin sensitive (penicillin susceptible) with zone diameter ≥ 20 millimeters.

[c] R, resistant; I, intermediate; S, susceptible.

[d] Susceptibility as determined by broth microdilution.

Table 8. **β-lactam resistance profiles and comparison of broth microdilution, broth macrodilution, E-test and oxacillin disk diffusion for the determination of penicillin susceptibility.**

PART II. Characterization of Canadian *S. pneumoniae* Isolates by Serotyping, AP-PCR and PFGE

1. Molecular Epidemiology of Penicillin-Nonsusceptible Pneumococci: Analysis by PFGE

a. Penicillin Resistance Level and PFGE Type

PFGE of *Sma*I-restricted chromosomal DNA generated 14 distinct DNA profiles, each with 9-17 well resolved 23-388 kb fragments for comparison between isolates. The results of PFGE for the five isolates of penicillin-susceptible *S. pneumoniae* characterized in this study are shown in Figure 6. Among these five isolates, five unique genotypes were identified. Five additional restriction patterns were likewise seen in the five penicillin-intermediate isolates (Figure 7), demonstrating exclusive heterogeneity amongst these two groups. In contrast, PFGE revealed greater homogeneity amongst the five penicillin-resistant *S. pneumoniae* isolates, as illustrated in Figure 8. While four of these five isolates produced nearly indistinguishable PFGE patterns, one isolate (2848, MIC; 2 µg/ml) appeared noticeably different after restriction by *Sma*I, with a PFGE profile most like that of penicillin-intermediate isolate 3455 (MIC; 1 µg/ml).

Figure 9 shows a dendrogram constructed by computer analysis of the DNA fingerprints. In general, the isolates appeared to segregate into two major groups. The predominant cluster consisted primarily of penicillin-susceptible and penicillin-intermediate isolates with highly variable banding patterns, suggesting that these isolates were most likely genetically unrelated. The second much smaller cluster of isolates, by comparison, was comprised entirely of penicillin-resistant isolates with multidrug-resistant phenotypes. Within this aggregate, isolates 6363 and 742 were found to be

genetically indistinguishable by PFGE. Isolates 6190 and 8111 were also found to be closely related subtypes of the aforementioned group, with a 96.8 and 94.5% coefficient of similarity, respectively. These results indicate that penicillin-resistant *S. pneumoniae* isolates from across Canada may share a common genetic background.

b. Genetic Diversity of Isolates in Relation to Geographical Distribution

In addition to penicillin resistance level, it was also of interest to analyze PFGE patterns in the context of the geographic areas (i.e., provinces) from which the isolates were obtained, the date on which the isolates were received by the coordinating laboratory and the specimen sources from which the isolates were recovered. Although the highest rates of nonsusceptibility to penicillin were found in isolates collected in the western Canadian provinces (Manitoba, Saskatchewan, Alberta and British Columbia), clustering was generally not observed for *S. pneumoniae* isolates originating in the same region. Similarly, no statistically significant relationship was observed in the latter two cases (data not shown).

c. PFGE Typing as an Epidemiological Tool

The discriminatory capacity of PFGE fingerprinting was determined in order to evaluate the suitability of this technique for the epidemiological analysis of *S. pneumoniae* isolates. It was possible to define 14 PFGE types for the 15 isolates, thereby producing an index of discrimination of 0.99.

Figure 6. ***Sma*I pulsed-field gel electrophoresis patterns of penicillin-susceptible *S. pneumoniae* isolates.** Preparation of chromosomal DNA, restriction by *Sma*I endonuclease and PFGE were performed as described in Materials and Methods. Lane L, lambda DNA ladder (molecular sizes [in kilobases] are indicated on the left); lane 1, isolate 8099; lane 2, isolate 14016; lane 3, isolate 12244; lane 4, isolate 11184; lane 5, isolate 3203; lane 6, *S. pneumoniae* ATCC 49619 (included for quality control).

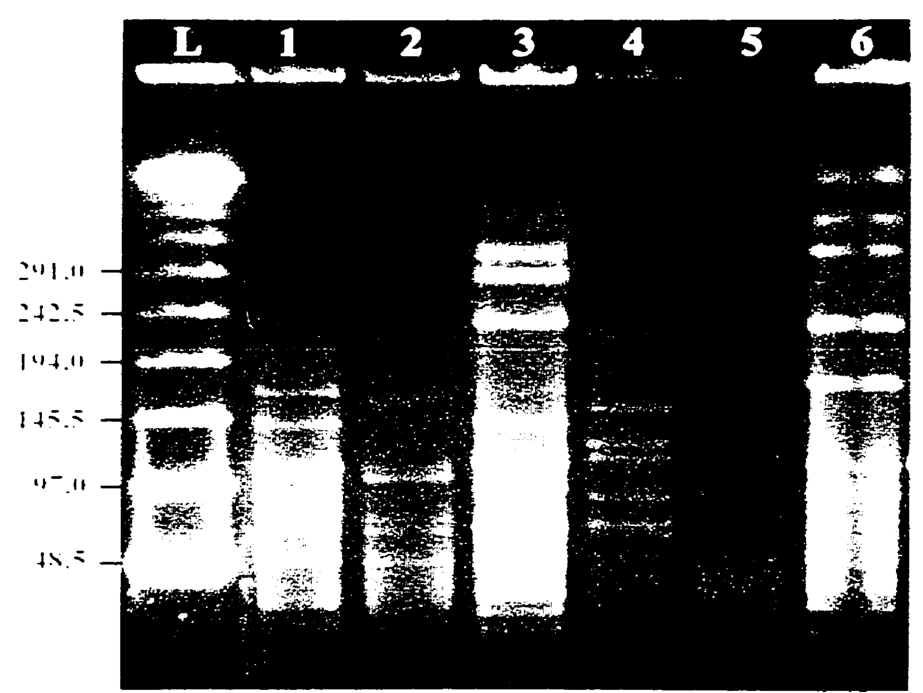


Figure 7. ***Sma*I pulsed-field gel electrophoresis patterns of penicillin-intermediate *S. pneumoniae* isolates.** Preparation of chromosomal DNA, restriction by *Sma*I endonuclease and PFGE were performed as described in Materials and Methods. Lane L, lambda DNA ladder (molecular sizes [in kilobases] are indicated on the left); lane 1, isolate 12276; lane 2, isolate 3996; lane 3, isolate 11413; lane 4, isolate 14126; lane 5, isolate 3455; lane 6, *S. pneumoniae* ATCC 49619 (included for quality control).

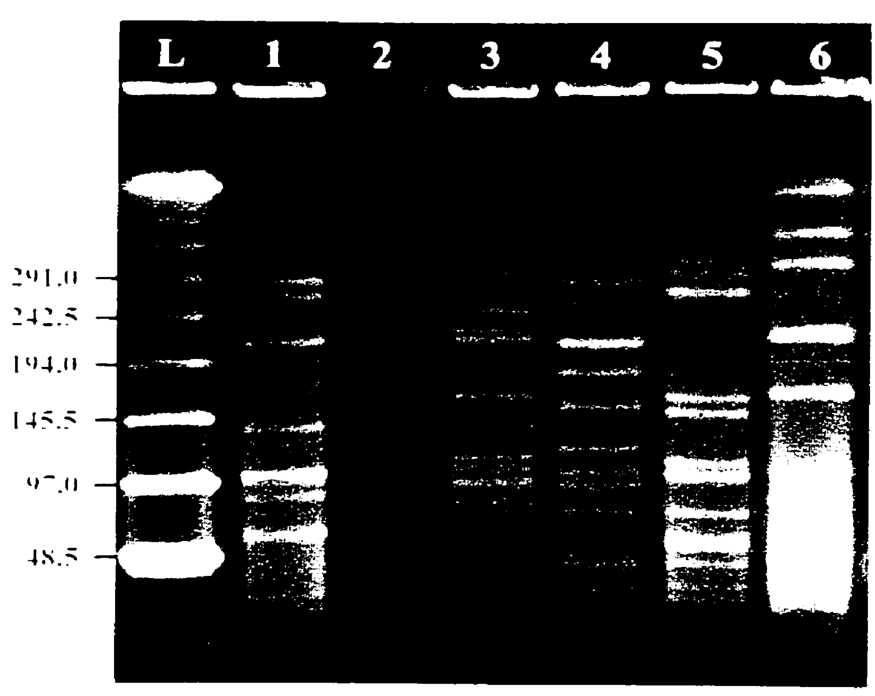


Figure 8. ***Sma*I pulsed-field gel electrophoresis patterns of penicillin-resistant *S. pneumoniae* isolates.** Preparation of chromosomal DNA, restriction by *Sma*I endonuclease and PFGE were performed as described in Materials and Methods. Lane L, lambda DNA ladder (molecular sizes [in kilobases] are indicated on the left); lane 1, isolate 2848; lane 2, isolate 6363; lane 3, isolate 742; lane 4, isolate 6190; lane 5, isolate 8111; lane 6, *S. pneumoniae* ATCC 49619 (included for quality control).

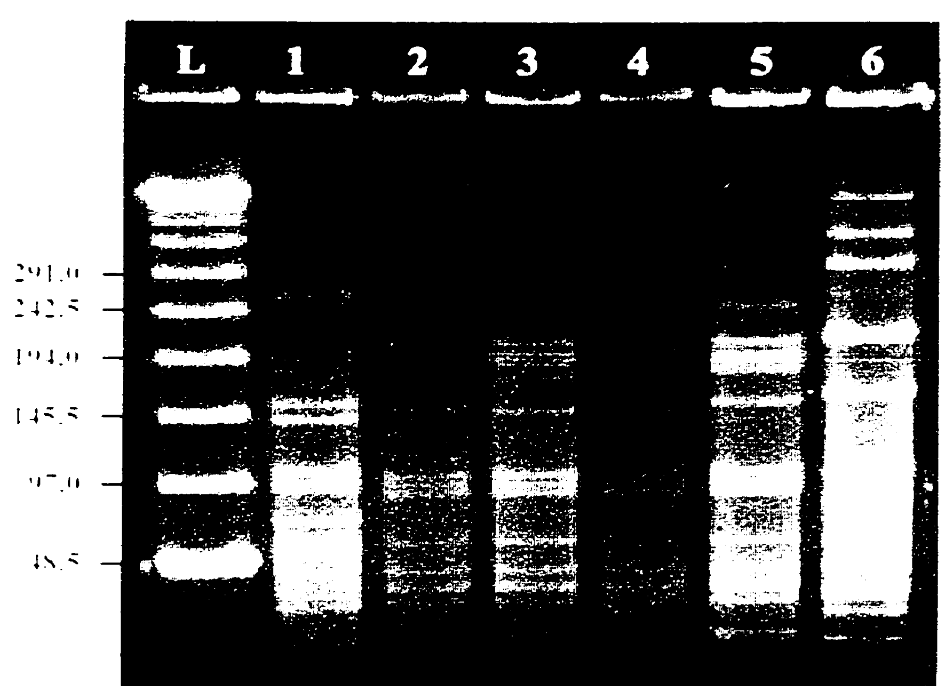
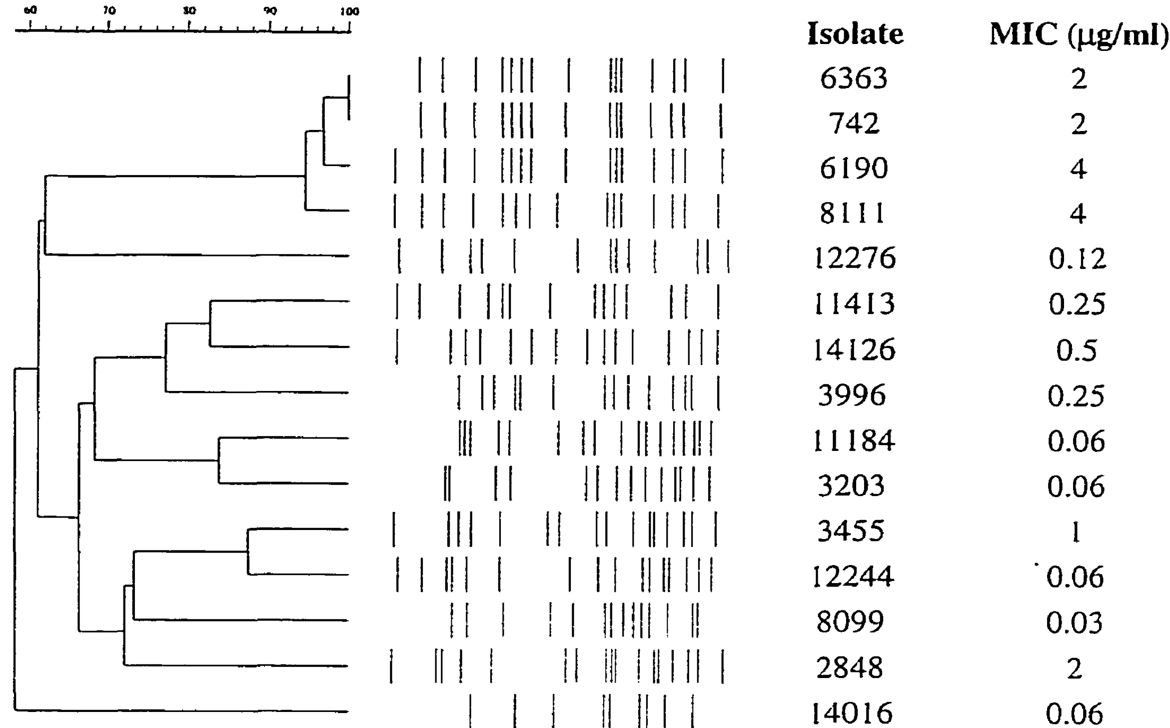


Figure 9. **Dendrogram of *Sma*I digestion electrophoretic patterns of 15 clinical *S. pneumoniae* isolates.** Cluster analysis was performed by the unweighted pair group method with arithmetic averages. Scale indicates the percentage of genetic similarity between isolates as determined on the basis of Dice coefficients.



2. Discrimination of *S. pneumoniae* by Arbitrarily Primed PCR

a. Penicillin Resistance Level and AP-PCR Type

AP-PCR using a single 10-mer generated 13 unique DNA profiles with 8-13 amplified products ranging from 0.25 to 2 kb in size. The stability and reproducibility of the AP-PCR patterns was established by repeated testing of each isolate on multiple occasions; all such tests yielded identical results (data not shown). Amongst our collection of 15 *S. pneumoniae* isolates, penicillin-susceptible pneumococci demonstrated the most heterogeneity. Molecular typing identified five distinct and highly variable banding patterns within these five susceptible isolates (Figure 10). DNA fingerprints arising from AP-PCR of the five penicillin-intermediate *S. pneumoniae* isolates are presented in Figure 11. Interestingly, each isolate expressed a distinguishable AP-PCR pattern, although a subsequent decrease in overall heterogeneity was observed with no apparent correlation between fingerprint type and antibiotic susceptibility. Comparison of five penicillin-resistant isolates, however, revealed a low level of genetic polymorphism. As shown in Figure 12, only three DNA typing profiles were present among these isolates, with two patterns differing by no more than a single band.

Computer-assisted cluster analysis was invaluable for discerning subtle variations between isolates and made comparisons easier than visual analysis alone. The genetic relationship among all AP-PCR profiles of *S. pneumoniae* is represented in the dendrogram shown in Figure 13. Most notably, four of the five penicillin-resistant isolates grouped into one of two closely related fingerprint subtypes. Within these clusters, isolates 6363 and 742 (MIC; 2 μg/ml) were found to be genetically indistinguishable by AP-PCR, as were isolates 6190 and 8111 (MIC; 4 μg/ml). With a

coefficient of similarity between these groups calculated at 95.7%, these results suggest a close genetic relatedness among Canadian isolates of penicillin-resistant *S. pneumoniae*. As with PFGE, one penicillin-resistant isolate (2848, MIC; 2 μg/ml) was found to be most closely related genetically to penicillin-intermediate isolate 3455 (MIC; 1 μg/ml). Among the remaining penicillin-intermediate and -susceptible *S. pneumoniae* isolates, cluster analysis revealed overall genetic diversity, with the percent similarity between isolates oscillating from 68 to 85.7%.

b. Genetic Diversity of Isolates in Relation to Geographical Distribution

In addition to penicillin susceptibility patterns, AP-PCR profiles were also evaluated for associations of locality (i.e., provinces from which the isolates were obtained), isolation date and specimen source. No ancestral delineation could be made on the basis of isolate origin.

c. AP-PCR Typing as an Epidemiological Tool

The discriminatory capacity of AP-PCR typing was determined in order to evaluate the suitability of this technique for the epidemiological analysis of *S. pneumoniae* isolates. It was possible to define 13 AP-PCR types for the 15 isolates, resulting in a 0.98 index of discrimination. Because PFGE was able to more extensively subtype isolates (in that identical AP-PCR profiles might have PFGE patterns that differed by one or two bands), AP-PCR was found to be slightly less discriminatory than PFGE.

Figure 10. **AP-PCR profiles of penicillin-susceptible *S. pneumoniae* isolates.** Preparation of chromosomal DNA and PCR amplification using a single oligonucleotide primer were performed as described in Materials and Methods. Lane L, 123-bp ladder; lane 1, isolate 8099; lane 2, isolate 14016; lane 3, isolate 12244; lane 4, isolate 11184; lane 5, isolate 3203; lane 6, *S. pneumoniae* ATCC 49619 (included for quality control); lane 7, H_2O contamination cotrol.

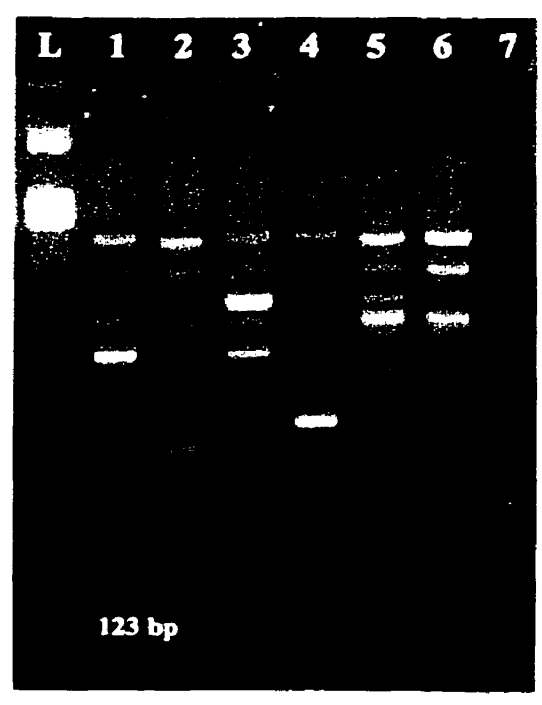


Figure 11. AP-PCR profiles of penicillin-intermediate *S. pneumoniae* isolates. Preparation of chromosomal DNA and PCR amplification using a single oligonucleotide primer were performed as described in Materials and Methods. Lane L, 123-bp ladder; lane 1, isolate 12276; lane 2, isolate 3996; lane 3, isolate 11413; lane 4, isolate 14126; lane 5, isolate 3455; lane 6, *S. pneumoniae* ATCC 49619 (included for quality control); lane 7, H_2O contamination control.

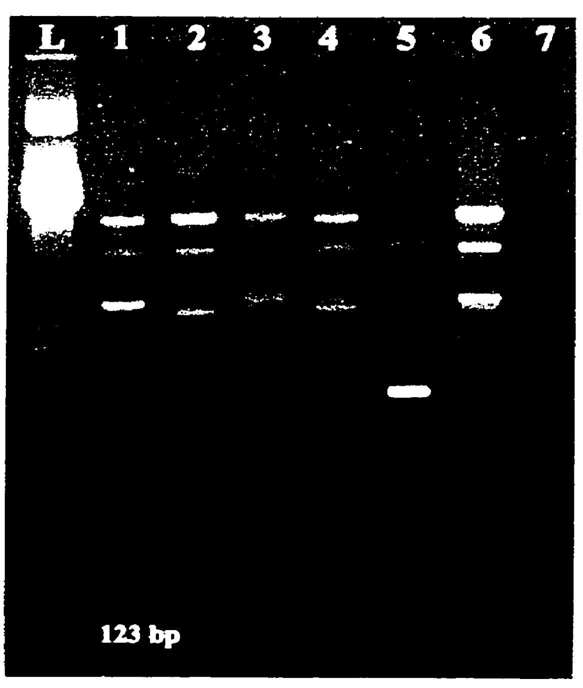


Figure 12. **AP-PCR profiles of penicillin-resistant *S. pneumoniae* isolates.** Preparation of chromosomal DNA and PCR amplification using a single oligonucleotide primer were performed as described in Materials and Methods. Lane L, 123-bp ladder; lane 1, isolate 2848; lane 2, isolate 6363; lane 3, isolate 742; lane 4, isolate 6190; lane 5, isolate 8111; lane 6, *S. pneumoniae* ATCC 49619 (included for quality control); lane 7, H_2O contamination control.

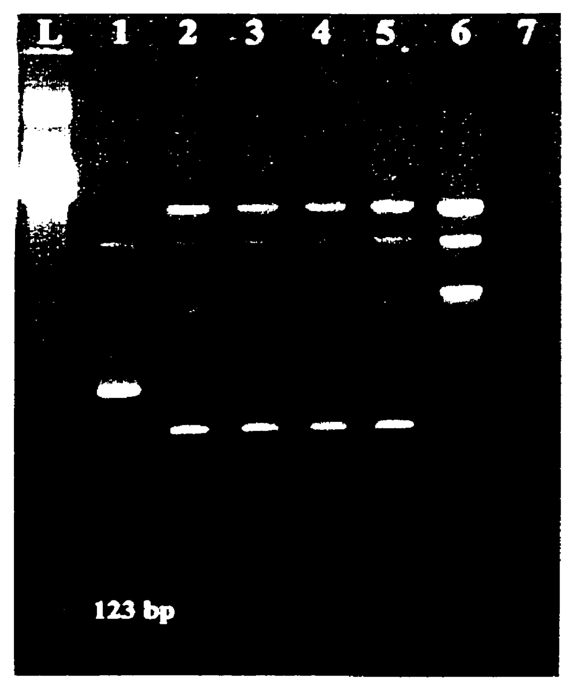


Figure 13. Dendrogram of AP-PCR profiles of 15 clinical *S. pneumoniae* isolates. Cluster analysis was performed by the unweighted pair group method with arithmetic averages. Scale indicates the percentage of genetic similarity between isolates as determined on the basis of Dice coefficients.

Isolate	MIC (μg/ml)
3996	0.25
8099	0.03
2848	2
3455	1
11413	0.25
12276	0.12
14126	0.5
14016	0.06
6190	4
8111	4
6363	2
742	2
12244	0.06
3203	0.06
11184	0.06

3. Serology

a. Genetic Relatedness Within and Between Serotypes

The relationship between serotype, PFGE pattern, AP-PCR profile and penicillin-susceptibility is observed in Table 9. Among the 15 *S. pneumoniae* isolates examined, a total of eight capsular types were identified. Serotypes (and the number of isolates belonging to each polysaccharide type) included: 19(4), 23F(3), 19A(2), 9V(2), 31(1), 14(1), 11A(1) and 6A(1). Molecular typing showed that isolates from most serotypes were genetically heterogeneous. For example, all three isolates of serotype 23F had unique fingerprint patterns and were subsequently scattered throughout the dendrogram(s). Of the four serotype 19F isolates, two (6363 and 742) were found to be genetically identical by both PFGE and AP-PCR. Conversely, the remaining two DNA profiles generated for penicillin-intermediate 19F isolates (14126 and 12276) were unrelated. For serotypes 19A and 9V, isolates within the same serotype did not appear to be more closely related to each other than to isolates of different serotypes. Interestingly, four penicillin-resistant isolates with near homogeneous typing profiles serotyped 19F(2), 23F(1) and 14(1). These results suggest that, within a given serotype, there may be both conservation and dispersion of genotypes. Consequently, associations of genotypic relatedness, serotype and penicillin MIC cannot be ascertained in Canadian *S. pneumoniae* isolates.

b. Serotyping as an Epidemiological Tool

The discriminatory capacity of serotyping was determined in order to evaluate the suitability of this technique for the epidemiological analysis of *S. pneumoniae* isolates.

With only eight different capsular serotypes expressed by the 15 *S. pneumoniae* isolates, the discriminatory index for serotyping was calculated to be 0.90.

Table 9. Typing characteristics of 15 *S. pneumoniae* isolates recovered in Canada.

Isolate	Penicillin MIC (μg/ml)	Serotype	PFGE Pattern[a,b]	AP-PCR Profile[a,b]
6190	4	14	A_2	A_2
8111	4	23F	A_3	a_2
6363	2	19F	A_1	a_1
742	2	19F	A_1	a_1
2848	2	9V	K	f_2
3455	1	9V	H	f_1
14126	0.5	19F	D	C
11413	0.25	19A	C	E
3996	0.12	19A	E	H
12276	0.12	19F	B	D
3203	0.06	11A	G	J
11184	0.06	31	F	K
12244	0.06	6A	I	I
14016	0.06	23F	L	B
8099	≤0.03	23F	J	G

[a] See Materials and Methods for explanation of type designation.
[b] Arbitrary assignment in order of decreasing genetic similarity. The most frequent DNA fingerprint was repored as type "A/a".

PART III. Molecular Diagnosis of Penicillin Resistance in *S. pneumoniae*

1. Identification of PBP Gene Mutations by PCR

a. Amplified DNA Profiles of Penicillin-Susceptible, -Intermediate and -Resistant Isolates

A multiplex PCR strategy was evaluated for its ability to determine the penicillin susceptibility of 15 clinical isolates of *S. pneumoniae*. To this end, regions of the penicillin-binding domains of *pbp1a*, *pbp2b* and *pbp2x* previously associated with β-lactam resistance were amplified using primers speciic for the unaltered genes of susceptible isolates. The 430, 77 and 292 bp fragments detected by this assay corresponded to products of the *pbp1a*, *pbp2b* and *pbp2x* genes, respectively. An additional primer pair derived from the pneumococcal autolysin (*lytA*) gene was also incorporated to permit positive species identification (through amplification of a 273 bp fragment) of *S. pneumoniae*. Simultaneous amplification of *pbp1a*, *pbp2b* and *pbp2x* gene fragments was indicative of homology between these PBP sequences and those of a penicillin-susceptible reference strain, R6. In contrast, the inability to detect DNA bands suggested that such isolates possessed gene sequences unlike those of the susceptible reference strain.

Figure 14 shows the PCR-amplified DNA profiles obtained from five isolates of penicillin-susceptible *S. pneumoniae*. Of the five isolates for which the penicillin MIC was ≤ 0.06 μg/ml, four were confirmed to be true susceptible isolates with no PBP gene mutations. One isolate (12244, MIC; 0.06 μg/ml), on the other hand, was found to possess a mutation in *pbp2x*. In contrast, each of the five penicillin-resistant (MIC; ≥ 2 μg/ml) isolates was shown to harbor alterations in all three PBP genes (Figure 15). As

shown in Figure 16, five penicillin-intermediate isolates with MICs between 0.12 and 1 μg/ml contained various combinations of PBP gene alterations. PBP profiles detected by PCR included alterations in each of the three genes (2 isolates, MICs; 0.5, 1 μg/ml), mutation of *pbp1a* and *pbp2x* (1 isolate, MIC; 0.25 μg/ml) and alteration of *pbp2b* and *pbp2x* (2 isolates, MICs; 0.12, 0.25 μg/ml).

The correlation between PCR results and the MIC of penicillin for our 15 clinical isolates of *S. pneumoniae* is presented in Table 10. As expected, PCR-amplified profiles readily differentiated between isolates of penicillin-susceptible and -resistant *S. pneumoniae*. Results also indicated that when amplification products were observed with only one of the three primer sets, isolates could be correctly classified as penicillin-intermediate with penicillin MICs of 0.12 to 0.25 μg/ml. On the other hand, using the PCR assay described herein, we were unable to differentiate between moderately (MICs of 0.5 to 1 μg/ml) and highly (MICs $\geq$ 2 μg/ml) resistant isolates of *S. pneumoniae*. Whether the design of *pbp1a* and/or *pbp2b* primers with increased specificity for resistant genes would allow for more accurate detection of intermediately resistant isolates remains to be determined.

Figure 14. **PCR detection of PBP gene mutations in penicillin-susceptible *S. pneumoniae* isolates.** PCR amplification of DNA fragments of the *lytA*, *pbp1a*, *pbp2b* and *pbp2x* genes was performed as described in Materials and Methods. PBP genes with sequences identical to those of a penicillin-susceptible R6 reference strain were amplified. Column A, 430-bp product of *pbp1a* and 273-bp product of *lytA*; column B, 292-bp product of *pbp2x* and 77-bp product of *pbp2b*. Lane L, 123-bp ladder; lane 1, isolate 8099; lane 2, isolate 14016; lane 3, isolate 11184; lane 4, isolate 12244; lane 5, isolate 3203; lane 6, *S. pneumoniae* ATCC 49619 (positive control); lane 7, H_2O contamination control.

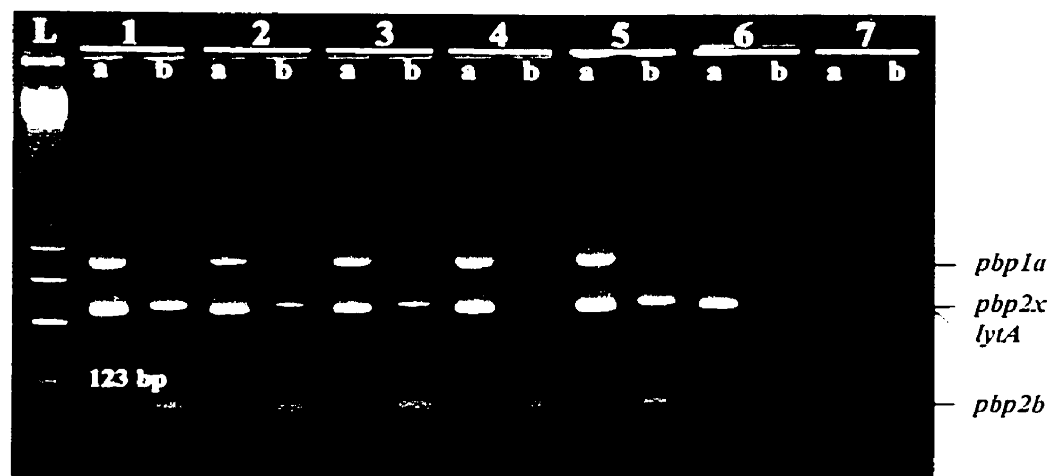


Figure 15. PCR detection of PBP gene mutations in penicillin-intermediate *S. pneumoniae* isolates. PCR amplification of DNA fragments of the *lytA*, *pbp1a*, *pbp2b* and *pbp2x* genes was performed as described in Materials and Methods. PBP genes with sequences identical to those of a penicillin-susceptible R6 reference strain were amplified. Column A, 430-bp product of *pbp1a* and 273-bp product of *lytA*; column B, 292-bp product of *pbp2x* and 77-bp product of *pbp2b*. Lane L, 123-bp ladder; lane 1, isolate 12276; lane 2, isolate 3996; lane 3, isolate 11413; lane 4, isolate 14126; lane 5, isolate 3455; lane 6, *S. pneumoniae* ATCC 49619 (positive control); lane 7, H_2O contamination control.

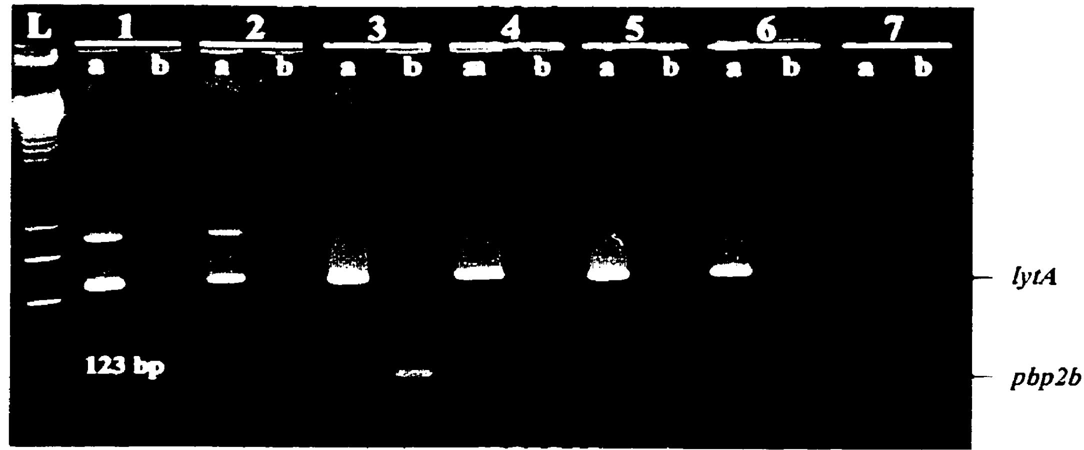


Figure 16. PCR detection of PBP gene mutations in penicillin-resistant *S. pneumoniae* isolates. PCR amplification of DNA fragments of the *lytA*, *pbp1a*, *pbp2b* and *pbp2x* genes was performed as described in Materials and Methods. PBP genes with sequences identical to those of a penicillin-susceptible R6 reference strain were amplified. Column A, 430-bp product of *pbp1a* and 273-bp product of *lytA*; column B, 292-bp product of *pbp2x* and 77-bp product of *pbp2b*. Lane L, 123-bp ladder; lane 1, isolate 2848; lane 2, isolate 6363; lane 3, isolate 742; lane 4, isolate 6190; lane 5, isolate 8111; lane 6, *S. pneumoniae* ATCC 49619 (positive control); lane 7, H_2O contamination control.

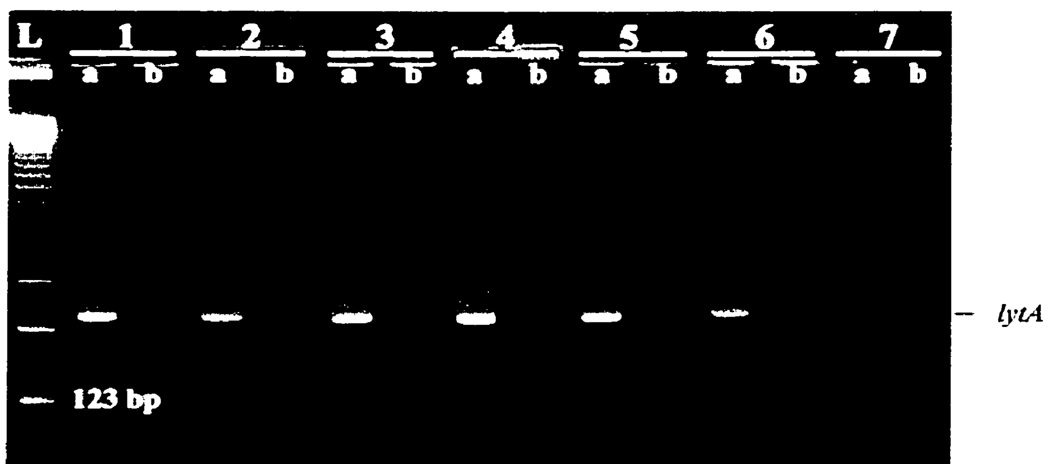


Table 10. Correlation between *S. pneumoniae* penicillin MICs and PBP gene alterations.

Isolate	Penicillin MIC (µg/ml)	PCR Results[a]		
		pbp1a	*pbp2b*	*Pbp2x*
6190	4	+	+	+
8111	4	+	+	+
742	2	+	+	+
2848	2	+	+	+
6363	2	+	+	+
3455	1	+	+	+
14126	0.5	+	+	+
3996	0.25	-	+	+
11413	0.25	+	-	+
12276	0.12	-	+	+
3203	0.06	-	-	-
11184	0.06	-	-	-
12244	0.06	-	-	+
14016	0.06	-	-	-
8099	≤0.03	-	-	-

[a] +, altered PBP gene sequence (PCR product not observed); –, unaltered PBP gene sequence (PCR product observed).

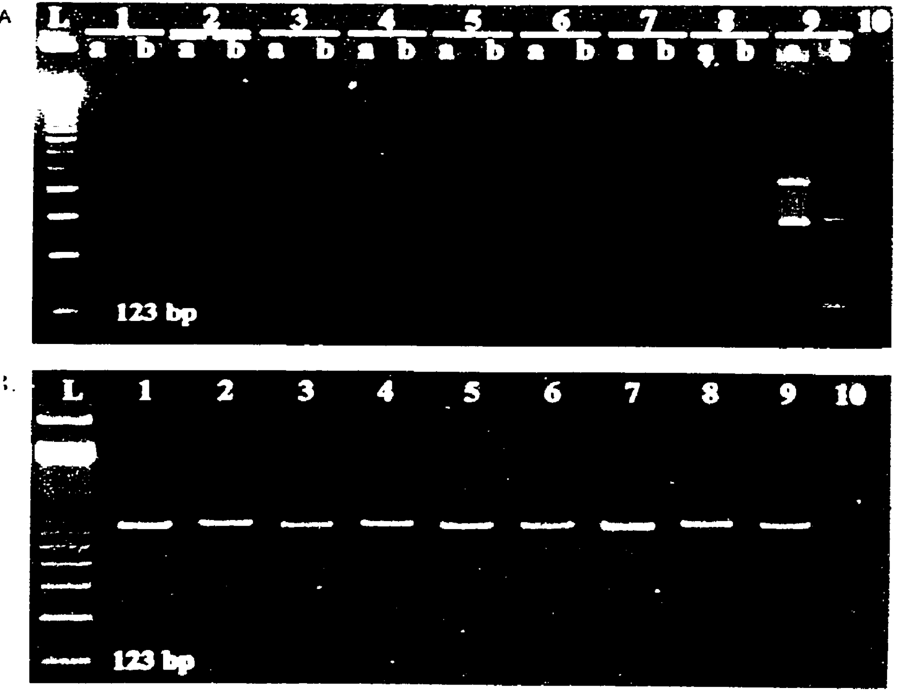
b. Specificity and Rapidity of the Method

To determine the specificities of the *S. pneumoniae* autolysin and PBP gene primers, the reactivities of DNA from eight nonpneumococcal organisms were tested in the PCR assay. No PCR amplification products were observed for any of the streptococcal isolates (Figure 17A). Negative reactions also occurred for *E. coli*, *E. faecalis* and *S. aureus*. A penicillin-susceptible isolate of *S. pneumoniae*, included as a positive control, produced the expected results.

To demonstrate that the absence of amplification products was indeed due to the absence of pneumococcus-specific genes rather than to an inadequate genomic DNA supply, the 8FPL/806R primer pair, which has broad specificity for the conserved 16S rDNA sequences that are present in bacteria, was used as an amplification control. An 800-bp 16S rDNA amplification product was detected in all organisms tested (Figure 17B).

A real time approach was used to determine the time required for susceptibility testing of *S. pneumoniae* by PCR. A typical experiment contained amplification reactions (two each) for five clinical isolates, one negative and one positive control. On average, it took 1 – 1.5 hours to extract the DNA and prepare amplification reactions, and 1.25 hours each for PCR amplification and agarose gel electrophoresis. Therefore, the penicillin-susceptible, -intermediate or -resistant genotypes of five primary culture isolates of *S. pneumoniae* could be identified within four hours. This compares with 24 hours by conventional methodology.

Figure 17. **Specificity of PCR for the detection of PBP gene mutations.** PCR amplification of *pbp1a* and *lytA* genes (column A) or *pbp2x* and *pbp2b* genes (column B) **(A)** and amplification of the 16S rRNA gene **(B)** was performed as described in Materials and Methods. Lane L, 123-bp ladder; lane 1, *Escherichia coli*; lane 2, *Enterococcus faecalis*; lane 3, *Staphylococcus aureus*; lane 4, *Streptococcus milleri*; lane 5, *Streptococcus mitis*; lane 6, *Streptococcus mutans*; lane 7, *Streptococcus oralis*; lane 8, *Streptococcus sanguis*; lane 9, penicillin-susceptible *Streptococcus pneumoniae* (positive control); lane 10, H_2O contamination control.



2. Influence of PBP Gene Mutations on Penicillin MIC

Multiple regression analysis was performed to determine if, and to what degree, the presence of PBP gene mutations influenced the MIC of penicillin. The following formula was obtained by multivariate analysis:

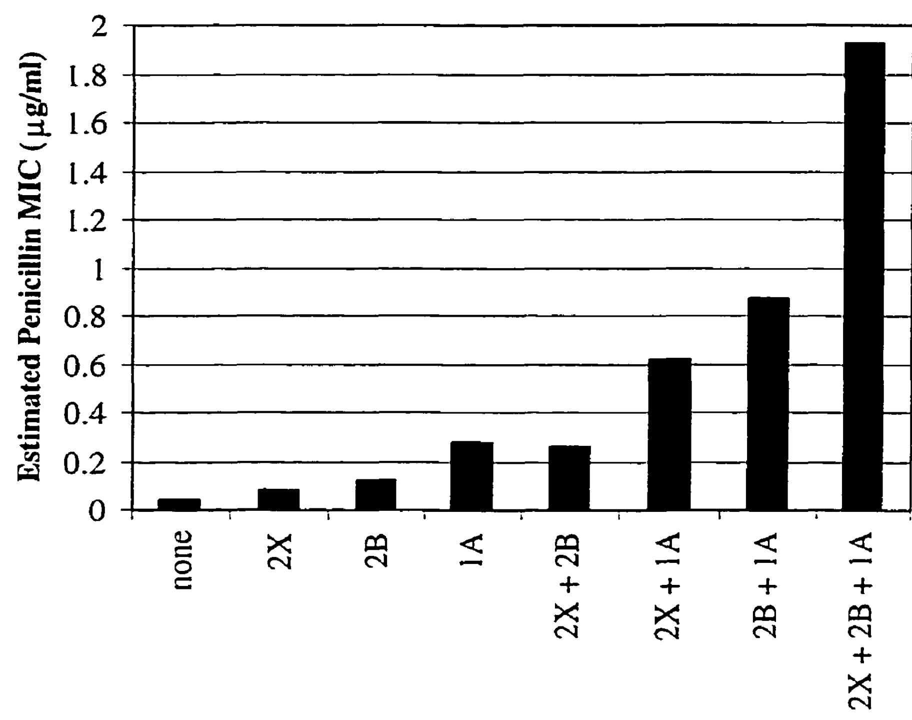
$$\text{MIC}(\log_{10}) = (\text{mutation of } pbp1a \times 0.862147) + (\text{mutation of } pbp2b \times 0.493525) + (\text{mutation of } pbp2x \times 0.34233) - 1.41298$$

Table 11 shows the estimated MIC values for penicillin that were calculated by entering the appropriate explanatory variables ('one' for the presence of mutation or 'zero' for the absence of mutation) into the above formula. Penicillin MIC values could be predicted with a high probability when PBP gene mutations were demonstrated definitely by PCR. Figure 18 provides a graphical representation of the relationship between *pbp1a*, *pbp2b* and *pbp2x* gene mutations and penicillin susceptibility. It was observed that the MIC of penicillin was affected more strongly by the mutation of *pbp1a* and *pbp2b* than by that of *pbp2x*. Furthermore, alteration of *pbp2x* and *pbp2b*, either alone or in combination, was found to contribute to low-level resistance. Thereafter, additional alteration of *pbp1a* clearly played a vital role in the development of full penicillin resistance.

Table 11. Estimated penicillin MIC values calculated from multiple regression formula.

Pattern of PBP Gene Mutation	Estimated Penicillin MIC (μg/ml)
No mutations	0.038638
pbp2x	0.084987
pbp2b	0.120377
pbp1a	0.281298
pbp2x + pbp2b	0.264774
pbp2x + pbp1a	0.618724
pbp2b + pbp1a	0.876379
pbp2x + pbp2b + pbp1a	1.927623

Figure 18. Influence of *pbp1a*, *pbp2b* and *pbp2x* gene mutations on penicillin susceptibility. Estimated penicillin MIC values were determined by multiple regression analysis as described in Materials and Methods.



PART IV. DNA Sequencing of *S. pneumoniae* PBP Genes

1. Analysis of *pbp2x*

The nucleotide sequences of a 999-bp region (from bp 1081 to 2079) encoding PBP 2X transpeptidase activity in 15 clinical isolates of *S. pneumoniae* were determined by direct sequencing. Figure 19 is a representation of a typical electropherogram generated by the ABI PRISM™ Sequence Analysis Software. The nucleotide and deduced amino acid sequences of the 15 isolates were subsequently aligned, along with the previously determined sequence of a susceptible strain (R6) (Appendix A). Three of five penicillin-susceptible isolates (8099, 3203 and 11184) differed from *S. pneumoniae* R6 by only three, one and one nucleotides, respectively, resulting in a single amino acid substitution (Table 12). PBP 2X of susceptible isolate 14016 also showed a low degree of sequence variation, with 99.7% nucleotide sequence homology and 100% amino acid sequence homology to the R6 reference strain. In contrast, susceptible isolate 12244 carried 57 (5.7%) nucleotide alterations, nine of which were nonsynonymous substitutions, and possessed a threonine-338 to alanine alteration within the serine-threonine-methionine-lyisne (STMK) motif (Table 13). By comparison, the amino acid sequences of PBP 2X in isolates for which the penicillin MICs were ≥ 0.12 µg/ml exhibited a variety of substitutions. On the basis of substitution patterns within or adjacent to the three conserved amino acid motifs (STMK, serine-serine-asparagine [SSN] and lysine-serine-glycine [KSG]), isolates 12276 (MIC; 0.12 µg/ml) and 3996 (MIC; 0.25 µg/ml) were found to be identical to penicillin-susceptible isolate 12244. In penicillin-intermediate isolate 11413 (MIC; 0.25 µg/ml), substitution of threonine-338 was not detected. A histidine-394 to leucine alteration just before the SSN motif was

observed instead. The homology of amino acid sequences between this isolate and the R6 strain was 96.4%. The remaining seven *S. pneumoniae* isolates requiring penicillin concentrations ≥ 0.5 μg/ml had identical *pbp2x* genes and revealed extensive sequence divergence from the R6 reference strain, differing by 177 (17.7%) nucleotide substitutions which resulted in 38 (11.4%) alterations within the 333 amino acid protein sequence. These isolates had altered *pbp1a*, *pbp2b* and *pbp2x* genes and two key amino acid substitutions, alanine for threonine-338 and valine for leucine-546, within the *pbp2x* gene product. Interestingly, the majority of amino acid changes within the PBP 2X transpeptidase domain of these isolates were found to lie between the STMK and SSN motifs and/or within the locality of the C-terminal KSG motif.

Figure 19. Sample electropherogram of sequencing data as generated by the ABI PRISM™ 310 Sequence Analysis Software. PCR amplification of the transpeptidase-encoding region of *S. pneumoniae* PBP genes and subsequent sequencing reactions were performed as described in Materials and Methods. A portion of the *pbp1a* gene sequence of penicillin-resistant *S. pneumoniae* isolate 2848 is shown below.

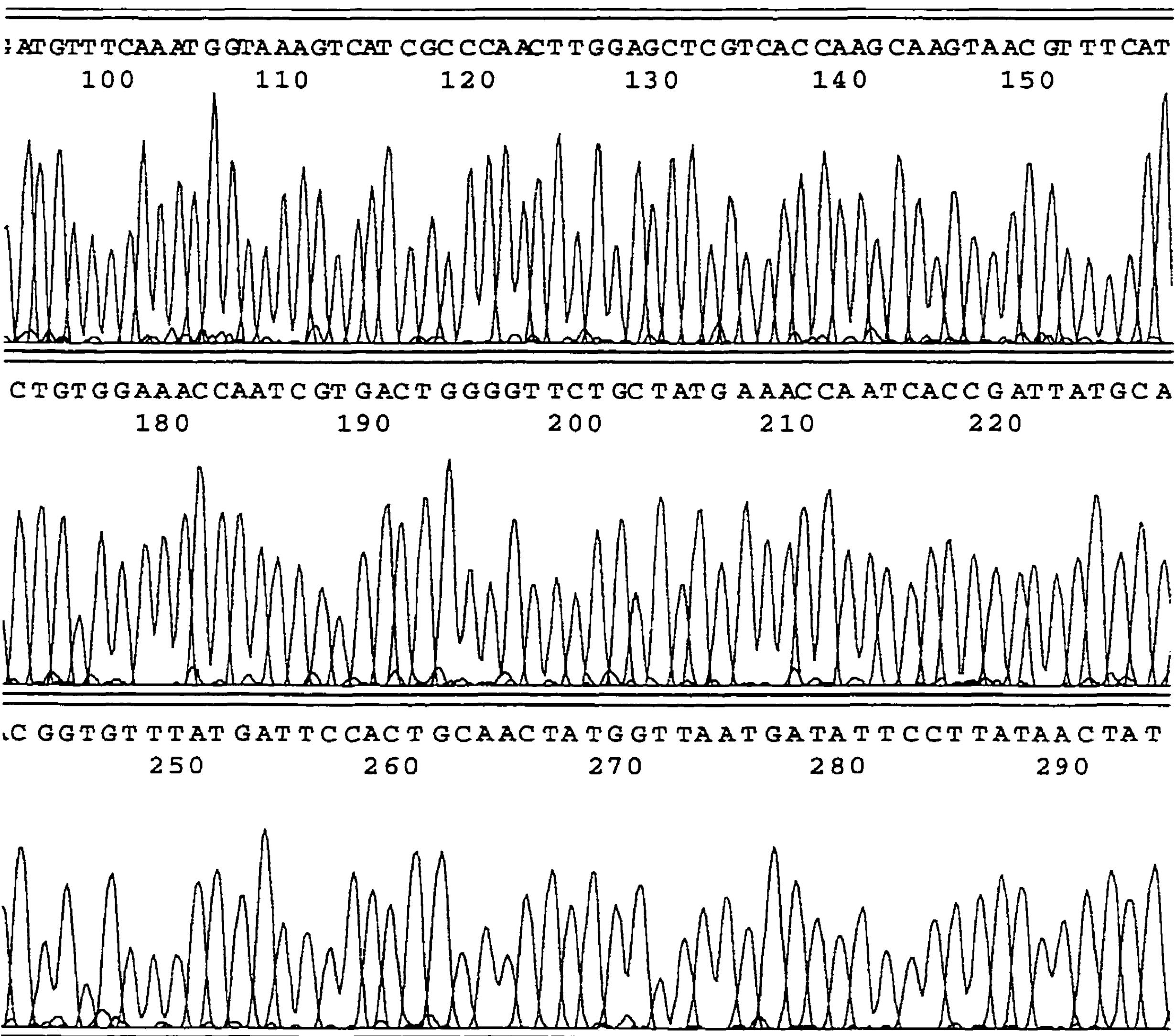


Table 12. **Divergence of *pbp2x* gene sequences in clinical isolates of *S. pneumoniae*.**

Isolate	Penicillin MIC (µg/ml)	Nucleotides Altered[a] (%)	Amino Acids Altered[a] (%)
6190	4	177 (17.7)	38 (11.4)
8111	4	177 (17.7)	38 (11.4)
6363	2	177 (17.7)	38 (11.4)
742	2	177 (17.7)	38 (11.4)
2848	2	177 (17.7)	38 (11.4)
3455	1	177 (17.7)	38 (11.4)
14126	0.5	177 (17.7)	38 (11.4)
11413	0.25	67 (6.7)	12 (3.6)
3996	0.12	57 (5.7)	9 (2.7)
12276	0.12	57 (5.7)	9 (2.7)
3203	0.06	1 (0.1)	1 (0.1)
11184	0.06	1 (0.1)	1 (0.1)
12244	0.06	57 (5.7)	9 (2.7)
14016	0.06	3 (0.3)	0
8099	≤0.03	3 (0.3)	1 (0.3)

[a] Published sequence of the penicillin-susceptible R6 reference strain was used for comparison.

Table 13. Distribution of amino acid substitutions in the penicillin-binding domain of PBP 2X from clinical isolates of *S. pneumoniae*.

Isolate	Penicillin MIC (μg/ml)	Amino Acid Motif [a]			PCR Results[b]		
		STMK	AHSSNV	LKSGT	*pbp1a*	*pbp2b*	*Pbp2x*
6190	4	–A – –	– – – – – –	V– – – –	+	+	+
8111	4	–A – –	– – – – – –	V– – – –	+	+	+
6363	2	–A – –	– – – – – –	V– – – –	+	+	+
742	2	–A – –	– – – – – –	V– – – –	+	+	+
2848	2	–A – –	– – – – – –	V– – – –	+	+	+
3455	1	–A – –	– – – – – –	V– – – –	+	+	+
14126	0.5	–A – –	– – – – – –	V– – – –	+	+	+
11413	0.25	– – – –	– L – – – –	– – – – –	-	+	+
3996	0.12	–A – –	– – – – – –	– – – – –	+	-	+
12276	0.12	–A – –	– – – – – –	– – – – –	-	+	+
3203	0.06	– – – –	– – – – – –	– – – – –	-	-	-
11184	0.06	– – – –	– – – – – –	– – – – –	-	-	-
12244	0.06	–A – –	– – – – – –	– – – – –	-	-	+
14016	0.06	– – – –	– – – – – –	– – – – –	-	-	-
8099	≤0.03	– – – –	– – – – – –	– – – – –	-	-	-

[a] Only amino acid residues differing from PBP 2X conserved motif sequences of the penicillin-susceptible R6 reference strain are shown. Conserved amino acid motifs are underlined.

[b] +, altered PBP gene sequence (PCR product not observed); –, unaltered PBP gene sequence (PCR product observed).

2. Analysis of *pbp2b*

The number of mutations in the PBP 2B genes from 15 clinical *S. pneumoniae* isolates was determined after direct sequencing of PCR-amplified chromosomal DNA. The previously determined sequence of the transpeptidase-encoding region from penicillin-susceptible reference strain R6 was used as the basis for comparison with these isolates (see Appendix B for complete sequence alignments). Penicillin-susceptible isolates (inhibited by penicillin concentrations ≤ 0.06 μg/ml) carried zero to three nucleotide substitutions within the 1056-bp PBP 2B penicillin-binding domain (Table 14). No alterations to the corresponding amino acid sequence of these proteins were observed. In contrast, a variety of nonsynonymous substitutions were identified in all ten penicillin-intermediate and -resistant isolates. The majority of these nucleotide and associated amino acid alterations occurred within a ± 250-bp area between asparagine-404 and threonine-488 and were located within the vicinity of two of the three conserved amino acid motifs, namely the serine-valine-valine-lysine (SVVK) tetrad housing the active-site serine residue and the serine-serine-asparagine (SSN) triad. Four substitutions appeared to be common to all isolates exhibiting an MIC of at least 0.12 μg/ml. These included the replacement of glutamic acid-332 by glycine, substitution of threonine-445 with alanine, alteration of glutamic acid-475 to glycine and replacement of threonine-488 by serine or alanine. In terms of amino acid substitution profiles, penicillin-intermediate isolates 12276 (MIC; 0.12 μg/ml) and 3996 (MIC; 0.25 μg/ml) were identical to one another, differing from strain R6 by 37 (3.5%) nucleotide alterations and 7 (2%) amino acid substitutions. The most prominent amino acid alterations in PBP 2B involved the substitution of six consecutive residues between glutamine-426 and phenylalanine-431, a

feature unique to isolate 11413 (MIC; 0.25 µg/ml). Interestingly, isolate 14126 (MIC; 0.5 µg/ml) contained the greatest number of overall changes, with 7.4 and 4.0% divergence in nucleotide and amino acid sequence, respectively. Nucleotide sequence analysis of the *pbp2b* gene revealed highly similar patterns of nucleotide and amino acid sequence variation amongst all resistant isolates (MICs; ≥ 2 µg/ml), including penicillin-intermediate isolate 3455 (MIC; 1 µg/ml). As seen in Table 15, such isolates showed simultaneous alterations in *pbp1a*, *pbp2b* and *pbp2x* together with substitution of alanine for threonine-445 immediately following the SSN motif. A similar substitution pattern within or adjacent to the three conserved amino acid motifs was likewise seen with a penicillin MIC of 0.5 µg/ml. By comparison, penicillin MICs were 0.12 to 0.25 µg/ml when this same threonine-445 to alanine substitution was detected but only two of three PBP genes were altered.

Table 14. **Divergence of *pbp2b* gene sequences in clinical isolates of *S. pneumoniae*.**

Isolate	Penicillin MIC (µg/ml)	Nucleotides Altered[a] (%)	Amino Acids Altered[a] (%)
6190	4	73 (6.9)	13 (3.7)
8111	4	73 (6.9)	13 (3.7)
6363	2	74 (7.0)	13 (3.7)
742	2	73 (6.9)	13 (3.7)
2848	2	73 (6.9)	13 (3.7)
3455	1	73 (6.9)	13 (3.7)
14126	0.5	78 (7.4)	14 (4.0)
11413	0.25	54 (5.1)	13 (3.7)
3996	0.12	37 (3.5)	7 (2.0)
12276	0.12	37 (3.5)	7 (2.0)
3203	0.06	3 (0.3)	0
11184	0.06	0	0
12244	0.06	2 (0.2)	0
14016	0.06	3 (0.3)	0
8099	≤0.03	1 (0.1)	0

[a] Published sequence of the penicillin-susceptible R6 reference strain was used for comparison.

Table 15. Distribution of amino acid substitutions in the penicillin-binding domain of PBP 2B from clinical isolates of *S. pneumoniae*.

Isolate	Penicillin MIC (μg/ml)	Amino Acid Motif [a]			PCR Results[b]		
		SVVK	SSNT	KTGTA	*pbp1a*	*pbp2b*	*Pbp2x*
6190	4	----	---A	-----	+	+	+
8111	4	----	---A	-----	+	+	+
6363	2	----	---A	-----	+	+	+
742	2	----	---A	-----	+	+	+
2848	2	----	---A	-----	+	+	+
3455	1	----	---A	-----	+	+	+
14126	0.5	----	---A	-----	+	+	+
11413	0.25	----	---A	-----	-	+	+
3996	0.12	----	---A	-----	+	-	+
12276	0.12	----	---A	-----	-	+	+
3203	0.06	----	----	-----	-	-	-
11184	0.06	----	----	-----	-	-	-
12244	0.06	----	----	-----	-	-	+
14016	0.06	----	----	-----	-	-	-
8099	≤0.03	----	----	-----	-	-	-

[a] Only amino acid residues differing from PBP 2B conserved motif sequences of the penicillin-susceptible R6 reference strain are shown. Conserved amino acid motifs are underlined.

[b] +, altered PBP gene sequence (PCR product not observed); –, unaltered PBP gene sequence (PCR product observed).

3. Analysis of *pbp1a*

Sequence variations within the structural *pbp1a* gene and amino acid substitutions in the deduced protein sequences of 15 clinical *S. pneumoniae* isolates were determined by comparison with penicillin-susceptible *S. pneumoniae* R6 (Appendix C). All sensitive (MIC; ≤ 0.06 μg/ml) genes and those of penicillin-intermediate isolates 12276 (MIC; 0.12 μg/ml), 3996 (MIC; 0.25 μg/ml) and 11413 (MIC; 0.25 μg/ml) showed a low degree of sequence variation (< 1%). The 930-bp PBD of *pbp1a* from these isolates revealed four to six nucleotide alterations and up to three amino acid substitutions (Table 16). One particular substitution, that of glutamic acid-388 by aspartic acid, occurred in all 15 clinical isolates analyzed. In isolates with MICs between 0.03 and 0.25 μg/ml, nucleotide and amino acid alterations were essentially confined to an area surrounding the lysine-577-threonine-glycine (KTG) motif. This included substitution of aspartic acid-533 by glutamic acid or replacement of serine-540 with threonine. Thereafter, as the level of penicillin resistance among isolates increased above MICs of 0.5 μg/ml, the number of nucleotide and amino acid alterations also increased such that the entire PBD was included. Penicillin-intermediate isolate 14126 (MIC; 0.5 μg/ml) revealed the most extensive nucleotide sequence divergence (21.8%) from strain R6, resulting in 43 (13.9%) alterations in the amino acid sequence of the protein. Widespread alterations in the transpeptidase-encoding region of *pbp1a* were likewise seen amongst five penicillin-resistant (MICs; ≥ 2 μg/ml) isolates and penicillin-intermediate isolate 3455 (MIC; 1 μg/ml), where nucleotide and amino acid sequences differed from those of strain R6 by 18.3 and 11.6%, respectively. In fact, only isolates with MICs ≥ 0.5 μg/ml had amino acid alterations within the locality of the serine-370-threonine-methionine-lysine (STMK)

and serine-428-arginine-asparagine (SRN) motifs. Two key changes within these regions included the substitution of serine or alanine for threonine-371 adjacent to the active-site serine residue, and that of threonine for proline-432 just after the SRN motif. Consequently, isolates with altered *pbp2x* and *pbp2b* genes in which threonine-371 was substituted by alanine in the PBP 1A STMK motif had penicillin MICs ≥ 1 μg/ml (Table 17). For isolate 14126, which likewise had altered *pbp2x* and *pbp2b* genes but which carried a threonine-371 to serine substitution in PBP 1A, the penicillin MIC was two-fold lower (0.5 μg/ml). For three isolates (11413, 3996 and 12276) with alterations in two PBP genes but not in the PBP 1A STMK or SRN motifs, penicillin MICs were 0.12 to 0.25 μg/ml. No substitutions within or adjacent to the conserved amino acid motifs of PBP 1A were observed in penicillin-susceptible isolates.

Table 16. **Divergence of *pbp1a* gene sequences in clinical isolates of *S. pneumoniae*.**

Isolate	Penicillin MIC (µg/ml)	Nucleotides Altered[a] (%)	Amino Acids Altered[a] (%)
6190	4	170 (18.3)	36 (11.6)
8111	4	170 (18.3)	36 (11.6)
6363	2	170 (18.3)	36 (11.6)
742	2	170 (18.3)	36 (11.6)
2848	2	170 (18.3)	36 (11.6)
3455	1	170 (18.3)	36 (11.6)
14126	0.5	203 (21.8)	43 (13.9)
11413	0.25	4 (0.4)	1 (0.1)
3996	0.12	6 (0.6)	2 (0.6)
12276	0.12	5 (0.5)	2 (0.6)
3203	0.06	6 (0.6)	1 (0.1)
11184	0.06	5 (0.5)	2 (0.6)
12244	0.06	6 (0.6)	3 (1.0)
14016	0.06	6 (0.6)	1 (0.1)
8099	≤0.03	4 (0.4)	2 (0.6)

[a] Published sequence of penicillin-susceptible R6 reference strain used as the basis of comparison with these isolates.

Table 17. Distribution of amino acid substitutions in the penicillin-binding domain of PBP 1A from clinical isolates of *S. pneumoniae*.

Isolate	Penicillin MIC (μg/ml)	Amino Acid Motif [a]			PCR Results[b]		
		STMK	SRNVP	KTG	*pbp1a*	*pbp2b*	*Pbp2x*
6190	4	–A – –	– – – – –T	– – –	+	+	+
8111	4	–A – –	– – – – –T	– – –	+	+	+
6363	2	–A – –	– – – – –T	– – –	+	+	+
742	2	–A – –	– – – – –T	– – –	+	+	+
2848	2	–A – –	– – – – –T	– – –	+	+	+
3455	1	–A – –	– – – – –T	– – –	+	+	+
14126	0.5	–S – –	– – – – –T	– – –	+	+	+
11413	0.25	– – – –	– – – – – –	– – –	-	+	+
3996	0.12	– – – –	– – – – – –	– – –	+	-	+
12276	0.12	– – – –	– – – – – –	– – –	-	+	+
3203	0.06	– – – –	– – – – – –	– – –	-	-	-
11184	0.06	– – – –	– – – – – –	– – –	-	-	-
12244	0.06	– – – –	– – – – – –	– – –	-	-	+
14016	0.06	– – – –	– – – – – –	– – –	-	-	-
8099	≤0.03	– – – –	– – – – – –	– – –	-	-	-

[a] Only amino acid residues differing from PBP 1A conserved motif sequences of the penicillin-susceptible R6 reference strain are shown. Conserved amino acid motifs are underlined.

[b] +, altered PBP gene sequence (PCR product not observed); –, unaltered PBP gene sequence (PCR product observed).

D. DISCUSSION

Previous studies have suggested that penicillin-resistant pneumococcal isolates (especially those with MICs > 1 μg/ml) usually are clonally related (56, 92, 115, 104, 116, 117, 118, 119, 120, 121, 122). To test this hypothesis, 15 clinical isolates of *S. pneumoniae* collected from across Canada were analyzed by capsular serotyping, pulsed-field gel electrophoresis and direct DNA sequencing. Arbitrarily-primed PCR of genomic DNA was also performed to determine its value in the epidemiological survey of pneumococcal infections. Both PFGE and AP-PCR revealed homogeneity amongst penicillin-resistant isolates and exclusive heterogeneity amongst penicillin-intermediate and penicillin-susceptible isolates. Sequence analysis of *pbp1a*, *pbp2b* and *pbp2x* genes revealed identical nucleotide and amino acid substitution patterns in all isolates for which MICs were ≥ 1 μg/ml. These data demonstrate the important contribution of clonal spread in the overall increase of penicillin resistance in this country.

To determine the penicillin susceptibility of clinical *S. pneumoniae* isolates by PCR, three sets of primers were used to differentially amplify PBP genes in penicillin-susceptible isolates. PCR correctly identified all five penicillin-susceptible isolates, three of five intermediately resistant isolates and each of the five highly resistant isolates. The susceptibility (i.e., intermediate vs. resistant) of two isolates could not be determined in this manner. Both of these isolates had a penicillin MIC ≤ 1 μg/ml. These findings suggest that the rapid identification of penicillin-susceptible and -resistant (MIC; ≥ 2 μg/ml) genotypes among clinical isolates of *S. pneumoniae* may be possible through the application of a multiplex-PCR assay.

PART I. Molecular Epidemiology of Penicillin-Resistant *S. pneumoniae*

The mechanism of penicillin resistance in clinical isolates of *S. pneumoniae* was first shown to involve the alteration of penicillin-binding proteins by the demonstration that PBPs from penicillin-resistant bacteria had radically reduced affinities and/or binding capacities for the antibiotic molecule (38). In addition, genetic transformation of resistance using clinical isolates as DNA donors demonstrated that high-level resistance to penicillin involved gradual remodeling of three to four of the five high-molecular-weight PBPs in parallel with a stepwise increase in resistance level (38). Next, cloning and sequencing of resistant PBP genes identified mosaic sequences, indicating that the origin of these PBP genes must have been heterologous recombination events in which nonpneumococcal bacteria may have served as DNA donors (123).

On the basis of what we know about the mechanism of penicillin resistance in *S. pneumoniae*, one can envision at least three processes that may have contributed to the striking increase in resistant pneumococci across Canada. First, penicillin resistance could have emerged on multiple occasions in unrelated strains of wild-type penicillin-susceptible *S. pneumoniae* at diverse geographic locales, most likely through acquisition of heterologous gene segments derived from taxonomically related streptococcal species (60). It is conceivable that random mutational events in the PBP genes also contributed to this process. Once acquired, resistant DNA sequences could then be redistributed via horizontal gene transfer from a penicillin-resistant pneumococcus to a genetically distinct, penicillin-susceptible pneumococcus (48). Third, the increased incidence of resistant pneumococci may have involved the multiplication and spread of one or more resistant pneumococcal 'clones'. To this end, it is possible that a small number of penicillin-

resistant *S. pneumoniae* clones were introduced into Canada almost simultaneously during the late 1980s or early 1990s, became established in restricted geographic areas under the selective pressures of β-lactam use, and then spread horizontally throughout the country via human-to-human transmission and travel, becoming secondarily established in widespread geographic areas again because of the selection effect of antibiotics. Such isolates may have had their origins in other countries, perhaps in the United States, or may have arisen indigenously in Canada.

1. Evidence of Clonal Dissemination

To examine whether the increase in penicillin-resistant *S. pneumoniae* observed in Canada could be attributed to *de novo* acquisition of resistance by genetic recombination or to clonal spread of one or more resistant isolates, PFGE and AP-PCR were used to determine the genetic relatedness of the isolates selected for this study. The PFGE restriction patterns and AP-PCR profiles from four of the five resistant isolates from diverse regions of Canada were nearly identical (Figures 8 and 12), suggesting a possible clonal origin. In contrast, penicillin-susceptible and penicillin-intermediate isolates were genetically diverse; 10 distinct lineages were distinguished amongst these 10 isolates (Figures 6, 7, 10 and 11). None of these lineages gave fingerprint patterns resembling those of the penicillin-resistant isolates. Resistant isolates are therefore not closely related to susceptible or intermediate isolates.

During genetic transformation of high-level penicillin resistance, transfer occurs in a stepwise fashion consistent with the involvement of multiple genetic elements (i.e., as many as four of the five high-molecular-weight PBP genes) (38). It is therefore reasonable to assume that the evolution of penicillin resistance in the clinical

environment also follows the stepwise direction of low (susceptible) to intermediate to high levels. Consequently, one would expect that the genetic background of highly resistant isolates should also be present among intermediate-level isolates, which would represent an evolutionary stage in the process that must have begun with a susceptible ancestral isolate of the same, or highly similar, genetic background. For the most part, however, no such potential precursor was evident among the limited number of penicillin-intermediate isolates included in this particular pneumococcal collection. Nevertheless, general similarities in the genetic background, PBP gene sequences, serotype and antibiotic susceptibility patterns of penicillin-intermediate isolate 3455 (MIC; 1 μg/ml) and penicillin-resistant isolate 2848 (MIC; 2 μg/ml) do not preclude the possibility that resistant isolates have indeed been derived locally from isolates with intermediate levels of resistance. It is possible, rather, that once high-level resistance was achieved, the superior and competitive survival of such isolates under the fluctuating selective pressure of β-lactam use led to the 'disappearance' of their evolutionary intermediates. Another important reason for these 'missing' intermediates may be the frequent presence of multidrug-resistant phenotypes among highly penicillin-resistant *S. pneumoniae*, again selecting in favor of these particular isolates which have much more versatility and fitness amidst the unstable pressures of the clinical environment. Although no explanation is currently available for the preferential appearance of multiple resistance traits among highly penicillin-resistant isolates of *S. pneumoniae*, is has been postulated that the high percentage of co-resistance may be the result of genetic linkage and efficient transfer of resistance markers (56, 124, 125, 126). As to the nature of the penicillin-intermediate isolates, the relatively large variation in genetic backgrounds, PBP

gene sequences and antibiotic susceptibility patterns, suggests that they may represent early stages of independently emerging penicillin-resistant lineages. Whether the sources of the penicillin resistance genes in these isolates are other pneumococci or hetero-specific donors is not known.

The apparently inverse relationship between penicillin MIC values and genetic variability has already been described in collections of pneumococcal isolates from around the world (56, 116, 117, 119, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134). In the present study, microbiological, serological and molecular analyses of 15 Canadian *S. pneumoniae* isolates revealed relative homogeneity amongst the more highly penicillin-resistant isolates and substantial genotypic and phenotypic variation amongst penicillin-intermediate and -susceptible isolates. Such diversity among the latter two groups suggests that these isolates must have originated *in situ* from a large variety of genetic backgrounds. As would be expected, most penicillin-susceptible *S. pneumoniae* isolates displayed relatively similar PBP gene sequences with very little (< 1%) genetic polymorphism (Tables 12, 14 and 16). The slight nucleotide and/or amino acid differences observed within these PBP genes could be attributed to a random mutation background, as isolates were recovered in distinct geographic areas between 1997 and 1999. Among penicillin-intermediate isolates, multiple interspecies recombinational events, gradual accumulation of point mutations and ensuing formation of 'mosaic' PBP genes appear to account for the acquisition of low-level resistance. On the other hand, the existence of identical PBP genes in two genetically unrelated penicillin-intermediate isolates (12276, MIC; 0.12 µg/ml and 3996, MIC; 0.25 µg/ml) argues for horizontal transfer of PBP genes between these bacteria. Interestingly, penicillin-susceptible isolate

12244 was likewise shown to harbor a highly polymorphic *pbp2x* gene identical to that found in intermediate isolates 12276 and 3996, suggesting similar horizontal gene transfer in susceptible isolates albeit without detectable alteration of penicillin susceptibility.

Molecular typing studies of penicillin-resistant pneumococci from several countries have demonstrated that, in general, the majority of isolates circulating within a geographic area are derivatives of a relatively small number of clonal lineages (65, 90, 91, 125, 127, 128, 133, 135, 136, 137, 138). Indeed, the most striking observation documented by molecular fingerprinting of Canadian isolates was the expression of a highly conserved chromosomal background amongst four of five penicillin-resistant isolates. Within this genetically related cluster, isolates 742 and 6363 (MICs; 2 μg/ml) could not be distinguished from one another by PFGE or AP-PCR (Figures 8 and 9). In addition, both isolates shared not only the same capsular determinants of serotype 19F (Table 9) but also exhibited identical resistance profiles. By comparison, isolates 6190 and 8111 likewise shared simple variants of this common genetic background, but differed from the former isolates by virtue of their penicillin MICs (4 μg/ml) and expression of capsular serotypes (14 and 23F, respectively). The results obtained by PFGE and AP-PCR were subsequently confirmed by studying the DNA sequences of three PBP genes (*pbp1a*, *pbp2b* and *pbp2x*). All penicillin-resistant pneumococci, including genetically unrelated isolate 2848, were found to possess homologous (mosaic) PBP alleles (Tables 12 through 17). If these variant DNA sequences were each harbored in a pneumococcus of distinct genotype, then the number of independent pathways by which pneumococcci could acquire resistance would be enormous indeed. Fortunately,

there was no evidence for this in our study. Therefore, on the basis of these observations, there are essentially two explanations for the dramatic genotypic similarity between penicillin-resistant isolates of *S. pneumoniae*. First, in clinical isolates of pneumococci, the structural remodeling of PBPs that resulted in reduced penicillin affinity and acquisition of resistance could have occurred multiple times through independent pathways in distinct isolates, with gradual evolutionary convergence to the same, or highly similar, genotype. Alternatively, extensive homogeneity may be linked to the geographic dissemination of a penicillin-resistant pneumococcal 'clone' having the selective advantage to spread in an environment in which antibiotics are often misused.

Because evolutionary convergence to the same genotype is highly unlikely, the simplest explanation for the repeated recovery of Canadian isolates with the same array of genetic polymorphisms is that they have recently descended from a common ancestor and hence constitute a clonal lineage. In support of this theory, genetic lineages that have achieved massive geographic spread across both national and continental boundaries have previously been identified through collaborative surveillance projects and DNA typing. The most widely spread of these is often referred to in the literature as the Spanish/USA serotype 23F clone and was isolated in Spain in the 1980s (139). It was soon recovered in the US and South Africa (63, 139), can now be isolated in virtually every western European and Latin American country and has recently crossed the border into Eastern Europe and Asia (124, 136, 139, 140, 141, 142). Pneumococci belonging to this clone are not only widespread in the geographic sense but can also represent a very large proportion of penicillin-resistant pneumococci in a given epidemiologic setting. A second and distinct pneumococcal clone (the French/Spanish clone), resistant to penicillin

and expressing either serotype 14 or 9, has also achieved massive geographic expansion on several continents (143). A third clone of *S. pneumoniae* expressing capsular type 6B and carrying multidrug-resistant genes has repeatedly been identified in Spain, the United Kingdom and (with particularly high frequency) in Iceland (128). Whether any of the isolates characterized in this study are identical to those described in previous reports remains to be determined.

Although penicillin resistance has been reported for several different capsular serotypes, certain pneumococcal serotypes are known to be more virulent than others with the distribution of such serotypes varying in different populations and geographic areas (67). In most countries, intermediate-level penicillin resistance is found in isolates of many serotypes, but high-level resistance and multiple antibiotic resistance are particularly associated with a limited number of serotypes; specifically 6B, 9V, 14, 19F and 23F (68, 144). In the present study, four penicillin-resistant isolates with nearly homogeneous typing profiles serotyped 19F (2 isolates), 23F (1 isolate) and 14 (1 isolate) (Table 9). Molecular analysis clearly showed that isolates expressing different capsular types can be closely related in genetic terms, whereas isolates of the same serotype are often diverse (Figures 9 and 13). Additional observations of clonally related organisms manifesting different capsular serotypes have been reported extensively throughout the literature (53, 65, 67, 90, 125, 127, 134, 135, 137, 142, 143, 145, 146, 147, 148, 149, 150, 151, 152, 153). Since only one set of type-specific capsular polysaccharide biosynthetic genes are present in a given organism (154), these pneumococcal isolates are most likely the products of spontaneous *in vivo* capsular transformation events, presumably mediated by the horizontal transfer and recombinational replacement of genes specifying capsular

type (145, 150). Indeed, multiple recombinational exchanges have been shown to occur at capsular biosynthetic loci with consequential serotype changes in penicillin-resistant *S. pneumoniae*. Barnes *et al.* (148), for example, surveyed the genetic characteristics of multidrug-resistant pneumococcal isolates from a research day-care centre and in so doing identified a serotype 14 variant of the multi-resistant 23F clone which emerged during antibiotic therapy. Coffey *et al.* (145) also showed horizontal transfer of capsular genes, resulting in a serotype 19 strain that was indistinguishable from this same serotype 23F penicillin-resistant clone. Interestingly, four of the five penicillin-resistant isolates characterized in our study expressed three different capsular serotypes but were otherwise nearly identical on the basis of PFGE/AP-PCR profiles and PBP gene sequences. Divergent capsular types among isolates with identical PBP gene sequences and PFGE types indicated several instances of probable capsular serotype switching. This thesis therefore includes suggestive evidence for *in vivo* pneumococcal capsular transformation between serotypes 14, 19 and 23F and provides further support for the hypothesis that the spread of resistant clones has contributed in part to the overall increase of penicillin resistance in Canada.

Whether transformation of capsular genes is a more likely event than that of genes encoding resistance determinants (PBP genes) depends on the number of genes involved and their localization on the chromosome. Thus far, knowledge about genes responsible for the biosynthesis of the pneumococcal polysaccharide capsule is very limited (134). In the absence of such information, it is difficult to investigate further the reason why isolates that are apparently indistinguishable will often have different serotypes. It is conceivable, nonetheless, that horizontal spread of capsular biosynthetic genes to

multidrug-resistant organisms may very well be a mechanism through which spread of the multi-resistance phenotype to additional serotypes can occur. Since multiple serotypes of *S. pneumoniae* are frequently carried concurrently in the human nasopharynx, such events may be a common occurrence. Finally, in addition to enhancing the spread of drug resistance among diverse capsular types, these exchanges may also alter tissue tropism of the bacteria and provide a temporary mechanism for evasion of serotype-specific host immune defenses. In light of selective pressures imposed by conjugate vaccines that focus entirely on the use of capsular polysaccharides representing a restricted number of capsular types (i.e., the commercial 23-valent polysaccharide vaccine targets only serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F), the use of multivalent conjugate vaccines may shift the capsular distribution toward capsular types not represented in these vaccines. Consequently, the capacity of multidrug-resistant pneumococci to undergo spontaneous capsular switch is an issue of obvious concern.

2. Evidence of Horizontal Transfer

In addition to clonal spread, intraspecies genetic exchange also appears to have influenced the acquisition and spread of β-lactam resistance (particularly that of penicillin) in isolates of *S. pneumoniae*. Evidence for horizontal transfer of penicillin resistance genes has already been documented (44, 55, 56, 59, 65, 116, 118, 120, 122, 125, 127, 140, 142, 149, 155). Among five Canadian isolates with penicillin MICs ≥ 2 μg/ml, one isolate (2848) was shown to harbor altered PBP genes identical to those of the other resistant isolates, but differed from the latter group by virtue of its serotype (9V),

antibiogram and PFGE/AP-PCR profiles. In this case, horizontal gene transfer is believed to have distributed the same resistance genes into a genetically unrelated pneumococcal isolate. Complementation experiments suggest that genes as far apart as 30 kb can be transformed in a single genetic event (59). Since both *pbp1a* and *pbp2x* are closely linked on the chromosome, simultaneous transfer to recipient isolates is thought to occur during transformation (140, 156). The prevalence of identical PBP gene sequences among isolates of several different genetic backgrounds suggests that the three resistance gene determinants are often transferred simultaneously during transformation. Upon close examination, it can also be postulated that this transfer was chronologically subsequent to the genomic divergence between these isolates. Had PBP gene transfer occurred prior to this divergence, the PBP gene sequences would be expected to display the same level of divergence as the overall genomic DNA. This, however, was not the case. On the other hand, isolate 2848 was indeed found to be sufficiently different for the divergence to have occurred before the emergence of penicillin resistance.

In summary, the development and dissemination of penicillin resistance in *S. pneumoniae* appears to be a complex process involving the acquisition of gene segments through homologous recombination with related streptococcal species, accumulation of point mutations, redistribution via horizontal transfer among genetically different pneumococcal isolates and geographic spread of resistant 'clones'. The relative contribution of these mechanisms to the recent increase in the incidence of penicillin-resistant pneumococci in Canada, however, remains to be established. Further studies on a more extensive panel of isolates with various serotypes and of various clinical and

geographic origins are therefore required to draw the definitive epidemiology of *S. pneumoniae* and their PBP genes.

PART II. Evaluation of DNA Fingerprint Techniques for Molecular Typing of *S. pneumoniae*

1. Suitability of AP-PCR as an Alternative Typing Scheme

Phenotyping and genotyping methods are increasingly being used to monitor the source and transmission of disease, as well as the emergence of strains with increased pathogenicity (86). While serotyping and antibiotic susceptibility testing have been the most common epidemiologic tools for typing *S. pneumoniae*, they have relatively limited discriminatory power and as such are being increasingly challenged by the use of DNA-based methods. These have included multilocus enzyme electrophoresis (127, 135), PBP profile analysis (65, 139), pneumococcal surface protein A typing (127), restriction endonuclease analysis of genomic DNA (66, 157), ribotyping (65, 66, 140) and PFGE (66, 135, 158). Recently, several PCR-based techniques (56, 66, 125, 159), including AP-PCR, have also been described. AP-PCR, alternately known as randomly amplified polymorphic DNA (RAPD), is based on the fact that short primers whose sequences are not directed at any particular genomic element will hybridize at random sites to initiate DNA polymerization (160, 161, 162). Since the proximity, number and location of these priming sites vary between isolates, the resulting PCR products are strain-specific DNA fingerprints that differ according to the degree of relatedness between the isolates under investigation. The main advantage of this technique over traditional phenotypic methods

and some of the more recent molecular methods lies in its rapidity, low expense, technical feasibility for most laboratories and theoretical applicability to any organism (162).

In the current study, PFGE and AP-PCR were evaluated for their ability to differentiate between Canadian isolates of *S. pneumoniae*. Although the discriminatory powers of both methods were comparable (0.99 and 0.98, respectively), similarity values among isolates varied significantly between the different techniques. Since different typing methods assess different parameters, some variation with respect to these parameters is not unexpected. Interestingly, a comparison of the genetic clustering of the 15 isolates showed a high degree of resemblance. Most importantly, both techniques invariably displayed clustering of the penicillin-resistant isolates with MICs ≥ 2 µg/ml. It was concluded, therefore, that, like PFGE, AP-PCR could be used to accurately type and discriminate between epidemiologically unrelated isolates of *S. pneumoniae*.

In response to criticisms leveled at the reproducibility of AP-PCR, standardization of the technique (including standard methods of DNA preparation, consistent volumes and concentrations of reagents, consistent use of the same DNA polymerase and equipment, and standard procedures for visualization of fingerprints) should be sufficient to circumvent this obstacle. To verify the reproducibility of DNA banding patterns under the conditions defined within this thesis, AP-PCR was repeated on at least three separate occasions for each isolate. Consistent generation of PCR products was observed when DNA preparations were freshly made (data not shown). Nevertheless, in order for AP-PCR to be considered as a definitive typing technique, automated systems for DNA preparation as well as for the reproducible generation and interpretation of DNA

fingerprints need to be developed. At the present moment, however, AP-PCR remains an effective comparative tool for the epidemiological survey of pneumococcal infections.

2. Interpreting *S. pneumoniae* Chromosomal DNA Restriction Patterns Produced by PFGE

Pulsed-field gel electrophoresis, created by Schwartz, Cantor and colleagues in 1982, allows large DNA fragments to be separated on an agarose gel by virtue of their molecular weights. Although criticized for being labor-intensive, time-consuming and allowing only limited throughput per gel, this technique is thought to be the most sensitive epidemiological method for studying the mechanism involved in the spread of penicillin-resistant pneumococci (163). Consequently, PFGE remains the gold standard typing method for defining genetic relatedness among clinical isolates of *S. pneumoniae*. Unfortunately, however, standardized criteria for analyzing DNA restriction patterns are currently unavailable. Interpretation of results, specifically the number of band differences needed to truly differentiate unrelated isolates, is therefore an issue of growing concern. In 1995, Tenover *et al.* (164) proposed a set of guidelines for interpretation of PFGE interrelationships that has subsequently been applied extensively throughout the literature. These suggest that isolates are indistinguishable if their restriction patterns have the same number of bands and the corresponding bands are the same apparent size. On the other hand, isolates that are closely or possibly related will differ from one another by changes consistent with one or two independent genetic events. Such changes typically result in two to three and four to six band differences, respectively. Seven or more band differences, by comparison, are definite evidence that

isolates are genetically unrelated. Typically, this implies that < 50% of the well-resolved fragments will be identical between such isolates. Although epidemiologically useful in analyzing discrete sets of isolates obtained during relatively short periods of time (one to three months), the criteria for strain identity are stringent and are therefore not appropriate for studies of organisms collected over extended periods of one year or longer. Other laboratories (67, 140, 165) have favored an interpretation loosely based on the above criteria in which isolates that are genetically unrelated differ by anywhere from three to five restriction fragments. For investigation of potential relationships among Canadian *S. pneumoniae* isolates collected over extended periods, the following guidelines and interpretive criteria were established. During visual comparisons, isolates with identical PFGE patterns were deemed genetically indistinguishable. Thereafter, isolates with two or three band shifts consistent with a single genetic event were defined as closely or possibly related and categorized as subtypes of one another. Finally, banding profiles that differed by four or more fragments were considered to be different and were thus indicative of genetically unrelated isolates. Although comparison of restriction patterns will remain, in part, a subjective process that cannot be totally reduced to rigid algorithms, the establishment of standardized interpretive criteria is desirable if the epidemiological significance of PFGE is to be more easily understood. As further modifications continue to simplify existing protocols, the attractiveness of PFGE for molecular typing will undoubtedly increase.

PART III. Characterization of PBP 1A, 2B and 2X Mutations in Penicillin-Resistant *S. pneumoniae*

The resistance of *S. pneumoniae* to β-lactam antibiotics has been shown to involve changes in the affinities of at least four of the five high-molecular-weight PBPs, namely 1A, 2A, 2B and 2X (32, 38, 51, 58, 123, 135, 166, 167, 168, 169, 170). Genetic analysis, however, has revealed that high-level penicillin resistance can be established by alterations in only PBPs 1A, 2B and 2X (38, 43, 46, 52, 55, 64, 101, 113, 115, 171, 172, 173, 174, 175, 176, 177). To obtain insight into the extent of the diversity of these genes in Canada's pneumococcal population, the PBD of PBPs 1A, 2B, and 2X from 15 clinical isolates were sequenced. These data were used to identify amino acid alterations which were common to all resistant isolates and which would appear to be essential for the development of resistance.

The evolution of penicillin resistance development begins with the acquisition of low-level resistance through alteration of PBP 2X. Although single amino acid changes in the gene encoding this protein can confer a slight increase in resistance, the elevated resistance levels typically observed in clinical isolates or in laboratory mutants are most commonly the result of multiple alterations (24, 115, 177, 178). Furthermore, analysis of *pbp2x* in laboratory mutants has revealed that an amazing variety of distinct mutational pathways can lead to such low-affinity variants (40, 179). This suggests that the development of resistance does not follow a strictly predetermined pathway. Consequently, the identification of specific amino acid alterations that produce resistance in clinical isolates is somewhat problematic (177) and the impact of many of these changes is still unknown.

Comparison of point mutations in several independently recovered isolates has revealed that three conserved amino acid motifs within the PBD of PBP 2X appear to be preferentially affected, suggesting that these areas are generally important for interaction with the antibiotic. Since mutations in the active-site are more likely to lead to changes in the specificity of this enzyme, the number of alterations conferring high-level resistance is presumably somewhat restricted (174). For example, substitution of alanine for threonine-550 just after the KSG motif has been identified as a major resistance factor whose involvement in resistance to cephalosporins is presumably due to the loss of a hydrogen bond between the threonine and the β-lactam as a consequence of this change (24, 40, 104, 174, 177, 179, 180). Interestingly, while providing increased resistance to expanded-spectrum cephalosporins, substitution at this residue has also been shown to concurrently result in a loss of resistance to penicillin (24, 104, 174). As expected, this threonine-550 to alanine replacement was not detected in PBP 2X from clinical isolates of penicillin-resistant *S. pneumoniae*. More recently, structural evidence linking penicillin resistance to the absence of a hydroxyl group following substitution of alanine for threonine-338 has been presented (30, 177, 180). Kinetic parameters of PBP 2X variants have suggested that the mutation of threonine-338 near the active-site serine residue significantly reduces the acylation efficiency of this resistance determinant by modifying the reactivity of serine-337 toward both the antibiotic and substrate analogues (30). In agreement with this theory, virtually all clinical isolates with reduced susceptibility (MIC > 0.06 μg/ml) contained the aforementioned alteration at position 338. Additional sites that contribute to affinity changes in PBP 2X have also been identified through site-directed mutagenesis or analysis of selected laboratory mutants

(24, 40, 167, 177, 179) and in Canadian clinical isolates include the exchange of leucine for histidine-394 and valine for leucine-546, to mention a few. Finally, substitutions at the extreme C-terminal end of the penicillin-binding domain are predicted to affect the active-site of this protein through an altered secondary structure and consequently confer low-level resistance not only to penicillin but to a wide variety of β-lactam antibiotics (40, 167, 177).

PBP 2B constitutes a primary resistance determinant whose alteration follows that of PBP 2X and confers intermediate levels of β-lactam resistance in clinical isolates of *S. pneumoniae*. Nucleotide sequence analysis of the *pbp2b* transpeptidase-encoding region in penicillin-resistant strains revealed extensive sequence divergence compared to penicillin-sensitive strains. In most resistant isolates, the area within the locality of the SVVK tetrad containing the active-site serine and the SSN triad housed the majority of all nucleotide and amino acid substitutions occurring within the PBD. It would therefore appear that amino acid substitutions occurring within the region between asparagine-404 and phenylalanine-508 may strongly influence penicillin resistance, at least up to an intermediate level. In addition, analysis of PBP 2B has revealed four particularly prominent alterations which occur amongst most isolates and which appear to be essentially associated with a decreased affinity of the protein for penicillin. These include the replacements of glutamic acid-332 by glycine, threonine-445 by alanine, glutamic acid-475 by glycine and threonine-488 by alanine or serine.

The importance of the exchange of alanine for threonine-445, which has similarly been identified in all resistant isolates analyzed to date (92, 174), has previously been noted by Dowson and coworkers (123) and occurs adjacent to the conserved SSN motif.

Because the asparagine residue of this motif has been proposed to form a hydrogen bond with the carbonyl group of the penicillin R1 side chain (181), the substitution of alanine for threonine-445 presumably disrupts this hydrogen bond. On the other hand, the significance of the remaining substitutions has not yet been determined. Interestingly, a second phenotype associated with this same threonine-445 to alanine alteration, i.e., a reduced lytic response, may also be of notable clinical significance by allowing prolonged survival during antibiotic treatment. In agreement with this theory, it has been noted before that in clinical isolates, penicillin resistance and defective lysis upon penicillin treatment are frequently associated (24, 58, 174). Another aspect concerning the biological impact of the reduced lytic response upon penicillin treatment is related to the fact that penicillin resistance in pneumococci is an acquired property that involves multiple occurrences of horizontal gene transfer. Lysis-defective strains may consequently display an enhanced capability for uptake and incorporation of the DNA fragments that encode resistance determinants. But despite the fact that changes within PBP 2B very likely play an essential role in the development of resistance, alteration of PBP 2B alone would presumably not dictate the final level of resistance in pneumococci (169). Instead, the final level of resistance would most likely be dependent on the collective action of multiple altered PBPs.

In the presence of low-affinity variants of PBP 2X and PBP 2B, alteration of PBP 1A plays a vital role in the development of high-level resistance. At present, however, the amino acid alterations in PBP 1A that are responsible for decreased penicillin affinity in clinical isolates of *S. pneumoniae* are not well defined (43). Nevertheless, widespread alterations in the PBD of PBP 1A were seen in isolates for which penicillin MICs were ≥

0.5 μg/ml. This suggests that an MIC of 0.5 to 1 μg/ml represents a breakpoint in resistance where PBP 1A starts participating in the development of resistance as a result of significant alterations in its PBD. These data can be compared to those from previous phenotypic studies in which the disappearance of PBP 1A from PBP profiles of transformants as they reached resistance levels of 0.4 μg of penicillin per ml has suggested that an altered PBP 1A with decreased affinity for penicillin occurs only in isolates for which MICs are 0.4 μg/ml and higher (38). Studies with clinical isolates of pneumococci, by comparison, have revealed that PBP 1A is absent from the fluorograms of isolates for which penicillin MICs are ≥ 0.25 μg/ml (46). Furthermore, Kell and coworkers (32) transformed a penicillin-resistant strain (MIC; 4 μg/ml) with inactivated PBP 1A DNA and successfully obtained growth of the transformant, revealing the tolerance of pneumococci to the loss of PBP 1A. The resultant MIC decrease to 0.5 μg/ml that accompanied the inactivation of PBP 1A supports the idea that PBP 1A plays a role in the development of penicillin resistance when MICs are > 0.5 μg/ml.

In Canadian *S. pneumoniae* isolates for which penicillin MICs were 0.03 to 0.25 μg/ml, nucleotide and amino acid alterations were essentially confined to an area preceding the KTG motif. This included substitution of asparagine-533 by glutamic acid or replacement of serine-540 with threonine. Thereafter, as the level of penicillin resistance increased above MICs of 0.5 μg/ml, the number of nucleotide and amino acid alterations also increased such that the entire PBD was included. Only these isolates had amino acid alterations within the locality of the STMK and SRN motifs of PBP 1A, including four consecutive alterations (threonine-574 to asparagine, serine-575 to threonine, glutamine-576 to glycine and phenylalanine-577 to tyrosine) which were

common to all. Interestingly, examination of the PBD of PBP 1A from resistant isolates has revealed that substitution of threonine-371 by serine or alanine is predominant and is furthermore associated with the level of resistance in strains having simultaneous alterations in PBP 2X and PBP 2B. It would therefore appear that this substitution may be of particular importance in mediating higher levels of resistance. Garcia-Bustos and Tomasz (182) have shown that penicillin-resistant pneumococcal strains produce cell walls with profoundly altered chemical compositions. It is possible that a substitution of threonine-371 adjacent to the active-site serine may change the three-dimensional structure of the transpeptidase domain and alter the enzymatic activity for peptidoglycan synthesis. Thus, amino acid residue 371, in addition to residues 574-577, is likely important with respect to the interaction of PBP 1A with penicillin (43). Since substitutions at 574-577 are common to all isolates with MICs > 0.25 μg/ml and have been shown to be critical to the development of penicillin resistance (43), it is quite possible that, in the presence of these four substitutions, an alteration at residue 371 would account for the development of full resistance.

In summary, altered PBP 2X appears to be essential for the recovery of isolates with altered PBPs 2B and 1A. This scenario at the breakpoint of resistance (0.06 to 0.12 μg/ml) may indicate that an isolate can acquire resistance to penicillin solely because of a *pbp2x* gene alteration while the *pbp2b* and *pbp1a* genes remain unaltered. However, this increase in resistance appears to be limited to a low level (i.e., MIC; ± 0.12 μg/ml) until additional mutations, including the initiation of *pbp2b* gene alteration, allow the expression of higher levels of resistance. The presence of a diverse *pbp2x* and uniform *pbp2b* gene profile amongst susceptible isolates thus supports the notion that *pbp2x* gene

alterations are associated with a lower level of penicillin resistance than are alterations in *pbp2b*. Variation in *pbp2x* is initiated at a low MIC while variation in *pbp2b* is seen only in isolates for which MICs are 0.12 μg/ml and greater, implying that an isolate requires changes in both its *pbp2x* and *pbp2b* genes to obtain high levels of resistance to penicillin. Only within this genetic background of altered *pbp2x* and *pbp2b* is the recovery of isolates with an altered *pbp1a* possible. These results suggest that if a stepwise alteration of PBPs with increasing levels of penicillin does occur, as first suggested by Zighelboim and Tomasz (38), then the order of the PBP change toward a lower affinity for penicillin is indeed 2X > 2B > 1A.

This apparent orderliness in which the antibiotic-binding capacities of the individual PBPs are reduced is thought to reflect the relative penicillin affinities of these pneumococcal proteins (18, 178, 183). In penicillin-susceptible *S. pneumoniae*, the order of penicillin reactivity of the PBPs is 1A > 2B > 2X (183). Consequently, one would expect that the most penicillin-sensitive PBP (i.e., PBP 1A) should be the primary resistance determinant. In clinical isolates of penicillin-nonsusceptible *S. pneumoniae*, however, exclusive alteration of PBP 2X has clearly been shown to accompany the acquisition of low-level penicillin resistance. This observation suggests that a low-affinity variant of PBP 1A may be unable to perform its physiological function of peptidoglycan synthesis when additional PBPs are present in their unaltered, highly reactive forms. Moreover, pneumococcal PBPs may associate into multiprotein complexes that operate in a concerted manner, as a kind of 'assembly line' in the synthesis of the bacterial cell wall (26, 184). It is conceivable, therefore, that extensive

alterations in only a single PBP, without appropriate modification in the reactivities of other PBPs, may make cooperative functioning impossible.

Changes in all streptococcal PBPs, including the low-molecular-weight PBP 3 (185), have been associated with resistance to β-lactams, documenting that the genetic background and function of other PBPs are important parameters that define the indispensable nature of a PBP. While the role of PBP 1A, PBP 2B and PBP 2X in their resistance to β-lactam antibiotics has been clearly established, the involvement of PBP 2A and particularly that of PBP 1B is not well understood (64, 175, 184). Characterization of the PBP sequence profiles of clinical isolates, however, has suggested that not all steps of resistance increase are mediated solely by mutations in the genes encoding PBPs 2X, 2B and 1A (177). In Canadian *S. pneumoniae* isolates, for example, identical mosaic PBP arrangements were found to occur at widely different MICs (from 1 to 4 μg/ml). This strongly suggests that, at least theoretically, additional PBP and non-PBP genes are also involved in resistance development.

PBP 1B variants with reduced affinity have been previously described in interspecies transformations to penicillin resistance (186), and a low-affinity PBP 2A has been noted in several penicillin-resistant clinical isolates of *S. pneumoniae* (51). Recent sequence analysis of the *pbp2a* gene has also revealed diverse profiles only for those strains whose MICs were 4 μg/ml (187). This indicates that *pbp2a* alterations may be common, although not necessarily present in all penicillin-resistant isolates. In addition, genetic studies have established that the *pbp2a* gene, in combination with other PBPs, is essential for viability of *S. pneumoniae* and that the *pbp2a*-encoded transglycosylase may play a major role in peptidoglycan polymerization in the cell (29, 188, 189). Given that

PBP 2A is a low-affinity protein compared with other PBPs in *S. pneumoniae*, it has been hypothesized that PBP 2A may be a naturally resistant PBP capable of taking over the activity of other PBPs in the presence of clinically relevant concentrations of β-lactam antibiotics. Further analysis will therefore determine whether amino acid substitutions in PBP 2A as well as in PBP 1B contribute to the development of penicillin resistance in *S. pneumoniae*.

It has been suggested that, in addition to the altered PBP genes, penicillin-resistant pneumococci may carry non-PBP traits that contribute to their successful and/or superior survival in the natural environment (190). For example, the slow autolysis and relatively high survival rate of penicillin-resistant isolates during stationary phase as described by Tarasi *et al.* (142) may very well be a novel feature of antibiotic-resistant pneumococci. The fact that mutations in non-PBP genes are selected by β-lactam treatment suggests that they counteract the β-lactam induced changes of the bacterial cell wall. Whether or not a relatively increased survival during stationary phase autolysis is frequently associated with increased levels of penicillin resistance, however, remains to be established. Clearly, the identification of resistance determinants in clinical isolates remains an ongoing process.

PART IV. PBPs as Penicillin Resistance Determinants in *S. pneumoniae*

Early detection of infection with penicillin-resistant *S. pneumoniae* is essential both to ensure effective treatment and to allow for early implementation of measures for the prevention of secondary cases. Current methods of detection based on successful culture take several days to complete and have poor sensitivity in patients treated with

antibiotics. Diagnosis of pneumococcal infections by antigen detection (i.e., latex agglutination) in clinical samples is rapid but this approach lacks sensitivity as well as specificity (191). Throughout the literature, numerous reports have described the identification of *S. pneumoniae* DNA in clinical samples using PCR (192, 193, 194, 195, 196, 197) and have highlighted the advantages of PCR compared with traditional culture methods. These include a more rapid diagnosis, combined with high sensitivity and specificity, and the potential for use in the diagnosis of pneumococcal infections in patients pre-treated with antibiotics.

Mosaic *pbp1a*, *pbp2b* and *pbp2x* genes have been associated with high-level penicillin resistance in *S. pneumoniae*, with the nucleotide sequences of these genes varying considerably among penicillin-susceptible and -resistant pneumococcal isolates. Although the precise role of this sequence variation in the development of resistance is still unclear, recent studies have indicated that some of these differential nucleotide sequences can act as markers for penicillin susceptibility. These findings suggest that rapid identification of penicillin-susceptible and penicillin-intermediate or -resistant genotypes may be possible through the detection of PBP gene mutations. Pursuing this possibility, the relationship between penicillin susceptibility and the *pbp1a*, *pbp2b* and *pbp2x* genes was investigated using a multiplex PCR strategy. To compensate for the multiple mutational pathways through which a PBP's active site may be remodeled, primers were chosen to target the genes of susceptible isolates but not those of resistant isolates. Negative PCR amplification was thus indicative of penicillin resistance due to the inability of PCR to amplify the specific determinants of β-lactam resistance in *S. pneumoniae*. A strong correlation was found between PCR products and the MIC data.

Among those isolates for which penicillin MICs were ≥ 0.5 μg/ml, only the 274-bp species-specific *lytA* (autolysin) product was observed (Figures 15 and 16). Isolates with intermediate levels of resistance between 0.12 and 0.25 μg/ml produced one of two additional amplification products, the 430-bp *pbp1a* fragment or the 77-bp *pbp2b* fragment (Figure 15). Concomitant detection of *pbp1a*, *pbp2b* and *pbp2x* occurred in four of five isolates for which the MIC of penicillin was ≤ 0.06 μg/ml (Figure 14). Amplification of the 292-bp *pbp2x* gene fragment was not observed in one susceptible isolate (12244). Interestingly, amplification of *pbp2b* but not of *pbp1a* was noted in penicillin-intermediate isolate 11413 (MIC; 0.25 μg/ml). This result was unexpected, considering that previous data have shown that the development of penicillin resistance occurs in a stepwise manner with an alteration of *pbp2b* occurring before that of *pbp1a*. This uncommon situation, however, was found only at the intermediate level of resistance. At a higher level of penicillin resistance, an altered *pbp2b* would undoubtedly be required.

PCR-based diagnosis of penicillin resistance is complicated by the participation of multiple PBPs. Furthermore, while PBP genes of penicillin-susceptible *S. pneumoniae* have very few mutations and those of highly resistant isolates contain numerous alterations, intermediately resistant *S. pneumoniae* fall somewhere in between. It is therefore not surprising that this study was most successful in determining the penicillin susceptibility of highly resistant and susceptible clinical isolates. Identification of intermediately resistant isolates was somewhat more problematic. Although targeting more than one PBP gene of penicillin-susceptible *S. pneumoniae* increased the specificity of this technique, we were still unable to differentiate between moderately (MIC; 0.5 – 1

μg/ml) and highly (MIC; ≥ 2 μg/ml) resistant isolates. An optimal PCR assay for resistance would therefore require continuous monitoring of new sequence data from resistant isolates in order to facilitate the design of primers with greater specificity. Nevertheless, PCR results clarified that the MIC of penicillin is indeed affected by mutations in the PBP genes of *S. pneumoniae*. Moreover, multivariate analysis showed that the influence of PBP gene mutations on the observed MIC value differs according to the combination of determinants involved.

In general, the assay described herein was simple, specific, reproducible, and rapid. Since simplicity and speed are important considerations if molecular diagnostic techniques are to be applied in clinical practice, a multiplex PCR format was preferentially employed while the use of nested PCR primers and probes was deliberately avoided. To test the reproducibility of this procedure, PCR was repeated on at least three separate occasions for each isolate. As equivocal results were not obtained, one series of amplification is thought to be sufficient for the determination of penicillin susceptibility in *S. pneumoniae*. The specificity of the assay was demonstrated by the inability of PCR to amplify DNA from eight nonpneumococcal organisms, including five related streptococcal species. Previous work has demonstrated that viridans group streptococci, in particular *S. sanguis* and *S. mitis*, have the potential to transfer resistance genes to pneumococci (and vice versa) at remarkably high frequencies (186, 198). None of the bacterial species included in this study gave amplification products that interfered with the interpretation of our results (Figure 17). By combining the species-specific *lytA* primers and three sets of primers designed for amplification of the *pbp1a*, *pbp2b* and *pbp2x* genes from penicillin-susceptible *S. pneumoniae*, we were able not only to confirm

the presence of *S. pneumoniae* but also to determine the penicillin susceptibility of pneumococcal isolates from clinical samples. We therefore propose that PCR could be extremely useful in the early detection of penicillin resistance (MIC $\geq$ 0.12 µg/ml) if two or more PBP primer sets fail to generate products but the autolysin gene is amplified. Finally, the results presented here are of sufficient value to merit further clinical development and to potentially extend the spectrum of the assay for the detection of resistance to additional β-lactam antibiotics as well.

PART V. Summary

In clinical isolates of *S. pneumoniae*, horizontal genetic exchange has influenced the emergence and spread of penicillin resistance through both the generation of novel alleles encoding low-affinity PBP variants and dissemination of these alleles among genetically diverse organisms. It appears, however, that the recent increase in the incidence of penicillin-resistant pneumococci in Canada can be largely attributed to the geographic spread of a small number of resistant 'clones'. The surprisingly predominant representation of a common chromosomal background among the majority of penicillin-resistant isolates suggests that these bacteria originated from a common ancestor with minor genetic variations most likely arising through independent mutation. The microbial and mechanistic factors likely to contribute to the remarkable epidemicity of these isolates are presently unknown. Molecular fingerprinting techniques will therefore play an increasingly important role in tracking evolutionary changes within the pneumococcus and also in identifying the epidemiologic and molecular forces that drive

the epidemic spread of resistant clones and resistant genes of this important human pathogen.

Due to the high morbidity and mortality associated with invasive *S. pneumoniae* infection, early implementation of appropriate antibiotic therapy requires prompt identification of both the organism and its antibiotic susceptibility pattern. Consequently, the utilization of PCR as a molecular-based diagnostic technique has become particularly attractive. Since penicillin is frequently advocated in the empiric treatment of pneumococcal disease, the early detection of isolates with decreased susceptibility to this agent is of paramount importance. PCR could therefore be used to guide therapy in the early stages of infection through the differential identification of susceptible and resistant genotypes. In primary culture isolates assumed to be *S. pneumoniae*, examination of the three PBP genes together with *lytA* gives predicted values for susceptibility within four hours and could be remarkably valuable for the treatment of infectious diseases caused by *S. pneumoniae*.

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APPENDIX A

Nucleotide and amino acid sequence alignments of the PBP 2X penicillin-binding domain from clinical isolates of *S. pneumoniae*.

The sequence of the *pbp2x* gene and the amino acid sequence of PBP 2X from penicillin-susceptible *S. pneumoniae* R6 are shown at the top. Nucleotide and amino acid sequences are numbered at the end of each line according to data published in reference 199. Amino acid residues differing from the R6 sequence are shaded. Conserved amino acid motifs are boxed and in boldface.

R6 *pbp2x*	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	**288**
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGA	AAG	TAC	**1116**
8099	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGA	AAG	TAC	
3203	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGA	AAG	TAC	
11184	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGA	AAG	TAC	
12244	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGT	AAG	TAC	
14016	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGA	AAG	TAC	
12276	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGT	AAG	TAC	
3996	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGT	AAG	TAC	
11413	Met	Asp	Ala	Phe	Gln	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCT	TTT	CAA	GAG	AAG	GTA	AAA	GGA	AAG	TAC	
14126	Met	Asp	Ala	Phe	Leu	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCC	TTT	CTA	GAA	AAA	GTA	AAA	GGT	AAG	TAT	
3455	Met	Asp	Ala	Phe	Leu	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCC	TTT	CTA	GAA	AAA	GTA	AAA	GGT	AAG	TAT	
742	Met	Asp	Ala	Phe	Leu	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCC	TTT	CTA	GAA	AAA	GTA	AAA	GGT	AAG	TAT	
2848	Met	Asp	Ala	Phe	Leu	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCC	TTT	CTA	GAA	AAA	GTA	AAA	GGT	AAG	TAT	
6363	Met	Asp	Ala	Phe	Leu	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCC	TTT	CTA	GAA	AAA	GTA	AAA	GGT	AAG	TAT	
6190	Met	Asp	Ala	Phe	Leu	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCC	TTT	CTA	GAA	AAA	GTA	AAA	GGT	AAG	TAT	
8111	Met	Asp	Ala	Phe	Leu	Glu	Lys	Val	Lys	Gly	Lys	Tyr	
	ATG	GAT	GCC	TTT	CTA	GAA	AAA	GTA	AAA	GGT	AAG	TAT	

R6 *pbp2x*	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	**300**
	ATG	ACA	GCG	ACT	TTG	GTC	AGT	GCT	AAA	ACA	GGG	GAA	**1152**
8099	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACA	GCG	ACT	TTG	GTC	AGT	GCT	AAA	ACA	GGG	GAA	
3203	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACA	GCG	ACT	TTG	GTC	AGT	GCT	AAA	ACA	GGG	GAA	
11184	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACA	GCG	ACT	TTG	GTC	AGT	GCT	AAA	ACA	GGG	GAA	
12244	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACT	GGT	GAA	
14016	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACA	GCG	ACT	TTG	GTC	AGT	GCT	AAA	ACA	GGG	GAA	
12276	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACT	GGT	GAA	
3996	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACT	GGT	GAA	
11413	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACA	GCA	ACT	TTG	GTC	AGT	GCT	AAA	ACG	GGG	GAA	
14126	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACC	GGT	GAA	
3455	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACC	GGT	GAA	
742	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACC	GGT	GAA	
2848	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACC	GGT	GAA	
6363	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACC	GGT	GAA	
6190	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACC	GGT	GAA	
8111	Met	Thr	Ala	Thr	Leu	Val	Ser	Ala	Lys	Thr	Gly	Glu	
	ATG	ACC	GCG	ACC	TTG	GTC	AGT	GCA	AAG	ACC	GGT	GAA	

R6 *pbp2x*	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	Asp	Ala	**312**
	ATT	CTG	GCA	ACA	ACG	CAA	CGA	CCG	ACC	TTT	GAT	GCA	**1188**
8099	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	Asp	Ala	
	ATT	CTG	GCA	ACA	ACG	CAA	CGA	CCG	ACC	TTT	GAT	GCA	
3203	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	Asp	Ala	
	ATT	CTG	GCA	ACA	ACG	CAA	CGA	CCG	ACC	TTT	GAT	GCA	
11184	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	Asp	Ala	
	ATT	CTG	GCA	ACA	ACG	CAA	CGA	CCG	ACC	TTT	GAT	GCA	
12244	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTT	GCT	ACC	ACC	CAA	CGA	CCG	ACC	TTT	**AAT**	GCA	
14016	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	Asp	Ala	
	ATT	CTG	GCA	ACA	ACG	CAA	CGA	CCG	ACC	TTT	GAT	GCA	
12276	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTT	GCT	ACC	ACC	CAA	CGA	CCG	ACC	TTT	**AAT**	GCA	
3996	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	Asn	Ala	
	ATC	CTT	GCT	ACC	ACC	CAA	CGA	CCG	ACC	TTT	AAT	GCA	
11413	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	Asp	Ala	
	ATT	CTT	GCA	ACG	ACG	CAG	AGA	CCA	ACC	TTC	GAT	GCT	
14126	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTC	GCT	ACC	ACC	CAA	CGA	CCT	ACC	TTT	**AAT**	GCA	
3455	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTC	GCT	ACC	ACC	CAA	CGA	CCT	ACC	TTT	**AAT**	GCA	
742	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTC	GCT	ACC	ACC	CAA	CGA	CCT	ACC	TTT	**AAT**	GCA	
2848	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTC	GCT	ACC	ACC	CAA	CGA	CCT	ACC	TTT	**AAT**	GCA	
6363	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTC	GCT	ACC	ACC	CAA	CGA	CCT	ACC	TTT	**AAT**	GCA	
6190	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTC	GCT	ACC	ACC	CAA	CGA	CCT	ACC	TTT	**AAT**	GCA	
8111	Ile	Leu	Ala	Thr	Thr	Gln	Arg	Pro	Thr	Phe	**Asn**	Ala	
	ATC	CTC	GCT	ACC	ACC	CAA	CGA	CCT	ACC	TTT	**AAT**	GCA	

R6 *pbp2x*	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	**324**
	GAT	ACA	AAA	GAA	GGC	ATT	ACA	GAG	GAC	TTT	GTT	TGG	**1224**
8099	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACA	AAA	GAA	GGC	ATT	ACA	GAG	GAC	TTT	GTT	TGG	
3203	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACA	AAA	GAA	GGC	ATT	ACA	GAG	GAC	TTT	GTT	TGG	
11184	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACA	AAA	GAA	GGC	ATT	ACA	GAG	GAC	TTT	GTT	TGG	
12244	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Lys	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	AAG	GAC	TTT	GTT	TGG	
14016	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACA	AAA	GAA	GGC	ATT	ACA	GAG	GAC	TTT	GTT	TGG	
12276	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Lys	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	AAG	GAC	TTT	GTT	TGG	
3996	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Lys	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	AAG	GAC	TTT	GTT	TGG	
11413	Asp	Thr	Lys	Glu	Gly	Leu	Thr	Lys	Asp	Phe	Val	Trp	
	GAT	ACT	AAG	GAA	GGG	CTG	ACT	AAG	GAC	TTT	GTT	TGG	
14126	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	GAG	GAC	TTT	GTT	TGG	
3455	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	GAG	GAC	TTT	GTT	TGG	
742	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	GAG	GAC	TTT	GTT	TGG	
2848	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	GAG	GAC	TTT	GTT	TGG	
6363	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	GAG	GAC	TTT	GTT	TGG	
6190	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	GAG	GAC	TTT	GTT	TGG	
8111	Asp	Thr	Lys	Glu	Gly	Ile	Thr	Glu	Asp	Phe	Val	Trp	
	GAT	ACT	AAA	GAA	GGA	ATC	ACT	GAG	GAC	TTT	GTT	TGG	

R6 *pbp2x*	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	**336**
	CGT	GAT	ATC	CTT	TAC	CAA	AGT	AAC	TAT	GAG	CCA	GGT	**1260**
8099	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTT	TAC	CAA	AGT	AAC	TAT	GAG	CCA	GGT	
3203	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTT	TAC	CAA	AGT	AAC	TAT	GAG	CCA	GGT	
11184	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTT	TAC	CAA	AGT	AAC	TAT	GAG	CCA	GGT	
12244	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGG	
14016	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTT	TAC	CAA	AGT	AAC	TAT	GAG	CCA	GGT	
12276	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGG	
3996	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGG	
11413	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATC	CTC	TAT	CAA	AGT	AAC	TAT	GAG	CCA	GGG	
14126	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATT	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGA	
3455	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATT	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGA	
742	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATT	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGA	
2848	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATT	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGA	
6363	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATT	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGA	
6190	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATT	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGA	
8111	Arg	Asp	Ile	Leu	Tyr	Gln	Ser	Asn	Tyr	Glu	Pro	Gly	
	CGT	GAT	ATT	CTT	TAT	CAA	AGT	AAC	TAT	GAA	CCA	GGA	

R6 *pbp2x*	**Ser**	**Thr**	**Met**	**Lys**	Val	Met	Met	Leu	Ala	Ala	Ala	Ile	**348**
	TCC	ACT	ATG	AAA	GTG	ATG	ATG	TTG	GCT	GCT	GCT	ATT	**1296**
8099	Ser	Thr	Met	Lys	Val	Met	Thr	Leu	Ala	Ala	Ala	Ile	
	TCC	ACT	ATG	AAA	GTG	ATG	ACG	TTG	GCT	GCT	GCT	ATT	
3203	Ser	Thr	Met	Lys	Val	Met	Met	Leu	Ala	Ala	Ala	Ile	
	TCC	ACT	ATG	AAA	GTG	ATG	ATG	TTG	GCT	GCT	GCT	ATT	
11184	Ser	Thr	Met	Lys	Val	Met	Met	Leu	Ala	Ala	Ala	Ile	
	TCC	ACT	ATG	AAA	GTG	ATG	ATG	TTG	GCT	GCT	GCT	ATT	
12244	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ala	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	GCT	TCT	ATT	
14016	Ser	Thr	Met	Lys	Val	Met	Met	Leu	Ala	Ala	Ala	Ile	
	TCC	ACT	ATG	AAA	GTG	ATG	ATG	TTG	GCT	GCT	GCT	ATT	
12276	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ala	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	GCT	TCT	ATT	
3996	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ala	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	GCT	TCT	ATT	
11413	Ser	Thr	Met	Lys	Val	Met	Thr	Leu	Ala	Ala	Ala	Ile	
	TCA	ACC	ATG	AAG	GTC	ATG	ACG	CTC	GCT	GCT	GCT	ATT	
14126	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ser	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	TCT	TCT	ATT	
3455	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ser	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	TCT	TCT	ATT	
742	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ser	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	TCT	TCT	ATT	
2848	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ser	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	TCT	TCT	ATT	
6363	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ser	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	TCT	TCT	ATT	
6190	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ser	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	TCT	TCT	ATT	
8111	Ser	Ala	Met	Lys	Val	Met	Thr	Leu	Ala	Ser	Ser	Ile	
	TCA	GCC	ATG	AAG	GTT	ATG	ACG	TTA	GCT	TCT	TCT	ATT	

R6 *pbp2x*	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Val	Phe	Asn	**360**
	GAT	AAT	AAT	ACC	TTT	CCA	GGA	GGA	GAA	GTC	TTT	AAT	**1332**
8099	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Val	Phe	Asn	
	GAT	AAT	AAT	ACC	TTT	CCA	GGA	GGA	GAA	GTC	TTT	AAT	
3203	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Val	Phe	Asn	
	GAT	AAT	AAT	ACC	TTT	CCA	GGA	GGA	GAA	GTC	TTT	AAT	
11184	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Val	Phe	Asn	
	GAT	AAT	AAT	ACC	TTT	CCA	GGA	GGA	GAA	GTC	TTT	AAT	
12244	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAC	AAT	ACC	TTC	CCA	GGT	GGA	GAA	TAT	TTC	AAT	
14016	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Val	Phe	Asn	
	GAT	AAT	AAT	ACC	TTT	CCA	GGA	GGA	GAA	GTC	TTT	AAT	
12276	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAC	AAT	ACC	TTC	CCA	GGT	GGA	GAA	TAT	TTC	AAT	
3996	Asp	Asn	Asn	Thr	Phe	Pro	Gly	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAC	AAT	ACC	TTC	CCA	GGT	GGA	GAA	TAT	TTC	AAT	
11413	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	
14126	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	
3455	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	
742	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	
2848	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	
6363	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	
6190	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	
8111	Asp	Asn	Asn	Thr	Phe	Pro	Ser	Gly	Glu	Tyr	Phe	Asn	
	GAT	AAT	AAT	ACC	TTC	CCA	AGT	GGA	GAA	TAC	TTC	AAT	

R6 *pbp2x*	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Ala	Thr	Ile	Arg	**372**
	AGT	AGT	GAG	TTA	AAA	ATT	GCA	GAT	GCC	ACG	ATT	CGA	**1368**
8099	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Ala	Thr	Ile	Arg	
	AGT	AGT	GAG	TTA	AAA	ATT	GCA	GAT	GCC	ACG	ATT	CGA	
3203	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Ala	Thr	Ile	Arg	
	AGT	AGT	GAG	TTA	AAA	ATT	GCA	GAT	GCC	ACG	ATT	CGA	
11184	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Ala	Thr	Ile	Arg	
	AGT	AGT	GAG	TTA	AAA	ATT	GCA	GAT	GCC	ACG	ATT	CGA	
12244	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Val	Thr	Ile	Arg	
	AGC	AGT	GAA	TTA	AAA	ATA	GCG	GAT	GTG	ACG	ATC	CGA	
14016	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Ala	Thr	Ile	Arg	
	AGT	AGT	GAG	TTA	AAA	ATT	GCA	GAT	GCC	ACG	ATT	CGA	
12276	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Val	Thr	Ile	Arg	
	AGC	AGT	GAA	TTA	AAA	ATA	GCG	GAT	GTG	ACG	ATC	CGA	
3996	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Val	Thr	Ile	Arg	
	AGC	AGT	GAA	TTA	AAA	ATA	GCG	GAT	GTG	ACG	ATC	CGA	
11413	Ser	Ser	Glu	Leu	Lys	Ile	Ala	Asp	Val	Thr	Ile	Arg	
	AGC	AGT	GAA	TTA	AAA	ATA	GCG	GAT	GTG	ACG	ATT	CGA	
14126	Ser	Ser	Glu	Phe	Lys	Ile	Ala	Asp	Ala	Thr	Thr	Arg	
	AGC	AGT	GAA	TTC	AAA	ATA	GCG	GAT	GCG	ACG	ACT	CGA	
3455	Ser	Ser	Glu	Phe	Lys	Ile	Ala	Asp	Ala	Thr	Thr	Arg	
	AGC	AGT	GAA	TTC	AAA	ATA	GCG	GAT	GCG	ACG	ACT	CGA	
742	Ser	Ser	Glu	Phe	Lys	Ile	Ala	Asp	Ala	Thr	Thr	Arg	
	AGC	AGT	GAA	TTC	AAA	ATA	GCG	GAT	GCG	ACG	ACT	CGA	
2848	Ser	Ser	Glu	Phe	Lys	Ile	Ala	Asp	Ala	Thr	Thr	Arg	
	AGC	AGT	GAA	TTC	AAA	ATA	GCG	GAT	GCG	ACG	ACT	CGA	
6363	Ser	Ser	Glu	Phe	Lys	Ile	Ala	Asp	Ala	Thr	Thr	Arg	
	AGC	AGT	GAA	TTC	AAA	ATA	GCG	GAT	GCG	ACG	ACT	CGA	
6190	Ser	Ser	Glu	Phe	Lys	Ile	Ala	Asp	Ala	Thr	Thr	Arg	
	AGC	AGT	GAA	TTC	AAA	ATA	GCG	GAT	GCG	ACG	ACT	CGA	
8111	Ser	Ser	Glu	Phe	Lys	Ile	Ala	Asp	Ala	Thr	Thr	Arg	
	AGC	AGT	GAA	TTC	AAA	ATA	GCG	GAT	GCG	ACG	ACT	CGA	

R6 *pbp2x*	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Arg	**384**
	GAT	TGG	GAC	GTT	AAT	GAA	GGA	TTG	ACT	GGT	GGC	AGA	**1404**
8099	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Arg	
	GAT	TGG	GAC	GTT	AAT	GAA	GGA	TTG	ACT	GGT	GGC	AGA	
3203	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Arg	
	GAT	TGG	GAC	GTT	AAT	GAA	GGA	TTG	ACT	GGT	GGC	AGA	
11184	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Arg	
	GAT	TGG	GAC	GTT	AAT	GAA	GGA	TTG	ACT	GGT	GGC	AGA	
12244	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Gly	
	GAT	TGG	GAC	GTT	AAT	GAA	GGT	TTG	ACC	GGC	GGT	GGT	
14016	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Arg	
	GAT	TGG	GAC	GTT	AAT	GAA	GGA	TTG	ACT	GGT	GGC	AGA	
12276	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Gly	
	GAT	TGG	GAC	GTT	AAT	GAA	GGT	TTG	ACC	GGC	GGT	GGT	
3996	Asp	Trp	Asp	Val	Asn	Glu	Gly	Leu	Thr	Gly	Gly	Gly	
	GAT	TGG	GAC	GTT	AAT	GAA	GGT	TTG	ACC	GGC	GGT	GGT	
11413	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Arg	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	AGG	
14126	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Gly	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	GGG	
3455	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Gly	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	GGG	
742	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Gly	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	GGG	
2848	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Gly	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	GGG	
6363	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Gly	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	GGG	
6190	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Gly	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	GGG	
8111	Asp	Trp	Asp	Val	Asn	Asp	Gly	Leu	Thr	Thr	Gly	Gly	
	GAT	TGG	GAT	GTT	AAT	GAT	GGT	TTG	ACT	ACT	GGT	GGG	

R6 *pbp2x*	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	396
	ATG	ATG	ACT	TTT	TCT	CAA	GGT	TTT	GCA	CAC	TCA	AGT	1440
8099	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTT	TCT	CAA	GGT	TTT	GCA	CAC	TCA	AGT	
3203	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTT	TCT	CAA	GGT	TTT	GCA	CAC	TCA	AGT	
11184	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTT	TCT	CAA	GGT	TTT	GCA	CAC	TCA	AGT	
12244	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACC	TTT	TCT	CAA	GGA	TTT	GCT	CAC	TCA	AGT	
14016	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTT	TCT	CAA	GGT	TTT	GCA	CAC	TCA	AGT	
12276	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACC	TTT	TCT	CAA	GGA	TTT	GCT	CAC	TCA	AGT	
3996	Met	Met	Thr	Phe	Ser	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACC	TTT	TCT	CAA	GGA	TTT	GCT	CAC	TCA	AGT	
11413	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	Leu	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CTC	TCC	AGT	
14126	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CAC	TCC	AGT	
3455	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CAC	TCC	AGT	
742	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CAC	TCC	AGT	
2848	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CAC	TCC	AGT	
6363	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CAC	TCC	AGT	
6190	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CAC	TCC	AGT	
8111	Met	Met	Thr	Phe	Leu	Gln	Gly	Phe	Ala	His	Ser	Ser	
	ATG	ATG	ACT	TTC	TTA	CAA	GGT	TTC	GCT	CAC	TCC	AGT	

R6 *pbp2x*	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	408
	AAC	GTT	GGG	ATG	ACC	CTC	CTT	GAG	CAA	AAG	ATG	GGA	1476
8099	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAC	GTT	GGG	ATG	ACC	CTC	CTT	GAG	CAA	AAG	ATG	GGA	
3203	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAC	GTT	GGG	ATG	ACC	CTC	CTT	GAG	CAA	AAG	ATG	GGA	
11184	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAC	GTT	GGG	ATG	ACC	CTC	CTT	GAG	CAA	AAG	ATG	GGA	
12244	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAC	GTT	GGG	ATG	ACC	CTC	CTT	GAG	CAA	AAG	ATG	GGA	
14016	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAC	GTT	GGG	ATG	ACT	CTC	CTT	GAG	CAA	AAG	ATG	GGA	
12276	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAC	GTT	GGG	ATG	ACC	CTC	CTT	GAG	CAA	AAG	ATG	GGA	
3996	Asn	Val	Gly	Met	Thr	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAC	GTT	GGG	ATG	ACC	CTC	CTT	GAG	CAA	AAG	ATG	GGA	
11413	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	
14126	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	
3455	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	
742	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	
2848	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	
6363	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	
6190	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	
8111	Asn	Val	Gly	Met	Ser	Leu	Leu	Glu	Gln	Lys	Met	Gly	
	AAT	GTT	GGA	ATG	AGT	CTA	CTT	GAA	CAA	AAA	ATG	GGA	

R6 *pbp2x*	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	**420**
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGT	TTT	AAA	**1512**
8099	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGT	TTT	AAG	
3203	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGT	TTT	AAA	
11184	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGT	TTT	AAA	
12244	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGC	TTT	AAA	
14016	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGT	TTT	AAA	
12276	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGC	TTT	AAA	
3996	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGC	TTT	AAA	
11413	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Asn	Arg	Phe	Lys	
	GAT	GCT	ACC	TGG	CTT	GAT	TAT	CTT	AAT	CGC	TTT	AAA	
14126	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Lys	Arg	Phe	Lys	
	GAT	GCT	ACT	TGG	TTG	GAT	TAT	CTA	AAA	CGC	TTT	AAA	
3455	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Lys	Arg	Phe	Lys	
	GAT	GCT	ACT	TGG	TTG	GAT	TAT	CTA	AAA	CGC	TTT	AAA	
742	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Lys	Arg	Phe	Lys	
	GAT	GCT	ACT	TGG	TTG	GAT	TAT	CTA	AAA	CGC	TTT	AAA	
2848	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Lys	Arg	Phe	Lys	
	GAT	GCT	ACT	TGG	TTG	GAT	TAT	CTA	AAA	CGC	TTT	AAA	
6363	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Lys	Arg	Phe	Lys	
	GAT	GCT	ACT	TGG	TTG	GAT	TAT	CTA	AAA	CGC	TTT	AAA	
6190	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Lys	Arg	Phe	Lys	
	GAT	GCT	ACT	TGG	TTG	GAT	TAT	CTA	AAA	CGC	TTT	AAA	
8111	Asp	Ala	Thr	Trp	Leu	Asp	Tyr	Leu	Lys	Arg	Phe	Lys	
	GAT	GCT	ACT	TGG	TTG	GAT	TAT	CTA	AAA	CGC	TTT	AAA	

R6 *pbp2x*	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	**432**
	TTT	GGT	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	**1548**
8099	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGA	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	
3203	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGT	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	
11184	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGT	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	
12244	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGT	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	
14016	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGA	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	
12276	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGT	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	
3996	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGT	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACG	GAT	GAG	
11413	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGT	GTT	CCG	ACC	CGT	TTC	GGT	TTG	ACA	GAT	GAG	
14126	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGG	GTT	CCA	ACT	CGC	TTT	GGC	TTG	ACA	GAT	GAA	
3455	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGG	GTT	CCA	ACT	CGC	TTT	GGC	TTG	ACA	GAT	GAA	
742	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGG	GTT	CCA	ACT	CGC	TTT	GGC	TTG	ACA	GAT	GAA	
2848	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGG	GTT	CCA	ACT	CGC	TTT	GGC	TTG	ACA	GAT	GAA	
6363	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGG	GTT	CCA	ACT	CGC	TTT	GGC	TTG	ACA	GAT	GAA	
6190	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGG	GTT	CCA	ACT	CGC	TTT	GGC	TTG	ACA	GAT	GAA	
8111	Phe	Gly	Val	Pro	Thr	Arg	Phe	Gly	Leu	Thr	Asp	Glu	
	TTT	GGG	GTT	CCA	ACT	CGC	TTT	GGC	TTG	ACA	GAT	GAA	

R6 *pbp2x*	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	**444**
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	**1584**
8099	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	
3203	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	
11184	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	
12244	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	
14016	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	
12276	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	
3996	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATT	GTC	AAC	
11413	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Asn	
	TAT	GCT	GGT	CAG	CTT	CCT	GCG	GAT	AAT	ATA	GTT	AAC	
14126	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Ser	
	TAC	GCT	GGT	CAA	CTT	CCA	GCT	GAT	AAT	ATT	GTT	AGT	
3455	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Ser	
	TAC	GCT	GGT	CAA	CTT	CCA	GCT	GAT	AAT	ATT	GTT	AGT	
742	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Ser	
	TAC	GCT	GGT	CAA	CTT	CCA	GCT	GAT	AAT	ATT	GTT	AGT	
2848	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Ser	
	TAC	GCT	GGT	CAA	CTT	CCA	GCT	GAT	AAT	ATT	GTT	AGT	
6363	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Ser	
	TAC	GCT	GGT	CAA	CTT	CCA	GCT	GAT	AAT	ATT	GTT	AGT	
6190	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Ser	
	TAC	GCT	GGT	CAA	CTT	CCA	GCT	GAT	AAT	ATT	GTT	AGT	
8111	Tyr	Ala	Gly	Gln	Leu	Pro	Ala	Asp	Asn	Ile	Val	Ser	
	TAC	GCT	GGT	CAA	CTT	CCA	GCT	GAT	AAT	ATT	GTT	AGT	

R6 *pbp2x*	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	**456**
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	**1620**
8099	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	
3203	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	
11184	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	
12244	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	
14016	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	
12276	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	
3996	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGA	CAA	GGG	ATT	TCA	GTG	
11413	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCG	CAA	AGC	TCA	TTT	GGG	CAA	GGG	ATT	TCA	GTG	
14126	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCT	CAA	AGC	TCA	TTT	GGG	CAA	GGA	ATT	TCA	GTG	
3455	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCT	CAA	AGC	TCA	TTT	GGG	CAA	GGA	ATT	TCA	GTG	
742	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCT	CAA	AGC	TCA	TTT	GGG	CAA	GGA	ATT	TCA	GTG	
2848	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCT	CAA	AGC	TCA	TTT	GGG	CAA	GGA	ATT	TCA	GTG	
6363	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCT	CAA	AGC	TCA	TTT	GGG	CAA	GGA	ATT	TCA	GTG	
6190	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCT	CAA	AGC	TCA	TTT	GGG	CAA	GGA	ATT	TCA	GTG	
8111	Ile	Ala	Gln	Ser	Ser	Phe	Gly	Gln	Gly	Ile	Ser	Val	
	ATT	GCT	CAA	AGC	TCA	TTT	GGG	CAA	GGA	ATT	TCA	GTG	

Strain													
R6 *pbp2x*	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	**468**
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACA	GCT	ATT	**1656**
8099	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACA	GCT	ATT	
3203	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACA	GCT	ATT	
11184	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACA	GCT	ATT	
12244	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACC	GCT	ATT	
14016	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACA	CAA	ATG	ATT	CGT	GCC	TTT	ACA	GCT	ATT	
12276	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACC	GCT	ATT	
3996	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACC	GCT	ATT	
11413	Thr	Gln	Thr	Gln	Met	Ile	Arg	Ala	Phe	Thr	Ala	Ile	
	ACC	CAG	ACG	CAA	ATG	ATT	CGT	GCC	TTT	ACA	GCT	ATT	
14126	Thr	Gln	Thr	Gln	Met	Leu	Arg	Ala	Phe	Thr	Ala	Ile	
	ACA	CAA	ACA	CAA	ATG	CTT	CGT	GCC	TTT	ACA	GCT	ATT	
3455	Thr	Gln	Thr	Gln	Met	Leu	Arg	Ala	Phe	Thr	Ala	Ile	
	ACA	CAA	ACA	CAA	ATG	CTT	CGT	GCC	TTT	ACA	GCT	ATT	
742	Thr	Gln	Thr	Gln	Met	Leu	Arg	Ala	Phe	Thr	Ala	Ile	
	ACA	CAA	ACA	CAA	ATG	CTT	CGT	GCC	TTT	ACA	GCT	ATT	
2848	Thr	Gln	Thr	Gln	Met	Leu	Arg	Ala	Phe	Thr	Ala	Ile	
	ACA	CAA	ACA	CAA	ATG	CTT	CGT	GCC	TTT	ACA	GCT	ATT	
6363	Thr	Gln	Thr	Gln	Met	Leu	Arg	Ala	Phe	Thr	Ala	Ile	
	ACA	CAA	ACA	CAA	ATG	CTT	CGT	GCC	TTT	ACA	GCT	ATT	
6190	Thr	Gln	Thr	Gln	Met	Leu	Arg	Ala	Phe	Thr	Ala	Ile	
	ACA	CAA	ACA	CAA	ATG	CTT	CGT	GCC	TTT	ACA	GCT	ATT	
8111	Thr	Gln	Thr	Gln	Met	Leu	Arg	Ala	Phe	Thr	Ala	Ile	
	ACA	CAA	ACA	CAA	ATG	CTT	CGT	GCC	TTT	ACA	GCT	ATT	

Strain													
R6 *pbp2x*	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	**480**
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	**1692**
8099	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
3203	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
11184	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
12244	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
14016	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
12276	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
3996	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
11413	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAC	GGT	GTC	ATG	CTG	GAG	CCT	AAA	TTT	ATT	
14126	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAT	GGA	GTT	ATG	CTG	GAG	CCA	AAA	TTT	ATA	
3455	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAT	GGA	GTT	ATG	CTG	GAG	CCA	AAA	TTT	ATA	
742	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAT	GGA	GTT	ATG	CTG	GAG	CCA	AAA	TTT	ATA	
2848	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAT	GGA	GTT	ATG	CTG	GAG	CCA	AAA	TTT	ATA	
6363	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAT	GGA	GTT	ATG	CTG	GAG	CCA	AAA	TTT	ATA	
6190	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAT	GGA	GTT	ATG	CTG	GAG	CCA	AAA	TTT	ATA	
8111	Ala	Asn	Asp	Gly	Val	Met	Leu	Glu	Pro	Lys	Phe	Ile	
	GCT	AAT	GAT	GGA	GTT	ATG	CTG	GAG	CCA	AAA	TTT	ATA	

Strain													
R6 *pbp2x*	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	**492**
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	**1728**
8099	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
3203	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
11184	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
12244	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
14016	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
12276	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
3996	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
11413	Ser	Ala	Ile	Tyr	Asp	Pro	Asn	Asp	Gln	Thr	Ala	Arg	
	AGT	GCC	ATT	TAT	GAT	CCA	AAT	GAT	CAA	ACT	GCT	CGG	
14126	Ser	Ala	Ile	Tyr	Asp	Thr	Asn	Asn	Gln	Ser	Val	Arg	
	AGT	GCT	ATT	TAT	GAT	ACT	AAC	AAT	CAG	TCT	GTA	CGT	
3455	Ser	Ala	Ile	Tyr	Asp	Thr	Asn	Asn	Gln	Ser	Val	Arg	
	AGT	GCT	ATT	TAT	GAT	ACT	AAC	AAT	CAG	TCT	GTA	CGT	
742	Ser	Ala	Ile	Tyr	Asp	Thr	Asn	Asn	Gln	Ser	Val	Arg	
	AGT	GCT	ATT	TAT	GAT	ACT	AAC	AAT	CAG	TCT	GTA	CGT	
2848	Ser	Ala	Ile	Tyr	Asp	Thr	Asn	Asn	Gln	Ser	Val	Arg	
	AGT	GCT	ATT	TAT	GAT	ACT	AAC	AAT	CAG	TCT	GTA	CGT	
6363	Ser	Ala	Ile	Tyr	Asp	Thr	Asn	Asn	Gln	Ser	Val	Arg	
	AGT	GCT	ATT	TAT	GAT	ACT	AAC	AAT	CAG	TCT	GTA	CGT	
6190	Ser	Ala	Ile	Tyr	Asp	Thr	Asn	Asn	Gln	Ser	Val	Arg	
	AGT	GCT	ATT	TAT	GAT	ACT	AAC	AAT	CAG	TCT	GTA	CGT	
8111	Ser	Ala	Ile	Tyr	Asp	Thr	Asn	Asn	Gln	Ser	Val	Arg	
	AGT	GCT	ATT	TAT	GAT	ACT	AAC	AAT	CAG	TCT	GTA	CGT	

Strain													
R6 *pbp2x*	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	**504**
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	**1764**
8099	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
3203	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
11184	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
12244	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
14016	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
12276	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
3996	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
11413	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAA	TCT	CAA	AAA	GAA	ATT	GTG	GGA	AAT	CCT	GTT	TCT	
14126	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAG	TCA	CAA	AAA	GAA	ATA	GTA	GGA	AAT	CCT	GTT	TCC	
3455	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAG	TCA	CAA	AAA	GAA	ATA	GTA	GGA	AAT	CCT	GTT	TCC	
742	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAG	TCA	CAA	AAA	GAA	ATA	GTA	GGA	AAT	CCT	GTT	TCC	
2848	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAG	TCA	CAA	AAA	GAA	ATA	GTA	GGA	AAT	CCT	GTT	TCC	
6363	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAG	TCA	CAA	AAA	GAA	ATA	GTA	GGA	AAT	CCT	GTT	TCC	
6190	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAG	TCA	CAA	AAA	GAA	ATA	GTA	GGA	AAT	CCT	GTT	TCC	
8111	Lys	Ser	Gln	Lys	Glu	Ile	Val	Gly	Asn	Pro	Val	Ser	
	AAG	TCA	CAA	AAA	GAA	ATA	GTA	GGA	AAT	CCT	GTT	TCC	

R6 *pbp2x*	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	**516**
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	**1800**
8099	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
3203	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
11184	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
12244	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
14016	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
12276	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
3996	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
11413	Lys	Asp	Ala	Ala	Ser	Leu	Thr	Arg	Thr	Asn	Met	Val	
	AAA	GAT	GCA	GCT	AGT	CTA	ACT	CGG	ACT	AAC	ATG	GTT	
14126	Lys	Gln	Ala	Ala	Ser	Thr	Thr	Arg	Asn	His	Met	Ile	
	AAA	GAG	GCA	GCA	AGC	ACA	ACT	CGA	AAT	CAC	ATG	ATC	
3455	Lys	Gln	Ala	Ala	Ser	Thr	Thr	Arg	Asn	His	Met	Ile	
	AAA	GAG	GCA	GCA	AGC	ACA	ACT	CGA	AAT	CAC	ATG	ATC	
742	Lys	Gln	Ala	Ala	Ser	Thr	Thr	Arg	Asn	His	Met	Ile	
	AAA	GAG	GCA	GCA	AGC	ACA	ACT	CGA	AAT	CAC	ATG	ATC	
2848	Lys	Gln	Ala	Ala	Ser	Thr	Thr	Arg	Asn	His	Met	Ile	
	AAA	GAG	GCA	GCA	AGC	ACA	ACT	CGA	AAT	CAC	ATG	ATC	
6363	Lys	Gln	Ala	Ala	Ser	Thr	Thr	Arg	Asn	His	Met	Ile	
	AAA	GAG	GCA	GCA	AGC	ACA	ACT	CGA	AAT	CAC	ATG	ATC	
6190	Lys	Gln	Ala	Ala	Ser	Thr	Thr	Arg	Asn	His	Met	Ile	
	AAA	GAG	GCA	GCA	AGC	ACA	ACT	CGA	AAT	CAC	ATG	ATC	
8111	Lys	Gln	Ala	Ala	Ser	Thr	Thr	Arg	Asn	His	Met	Ile	
	AAA	GAG	GCA	GCA	AGC	ACA	ACT	CGA	AAT	CAC	ATG	ATC	

R6 *pbp2x*	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	**528**
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	**1836**
8099	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
3203	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
11184	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
12244	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
14016	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
12276	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
3996	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
11413	Leu	Val	Gly	Thr	Asp	Pro	Val	Tyr	Gly	Thr	Met	Tyr	
	TTG	GTA	GGG	ACG	GAT	CCG	GTT	TAT	GGA	ACC	ATG	TAT	
14126	Leu	Val	Gly	Thr	Asp	Pro	Leu	Tyr	Gly	Thr	Met	Tyr	
	TTA	GTT	GGG	ACG	GAC	CCT	CTA	TAT	GGA	ACT	ATG	TAT	
3455	Leu	Val	Gly	Thr	Asp	Pro	Leu	Tyr	Gly	Thr	Met	Tyr	
	TTA	GTT	GGG	ACG	GAC	CCT	CTA	TAT	GGA	ACT	ATG	TAT	
742	Leu	Val	Gly	Thr	Asp	Pro	Leu	Tyr	Gly	Thr	Met	Tyr	
	TTA	GTT	GGG	ACG	GAC	CCT	CTA	TAT	GGA	ACT	ATG	TAT	
2848	Leu	Val	Gly	Thr	Asp	Pro	Leu	Tyr	Gly	Thr	Met	Tyr	
	TTA	GTT	GGG	ACG	GAC	CCT	CTA	TAT	GGA	ACT	ATG	TAT	
6363	Leu	Val	Gly	Thr	Asp	Pro	Leu	Tyr	Gly	Thr	Met	Tyr	
	TTA	GTT	GGG	ACG	GAC	CCT	CTA	TAT	GGA	ACT	ATG	TAT	
6190	Leu	Val	Gly	Thr	Asp	Pro	Leu	Tyr	Gly	Thr	Met	Tyr	
	TTA	GTT	GGG	ACG	GAC	CCT	CTA	TAT	GGA	ACT	ATG	TAT	
8111	Leu	Val	Gly	Thr	Asp	Pro	Leu	Tyr	Gly	Thr	Met	Tyr	
	TTA	GTT	GGG	ACG	GAC	CCT	CTA	TAT	GGA	ACT	ATG	TAT	

R6 *pbp2x*	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	**540**
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	**1872**
8099	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
3203	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
11184	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
12244	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
14016	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
12276	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
3996	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
11413	Asn	His	Ser	Thr	Gly	Lys	Pro	Thr	Val	Thr	Val	Pro	
	AAC	CAC	AGC	ACA	GGC	AAG	CCA	ACT	GTA	ACT	GTT	CCT	
14126	Asn	His	Tyr	Thr	Gly	Lys	Pro	Ile	Ile	Thr	Val	Pro	
	AAT	CAC	TAC	ACA	GGA	AAG	CCA	ATT	ATA	ACA	GTT	CCT	
3455	Asn	His	Tyr	Thr	Gly	Lys	Pro	Ile	Ile	Thr	Val	Pro	
	AAT	CAC	TAC	ACA	GGA	AAG	CCA	ATT	ATA	ACA	GTT	CCT	
742	Asn	His	Tyr	Thr	Gly	Lys	Pro	Ile	Ile	Thr	Val	Pro	
	AAT	CAC	TAC	ACA	GGA	AAG	CCA	ATT	ATA	ACA	GTT	CCT	
2848	Asn	His	Tyr	Thr	Gly	Lys	Pro	Ile	Ile	Thr	Val	Pro	
	AAT	CAC	TAC	ACA	GGA	AAG	CCA	ATT	ATA	ACA	GTT	CCT	
6363	Asn	His	Tyr	Thr	Gly	Lys	Pro	Ile	Ile	Thr	Val	Pro	
	AAT	CAC	TAC	ACA	GGA	AAG	CCA	ATT	ATA	ACA	GTT	CCT	
6190	Asn	His	Tyr	Thr	Gly	Lys	Pro	Ile	Ile	Thr	Val	Pro	
	AAT	CAC	TAC	ACA	GGA	AAG	CCA	ATT	ATA	ACA	GTT	CCT	
8111	Asn	His	Tyr	Thr	Gly	Lys	Pro	Ile	Ile	Thr	Val	Pro	
	AAT	CAC	TAC	ACA	GGA	AAG	CCA	ATT	ATA	ACA	GTT	CCT	

R6 *pbp2x*	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	**552**
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	**1908**
8099	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
3203	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
11184	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
12244	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
14016	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
12276	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
3996	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
11413	Gly	Gln	Asn	Val	Ala	Leu	Lys	Ser	Gly	Thr	Ala	Gln	
	GGG	CAA	AAT	GTA	GCC	CTC	AAG	TCT	GGT	ACG	GCT	CAG	
14126	Gly	Gln	Asn	Val	Ala	Val	Lys	Ser	Gly	Thr	Ala	Gln	
	GGA	CAA	AAT	GTA	GCA	GTT	AAA	TCC	GGT	ACG	GCT	CAA	
3455	Gly	Gln	Asn	Val	Ala	Val	Lys	Ser	Gly	Thr	Ala	Gln	
	GGA	CAA	AAT	GTA	GCA	GTT	AAA	TCC	GGT	ACG	GCT	CAA	
742	Gly	Gln	Asn	Val	Ala	Val	Lys	Ser	Gly	Thr	Ala	Gln	
	GGA	CAA	AAT	GTA	GCA	GTT	AAA	TCC	GGT	ACG	GCT	CAA	
2848	Gly	Gln	Asn	Val	Ala	Val	Lys	Ser	Gly	Thr	Ala	Gln	
	GGA	CAA	AAT	GTA	GCA	GTT	AAA	TCC	GGT	ACG	GCT	CAA	
6363	Gly	Gln	Asn	Val	Ala	Val	Lys	Ser	Gly	Thr	Ala	Gln	
	GGA	CAA	AAT	GTA	GCA	GTT	AAA	TCC	GGT	ACG	GCT	CAA	
6190	Gly	Gln	Asn	Val	Ala	Val	Lys	Ser	Gly	Thr	Ala	Gln	
	GGA	CAA	AAT	GTA	GCA	GTT	AAA	TCC	GGT	ACG	GCT	CAA	
8111	Gly	Gln	Asn	Val	Ala	Val	Lys	Ser	Gly	Thr	Ala	Gln	
	GGA	CAA	AAT	GTA	GCA	GTT	AAA	TCC	GGT	ACG	GCT	CAA	

R6 *pbp2x*	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	**564**
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	**1944**
8099	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
3203	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
11184	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
12244	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
14016	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
12276	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
3996	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
11413	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATT	GCT	GAC	GAG	AAA	AAT	GGT	GGT	TAT	CTA	GTC	GGG	
14126	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATC	GCT	GAT	GAG	AAA	AAT	GGA	GGA	TAC	TTG	GTT	GGT	
3455	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATC	GCT	GAT	GAG	AAA	AAT	GGA	GGA	TAC	TTG	GTT	GGT	
742	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATC	GCT	GAT	GAG	AAA	AAT	GGA	GGA	TAC	TTG	GTT	GGT	
2848	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATC	GCT	GAT	GAG	AAA	AAT	GGA	GGA	TAC	TTG	GTT	GGT	
6363	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATC	GCT	GAT	GAG	AAA	AAT	GGA	GGA	TAC	TTG	GTT	GGT	
6190	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATC	GCT	GAT	GAG	AAA	AAT	GGA	GGA	TAC	TTG	GTT	GGT	
8111	Ile	Ala	Asp	Glu	Lys	Asn	Gly	Gly	Tyr	Leu	Val	Gly	
	ATC	GCT	GAT	GAG	AAA	AAT	GGA	GGA	TAC	TTG	GTT	GGT	

R6 *pbp2x*	Leu	Thr	Asp	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	**576**
	TTA	ACC	GAC	TAT	ATT	TTC	TCG	GCT	GTA	TCG	ATG	AGT	**1980**
8099	Leu	Thr	Asp	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	TTA	ACC	GAC	TAT	ATT	TTC	TCG	GCT	GTA	TCG	ATG	AGT	
3203	Val	Thr	Asp	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	GTA	ACC	GAC	TAT	ATT	TTC	TCG	GCT	GTA	TCG	ATG	AGT	
11184	Val	Thr	Asp	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	GTA	ACC	GAC	TAT	ATT	TTC	TCG	GCT	GTA	TCG	ATG	AGT	
12244	Leu	Thr	Asn	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	TTA	ACT	AAC	TAT	ATT	TTT	TCG	GCT	GTA	TCG	ATG	AGT	
14016	Leu	Thr	Asp	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	TTA	ACC	GAC	TAT	ATT	TTC	TCG	GCT	GTA	TCG	ATG	AGT	
12276	Leu	Thr	Asn	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	TTA	ACT	AAC	TAT	ATT	TTT	TCG	GCT	GTA	TCG	ATG	AGT	
3996	Leu	Thr	Asn	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	TTA	ACT	AAC	TAT	ATT	TTT	TCG	GCT	GTA	TCG	ATG	AGT	
11413	Leu	Thr	Asn	Tyr	Ile	Phe	Ser	Ala	Val	Ser	Met	Ser	
	TTA	ACC	AAC	TAT	ATT	TTC	TCG	GCT	GTA	TCG	ATG	AGT	
14126	Ser	Thr	Asn	Tyr	Ile	Phe	Ser	Val	Val	Thr	Met	Asn	
	TCT	ACC	AAT	TAT	ATT	TTC	TCA	GTT	GTG	ACT	ATG	AAT	
3455	Ser	Thr	Asn	Tyr	Ile	Phe	Ser	Val	Val	Thr	Met	Asn	
	TCT	ACC	AAT	TAT	ATT	TTC	TCA	GTT	GTG	ACT	ATG	AAT	
742	Ser	Thr	Asn	Tyr	Ile	Phe	Ser	Val	Val	Thr	Met	Asn	
	TCT	ACC	AAT	TAT	ATT	TTC	TCA	GTT	GTG	ACT	ATG	AAT	
2848	Ser	Thr	Asn	Tyr	Ile	Phe	Ser	Val	Val	Thr	Met	Asn	
	TCT	ACC	AAT	TAT	ATT	TTC	TCA	GTT	GTG	ACT	ATG	AAT	
6363	Ser	Thr	Asn	Tyr	Ile	Phe	Ser	Val	Val	Thr	Met	Asn	
	TCT	ACC	AAT	TAT	ATT	TTC	TCA	GTT	GTG	ACT	ATG	AAT	
6190	Ser	Thr	Asn	Tyr	Ile	Phe	Ser	Val	Val	Thr	Met	Asn	
	TCT	ACC	AAT	TAT	ATT	TTC	TCA	GTT	GTG	ACT	ATG	AAT	
8111	Ser	Thr	Asn	Tyr	Ile	Phe	Ser	Val	Val	Thr	Met	Asn	
	TCT	ACC	AAT	TAT	ATT	TTC	TCA	GTT	GTG	ACT	ATG	AAT	

R6 *pbp2x*	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	**588**
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	**2016**
8099	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
3203	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
11184	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
12244	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
14016	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
12276	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
3996	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
11413	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCG	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTG	ACG	
14126	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCT	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTA	ACG	
3455	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCT	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTA	ACG	
742	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCT	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTA	ACG	
2848	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCT	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTA	ACG	
6363	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCT	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTA	ACG	
6190	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCT	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTA	ACG	
8111	Pro	Ala	Glu	Asn	Pro	Asp	Phe	Ile	Leu	Tyr	Val	Thr	
	CCT	GCT	GAA	AAT	CCT	GAT	TTT	ATC	TTG	TAT	GTA	ACG	

R6 *pbp2x*	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	**600**
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	**2052**
8099	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
3203	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
11184	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
12244	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
14016	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
12276	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
3996	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
11413	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTC	CAA	CAA	CCT	GAA	CAT	TAT	TCA	GGT	ATT	CAG	TTG	
14126	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTT	CAA	CAG	CCT	GAG	CAT	TAT	TCA	GGT	ATC	CAG	TTG	
3455	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTT	CAA	CAG	CCT	GAG	CAT	TAT	TCA	GGT	ATC	CAG	TTG	
742	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTT	CAA	CAG	CCT	GAG	CAT	TAT	TCA	GGT	ATC	CAG	TTG	
2848	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTT	CAA	CAG	CCT	GAG	CAT	TAT	TCA	GGT	ATC	CAG	TTG	
6363	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTT	CAA	CAG	CCT	GAG	CAT	TAT	TCA	GGT	ATC	CAG	TTG	
6190	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTT	CAA	CAG	CCT	GAG	CAT	TAT	TCA	GGT	ATC	CAG	TTG	
8111	Val	Gln	Gln	Pro	Glu	His	Tyr	Ser	Gly	Ile	Gln	Leu	
	GTT	CAA	CAG	CCT	GAG	CAT	TAT	TCA	GGT	ATC	CAG	TTG	

R6 *pbp2x*	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	**609**
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	**2079**
8099	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
3203	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
11184	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
12244	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
14016	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
12276	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
3996	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
11413	Gly	Glu	Phe	Ala	Asn	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	AAT	CCT	ATC	TTG	GAG	
14126	Gly	Glu	Phe	Ala	Thr	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	ACC	CCA	ATC	TTG	GAG	
3455	Gly	Glu	Phe	Ala	Thr	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	ACC	CCA	ATC	TTG	GAG	
742	Gly	Glu	Phe	Ala	Thr	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	ACC	CCA	ATC	TTG	GAG	
2848	Gly	Glu	Phe	Ala	Thr	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	ACC	CCA	ATC	TTG	GAG	
6363	Gly	Glu	Phe	Ala	Thr	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	ACC	CCA	ATC	TTG	GAG	
6190	Gly	Glu	Phe	Ala	Thr	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	ACC	CCA	ATC	TTG	GAG	
8111	Gly	Glu	Phe	Ala	Thr	Pro	Ile	Leu	Glu	
	GGA	GAA	TTT	GCC	ACC	CCA	ATC	TTG	GAG	

APPENDIX B

Nucleotide and amino acid sequence alignments of the PBP 2B penicillin-binding domain from clinical isolates of *S. pneumoniae*.

The sequence of the *pbp2b* gene and the amino acid sequence of PBP 2B from penicillin-susceptible *S. pneumoniae* R6 are shown at the top. Nucleotide and amino acid sequences are numbered at the end of each line according to data published in reference 200. Amino acid residues differing from the R6 sequence are shaded. Conserved amino acid motifs are boxed and in boldface.

R6 *pbp2b*	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Glu	Asn	Gly	Gly	Ala	**336**
	AGT	TAT	TTC	AAT	TCT	GAG	CTA	GAA	AAT	GGT	GGA	GCC	**1240**
8099	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Glu	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GAA	AAT	GGT	GGA	GCC	
3203	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Glu	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GAA	AAT	GGT	GGA	GCC	
11184	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Glu	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCT	GAG	CTA	GAA	AAT	GGT	GGA	GCC	
12244	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Glu	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GAA	AAT	GGT	GGA	GCC	
14016	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Glu	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCT	GAG	CTA	GAA	AAT	GGT	GGA	GCC	
12276	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCA	GAG	TTG	GGA	AAT	GGT	GGA	GCC	
3996	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCA	GAG	TTG	GGA	AAT	GGT	GGA	GCC	
11413	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCA	GAG	TTG	GGA	AAT	GGT	GGA	GCC	
14126	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GGA	AAT	GGT	GGA	GCC	
3455	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GGA	AAT	GGT	GGA	GCT	
742	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GGA	AAT	GGT	GGA	GCT	
2848	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GGA	AAT	GGT	GGA	GCT	
6363	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GGA	AAT	GGT	GGA	GCT	
6190	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GGA	AAT	GGT	GGA	GCT	
8111	Ser	Tyr	Phe	Asn	Ser	Glu	Leu	Gly	Asn	Gly	Gly	Ala	
	AGT	TAT	TTC	AAT	TCC	GAG	CTA	GGA	AAT	GGT	GGA	GCT	

R6 *pbp2b*	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	**348**
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	**1276**
8099	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
3203	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
11184	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
12244	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
14016	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
12276	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
3996	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
11413	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
14126	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAG	TAT	TCT	GAG	GGT	GTG	TAT	GCA	GTC	GCC	CTT	AAC	
3455	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAA	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
742	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAA	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
2848	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAA	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
6363	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAA	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
6190	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAA	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	
8111	Lys	Tyr	Ser	Glu	Gly	Val	Tyr	Ala	Val	Ala	Leu	Asn	
	AAA	TAT	TCT	GAA	GGT	GTC	TAT	GCA	GTC	GCC	CTT	AAC	

R6 *pbp2b*	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	**360**
	CCA	AAA	ACA	GGT	GCG	GTT	TTG	TCT	ATG	TCA	GGG	ATT	**1312**
8099	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCG	GTT	TTG	TCT	ATG	TCA	GGG	ATT	
3203	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCG	GTT	TTG	TCT	ATG	TCA	GGG	ATT	
11184	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCG	GTT	TTG	TCT	ATG	TCA	GGG	ATT	
12244	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCG	GTT	TTG	TCT	ATG	TCA	GGG	ATT	
14016	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCG	GTT	TTG	TCT	ATG	TCA	GGG	ATT	
12276	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	
3996	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	
11413	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTG	TCT	ATG	TCA	GGG	ATT	
14126	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Leu	
	CCC	AAA	ACA	GGT	GCT	GTT	TTG	TCT	ATG	TCA	GGA	CTC	
3455	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	
742	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	
2848	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	
6363	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	
6190	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	
8111	Pro	Lys	Thr	Gly	Ala	Val	Leu	Ser	Met	Ser	Gly	Ile	
	CCA	AAA	ACA	GGT	GCT	GTT	TTA	TCC	ATG	TCA	GGG	ATC	

R6 *pbp2b*	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	**372**
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCT	GAT	**1348**
8099	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCT	GAT	
3203	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCT	GAT	
11184	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCT	GAT	
12244	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCT	GAT	
14016	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCT	GAT	
12276	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCA	GAT	
3996	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCA	GAT	
11413	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	TTG	AAA	ACG	GGA	GAG	TTG	ACG	CCG	GAT	
14126	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	CTG	AAA	ACG	GGA	GAG	TTG	ACT	CCT	GAT	
3455	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	CTG	AAA	ACG	GGA	GAG	TTG	ACT	CCT	GAT	
742	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	CTG	AAA	ACG	GGA	GAG	TTG	ACT	CCT	GAT	
2848	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	CTG	AAA	ACG	GGA	GAG	TTG	ACT	CCT	GAT	
6363	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	CTG	AAA	ACG	GGA	GAG	TTG	ACT	CCT	GAT	
6190	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	CTG	AAA	ACG	GGA	GAG	TTG	ACT	CCT	GAT	
8111	Lys	His	Asp	Leu	Lys	Thr	Gly	Glu	Leu	Thr	Pro	Asp	
	AAA	CAT	GAC	CTG	AAA	ACG	GGA	GAG	TTG	ACT	CCT	GAT	

R6 *pbp2b*	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	**384**
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	**1384**
8099	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
3203	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
11184	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
12244	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
14016	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
12276	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
3996	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
11413	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTT	CCA	GGT	
14126	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTC	CCA	GGT	
3455	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTC	CCA	GGT	
742	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTC	CCA	GGT	
2848	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTC	CCA	GGT	
6363	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTC	CCA	GGT	
6190	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTC	CCA	GGT	
8111	Ser	Leu	Gly	Thr	Val	Thr	Asn	Val	Phe	Val	Pro	Gly	
	TCC	TTG	GGA	ACG	GTA	ACC	AAT	GTC	TTT	GTC	CCA	GGT	

R6 *pbp2b*	**Ser**	**Val**	**Val**	**Lys**	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	**396**
	TCG	GTT	GTC	AAG	GCG	GCG	ACC	ATC	AGC	TCA	GGT	TGG	**1420**
8099	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
3203	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
11184	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
12244	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
14016	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
12276	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACT	ATC	AGC	TCA	GGT	TGG	
3996	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACT	ATC	AGC	TCA	GGT	TGG	
11413	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCG	GCG	ACC	ATC	AGC	TCA	GGC	TGG	
14126	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTT	AAG	GCC	GCT	ACC	ATC	AGC	TCA	GGT	TGG	
3455	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCT	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
742	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCT	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
2848	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCT	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
6363	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCT	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
6190	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCT	GCG	ACC	ATC	AGC	TCA	GGT	TGG	
8111	Ser	Val	Val	Lys	Ala	Ala	Thr	Ile	Ser	Ser	Gly	Trp	
	TCG	GTT	GTC	AAG	GCT	GCG	ACC	ATC	AGC	TCA	GGT	TGG	

R6 *pbp2b*	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	**408**
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAC	CAG	ACC	TTG	ACA	**1456**
8099	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAC	CAG	ACC	TTG	ACA	
3203	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAC	CAG	ACC	TTG	ACA	
11184	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAC	CAG	ACC	TTG	ACA	
12244	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAC	CAG	ACC	TTG	ACA	
14016	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAC	CAG	ACC	TTG	ACA	
12276	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAT	CAG	ACC	TTG	ACA	
3996	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAT	CAG	ACC	TTG	ACA	
11413	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGA	GTC	TTG	TCA	GGA	AAT	CAG	ACC	TTG	ACA	
14126	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGT	GTT	TTA	TCA	GGA	AAC	CAA	ACC	TTA	ACA	
3455	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGT	GTT	TTA	TCA	GGA	AAC	CAA	ACC	TTA	ACA	
742	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGT	GTT	TTA	TCA	GGA	AAC	CAA	ACC	TTA	ACA	
2848	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGT	GTT	TTA	TCA	GGA	AAC	CAA	ACC	TTA	ACA	
6363	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGT	GTT	TTA	TCA	GGA	AAC	CAA	ACC	TTA	ACA	
6190	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGT	GTT	TTA	TCA	GGA	AAC	CAA	ACC	TTA	ACA	
8111	Glu	Asn	Gly	Val	Leu	Ser	Gly	Asn	Gln	Thr	Leu	Thr	
	GAA	AAT	GGT	GTT	TTA	TCA	GGA	AAC	CAA	ACC	TTA	ACA	

R6 *pbp2b*	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	**420**
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	**1492**
8099	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	
3203	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	
11184	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	
12244	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	
14016	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	
12276	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	
3996	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCC	ATC	
11413	Asp	Gln	Ser	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAC	CAG	TCC	ATT	GTC	TTC	CAA	GGT	TCA	GCT	CCA	ATT	
14126	Asp	Gln	Pro	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAT	CAG	CCT	ATT	GTT	TTC	CAA	GGT	TCA	GCT	CCA	ATT	
3455	Asp	Gln	Pro	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAT	CAG	CCT	ATT	GTT	TTC	CAA	GGT	TCA	GCT	CCA	ATT	
742	Asp	Gln	Pro	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAT	CAG	CCT	ATT	GTT	TTC	CAA	GGT	TCA	GCT	CCA	ATT	
2848	Asp	Gln	Pro	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAT	CAG	CCT	ATT	GTT	TTC	CAA	GGT	TCA	GCT	CCA	ATT	
6363	Asp	Gln	Pro	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAT	CAG	CCT	ATT	GTT	TTC	CAA	GGT	TCA	GCT	CCA	ATT	
6190	Asp	Gln	Pro	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAT	CAG	CCT	ATT	GTT	TTC	CAA	GGT	TCA	GCT	CCA	ATT	
8111	Asp	Gln	Pro	Ile	Val	Phe	Gln	Gly	Ser	Ala	Pro	Ile	
	GAT	CAG	CCT	ATT	GTT	TTC	CAA	GGT	TCA	GCT	CCA	ATT	

R6 *pbp2b*	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	**432**
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	**1528**
8099	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	
3203	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	
11184	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	
12244	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	
14016	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	
12276	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	
3996	Asn	Ser	Trp	Tyr	Thr	Gln	Ala	Tyr	Gly	Ser	Phe	Pro	
	AAT	TCT	TGG	TAT	ACT	CAG	GCT	TAC	GGT	TCA	TTC	CCT	
11413	Asn	Ser	Trp	Tyr	Thr	Ala	Phe	Ser	Arg	Pro	Met	Pro	
	AAT	TCT	TGG	TAT	ACT	GCC	TTT	TCT	AGG	CCA	ATG	CCG	
14126	Tyr	Ser	Trp	Tyr	Lys	Ser	Ala	Tyr	Gly	Ser	Phe	Pro	
	TAT	TCT	TGG	TAT	AAA	TCG	GCA	TAT	GGA	TCT	TTT	CCT	
3455	Tyr	Ser	Trp	Tyr	Lys	Leu	Ala	Tyr	Gly	Ser	Phe	Pro	
	TAT	TCT	TGG	TAT	AAA	TTG	GCA	TAT	GGA	TCT	TTT	CCT	
742	Tyr	Ser	Trp	Tyr	Lys	Ser	Ala	Tyr	Gly	Ser	Phe	Pro	
	TAT	TCT	TGG	TAT	AAA	TCG	GCA	TAT	GGA	TCT	TTT	CCT	
2848	Tyr	Ser	Trp	Tyr	Lys	Leu	Ala	Tyr	Gly	Ser	Phe	Pro	
	TAT	TCT	TGG	TAT	AAA	TTG	GCA	TAT	GGA	TCT	TTT	CCT	
6363	Tyr	Ser	Trp	Tyr	Lys	Leu	Ala	Tyr	Gly	Ser	Phe	Pro	
	TAT	TCT	TGG	TAT	AAA	TTG	GCA	TAT	GGA	TCT	TTT	CCT	
6190	Tyr	Ser	Trp	Tyr	Lys	Leu	Ala	Tyr	Gly	Ser	Phe	Pro	
	TAT	TCT	TGG	TAT	AAA	TTG	GCA	TAT	GGA	TCT	TTT	CCT	
8111	Tyr	Ser	Trp	Tyr	Lys	Leu	Ala	Tyr	Gly	Ser	Phe	Pro	
	TAT	TCT	TGG	TAT	AAA	TTG	GCA	TAT	GGA	TCT	TTT	CCT	

Strain													
R6 *pbp2b*	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	444
	ATC	ACA	GCG	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCA	AAT	1564
8099	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATC	ACA	GCG	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCA	AAT	
3203	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATC	ACA	GCG	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCA	AAT	
11184	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATC	ACA	GCG	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCA	AAT	
12244	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATC	ACA	GCG	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCA	AAT	
14016	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATC	ACA	GCG	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCT	AAT	
12276	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCA	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCT	AAT	
3996	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCA	GTC	CAA	GCT	CTG	GAG	TAT	TCA	TCT	AAT	
11413	Ile	Thr	Ala	Val	Gln	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACG	GCA	GTT	CAG	GCT	CTA	GAG	TAT	TCA	TCC	AAT	
14126	Ile	Thr	Ala	Val	Glu	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCT	GTG	GAA	GCC	TTG	GAG	TAT	TCA	TCC	AAT	
3455	Ile	Thr	Ala	Val	Glu	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCT	GTG	GAA	GCC	TTG	GAG	TAT	TCA	TCC	AAT	
742	Ile	Thr	Ala	Val	Glu	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCT	GTG	GAA	GCC	TTG	GAG	TAT	TCA	TCC	AAT	
2848	Ile	Thr	Ala	Val	Glu	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCT	GTG	GAA	GCC	TTG	GAG	TAT	TCA	TCC	AAT	
6363	Ile	Thr	Ala	Val	Glu	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCT	GTG	GAA	GCC	TTG	GAG	TAT	TCA	TCC	AAT	
6190	Ile	Thr	Ala	Val	Glu	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCT	GTG	GAA	GCC	TTG	GAG	TAT	TCA	TCC	AAT	
8111	Ile	Thr	Ala	Val	Glu	Ala	Leu	Glu	Tyr	Ser	Ser	Asn	
	ATT	ACA	GCT	GTG	GAA	GCC	TTG	GAG	TAT	TCA	TCC	AAT	

Strain													
R6 *pbp2b*	Thr	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	456
	ACC	TAT	ATG	GTC	CAA	ACA	GCC	TTA	GGT	CTT	ATG	GGG	1600
8099	Thr	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	ACC	TAT	ATG	GTC	CAA	ACA	GCC	TTA	GGT	CTT	ATG	GGG	
3203	Thr	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	ACC	TAT	ATG	GTC	CAA	ACA	GCC	TTA	GGT	CTT	ATG	GGG	
11184	Thr	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	ACC	TAT	ATG	GTC	CAA	ACA	GCC	TTA	GGT	CTT	ATG	GGG	
12244	Thr	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	ACC	TAT	ATG	GTC	CAA	ACA	GCC	TTA	GGT	CTT	ATG	GGG	
14016	Thr	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	ACC	TAT	ATG	GTC	CAA	ACA	GCC	TTA	GGT	CTT	ATG	GGG	
12276	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	GCC	TAT	ATG	GTC	CAA	ACA	GCC	CTA	GGT	CTT	ATG	GGG	
3996	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	GCC	TAT	ATG	GTC	CAA	ACA	GCC	CTA	GGT	CTT	ATG	GGG	
11413	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Leu	Met	Gly	
	GCT	TAT	ATG	GTC	CAA	ACA	GCC	TTA	GGT	CTT	ATG	GGG	
14126	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Ile	Met	Gly	
	GCT	TAC	ATG	GTT	CAA	ACC	GCT	CTT	GGA	ATC	ATG	GGC	
3455	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Ile	Met	Gly	
	GCT	TAC	ATG	GTT	CAA	ACC	GCT	CTT	GGA	ATC	ATG	GGC	
742	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Ile	Met	Gly	
	GCT	TAC	ATG	GTT	CAA	ACC	GCT	CTT	GGA	ATC	ATG	GGC	
2848	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Ile	Met	Gly	
	GCT	TAC	ATG	GTT	CAA	ACC	GCT	CTT	GGA	ATC	ATG	GGC	
6363	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Ile	Met	Gly	
	GCT	TAC	ATG	GTT	CAA	ACC	GCT	CTT	GGA	ATC	ATG	GGC	
6190	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Ile	Met	Gly	
	GCT	TAC	ATG	GTT	CAA	ACC	GCT	CTT	GGA	ATC	ATG	GGC	
8111	Ala	Tyr	Met	Val	Gln	Thr	Ala	Leu	Gly	Ile	Met	Gly	
	GCT	TAC	ATG	GTT	CAA	ACC	GCT	CTT	GGA	ATC	ATG	GGC	

R6 *pbp2b*	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	**468**
	CAA	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	**1636**
8099	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAA	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
3203	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAA	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
11184	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAA	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
12244	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAA	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
14016	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAA	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
12276	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
3996	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCC	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
11413	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAA	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTC	GGC	ACC	AGC	
14126	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAA	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTT	GGA	ACC	AGC	
3455	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTT	GGA	ACC	AGC	
742	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTT	GGA	ACC	AGC	
2848	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTT	GGA	ACC	AGC	
6363	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTT	GGA	ACC	AGC	
6190	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTT	GGA	ACC	AGC	
8111	Gln	Thr	Tyr	Gln	Pro	Asn	Met	Phe	Val	Gly	Thr	Ser	
	CAG	ACC	TAT	CAA	CCA	AAT	ATG	TTT	GTT	GGA	ACC	AGC	

R6 *pbp2b*	Asn	Leu	Glu	Ser	Ala	Met	Glu	Lys	Leu	Arg	Ser	Thr	**480**
	AAT	CTA	GAG	TCT	GCT	ATG	GAG	AAA	CTG	CGT	TCA	ACC	**1672**
8099	Asn	Leu	Glu	Ser	Ala	Met	Glu	Lys	Leu	Arg	Ser	Thr	
	AAT	CTA	GAG	TCT	GCT	ATG	GAG	AAA	CTG	CGT	TCA	ACC	
3203	Asn	Leu	Glu	Ser	Ala	Met	Glu	Lys	Leu	Arg	Ser	Thr	
	AAT	CTA	GAG	TCT	GCT	ATG	GAG	AAA	CTG	CGT	TCA	ACC	
11184	Asn	Leu	Glu	Ser	Ala	Met	Glu	Lys	Leu	Arg	Ser	Thr	
	AAT	CTA	GAG	TCT	GCT	ATG	GAG	AAA	CTG	CGT	TCA	ACC	
12244	Asn	Leu	Glu	Ser	Ala	Met	Glu	Lys	Leu	Arg	Ser	Thr	
	AAT	CTA	GAG	TCT	GCT	ATG	GAG	AAA	CTG	CGT	TCA	ACC	
14016	Asn	Leu	Glu	Ser	Ala	Met	Glu	Lys	Leu	Arg	Ser	Thr	
	AAT	CTA	GAG	TCT	GCT	ATG	GAG	AAA	CTG	CGT	TCA	ACC	
12276	Lys	Leu	Glu	Ser	Ala	Met	Gly	Lys	Leu	Arg	Ser	Thr	
	AAG	CTA	GAG	TCT	GCT	ATG	GGG	AAA	TTG	CGT	TCA	ACC	
3996	Lys	Leu	Glu	Ser	Ala	Met	Gly	Lys	Leu	Arg	Ser	Thr	
	AAG	CTA	GAG	TCT	GCT	ATG	GGG	AAA	TTG	CGT	TCA	ACC	
11413	Asn	Leu	Glu	Ser	Ala	Met	Gly	Lys	Leu	Arg	Ser	Thr	
	AAT	CTA	GAG	TCT	GCT	ATG	GGT	AAA	TTG	CGT	TCA	ACC	
14126	Asn	Leu	Glu	Thr	Ala	Met	Gly	Lys	Leu	Arg	Ala	Thr	
	AAT	TTG	GAA	ACA	GCT	ATG	GGA	AAA	CTT	CGT	GCG	ACC	
3455	Asn	Leu	Glu	Thr	Ala	Met	Gly	Lys	Leu	Arg	Ala	Thr	
	AAT	TTG	GAA	ACA	GCT	ATG	GGA	AAA	CTT	CGT	GCG	ACC	
742	Asn	Leu	Glu	Thr	Ala	Met	Gly	Lys	Leu	Arg	Ala	Thr	
	AAT	TTG	GAA	ACA	GCT	ATG	GGA	AAA	CTT	CGT	GCG	ACC	
2848	Asn	Leu	Glu	Thr	Ala	Met	Gly	Lys	Leu	Arg	Ala	Thr	
	AAT	TTG	GAA	ACA	GCT	ATG	GGA	AAA	CTT	CGT	GCG	ACC	
6363	Asn	Leu	Glu	Thr	Ala	Met	Gly	Lys	Leu	Arg	Ala	Thr	
	AAT	TTG	GAA	ACA	GCT	ATG	GGA	AAA	CTT	CGT	GCG	ACC	
6190	Asn	Leu	Glu	Thr	Ala	Met	Gly	Lys	Leu	Arg	Ala	Thr	
	AAT	TTG	GAA	ACA	GCT	ATG	GGA	AAA	CTT	CGT	GCG	ACC	
8111	Asn	Leu	Glu	Thr	Ala	Met	Gly	Lys	Leu	Arg	Ala	Thr	
	AAT	TTG	GAA	ACA	GCT	ATG	GGA	AAA	CTT	CGT	GCG	ACC	

Strain													
R6 *pbp2b*	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Thr	Ala	Thr	Gly	Ile	**492**
	TTT	GGC	GAA	TAT	GGC	TTG	GGT	ACT	GCG	ACA	GGA	ATT	**1708**
8099	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Thr	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGT	ACT	GCG	ACA	GGA	ATT	
3203	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Thr	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGT	ACT	GCG	ACA	GGA	ATT	
11184	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Thr	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGT	ACT	GCG	ACA	GGA	ATT	
12244	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Thr	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGT	ACT	GCG	ACA	GGA	ATT	
14016	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Thr	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGT	ACT	GCG	ACA	GGA	ATT	
12276	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ser	Ala	Thr	Gly	Ile	
	TTT	GGT	GAA	TAT	GGT	TTG	GGT	TCT	GCG	ACC	GGG	ATT	
3996	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ser	Ala	Thr	Gly	Ile	
	TTT	GGT	GAA	TAT	GGT	TTG	GGT	TCT	GCG	ACC	GGG	ATT	
11413	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ser	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGT	TCT	GCG	ACC	GGA	ATT	
14126	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ala	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGG	GCT	GCG	ACC	GGA	ATT	
3455	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ala	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGG	GCT	GCG	ACC	GGA	ATT	
742	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ala	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGG	GCT	GCG	ACC	GGA	ATT	
2848	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ala	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGG	GCT	GCG	ACC	GGA	ATT	
6363	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ala	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGG	GCT	GCG	ACC	GGA	ATT	
6190	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ala	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGG	GCT	GCG	ACC	GGA	ATT	
8111	Phe	Gly	Glu	Tyr	Gly	Leu	Gly	Ala	Ala	Thr	Gly	Ile	
	TTT	GGC	GAA	TAT	GGC	TTG	GGG	GCT	GCG	ACC	GGA	ATT	

Strain													
R6 *pbp2b*	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	**504**
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	**1744**
8099	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
3203	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
11184	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
12244	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
14016	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
12276	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
3996	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
11413	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Ile	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	ATT	CCC	AAA	
14126	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
3455	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
742	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
2848	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
6363	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
6190	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	
8111	Asp	Leu	Pro	Asp	Glu	Ser	Thr	Gly	Phe	Val	Pro	Lys	
	GAC	CTA	CCA	GAT	GAA	TCT	ACT	GGA	TTT	GTT	CCC	AAA	

R6 *pbp2b*	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	516
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	1780
8099	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
3203	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
11184	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
12244	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
14016	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
12276	Asp	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAC	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
3996	Asp	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAC	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
11413	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATT	ACT	AAT	GCC	TTT	
14126	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ala	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATC	ACC	AAT	GCC	TTT	
3455	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ser	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATC	ACC	AAT	TCC	TTT	
742	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ser	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATC	ACC	AAT	TCC	TTT	
2848	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ser	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATC	ACC	AAT	TCC	TTT	
6363	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ser	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATC	ACC	AAT	TCC	TTT	
6190	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ser	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATC	ACC	AAT	TCC	TTT	
8111	Glu	Tyr	Ser	Phe	Ala	Asn	Tyr	Ile	Thr	Asn	Ser	Phe	
	GAG	TAT	AGC	TTT	GCT	AAT	TAC	ATC	ACC	AAT	TCC	TTT	

R6 *pbp2b*	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	528
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCG	ATG	CAG	TTG	GCT	1816
8099	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCG	ATG	CAG	TTG	GCT	
3203	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCG	ATG	CAG	TTG	GCT	
11184	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCG	ATG	CAG	TTG	GCT	
12244	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCG	ATG	CAG	TTG	GCT	
14016	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCG	ATG	CAG	TTG	GCT	
12276	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
3996	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
11413	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACC	CCA	ATG	CAA	TTG	GCT	
14126	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
3455	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
742	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
2848	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
6363	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
6190	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	
8111	Gly	Gln	Phe	Asp	Asn	Tyr	Thr	Pro	Met	Gln	Leu	Ala	
	GGG	CAG	TTT	GAT	AAC	TAT	ACG	CCC	ATG	CAG	TTG	GCT	

R6 *pbp2b*	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	**540**
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	**1852**
8099	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
3203	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
11184	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
12244	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
14016	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
12276	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asp	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	GAT	GGT	GTT	CGT	
3996	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asp	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	GAT	GGT	GTT	CGT	
11413	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asp	Gly	Val	Arg	
	CAG	TAT	GTG	GCA	ACT	ATT	GCA	AAT	GAT	GGT	GTT	CGT	
14126	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asp	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	GAT	GGT	GTT	CGT	
3455	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
742	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
2848	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
6363	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
6190	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	
8111	Gln	Tyr	Val	Ala	Thr	Ile	Ala	Asn	Asn	Gly	Val	Arg	
	CAG	TAT	GTA	GCA	ACT	ATT	GCA	AAT	AAT	GGT	GTT	CGT	

R6 *pbp2b*	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	**552**
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	**1888**
8099	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
3203	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
11184	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
12244	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
14016	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
12276	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGC	AAT	
3996	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGC	AAT	
11413	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGC	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
14126	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
3455	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
742	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
2848	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
6363	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGC	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
6190	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	
8111	Val	Ala	Pro	Arg	Ile	Val	Glu	Gly	Ile	Tyr	Gly	Asn	
	GTG	GCT	CCT	CGT	ATT	GTT	GAA	GGC	ATT	TAT	GGT	AAT	

R6 *pbp2b*	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	**564**
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	**1924**
8099	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	TTG	GGT	GAC	TTG	ATT	CAG	CAA	
3203	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
11184	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
12244	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	TTG	GGT	GAC	TTG	ATT	CAG	CAA	
14016	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
12276	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGC	CTG	GGC	GAC	TTG	ATT	CAG	CAA	
3996	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGC	CTG	GGC	GAC	TTG	ATT	CAG	CAA	
11413	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGC	GAC	TTG	ATT	CAG	CAA	
14126	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
3455	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
742	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
2848	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
6363	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
6190	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	
8111	Asn	Asp	Lys	Gly	Gly	Leu	Gly	Asp	Leu	Ile	Gln	Gln	
	AAT	GAT	AAG	GGA	GGA	CTG	GGT	GAC	TTG	ATT	CAG	CAA	

R6 *pbp2b*	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	**576**
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	**1960**
8099	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
3203	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
11184	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
12244	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
14016	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
12276	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
3996	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
11413	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
14126	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
3455	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
742	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
2848	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
6363	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
6190	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	
8111	Leu	Gln	Pro	Thr	Glu	Met	Asn	Lys	Val	Asn	Ile	Ser	
	CTG	CAA	CCG	ACA	GAG	ATG	AAT	AAG	GTC	AAT	ATA	TCC	

R6 *pbp2b*	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	**588**
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	**1996**
8099	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
3203	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
11184	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
12244	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
14016	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
12276	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
3996	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
11413	Asp	Ser	Asp	Met	Ser	Val	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGT	GTC	TTG	CAC	CAA	GGT	TTT	TAT	
14126	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGT	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
3455	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
742	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
2848	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
6363	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
6190	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	
8111	Asp	Ser	Asp	Met	Ser	Ile	Leu	His	Gln	Gly	Phe	Tyr	
	GAC	TCC	GAT	ATG	AGC	ATC	TTG	CAC	CAA	GGT	TTT	TAT	

R6 *pbp2b*	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	**600**
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	**2032**
8099	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
3203	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
11184	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
12244	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
14016	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
12276	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCT	CAT	GGG	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
3996	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCT	CAT	GGG	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
11413	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCT	CAT	GGG	ACT	AGC	GGA	TTG	ACA	ACT	GGA	
14126	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
3455	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
742	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
2848	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
6363	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
6190	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	
8111	Gln	Val	Ala	His	Gly	Thr	Ser	Gly	Leu	Thr	Thr	Gly	
	CAG	GTT	GCC	CAT	GGT	ACT	AGT	GGA	TTG	ACA	ACT	GGA	

R6 *pbp2b*	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	**612**
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	**2068**
8099	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
3203	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGC	GCC	TTG	GTA	TCC	ATT	AGC	
11184	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
12244	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
14016	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
12276	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
3996	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
11413	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
14126	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGC	GCC	TTG	GTA	TCC	ATT	AGC	
3455	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
742	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
2848	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
6363	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
6190	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	
8111	Arg	Ala	Phe	Ser	Asn	Gly	Ala	Leu	Val	Ser	Ile	Ser	
	CGT	GCC	TTT	TCA	AAT	GGT	GCC	TTG	GTA	TCC	ATT	AGC	

R6 *pbp2b*	Gly	**Lys**	**Thr**	**Gly**	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	**624**
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	**2104**
8099	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
3203	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
11184	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
12244	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
14016	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
12276	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACG	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
3996	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACG	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
11413	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACG	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
14126	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
3455	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
742	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
2848	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
6363	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
6190	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	
8111	Gly	Lys	Thr	Gly	Thr	Ala	Glu	Ser	Tyr	Val	Ala	Asp	
	GGA	AAA	ACA	GGT	ACA	GCC	GAA	AGC	TAT	GTG	GCA	GAT	

R6 *pbp2b*	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	**636**
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	**2140**
8099	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
3203	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
11184	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
12244	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
14016	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
12276	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACT	AAT	GCG	GTG	GCC	TAT	
3996	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACT	AAT	GCG	GTG	GCC	TAT	
11413	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
14126	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
3455	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
742	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
2848	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
6363	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
6190	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	
8111	Gly	Gln	Gln	Ala	Thr	Asn	Thr	Asn	Ala	Val	Ala	Tyr	
	GGT	CAG	CAA	GCA	ACC	AAT	ACC	AAT	GCG	GTG	GCC	TAT	

R6 *pbp2b*	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	**648**
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	**2176**
8099	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
3203	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTT	GCA	GTG	
11184	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
12244	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
14016	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
12276	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTT	GCA	GTG	
3996	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTT	GCA	GTG	
11413	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTT	GCA	GTG	
14126	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTT	GCA	GTG	
3455	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
742	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
2848	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
6363	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
6190	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	
8111	Ala	Pro	Ser	Asp	Asn	Pro	Gln	Ile	Ala	Val	Ala	Val	
	GCC	CCA	TCT	GAT	AAT	CCC	CAA	ATC	GCT	GTC	GCA	GTG	

R6 *pbp2b*	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	**660**
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	**2212**
8099	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
3203	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
11184	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
12244	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
14016	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
12276	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
3996	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
11413	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
14126	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
3455	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
742	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
2848	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
6363	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
6190	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	
8111	Val	Phe	Pro	His	Asn	Thr	Asn	Leu	Thr	Asn	Gly	Val	
	GTC	TTT	CCT	CAT	AAT	ACC	AAT	CTA	ACA	AAT	GGT	GTA	

R6 *pbp2b*	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	**672**
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	**2248**
8099	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
3203	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
11184	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
12244	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
14016	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
12276	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAT	ATT	ATC	AAT	CTG	TAT	
3996	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAT	ATT	ATC	AAT	CTG	TAT	
11413	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGG	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
14126	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
3455	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
742	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
2848	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
6363	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
6190	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	
8111	Gly	Pro	Ser	Ile	Ala	Arg	Asp	Ile	Ile	Asn	Leu	Tyr	
	GGA	CCT	TCC	ATT	GCG	CGT	GAC	ATT	ATC	AAT	CTG	TAT	

R6 *pbp2b*	Gln	Lys	Tyr	His	**676**
	CAA	AAA	TAC	CAT	**2260**
8099	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
3203	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
11184	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
12244	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
14016	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
12276	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
3996	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
11413	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
14126	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
3455	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
742	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
2848	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
6363	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
6190	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	
8111	Gln	Lys	Tyr	His	
	CAA	AAA	TAC	CAT	

APPENDIX C

Nucleotide and amino acid sequence alignments of the PBP 1A penicillin-binding domain from clinical isolates of *S. pneumoniae*.

The sequence of the *pbp1a* gene and the amino acid sequence of PBP 1A from penicillin-susceptible *S. pneumoniae* R6 are shown at the top. Nucleotide and amino acid sequences are numbered at the end of each line according to data published in reference 201. Amino acid residues differing from the R6 sequence are shaded. Conserved amino acid motifs are boxed and in boldface.

R6 *pbp1a*	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	**321**
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	**1908**
8099	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
3203	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
11184	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
12244	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
14016	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
12276	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
3996	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
11413	Leu	Trp	Asp	Ile	Tyr	Asn	Thr	Asp	Glu	Tyr	Val	Ala	
	CTG	TGG	GAT	ATT	TAC	AAT	ACA	GAC	GAA	TAC	GTT	GCC	
14126	Leu	Trp	Asp	Ile	Tyr	Asn	Ser	Asp	Gln	Tyr	Val	Ser	
	CTG	TGG	GAT	ATC	TAC	AAC	TCC	GAT	GAA	TAC	GTC	TCT	
3455	Leu	Trp	Asp	Ile	Tyr	Asn	Ser	Asp	Gln	Tyr	Val	Ser	
	CTG	TGG	GAT	ATC	TAC	AAC	TCC	GAT	GAA	TAC	GTC	TCT	
742	Leu	Trp	Asp	Ile	Tyr	Asn	Ser	Asp	Gln	Tyr	Val	Ser	
	CTG	TGG	GAT	ATC	TAC	AAC	TCC	GAT	GAA	TAC	GTC	TCT	
2848	Leu	Trp	Asp	Ile	Tyr	Asn	Ser	Asp	Gln	Tyr	Val	Ser	
	CTG	TGG	GAT	ATC	TAC	AAC	TCC	GAT	GAA	TAC	GTC	TCT	
6363	Leu	Trp	Asp	Ile	Tyr	Asn	Ser	Asp	Gln	Tyr	Val	Ser	
	CTG	TGG	GAT	ATC	TAC	AAC	TCC	GAT	GAA	TAC	GTC	TCT	
6190	Leu	Trp	Asp	Ile	Tyr	Asn	Ser	Asp	Gln	Tyr	Val	Ser	
	CTG	TGG	GAT	ATC	TAC	AAC	TCC	GAT	GAA	TAC	GTC	TCT	
8111	Leu	Trp	Asp	Ile	Tyr	Asn	Ser	Asp	Gln	Tyr	Val	Ser	
	CTG	TGG	GAT	ATC	TAC	AAC	TCC	GAT	GAA	TAC	GTC	TCT	

R6 *pbp1a*	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	**333**
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	**1944**
8099	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	
3203	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	
11184	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	
12244	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATC	
14016	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	
12276	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	
3996	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	
11413	Tyr	Pro	Asp	Asp	Glu	Leu	Gln	Val	Ala	Ser	Thr	Ile	
	TAT	CCA	GAC	GAT	GAA	TTG	CAA	GTC	GCT	TCT	ACC	ATT	
14126	Tyr	Pro	Asp	Asp	Asp	Leu	Gln	Val	Ala	Ser	Thr	Val	
	TAC	CCT	GAC	GAT	GAT	TTG	CAA	GTC	GCA	TCT	ACG	GTC	
3455	Tyr	Pro	Asp	Asp	Asp	Leu	Gln	Val	Ala	Ser	Thr	Val	
	TAC	CCT	GAC	GAT	GAT	TTG	CAA	GTC	GCA	TCT	ACG	GTC	
742	Tyr	Pro	Asp	Asp	Asp	Leu	Gln	Val	Ala	Ser	Thr	Val	
	TAC	CCT	GAC	GAT	GAT	TTG	CAA	GTC	GCA	TCT	ACG	GTC	
2848	Tyr	Pro	Asp	Asp	Asp	Leu	Gln	Val	Ala	Ser	Thr	Val	
	TAC	CCT	GAC	GAT	GAT	TTG	CAA	GTC	GCA	TCT	ACG	GTC	
6363	Tyr	Pro	Asp	Asp	Asp	Leu	Gln	Val	Ala	Ser	Thr	Val	
	TAC	CCT	GAC	GAT	GAT	TTG	CAA	GTC	GCA	TCT	ACG	GTC	
6190	Tyr	Pro	Asp	Asp	Asp	Leu	Gln	Val	Ala	Ser	Thr	Val	
	TAC	CCT	GAC	GAT	GAT	TTG	CAA	GTC	GCA	TCT	ACG	GTC	
8111	Tyr	Pro	Asp	Asp	Asp	Leu	Gln	Val	Ala	Ser	Thr	Val	
	TAC	CCT	GAC	GAT	GAT	TTG	CAA	GTC	GCA	TCT	ACG	GTC	

R6 *pbp1a*	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	**345**
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	**1980**
8099	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
3203	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
11184	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
12244	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
14016	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
12276	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
3996	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
11413	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTT	GAT	GTT	TCT	AAC	GGT	AAA	GTC	ATT	GCC	CAG	CTA	
14126	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTA	GAT	GTT	TCA	AAT	GGT	AAA	GTC	ATC	GCA	CAA	CTT	
3455	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTA	GAT	GTT	TCA	AAT	GGT	AAA	GTC	ATC	GCC	CAA	CTT	
742	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTA	GAT	GTT	TCA	AAT	GGT	AAA	GTC	ATC	GCC	CAA	CTT	
2848	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTA	GAT	GTT	TCA	AAT	GGT	AAA	GTC	ATC	GCC	CAA	CTT	
6363	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTA	GAT	GTT	TCA	AAT	GGT	AAA	GTC	ATC	GCC	CAA	CTT	
6190	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTA	GAT	GTT	TCA	AAT	GGT	AAA	GTC	ATC	GCC	CAA	CTT	
8111	Val	Asp	Val	Ser	Asn	Gly	Lys	Val	Ile	Ala	Gln	Leu	
	GTA	GAT	GTT	TCA	AAT	GGT	AAA	GTC	ATC	GCC	CAA	CTT	

R6 *pbp1a*	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	**357**
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	**2016**
8099	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
3203	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
11184	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
12244	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
14016	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
12276	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
3996	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
11413	Gly	Ala	Arg	His	Gln	Ser	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCA	CGC	CAT	CAG	TCA	AGT	AAT	GTT	TCC	TTC	GGA	
14126	Gly	Ala	Arg	His	Gln	Ala	Ser	Asn	Val	Ser	Phe	Gly	
	GGT	GCT	CGT	CAT	CAA	GCA	AGT	AAT	GTT	TCA	TTC	GGT	
3455	Gly	Ala	Arg	His	Gln	Ala	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCT	CGT	CAC	CAA	GCA	AGT	AAC	GTT	TCA	TTT	GGT	
742	Gly	Ala	Arg	His	Gln	Ala	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCT	CGT	CAC	CAA	GCA	AGT	AAC	GTT	TCA	TTT	GGT	
2848	Gly	Ala	Arg	His	Gln	Ala	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCT	CGT	CAC	CAA	GCA	AGT	AAC	GTT	TCA	TTT	GGT	
6363	Gly	Ala	Arg	His	Gln	Ala	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCT	CGT	CAC	CAA	GCA	AGT	AAC	GTT	TCA	TTT	GGT	
6190	Gly	Ala	Arg	His	Gln	Ala	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCT	CGT	CAC	CAA	GCA	AGT	AAC	GTT	TCA	TTT	GGT	
8111	Gly	Ala	Arg	His	Gln	Ala	Ser	Asn	Val	Ser	Phe	Gly	
	GGA	GCT	CGT	CAC	CAA	GCA	AGT	AAC	GTT	TCA	TTT	GGT	

R6 pbp1a	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	**369**
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	**2052**
8099	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
3203	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
11184	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
12244	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
14016	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
12276	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
3996	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
11413	Ile	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ATT	AAC	CAA	GCA	GTA	GAA	ACA	AAC	CGC	GAC	TGG	GGA	
14126	Thr	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ACC	AAC	CAG	GCC	GTA	GAA	ACC	AAT	CGT	GAC	TGG	GGA	
3455	Thr	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ACC	AAC	CAA	GCT	GTG	GAA	ACC	AAT	CGT	GAC	TGG	GGT	
742	Thr	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ACC	AAC	CAA	GCT	GTG	GAA	ACC	AAT	CGT	GAC	TGG	GGT	
2848	Thr	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ACC	AAC	CAA	GCT	GTG	GAA	ACC	AAT	CGT	GAC	TGG	GGT	
6363	Thr	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ACC	AAC	CAA	GCT	GTG	GAA	ACC	AAT	CGT	GAC	TGG	GGT	
6190	Thr	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ACC	AAC	CAA	GCT	GTG	GAA	ACC	AAT	CGT	GAC	TGG	GGT	
8111	Thr	Asn	Gln	Ala	Val	Glu	Thr	Asn	Arg	Asp	Trp	Gly	
	ACC	AAC	CAA	GCT	GTG	GAA	ACC	AAT	CGT	GAC	TGG	GGT	

R6 *pbp1a*	**Ser**	**Thr**	**Met**	**Lys**	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	**381**
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	**2088**
8099	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
3203	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
11184	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
12244	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
14016	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
12276	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
3996	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
11413	Ser	Thr	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	ACT	ATG	AAA	CCG	ATC	ACA	GAC	TAT	GCT	CCT	GCC	
14126	Ser	Ser	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCA	TCA	ATG	AAA	CCA	ATC	ACT	GAC	TAT	GCT	CCC	GCT	
3455	Ser	Ala	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCT	GCT	ATG	AAA	CCA	ATC	ACC	GAT	TAT	GCA	CCT	GCC	
742	Ser	Ala	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCT	GCT	ATG	AAA	CCA	ATC	ACC	GAT	TAT	GCA	CCT	GCC	
2848	Ser	Ala	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCT	GCT	ATG	AAA	CCA	ATC	ACC	GAT	TAT	GCA	CCT	GCC	
6363	Ser	Ala	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCT	GCT	ATG	AAA	CCA	ATC	ACC	GAT	TAT	GCA	CCT	GCC	
6190	Ser	Ala	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCT	GCT	ATG	AAA	CCA	ATC	ACC	GAT	TAT	GCA	CCT	GCC	
8111	Ser	Ala	Met	Lys	Pro	Ile	Thr	Asp	Tyr	Ala	Pro	Ala	
	TCT	GCT	ATG	AAA	CCA	ATC	ACC	GAT	TAT	GCA	CCT	GCC	

R6 *pbp1a*	Leu	Glu	Tyr	Gly	Val	Tyr	Glu	Ser	Thr	Ala	Thr	Ile	**393**
	TTG	GAG	TAC	GGT	GTC	TAC	GAG	TCA	ACT	GCC	ACT	ATC	**2124**
8099	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	GTC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
3203	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	GTC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
11184	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	GTC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
12244	Leu	Glu	Tyr	Gly	Ile	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	ATC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
14016	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	GTC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
12276	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	GTC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
3996	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	GTC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
11413	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Ile	
	TTG	GAG	TAC	GGT	GTC	TAC	GAT	TCA	ACT	GCT	ACT	ATC	
14126	Leu	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Ser	Ile	
	TTA	GAA	TAT	GGA	GTC	TAT	GAC	TCT	ACT	GCT	TCT	ATT	
3455	Ile	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Met	
	ATA	GAA	TAC	GGT	GTT	TAT	GAT	TCC	ACT	GCA	ACT	ATG	
742	Ile	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Met	
	ATA	GAA	TAC	GGT	GTT	TAT	GAT	TCC	ACT	GCA	ACT	ATG	
2848	Ile	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Met	
	ATA	GAA	TAC	GGT	GTT	TAT	GAT	TCC	ACT	GCA	ACT	ATG	
6363	Ile	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Met	
	ATA	GAA	TAC	GGT	GTT	TAT	GAT	TCC	ACT	GCA	ACT	ATG	
6190	Ile	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Met	
	ATA	GAA	TAC	GGT	GTT	TAT	GAT	TCC	ACT	GCA	ACT	ATG	
8111	Ile	Glu	Tyr	Gly	Val	Tyr	Asp	Ser	Thr	Ala	Thr	Met	
	ATA	GAA	TAC	GGT	GTT	TAT	GAT	TCC	ACT	GCA	ACT	ATG	

Strain													
R6 *pbp1a*	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	**405**
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	**2160**
8099	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
3203	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
11184	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
12244	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
14016	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
12276	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
3996	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
11413	Val	His	Asp	Glu	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asn	
	GTT	CAC	GAT	GAG	CCC	TAT	AAC	TAC	CCT	GGG	ACA	AAT	
14126	Val	His	Asp	Val	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Asp	
	GTA	CAT	GAT	GTC	CCT	TAT	AAC	TAT	CCT	GGC	ACT	GAT	
3455	Val	Asn	Asp	Ile	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Ser	
	GTT	AAT	GAT	ATT	CCT	TAT	AAC	TAT	CCG	GGA	ACA	AGC	
742	Val	Asn	Asp	Ile	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Ser	
	GTT	AAT	GAT	ATT	CCT	TAT	AAC	TAT	CCG	GGA	ACA	AGC	
2848	Val	Asn	Asp	Ile	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Ser	
	GTT	AAT	GAT	ATT	CCT	TAT	AAC	TAT	CCG	GGA	ACA	AGC	
6363	Val	Asn	Asp	Ile	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Ser	
	GTT	AAT	GAT	ATT	CCT	TAT	AAC	TAT	CCG	GGA	ACA	AGC	
6190	Val	Asn	Asp	Ile	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Ser	
	GTT	AAT	GAT	ATT	CCT	TAT	AAC	TAT	CCG	GGA	ACA	AGC	
8111	Val	Asn	Asp	Ile	Pro	Tyr	Asn	Tyr	Pro	Gly	Thr	Ser	
	GTT	AAT	GAT	ATT	CCT	TAT	AAC	TAT	CCG	GGA	ACA	AGC	

Strain													
R6 *pbp1a*	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	**417**
	ACC	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	**2196**
8099	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACC	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
3203	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACT	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
11184	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACC	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
12244	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACC	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
14016	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACT	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
12276	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACT	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
3996	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACT	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
11413	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Gly	Tyr	Phe	Gly	
	ACC	CCT	GTT	TAT	AAC	TGG	GAT	AGG	GGC	TAC	TTT	GGC	
14126	Thr	Pro	Leu	Tyr	Asn	Trp	Asp	His	Val	Tyr	Phe	Gly	
	ACT	CCA	GTC	TAC	AAC	TGG	GAT	CAT	GTC	TAC	TTT	GGA	
3455	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Ala	Tyr	Phe	Gly	
	ACA	CCT	GTC	TAC	AAC	TGG	GAT	AGA	GCA	TAT	TTC	GGT	
742	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Ala	Tyr	Phe	Gly	
	ACA	CCT	GTC	TAC	AAC	TGG	GAT	AGA	GCA	TAT	TTC	GGT	
2848	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Ala	Tyr	Phe	Gly	
	ACA	CCT	GTC	TAC	AAC	TGG	GAT	AGA	GCA	TAT	TTC	GGT	
6363	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Ala	Tyr	Phe	Gly	
	ACA	CCT	GTC	TAC	AAC	TGG	GAT	AGA	GCA	TAT	TTC	GGT	
6190	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Ala	Tyr	Phe	Gly	
	ACA	CCT	GTC	TAC	AAC	TGG	GAT	AGA	GCA	TAT	TTC	GGT	
8111	Thr	Pro	Val	Tyr	Asn	Trp	Asp	Arg	Ala	Tyr	Phe	Gly	
	ACA	CCT	GTC	TAC	AAC	TGG	GAT	AGA	GCA	TAT	TTC	GGT	

R6 *pbp1a*	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	**Ser**	**Arg**	**429**
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	**2232**
8099	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
3203	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
11184	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
12244	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
14016	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
12276	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
3996	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
11413	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATC	ACC	TTG	CAA	TAC	GCC	CTG	CAA	CAA	TCG	CGA	
14126	Asn	Ile	Thr	Ile	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAC	ATT	ACA	ATC	CAG	TAT	GCT	CTT	CAA	CAA	TCA	CGA	
3455	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAT	ATT	ACT	CTG	CAA	TAT	GCT	CTT	CAA	CAA	TCA	CGA	
742	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAT	ATT	ACT	CTG	CAA	TAT	GCT	CTT	CAA	CAA	TCA	CGA	
2848	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAT	ATT	ACT	CTG	CAA	TAT	GCT	CTT	CAA	CAA	TCA	CGA	
6363	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAT	ATT	ACT	CTG	CAA	TAT	GCT	CTT	CAA	CAA	TCA	CGA	
6190	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAT	ATT	ACT	CTG	CAA	TAT	GCT	CTT	CAA	CAA	TCA	CGA	
8111	Asn	Ile	Thr	Leu	Gln	Tyr	Ala	Leu	Gln	Gln	Ser	Arg	
	AAT	ATT	ACT	CTG	CAA	TAT	GCT	CTT	CAA	CAA	TCA	CGA	

R6 *pbp1a*	**Asn**	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	**441**
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	**2268**
8099	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
3203	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
11184	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
12244	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
14016	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
12276	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
3996	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
11413	Asn	Val	Pro	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAC	GTC	CCA	GCC	GTG	GAA	ACT	CTA	AAC	AAG	GTC	GGA	
14126	Asn	Val	Thr	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAT	GTC	ACA	GCC	GTT	GAG	ACT	TTG	AAT	AAG	GTC	GGT	
3455	Asn	Val	Thr	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAT	GTC	ACA	GCC	GTT	GAG	ACT	TTG	AAT	AAG	GTC	GGT	
742	Asn	Val	Thr	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAT	GTC	ACA	GCC	GTT	GAG	ACT	TTG	AAT	AAG	GTC	GGT	
2848	Asn	Val	Thr	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAT	GTC	ACA	GCC	GTT	GAG	ACT	TTG	AAT	AAG	GTC	GGT	
6363	Asn	Val	Thr	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAT	GTC	ACA	GCC	GTT	GAG	ACT	TTG	AAT	AAG	GTC	GGT	
6190	Asn	Val	Thr	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAT	GTC	ACA	GCC	GTT	GAG	ACT	TTG	AAT	AAG	GTC	GGT	
8111	Asn	Val	Thr	Ala	Val	Glu	Thr	Leu	Asn	Lys	Val	Gly	
	AAT	GTC	ACA	GCC	GTT	GAG	ACT	TTG	AAT	AAG	GTC	GGT	

R6 *pbp1a*	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	**453**
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	**2304**
8099	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	
3203	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTA	GGA	
11184	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	
12244	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	
14016	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	
12276	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	
3996	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	
11413	Leu	Asn	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTC	AAC	CGC	GCC	AAG	ACT	TTC	CTA	AAT	GGT	CTC	GGA	
14126	Leu	Asp	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTA	GAT	AGA	GCT	AAA	ACC	TTC	CTT	AAT	GGT	CTT	GGT	
3455	Leu	Asp	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTA	GAT	AGA	GCT	AAA	ACC	TTC	CTT	AAT	GGT	CTT	GGT	
742	Leu	Asp	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTA	GAT	AGA	GCT	AAA	ACC	TTC	CTT	AAT	GGT	CTT	GGT	
2848	Leu	Asp	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTA	GAT	AGA	GCT	AAA	ACC	TTC	CTT	AAT	GGT	CTT	GGT	
6363	Leu	Asp	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTA	GAT	AGA	GCT	AAA	ACC	TTC	CTT	AAT	GGT	CTT	GGT	
6190	Leu	Asp	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTA	GAT	AGA	GCT	AAA	ACC	TTC	CTT	AAT	GGT	CTT	GGT	
8111	Leu	Asp	Arg	Ala	Lys	Thr	Phe	Leu	Asn	Gly	Leu	Gly	
	CTA	GAT	AGA	GCT	AAA	ACC	TTC	CTT	AAT	GGT	CTT	GGT	

R6 *pbp1a*	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	**465**
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	**2340**
8099	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
3203	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
11184	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
12244	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
14016	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
12276	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
3996	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
11413	Ile	Asp	Tyr	Pro	Ser	Ile	His	Tyr	Ser	Asn	Ala	Ile	
	ATC	GAC	TAC	CCA	AGT	ATT	CAC	TAC	TCA	AAT	GCC	ATT	
14126	Ile	Asp	Tyr	Pro	Ser	Met	His	Tyr	Ala	Asn	Ala	Ile	
	ATC	GAC	TAT	CCA	AGC	ATG	CAT	TAT	GCA	AAC	GCC	ATT	
3455	Ile	Asp	Tyr	Pro	Ser	Met	His	Tyr	Ala	Asn	Ala	Ile	
	ATC	GAC	TAT	CCA	AGC	ATG	CAT	TAT	GCA	AAC	GCC	ATT	
742	Ile	Asp	Tyr	Pro	Ser	Met	His	Tyr	Ala	Asn	Ala	Ile	
	ATC	GAC	TAT	CCA	AGC	ATG	CAT	TAT	GCA	AAC	GCC	ATT	
2848	Ile	Asp	Tyr	Pro	Ser	Met	His	Tyr	Ala	Asn	Ala	Ile	
	ATC	GAC	TAT	CCA	AGC	ATG	CAT	TAT	GCA	AAC	GCC	ATT	
6363	Ile	Asp	Tyr	Pro	Ser	Met	His	Tyr	Ala	Asn	Ala	Ile	
	ATC	GAC	TAT	CCA	AGC	ATG	CAT	TAT	GCA	AAC	GCC	ATT	
6190	Ile	Asp	Tyr	Pro	Ser	Met	His	Tyr	Ala	Asn	Ala	Ile	
	ATC	GAC	TAT	CCA	AGC	ATG	CAT	TAT	GCA	AAC	GCC	ATT	
8111	Ile	Asp	Tyr	Pro	Ser	Met	His	Tyr	Ala	Asn	Ala	Ile	
	ATC	GAC	TAT	CCA	AGC	ATG	CAT	TAT	GCA	AAC	GCC	ATT	

Strain													
R6 *pbp1a*	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	**477**
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	**2376**
8099	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
3203	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
11184	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
12244	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
14016	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
12276	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
3996	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
11413	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asp	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACC	GAA	TCA	GAC	AAA	AAA	TAT	GGA	
14126	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asn	Lys	Lys	Tyr	Gly	
	TCA	AGT	AAC	ACA	ACT	GAA	TCC	AAC	AAA	AAA	TAT	GGT	
3455	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asn	Lys	Gln	Tyr	Gly	
	TCA	AGT	AAT	ACA	ACA	GAA	TCT	AAT	AAA	CAA	TAC	GGA	
742	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asn	Lys	Gln	Tyr	Gly	
	TCA	AGT	AAT	ACA	ACA	GAA	TCT	AAT	AAA	CAA	TAC	GGA	
2848	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asn	Lys	Gln	Tyr	Gly	
	TCA	AGT	AAT	ACA	ACA	GAA	TCT	AAT	AAA	CAA	TAC	GGA	
6363	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asn	Lys	Gln	Tyr	Gly	
	TCA	AGT	AAT	ACA	ACA	GAA	TCT	AAT	AAA	CAA	TAC	GGA	
6190	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asn	Lys	Gln	Tyr	Gly	
	TCA	AGT	AAT	ACA	ACA	GAA	TCT	AAT	AAA	CAA	TAC	GGA	
8111	Ser	Ser	Asn	Thr	Thr	Glu	Ser	Asn	Lys	Gln	Tyr	Gly	
	TCA	AGT	AAT	ACA	ACA	GAA	TCT	AAT	AAA	CAA	TAC	GGA	

Strain													
R6 *pbp1a*	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	**489**
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	**2412**
8099	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
3203	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
11184	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
12244	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
14016	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
12276	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
3996	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
11413	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAG	ATG	GCT	GCT	GCT	TAC	GCT	GCC	
14126	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAA	ATG	GCT	GCT	GCC	TAC	GCT	GCT	
3455	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAA	ATG	GCT	GCT	GCT	TAT	GCT	GCC	
742	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAA	ATG	GCT	GCT	GCT	TAT	GCT	GCC	
2848	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAA	ATG	GCT	GCT	GCT	TAT	GCT	GCC	
6363	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAA	ATG	GCT	GCT	GCT	TAT	GCT	GCC	
6190	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAA	ATG	GCT	GCT	GCT	TAT	GCT	GCC	
8111	Ala	Ser	Ser	Glu	Lys	Met	Ala	Ala	Ala	Tyr	Ala	Ala	
	GCA	AGT	AGT	GAA	AAA	ATG	GCT	GCT	GCT	TAT	GCT	GCC	

R6 *pbp1a*	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	**501**
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	**2448**
8099	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
3203	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
11184	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
12244	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
14016	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
12276	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
3996	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
11413	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGA	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
14126	Phe	Ala	Asn	Gly	Gly	Ile	Tyr	His	Lys	Pro	Met	Tyr	
	TTT	GCT	AAT	GGT	GGT	ATT	TAT	CAC	AAA	CCA	ATG	TAT	
3455	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGC	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
742	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGC	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
2848	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGC	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
6363	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGC	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
6190	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGC	ACT	TAC	TAT	AAA	CCA	ATG	TAT	
8111	Phe	Ala	Asn	Gly	Gly	Thr	Tyr	Tyr	Lys	Pro	Met	Tyr	
	TTT	GCA	AAT	GGT	GGC	ACT	TAC	TAT	AAA	CCA	ATG	TAT	

R6 *pbp1a*	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	**513**
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	**2484**
8099	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
3203	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
11184	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
12244	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
14016	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
12276	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
3996	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
11413	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	CAT	AAA	GTC	GTC	TTT	AGT	GAT	GGG	AGT	GAA	AAA	
14126	Ile	Asn	Lys	Ile	Val	Phe	Ser	Asp	Gly	Ser	Glu	Lys	
	ATC	AAT	AAA	ATC	GTC	TTT	AGT	GAT	GGT	AGC	GAA	AAA	
3455	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Lys	Lys	
	ATC	CAT	AAA	GTC	GTC	TTC	AGT	GAT	GGA	AGT	AAA	AAA	
742	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Lys	Lys	
	ATC	CAT	AAA	GTC	GTC	TTC	AGT	GAT	GGA	AGT	AAA	AAA	
2848	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Lys	Lys	
	ATC	CAT	AAA	GTC	GTC	TTC	AGT	GAT	GGA	AGT	AAA	AAA	
6363	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Lys	Lys	
	ATC	CAT	AAA	GTC	GTC	TTC	AGT	GAT	GGA	AGT	AAA	AAA	
6190	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Lys	Lys	
	ATC	CAT	AAA	GTC	GTC	TTC	AGT	GAT	GGA	AGT	AAA	AAA	
8111	Ile	His	Lys	Val	Val	Phe	Ser	Asp	Gly	Ser	Lys	Lys	
	ATC	CAT	AAA	GTC	GTC	TTC	AGT	GAT	GGA	AGT	AAA	AAA	

R6 *pbp1a*	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	**525**
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAA	GAA	**2520**
8099	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAA	GAA	
3203	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
11184	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
12244	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
14016	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
12276	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAA	GAA	
3996	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
11413	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
14126	Glu	Phe	Ser	Asp	Ala	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAA	TTT	TCT	GAT	GCT	GGT	ACA	CGA	GCT	ATG	AAA	GAG	
3455	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
742	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
2848	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
6363	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
6190	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	
8111	Glu	Phe	Ser	Asn	Val	Gly	Thr	Arg	Ala	Met	Lys	Glu	
	GAG	TTC	TCT	AAT	GTC	GGA	ACT	CGT	GCC	ATG	AAG	GAA	

R6 *pbp1a*	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	**537**
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	**2556**
8099	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
3203	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
11184	Thr	Thr	Ala	Tyr	Met	Met	Thr	Glu	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAA	ATG	ATG	AAA	ACA	
12244	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
14016	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACA	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
12276	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
3996	Thr	Thr	Ala	Tyr	Met	Met	Thr	Glu	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAA	ATG	ATG	AAA	ACA	
11413	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
14126	Thr	Thr	Ala	Tyr	Met	Met	Thr	Glu	Met	Met	Lys	Thr	
	ACT	ACT	GCC	TAT	ATG	ATG	ACT	GAA	ATG	ATG	AAA	ACT	
3455	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
742	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
2848	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
6363	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
6190	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	
8111	Thr	Thr	Ala	Tyr	Met	Met	Thr	Asp	Met	Met	Lys	Thr	
	ACG	ACA	GCC	TAT	ATG	ATG	ACC	GAC	ATG	ATG	AAA	ACA	

R6 *pbp1a*	Val	Leu	Ser	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	**549**
	GTC	TTG	AGT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	**2592**
8099	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
3203	Val	Leu	Ser	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	AGT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
11184	Val	Leu	Ser	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	AGT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
12244	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
14016	Val	Leu	Ser	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	AGT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
12276	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
3996	Val	Leu	Ser	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	AGT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
11413	Val	Leu	Ser	Tyr	Gly	Thr	Gly	Arg	Asn	Ala	Tyr	Leu	
	GTC	TTG	AGT	TAT	GGA	ACT	GGA	CGA	AAT	GCC	TAT	CTT	
14126	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Gly	Ala	Tyr	Leu	
	GTT	TTA	ACT	TAC	GGA	ACA	GGA	CGT	GGA	GCC	TAC	CTA	
3455	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Gly	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGG	CGT	GGA	GCC	TAT	CTT	
742	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Gly	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGG	CGT	GGA	GCC	TAT	CTT	
2848	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Gly	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGG	CGT	GGA	GCC	TAT	CTT	
6363	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Gly	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGG	CGT	GGA	GCC	TAT	CTT	
6190	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Gly	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGG	CGT	GGA	GCC	TAT	CTT	
8111	Val	Leu	Thr	Tyr	Gly	Thr	Gly	Arg	Gly	Ala	Tyr	Leu	
	GTC	TTG	ACT	TAT	GGA	ACT	GGG	CGT	GGA	GCC	TAT	CTT	

R6 *pbp1a*	Ala	Trp	Leu	Pro	Gln	Ala	Gly	**Lys**	**Thr**	**Gly**	Thr	Ser	**561**
	GCT	TGG	CTC	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	**2628**
8099	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTT	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
3203	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTC	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
11184	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTC	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
12244	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTC	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
14016	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTC	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
12276	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTT	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
3996	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTC	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
11413	Ala	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	GCT	TGG	CTC	CCT	CAG	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
14126	Pro	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	CCA	TGG	CTT	CCA	CAA	GCA	GGT	AAG	ACA	GGT	ACT	TCT	
3455	Pro	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	CCT	TGG	CTT	CCT	CAA	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
742	Pro	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	CCT	TGG	CTT	CCT	CAA	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
2848	Pro	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	CCT	TGG	CTT	CCT	CAA	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
6363	Pro	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	CCT	TGG	CTT	CCT	CAA	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
6190	Pro	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	CCT	TGG	CTT	CCT	CAA	GCT	GGT	AAA	ACA	GGA	ACC	TCT	
8111	Pro	Trp	Leu	Pro	Gln	Ala	Gly	Lys	Thr	Gly	Thr	Ser	
	CCT	TGG	CTT	CCT	CAA	GCT	GGT	AAA	ACA	GGA	ACC	TCT	

R6 *pbp1a*	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	**573**
	AAC	TAT	ACA	GAC	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	**2664**
8099	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAC	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
3203	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAC	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
11184	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
12244	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAC	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
14016	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAC	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
12276	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAC	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
3996	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
11413	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAC	GAG	GAA	ATT	GAA	AAC	CAC	ATC	AAG	
14126	Asn	Tyr	Thr	Asp	Glu	Glu	Ile	Glu	Lys	Tyr	Ile	Lys	
	AAC	TAT	ACT	GAC	GAA	GAA	ATT	GAA	AAG	TAT	ATC	AAG	
3455	Asn	Tyr	Thr	Asp	Glu	Glu	Val	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	GTT	GAA	AAC	CAC	ATC	AAG	
742	Asn	Tyr	Thr	Asp	Glu	Glu	Val	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	GTT	GAA	AAC	CAC	ATC	AAG	
2848	Asn	Tyr	Thr	Asp	Glu	Glu	Val	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	GTT	GAA	AAC	CAC	ATC	AAG	
6363	Asn	Tyr	Thr	Asp	Glu	Glu	Val	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	GTT	GAA	AAC	CAC	ATC	AAG	
6190	Asn	Tyr	Thr	Asp	Glu	Glu	Val	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	GTT	GAA	AAC	CAC	ATC	AAG	
8111	Asn	Tyr	Thr	Asp	Glu	Glu	Val	Glu	Asn	His	Ile	Lys	
	AAC	TAT	ACA	GAT	GAG	GAA	GTT	GAA	AAC	CAC	ATC	AAG	

R6 *pbp1a*	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	**585**
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAT	GAA	CTA	TTT	GCT	**2700**
8099	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAT	GAA	CTA	TTT	GCT	
3203	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAC	GAA	CTA	TTT	GCT	
11184	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAT	GAA	CTA	TTT	GCT	
12244	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAT	GAA	CTA	TTT	GCT	
14016	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAC	GAA	CTA	TTT	GCT	
12276	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAT	GAA	CTA	TTT	GCT	
3996	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAT	GAA	CTA	TTT	GCT	
11413	Thr	Ser	Gln	Phe	Val	Ala	Pro	Asp	Glu	Leu	Phe	Ala	
	ACC	TCT	CAA	TTT	GTA	GCA	CCT	GAC	GAA	CTA	TTT	GCT	
14126	Asn	Thr	Gly	Tyr	Val	Ala	Pro	Asp	Glu	Met	Phe	Val	
	AAC	ACT	GGC	TAC	GTA	GCT	CCA	GAT	GAA	ATG	TTT	GTG	
3455	Asn	Thr	Gly	Tyr	Val	Ala	Pro	Asp	Glu	Met	Phe	Val	
	AAC	ACT	GGC	TAT	GTA	GCT	CCA	GAT	GAA	ATG	TTT	GTT	
742	Asn	Thr	Gly	Tyr	Val	Ala	Pro	Asp	Glu	Met	Phe	Val	
	AAC	ACT	GGC	TAT	GTA	GCT	CCA	GAT	GAA	ATG	TTT	GTT	
2848	Asn	Thr	Gly	Tyr	Val	Ala	Pro	Asp	Glu	Met	Phe	Val	
	AAC	ACT	GGC	TAT	GTA	GCT	CCA	GAT	GAA	ATG	TTT	GTT	
6363	Asn	Thr	Gly	Tyr	Val	Ala	Pro	Asp	Glu	Met	Phe	Val	
	AAC	ACT	GGC	TAT	GTA	GCT	CCA	GAT	GAA	ATG	TTT	GTT	
6190	Asn	Thr	Gly	Tyr	Val	Ala	Pro	Asp	Glu	Met	Phe	Val	
	AAC	ACT	GGC	TAT	GTA	GCT	CCA	GAT	GAA	ATG	TTT	GTT	
8111	Asn	Thr	Gly	Tyr	Val	Ala	Pro	Asp	Glu	Met	Phe	Val	
	AAC	ACT	GGC	TAT	GTA	GCT	CCA	GAT	GAA	ATG	TTT	GTT	

Strain													
R6 *pbp1a*	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	**597**
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	**2736**
8099	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
3203	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
11184	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
12244	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
14016	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
12276	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
3996	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
11413	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGC	TAT	ACG	CGT	AAA	TAT	TCA	ATG	GCT	GTA	TGG	ACA	
14126	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGT	TAT	ACT	CGT	AAG	TAT	TCT	ATG	GCT	GTA	TGG	ACA	
3455	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGT	TAT	ACT	CGT	AAG	TAT	TCT	ATG	GCT	GTA	TGG	ACA	
742	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGT	TAT	ACT	CGT	AAG	TAT	TCT	ATG	GCT	GTA	TGG	ACA	
2848	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGT	TAT	ACT	CGT	AAG	TAT	TCT	ATG	GCT	GTA	TGG	ACA	
6363	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGT	TAT	ACT	CGT	AAG	TAT	TCT	ATG	GCT	GTA	TGG	ACA	
6190	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGT	TAT	ACT	CGT	AAG	TAT	TCT	ATG	GCT	GTA	TGG	ACA	
8111	Gly	Tyr	Thr	Arg	Lys	Tyr	Ser	Met	Ala	Val	Trp	Thr	
	GGT	TAT	ACT	CGT	AAG	TAT	TCT	ATG	GCT	GTA	TGG	ACA	

Strain													
R6 *pbp1a*	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	**609**
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	**2772**
8099	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
3203	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
11184	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
12244	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
14016	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
12276	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
3996	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
11413	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Leu	Val	Gly	Asn	
	GGC	TAT	TCT	AAC	CGT	CTG	ACA	CCA	CTT	GTA	GGC	AAT	
14126	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Ile	Val	Gly	Asp	
	GGT	TAT	TCG	AAT	CGT	TTA	ACT	CCT	ATC	GTT	GGA	GAT	
3455	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Ile	Val	Gly	Asp	
	GGT	TAT	TCG	AAT	CGT	TTA	ACT	CCT	ATC	GTT	GGA	GAT	
742	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Ile	Val	Gly	Asp	
	GGT	TAT	TCG	AAT	CGT	TTA	ACT	CCT	ATC	GTT	GGA	GAT	
2848	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Ile	Val	Gly	Asp	
	GGT	TAT	TCG	AAT	CGT	TTA	ACT	CCT	ATC	GTT	GGA	GAT	
6363	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Ile	Val	Gly	Asp	
	GGT	TAT	TCG	AAT	CGT	TTA	ACT	CCT	ATC	GTT	GGA	GAT	
6190	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Ile	Val	Gly	Asp	
	GGT	TAT	TCG	AAT	CGT	TTA	ACT	CCT	ATC	GTT	GGA	GAT	
8111	Gly	Tyr	Ser	Asn	Arg	Leu	Thr	Pro	Ile	Val	Gly	Asp	
	GGT	TAT	TCG	AAT	CGT	TTA	ACT	CCT	ATC	GTT	GGA	GAT	

R6 *pbp1a*	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	**619**
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	**2802**
8099	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
3203	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
11184	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
12244	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
14016	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
12276	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
3996	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
11413	Gly	Leu	Thr	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGC	CTT	ACG	GTC	GCT	GCC	AAA	GTT	TAC	CGC	
14126	Gly	Phe	Leu	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGT	TTC	CTA	GTT	GCA	GCT	AAA	GTT	TAT	CGC	
3455	Gly	Phe	Leu	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGT	TTC	CTA	GTT	GCA	GCT	AAA	GTT	TAT	CGC	
742	Gly	Phe	Leu	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGT	TTC	CTA	GTT	GCA	GCT	AAA	GTT	TAT	CGC	
2848	Gly	Phe	Leu	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGT	TTC	CTA	GTT	GCA	GCT	AAA	GTT	TAT	CGC	
6363	Gly	Phe	Leu	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGT	TTC	CTA	GTT	GCA	GCT	AAA	GTT	TAT	CGC	
6190	Gly	Phe	Leu	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGT	TTC	CTA	GTT	GCA	GCT	AAA	GTT	TAT	CGC	
8111	Gly	Phe	Leu	Val	Ala	Ala	Lys	Val	Tyr	Arg	
	GGT	TTC	CTA	GTT	GCA	GCT	AAA	GTT	TAT	CGC	