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(54) Title: METHODS AND COMPOSITION TO IDENTIFY SWINE GENETICALLY RESISTANT TO F18 <i>E. COLI</i> ASSOCIATED DISEASES		
(57) Abstract The present invention provides non-invasive methods and compositions to differentiate, with a high level of sensitivity and specificity, swine that are genetically susceptible to diseases associated with F18 <i>E. coli</i> infection, from resistant swine. DNA polymorphisms in the swine alpha (1,2) fucosyltransferase 1 (<i>FUT1</i>) gene were used to differentiate resistant from susceptible swine. The invention includes a polypeptide with amino acid substitutions, encoded by the nucleotide polymorphisms, a molecular diagnostic assay, and a kit for the differentiation of <i>E. coli</i> F18–adhesion resistant, heterozygous (carrier) and homozygous susceptible pigs. The molecular test identifies susceptible to oedema disease and postweaning diarrhea with high sensitivity and specificity, therefore, is useful to swine breeders in their efforts to enhance for resistance. Information on the polymorphisms of the present invention provides insight into causation and treatment of <i>E. coli</i> associated intestinal disorders.		

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METHODS AND COMPOSITION TO IDENTIFY
SWINE GENETICALLY RESISTANT TO
F18 *E. COLI* ASSOCIATED DISEASES

5 Compositions and non-invasive methods are provided for the
identification of swine genetically resistant to *E. coli* related diseases, in
particular, intestinal diseases associated with a strain of *E. coli* bacteria
supplied with F18 fimbriae. DNA polymorphisms in the swine alpha (1,2)
fucosyltransferase (*FUT1*) gene were identified that differentiate resistant from
10 susceptible swine and provide a diagnostic test useful for swine breeders.

 A major problem in breeding swine is to keep them disease-free.
Intestinal disorders postweaning are a particular problem. A limited number of
serotypes of toxigenic *Escherichia (E.) coli* strains are the causative agents of
oedema disease and postweaning diarrhea in swine which induce serious
15 economic losses, especially among piglets aged 4 to 12 weeks, in swine
breeding farms all over the world. The typical clinical symptoms of oedema
disease are neurological signs such as ataxia, convulsions and paralysis. At
post mortem examination, oedema is typically present at characteristic sites
such as eyelids and forehead, stomach wall and mesocolon. The diseases are
20 caused by Shiga-like toxin-II variant and enterotoxins LT, STa, STb
respectively, produced by *E. coli* that colonize the surface of the small intestine
without effecting major morphological changes of the enterocytes (cells in the
intestine). Types of bacterial *E. coli* strains, such as F4, F18 and K88 are
major lethal villains in this regard. "Oedema disease of pigs is an
25 enterotoxaemia characterized by generalized vascular damage. The latter is
caused by a toxin, Shiga-like toxin II variant, produced by certain strains of
E. coli" (Bertschinger *et al.*, 1993). The *E. coli* are distinguished by their pili

types, a group of adhesive fimbriae that are related are designated *e.g.*, K88 or F18 (Vögeli *et al.*, 1996).

Not all swine succumb to *E. coli* infections. Colonization depends on adherence of the bacteria to the enterocytes which is mediated by the bacterial fimbriae designated *e.g.*, K88 or F18. Susceptibility to adhesion, *i.e.* expression of receptors in swine for binding the fimbriae, has been shown to be genetically controlled by the host and is inherited as a dominant trait with, in the case of F18, *B* being the susceptibility allele and *b* the resistance allele. (Vögeli *et al.*, 1996; Meijerink *et al.*, 1996). The genetic locus for this *E. coli* F18-receptor (*ECF18R*) has been mapped to porcine chromosome 6 (SSC6), based on its close genetic linkage to the *S* locus and other loci of the halothane (HAL) linkage group on chromosome 6. The receptor for K88 *E. coli* is on chromosome 13.

The mechanism for resistance appears to be that intestinal borders in resistant animals are not colonized by *E. coli*, *i.e.*, the bacteria do not adhere to intestinal walls of resistant swine. Glycoprotein receptors in the brush border membrane of the intestine were shown to be responsible for the differences between adhesive and non-adhesive phenotypes related to some *E. coli*, therefore, the genotype of the host swine determines resistance. The fimbriated bacteria also have been studied. (WO 9413811)

Current methods of identifying swine that are resistant to F18 *E. coli* associated diseases are either to 1) collect intestinal samples from swine at slaughter and perform the microscopic adhesion test, 2) challenge the animals with virulent *E. coli* ("colonization test"), or 3) perform blood typing of the A-O(S) blood group system. The first two methods are not practical for identifying resistant animals for use as breeding stock. Although the blood typing method does identify resistant animals, the test is unable to determine whether susceptible animals are homozygous or heterozygous for susceptibility. At least two alleles (condition of a gene) at the receptor locus

control either susceptibility (dominant) or resistance (recessive). Knowledge of the genotype of animals with regard to these alleles is essential to develop a successful breeding program. The purpose of the breeding program is to produce swine that are resistant to F18 *E. coli* associated diseases that decimate stock post-weaning.

In one publication the authors stated, in reference to oedema disease in swine, that "Searches are underway for appropriate genetic markers. . ." (Bertschinger *et al.*, 1993, page 87) and, citing Walters and Sellwood, 1982:

Breeding resistant swine is an attractive method for prevention of diseases for which an effective prophylaxis is not available. The feasibility of this approach will depend on the prevalence of the gene(s) encoding resistance in the pig population, improved methods for the detection of resistant pigs, and absence of negative genetic traits co-selected with this resistance.

A genetic "marker" locus is a coding or non-coding locus that is close to a genetic locus of interest, but is not necessarily the locus itself. Detectable phenotypes include restriction length fragment polymorphisms, colorimetric or enzymatic reactions, and antibiotic resistance. The *S* locus controls expression of the A and O blood group antigens. Swine homozygous recessive at the *S* locus do not express either A or O blood group antigens. A similar condition exists in humans and is due to mutations in the alpha (1,2) fucosyltransferase gene which encodes the human blood group H (Kelly *et al.*, 1994; *see also* WO 9628967). The porcine alpha (1,2) fucosyltransferase gene of swine has recently been sequenced (Cohnen *et al.*, 1996). This gene is very likely the gene present at the *S* locus in swine.

The blood group *H* and *Se* loci have been mapped genetically and physically to human chromosome 19q13.3. This region is evolutionarily conserved, containing genes homologous to the HAL linkage group of genes in pigs. The blood group H encoding gene is the so called *FUT1* whereas the *Se* gene is equivalent to the *FUT2* gene. *FUT1* determines H antigen expression

in the erythroid cell lineage, whereas *FUT2* regulates expression of the H antigen in the secretory epithelia and saliva. Conservation of the *FUT1* gene has been shown in lower mammals such as rat and rabbit, and mRNA expression has been shown in rabbit brain tissue and rat colon. In all these species two types of alpha (1,2) fucosyltransferase genes have been reported which are structurally very similar to the human *FUT1* and *FUT2* genes, but in particular the *FUT1* homologous genes show a species specific expression pattern. In humans the *FUT1* gene is responsible for synthesis of H antigens in the precursors of erythrocytes. However, in pigs erythrocytes passively adsorb H-like antigens from the serum, as is the case for the human *Lewis* antigens. In pigs all H-like antigens are related to exocrine secretory tissues, and expression of the *FUT2* (*Secretor*) gene is seen in secretory tissue of other animal species. Therefore, expression of the porcine A-O blood group determinants which cross-react with anti-human blood group H and A antibodies might be influenced by the *FUT2* gene.

Further information about blood groups and *E. coli* swine diseases include that carbohydrate structures of blood group antigens mediate the adhesion of some pathogenic microorganisms to host tissues, *e.g. Helicobacter pylori* adhere to Lewis^b blood group antigens, and *E. coli* causing urinary tract infections adhere to blood group P substance. Genes encoding glycosyltransferases that are responsible for the formation of the blood group specific carbohydrate structures, therefore, represent candidate genes for the control of bacterial colonization by the host. The localization of these genes is in the same chromosomal region as the locus responsible for adhesion/non-adhesion of F18 positive *E. coli* in the swine small intestine. Swine do not express blood group antigens A and O until after weaning, this is the same time that they become susceptible to disease caused by F18 *E. coli*.

New methods of diagnosis and treatment are needed for *E. coli* related intestinal diseases in swine. Detection of a genetic mutation was proposed as a

diagnostic test for some swine disorders (malignant hyperthermia) (Fujii *et al.*, 1991; U.S. Pat. No. 5,358,649), but polymorphic markers were not reported for diagnosis. Vaccines to develop resistance to *E. coli* colonization were described (U.S. Pat. No. 5,552,144; WO 8604604), but are unlikely to be a preferred method to prevent the *E. coli* disease because of difficulties in administering live vaccine orally to newborn swine, and because of regulatory restrictions. Antibiotics are available for treatment, but there is no successful prophylaxis.

SUMMARY OF THE INVENTION

The compositions and non-invasive methods of the present invention provide detection and elimination of swine that are susceptible to *E. coli* associated diseases. A non-invasive method for identifying a swine that is resistant to intestinal colonization by *E. coli* includes the following steps: determining whether a genetic polymorphism associated with resistance to colonization is in a biological sample from the swine; and inferring that the swine is resistant if the swine is homozygous for the polymorphism.

More particularly, the method is determining in a biological sample from the swine whether the only nitrogen base at position 307, starting with the ATG codon as position, in the alpha (1,2) fucosyltransferase gene of the swine is adenine; and identifying the swine as resistant if the only nitrogen base at position 307 is adenine.

To determine whether a polymorphism is present in a biological sample, restriction fragment length polymorphisms are analyzed on a gel that separates them by molecular weight. Restriction endonucleases are enzymes that reproducibly cut nucleic acid molecules at specific sites, resulting in nucleic acid fragments of different molecular weights, depending on the location of the cuts.

The invention also relates to a method for breeding swine to be resistant to *E. coli* associated diseases by selecting for breeding swine that have a genetic polymorphism in the alpha (1,2) fucosyltransferase 1 gene that identifies them as swine that are resistant to *E. coli* related intestinal diseases; and breeding the selected swine by methods known to those of skill in the art.

An aspect of the invention is a DNA molecule which is polymorphic for the alpha (1,2) fucosyltransferase 1 gene in swine, in particular a sequence in accordance with FIG. 1. Other aspects of the invention are molecules with nucleotide sequences complementary to that of FIG. 1 with the variation at position 307.

Polypeptides encoded by the polymorphic DNA molecules of the present invention and having alpha (1,2) fucosyltransferase activity are also aspects of the invention.

A molecular assay for detecting *E. coli* F18 receptors in swine is to (a) isolate DNA from porcine nucleated cells; (b) amplify the DNA in a polymerase chain reaction (PCR) using oligonucleotides as primers which are complementary to a DNA sequence of the porcine alpha (1,2) fucosyltransferase gene 1; (c) perform a restriction enzyme digest with at least one restriction enzyme *e.g.*, HinPI; (d) separate the resulting fragments by gel electrophoresis; and (e) determine the respective numbers and lengths of fragments on the gel; and (f) determine from the numbers and length of fragments, which receptors are present in the porcine cells. Use of the larger amplified fragments disclosed herein for restriction length polymorphism analysis (RFLP), rather than smaller fragments, is less expensive because the DNA bands can be run on agarose gels of relatively low concentration. Also, to produce some of the fragments, only one restriction enzyme is needed for a constant restriction site adjacent to the variable diagnostic site.

A kit for detecting polymorphisms associated with *E. coli* F18 receptors uses oligonucleotides in separate containers which are complementary to a

DNA sequence of the porcine alpha (1,2) fucosyltransferase gene 1 that distinguishes resistant from sensitive swine. The test can be performed on swine of any age. The kit includes in separate containers, enzymes and primers to amplify DNA, *e.g.*, by PCR, restriction enzymes, positive and negative control DNAs, and the *FUT1* gene.

The polymorphisms are also useful to develop drugs to treat swine that have *E. coli*-associated disease. A mutated form of porcine alpha 1,2 fucosyltransferase could interfere with the normal enzyme, preventing it from producing the intestinal receptor for F18.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 The nucleotide sequence of pHT83xF (below) and predicted amino acid sequence (above) of the swine $\alpha 1 \rightarrow 2$ fucosyltransferase polymorphism of the present invention using the *one-letter* amino acid code. The *solid double line* below the amino acid sequences (=) is the putative transmembrane region; the *dotted line* below the amino acid sequence shows two potential N-linked glycosylation sites (...).

□ is where an adenine (A) is substituted for guanine (G) in resistant swine.

*Indicates the termination codon.

Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Molecular analysis of DNA polymorphisms associated with resistance of swine to *E. coli* associated diseases facilitated diagnostic assays to select resistant pigs for breeding. Resistant pigs differ from sensitive pigs at the

E. coli F18 receptor locus as identified by the polymorphic markers of the present invention.

The present invention provides non-invasive methods and compositions to distinguish with a high level of sensitivity and specificity, swine that are genetically susceptible to diseases associated with F18 *E. coli* infection from resistant swine. A DNA polymorphism in the swine alpha (1,2) fucosyltransferase (*FUT1*) gene was identified that differentiates resistant from susceptible swine. The polymorphism arose by a mutation (change) in a nucleotide sequence leading to a new allele. An allele is a condition of a gene. In a population there may be many alleles of a gene differing by nitrogen base substitutions, presumably caused by mutations in an ancestral DNA molecule. The coexistence in a population of more than one allele (sometimes referred to as a "variant") is called a genetic polymorphism. Loci at which more than one allele may exist as apparently stable components of a population, is a polymorphic locus. Usually, one of the polymorphic loci is at a low frequency in the population.

As determined from a biological sample, preferably blood, the resistant swine have a polymorphism in their genomes in which the only base detected at position 307 (see FIG. 1) in the nucleotide sequence is adenine, whereas the base in the same position in homozygous susceptible swine is guanine. Heterozygous swine will show both types of DNA and will be susceptible. The polymorphism is a variation of a porcine gene sequence (Cohney *et al.* 1996).

The detection of the close association of the mutation at *FUT1* and *ECF18R* allowed a molecular test to be developed for the identification of *E. coli* F18 adhesion resistant, heterozygous (carrier) and homozygous susceptible pigs. This diagnostic test identifies, with high sensitivity and specificity, pigs that are susceptible to oedema disease and postweaning diarrhea. The incidence of the polymorphisms of the present invention differs

among swine breeds. The availability of the diagnostic test for the polymorphism of the present invention provides breeders with the opportunity to effectively eliminate the *ECF18R* susceptible allele from their swine herds, thereby eliminating a prerequisite for *E. coli* F18 bacterial adhesion causing oedema disease and postweaning diarrhea.

The present invention further includes nucleotide sequences of alpha (1,2) fucosyltransferase gene 1 representing the various polymorphisms at bp 307, and diagnostic molecular based kits to identify polymorphisms in the alpha (1,2) fucosyltransferase gene.

In order to obtain candidate genes for the *E. coli* F18 receptor locus (*ECF18R*) one genomic clone containing the gene was isolated containing the alpha (1,2) fucosyltransferase genes, *FUT1* and *FUT2*, from a porcine genomic library. Mapping by direct PCR placed the gene in band q11 of porcine chromosome 6 (SSC6q11). Sequence analysis of the cosmids resulted in the characterization of (a) an open reading frame (ORF), 1098 base pairs in length, that is 82.3% identical to the human *FUT1* sequence, and (b) a second ORF, 1023 base pairs in length which is 85% identical to human *FUT2* sequence. The *FUT1* and *FUT2* loci therefore seem to be porcine equivalents of the human blood group *H* and the *Secretor* locus. Direct sequencing of the two ORFs in swine either susceptible or resistant to adhesion and colonization by F18 fimbriated *E. coli* (*ECF18R*) revealed two polymorphisms at base pair 307 (M307) and base pair 857 (M857) of the *FUT1* ORF. The M307 mutation is a good marker for marker-assisted selection of *E. coli* F18 adhesion resistant animals. No polymorphisms were identified in *FUT2* that differentiated susceptible and resistant pigs.

Examples

The following examples provide embodiments of the invention.

Example 1: An Assay For Resistant Swine

The polymorphisms of the present invention are easily identified using PCR-RFLP tests. One embodiment of the tests used a 160bp fragment of porcine alpha (1,2) fucosyltransferase 1 amplified using PCR with the following primers; 5'CCAACGCCTCCGATTCCTGT3' and 5'GTGCATGGCAGGCTGGATGA3'. Preferred PCR conditions for this embodiment are 25 cycles at the following times and temperatures: 94°C, 30 sec; 60°C, 45 sec; 72°C, 90 sec. The amplified DNA from resistant swine was digested by the restriction enzyme HgaI, but was not digested by the restriction enzyme HinfI. The amplified DNA from homozygous susceptible swine was digested by the restriction enzyme HinfI. The amplified DNA from heterozygous susceptible swine was partially digested by both enzymes.

Example 2: Sensitivity And Specificity Of An Assay Using Alpha (1,2) Fucosyltransferase In Detecting Swine Resistant To F18 *E. Coli*

A study was conducted to determine the association between disease resistance and the polymorphism at position 307 of the FUT1 gene. 183 weaned swine (ranging in ages 2-6 months) were obtained from six different breeding herds. Only one of these herds was known to contain resistant animals before the start of the study, and this herd is known to have a high incidence of porcine stress syndrome. The other 5 herds had no evidence of porcine stress syndrome, and the incidence of disease resistance was unknown. Swine from each herd were randomly selected, humanely euthanized and spleens and samples of small intestine were removed. DNA was extracted from splenic tissue and used in a PCR-RFLP assay described in Example 1. Intestinal cells were purified by scraping the mucosal surface off the intestine, lysing the cells in a hypotonic EDTA solution and washing by centrifugation. The purified intestinal cell brush borders were incubated with F18 *E. coli*. This mixture was examined by phase contrast microscopy. This assay

determined if swine were susceptible (intestinal samples had adhering bacteria) or resistant (intestinal samples had no adhering bacteria). The PCR-RFLP assay for the polymorphism correlated with the bacteria-intestinal cell binding assay in 53 of 53 resistant swine and 128 of 130 susceptible swine. Two swine that were determined susceptible using the bacteria-intestinal cell binding assay were incorrectly predicted to be resistant using the PCR-RFLP assay. Two of the six herds examined contained resistant pigs, while only one herd had porcine stress syndrome, demonstrating that the PCR-RFLP assay can identify disease resistant animals in animals that do not have porcine stress syndrome.

METHODS

1. Primers

Primers derived from the human *FUT1* gene were used for the amplification of its porcine counterpart from genomic DNA. From the resulting porcine sequences specific primers were designed which were used in further amplification and sequencing reactions.

2. Screening of a Porcine Genomic Library

Porcine genomic libraries were obtained from Clontech and screened with porcine FUT1 cDNA. After hybridization of replica filters at 42°C for 15h (50% formamide, 6 x SSC, 5 x Denhardt's, 0.5% SDS, 0.1 mg/ml Salmon Sperm) and washing twice at 65°C for 30 min. (1 x SSC, 0.1% SDS), positive colonies were identified after exposure (15h, -80°C) to X-ray film.

3. In situ Hybridization of Porcine Metaphase Chromosomes

Clones are subjected to fluorescence *in situ* hybridization (FISH) or direct *in situ* chromosomal PCT (DISC PCR) on porcine metaphases. Metaphase chromosomes were Q-banded and photographed before

hybridization. The probes were labeled by random priming using
biotin-16-dUTP. Signal detection and amplification was performed using
avidin-FITC and biotinylated anti-avidin. The chromosomes were
counterstained with 4,6-diamidino-2-phenylindole, and the relative positions of
the cosmids were determined as described by Solinas Toldo, 1993.

4. Subcloning

Enzymatic digests of probe positive genomic colonies were separated
on agarose gel, transferred to a nylon membrane, and probe positive bands
were subcloned into plasmids for FUT sequencing. The sequence of FUT1
derived from this subclones is shown in FIG. 1.

5. Polymerase Chain Reaction and Direct Sequencing

Using the Perkin Elmer Ready Reaction Dye Terminator kit (Perkin
Elmer Cetus, Norwalk, CT, USA) and 10 pmol of primer, cycle sequencing
was performed with a thermal program consisting of an initial denaturation of
5 min at 95°C, followed by 25 cycles of 30 sec 95°C, 15 sec 50°C and 4 min
60°C. Samples were analyzed on a 373A ABI sequencer (Applied Biosystems
Inc.) and sequence analysis was performed with the GCG package (Devereux,
1984).

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WO 9628967, Inventor: Koike, C.

WE CLAIM

1. A method for identifying a swine that is resistant to intestinal colonization by *E. coli*, said method comprising:

- 5 a. determining whether a genetic polymorphism associated with resistance to colonization is in a biological sample from the swine; and
- b. inferring that the swine is resistant if the swine is homozygous for the polymorphism.

2. A method for identifying a swine that is resistant to *E. coli* associated intestinal disorders, said method comprising:

- 10 a. determining in a biological sample from the swine whether the only nitrogen base at position 307 in the alpha (1,2) fucosyltransferase gene 1 of the swine is adenine ;and
- b. identifying the swine as resistant if the only nitrogen base at position 307 is adenine.

15 3. A method for breeding swine that are resistant to *E. coli* associated diseases, said method comprising:

- a. selecting for breeding swine that have a genetic polymorphism in the alpha (1,2) fucosyltransferase 1 gene that identifies them as swine that are resistant to *E. coli* related intestinal diseases; and
- 20 b. breeding the selected swine.

4. The method of claim 1, 2 or 3, wherein *E. coli* is strain F18.

5. An isolated DNA molecule which is polymorphic for the alpha (1,2) fucosyltransferase gene 1 in swine.

6. An isolated DNA molecule having a nucleotide sequence in accordance with FIG. 1.

7. The isolated DNA molecule of claim 6 wherein adenine is in nucleotide position 307 instead of guanine.

5 8. An isolated DNA molecule which is complementary to the nucleotide sequence of claim 6 or claim 7.

9. A polypeptide encoded by the DNA molecule of claims 6 or 7, said molecule having alpha (1,2) fucosyltransferase activity.

10 10. A portion of the isolated DNA molecule of claims 6 or 7, said portion including the nucleotide sequence that distinguishes *E. coli* colonization resistant from sensitive swine.

15 11. A molecular assay for detecting *E. coli* F18 receptors, said assay comprising isolating DNA from porcine nucleated cells, amplifying said DNA in a polymerase chain reaction using oligonucleotides as primers which are complementary to a DNA sequence of the porcine alpha (1,2) fucosyltransferase 1 gene, performing a restriction enzyme digest with at least one restriction enzyme, separating the resulting fragments by gel electrophoresis, determining the respective numbers and lengths of fragments, and determining from the numbers and lengths of fragments, which receptors
20 are present.

12. The molecular assay of claim 13 wherein the restriction enzyme is *HinPI*.

13. A kit for detecting *E. coli* F18 receptors, said kit comprising in separate containers oligonucleotides which are complementary to a DNA sequence of polymorphisms of the porcine alpha (1,2) fucosyltransferase gene 1.

5 14. A porcine polypeptide having alpha (1,2) fucosyltransferase activity and an amino acid substitution in position 103.

15. Use of polymorphisms of porcine alpha (1,2) fucosyltransferase to develop drugs to treat swine that have *E. coli*-associated disease.

				M	W	V	P	S	R	R	H	L	C	L	T	F	L	L	V	C	17
CT	CGA	GCC	ATG	TGG	GTC	CCC	AGC	CGC	CGC	CAC	CTC	TGT	CTG	ACC	TTC	CTG	CTA	GTC	TGT	59	
V	L	A	A	I	F	F	L	N	V	Y	Q	D	L	F	Y	S	G	L	D	37	
GTT	TTA	GCA	GCA	ATT	TTC	TTC	CTG	AAC	GTC	TAT	CAA	GAC	CTC	TTT	TAC	AGT	GGC	TTA	GAC	119	
L	L	A	L	C	P	D	H	N	V	V	S	S	P	V	A	I	F	C	L	57	
CTG	CTG	GCC	CTG	TGT	CCA	GAC	CAT	AAC	GTG	GTA	TCA	TCT	CCC	GTG	GCC	ATA	TTC	TGC	CTG	179	
A	G	T	P	V	H	P	N	A	S	D	S	C	P	K	H	P	A	S	F	77	
GCG	GGC	ACG	CCG	GTA	CAC	CCC	AAC	GCC	TCC	GAT	TCC	TGT	CCC	AAG	CAT	CCT	GCC	TCC	TTT	239	
S	G	T	W	T	I	Y	P	D	G	R	F	G	N	Q	M	G	Q	Y	A	97	
TCC	GGG	ACC	TGG	ACT	ATT	TAC	CCG	GAT	GGC	CGG	TTT	GGG	AAC	CAG	ATG	GGA	CAG	TAT	GCC	299	
T	L	L	A	L	A	Q	L	N	G	R	Q	A	F	I	Q	P	A	M	H	117	
ACG	CTG	CTG	GCC	CTG	GCG	CAG	CTC	AAC	GGC	CGC	CAG	GCC	TTC	ATC	CAG	CCT	GCC	ATG	CAC	359	
A	V	L	A	P	V	F	R	I	T	L	P	V	L	A	P	E	V	D	R	137	
GCC	GTC	CTG	GCC	CCC	GTG	TTC	CGC	ATC	ACG	CTG	CCT	GTC	CTG	GCG	CCC	GAG	GTA	GAC	AGG	419	
H	A	P	W	R	E	L	E	L	H	D	W	M	S	E	D	Y	A	H	L	157	
CAC	GCT	CCT	TGG	CGG	GAG	CTG	GAG	CTT	CAC	GAG	TGG	ATG	TCC	GAG	GAT	TAT	GCC	CAC	TTA	479	
K	E	P	W	L	K	L	T	G	F	P	C	S	W	T	F	F	H	H	L	177	
AAG	GAG	CCC	TGG	CTG	AAG	CTC	ACC	GGC	TTC	CCC	TGC	TCC	TGG	ACC	TTC	TTC	CAC	CAC	CTC	539	
R	E	Q	I	R	S	E	F	T	L	H	D	H	L	R	Q	E	A	Q	G	197	
CGG	GAG	CAG	ATC	CGC	AGC	GAG	TTC	ACC	CTG	CAC	GAC	CAC	CTT	CGG	CAA	GAG	GCC	CAG	GGG	599	
V	L	S	Q	F	R	L	P	R	T	G	D	R	P	S	T	F	V	G	V	217	
GTA	CTG	AGT	CAG	TTC	CGT	CTA	CCC	CGC	ACA	GGG	GAC	CGC	CCC	AGC	ACC	TTC	GTG	GGG	GTC	659	
H	V	R	R	G	D	Y	L	R	V	M	P	K	R	W	K	G	V	V	G	237	
CAC	GTG	CGC	CGC	GGG	GAC	TAT	CTG	CGT	GTG	ATG	CCC	AAG	CGC	TGG	AAG	GGG	GTG	GTG	GGT	719	
D	G	G	A	Y	L	Q	Q	A	M	D	W	F	R	A	R	Y	E	A	P	257	
GAC	GCG	CGT	TAT	CTC	CAG	QAC	GCT	ATG	GAC	TGG	TTC	CGG	GCC	CGA	TAC	GAA	GCC	CCC	GTC	779	
F	V	V	T	S	N	G	M	E	W	C	R	K	N	I	D	T	S	R	G	277	
TTT	GTG	GTC	ACC	AGC	AAC	GGC	ATG	GAG	TGG	TGC	CGG	AAG	AAC	ATC	GAC	ACC	TCC	CGG	GGG	839	
D	V	I	F	A	G	D	G	R	E	A	A	P	A	R	D	F	A	L	L	297	
GAC	GTG	ATC	TTT	GCT	GGC	GAT	GGG	CGG	GAG	GCC	GCG	CCC	GCC	AGG	GAC	TTT	GCG	CTG	CTG	899	
V	Q	C	N	H	T	I	M	T	I	G	T	F	G	F	W	A	A	Y	L	317	
GTG	CAG	TGC	AAC	CAC	ACC	ATC	ATG	ACC	ATT	GGC	ACC	TTC	GGC	TTC	TGG	GCC	GCC	TAC	CTG	959	
A	G	G	D	T	I	Y	L	A	N	F	T	L	P	T	S	S	F	L	K	337	
GCT	GGT	GGA	GAT	ACC	ATC	TAC	TTG	GCT	AAC	TTC	ACC	CTG	CCC	ACT	TCC	AGC	TTC	CTG	AAG	1019	
I	F	K	P	E	A	A	F	L	P	E	W	V	G	I	N	A	D	L	S	357	
ATC	TTT	AAA	CCC	GAG	GCT	GCC	TTC	CTG	CCC	GAG	TGG	GTG	GGC	ATT	AAT	GCA	GAC	TTG	TCT	1079	
P	L	Q	M	L	A	G	P	*												365	
CCA	CTC	CAG	ATG	TTG	GCT	GGG	CCT	TGA	ACC	AGC	CAG	GAG	CCT	TTC	TGG	AAT	AGC	CTC	GGT	1139	
CAA	CCC	AGG	GCC	AGC	GTT	ATG	GGT	CTC	CGG	AAG	CCC	GAG	TAA	CTT	CCG	GAG	ATG	CTG	GTG	1199	
GTC	CTG	TAG	CAG	GCT	GGA	CAC	TTA	TTT	CAA	GAG	TGA	TTC	TAA	TTG	GCT	GGA	CTC	AGA	GGA	1259	
AAC	CCT	GCA	G																	1269	