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ORIGINAL ARTICLE

Genomic gene encoding manganese peroxidase from a white-rot fungus *Phanerochaete crassa* WD1694

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Abstract The gene encoding manganese peroxidase of a white-rot fungus *Phanerochaete crassa* WD1694 was cloned and sequenced. Four genomic clones were sequenced in which 3 clones were existed as alleles. The analysis of intron–exon structures divided the 4 clones into three subfamilies that corresponded to *mnp2* and *mnp3* of *Phanerochaete chrysosporium*, and a new subfamily possessing only five introns. The purified *P. crassa* WD1694 MnP consisted of 4 isozymes with same molecular weight, same N-terminal sequence, and different pI. N-terminal sequence of deduced protein of *P. crassa mnpB3* gene was identical to those of 4 MnP isozymes; however, the other 3 *mnp* genes had different N-terminal sequence. The molecular weight of encoded mature protein of *mnpB3* gene and purified MnP had a gap that could be difference between MnP proteins encoded by single gene. The results suggested that 4 MnP isozymes of *P. crassa* WD1694 arose from single gene.

Keywords Lignin · *Phanerochaete crassa* · Manganese peroxidase

Introduction

White-rot fungus is the only organism that can effectively break down lignin, which is very resistant to general microbial attack. It is well known that extracellular peroxidases, such as LiP and MnP play major roles in the lignin biodegradation process of white-rot fungi [1–4]. The catalytic mechanisms and molecular genetics of these ligninolytic peroxidases have been studied and have revealed their structural and functional properties [5–10]. It has also been reported that plant and fungal peroxidases may be arranged in a superfamily of three distinct classes; namely class I of bacterial and intracellular peroxidases, class II of fungal secretory peroxidases, and class III of secretory plant peroxidases, respectively [11]. The lignin-degrading peroxidases in class II of fungal secretory peroxidases have been further subdivided into three groups, LiP, MnP, and VP, based on the genetic and protein structural evidence [12]. Recently, phylogenetic analysis of fungal ligninolytic peroxidases was reported, showing the ubiquity and diversity of these enzymes among a wide range of ectomycorrhizal fungi, basidiomycetes and agaricomycetes [13–15].

We have studied the distribution of the extracellular peroxidase reaction of a white-rot fungus *P. crassa* WD1694 in detail, and showed how the MnP reaction occurred at the hyphal tips [16, 17]. Previously, we reported on the purification and characterization of MnP from *P. crassa* WD1694 [18]. In this report, we studied the cloning and sequence of genomic mnp genes of *P. crassa* WD1694 as an initial step toward understanding the molecular genetics of *P. crassa* WD1694.

Materials and methods

Strains

The white-rot fungus *Phanerochaete crassa* WD1694 [MAFF420737, *Phanerochaete crassa* (Lev.) Burdsall] was obtained from the culture collection of the Forestry

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and Forest Products Research Institute. *Escherichia coli* strain DH5α was used for transfections with pTA2 vector (Toyobo, Osaka, Japan).

Cultivation conditions

Cultivation was conducted as described previously [16–18].

Purification step

After cultivation for 4 days, culture filtrate was recovered and adsorbed on DEAE-Sepharose CL-6B and further extracted with 0.5 M NaCl in 10 mM acetate buffer (pH 5.5). Eluate was desalted and loaded on a DEAE-Toyopearl column (1 × 5 cm), equilibrated with 10 mM phosphate buffer (pH 6.0) and eluted with a linear 0–0.5 M NaCl gradient in 20 mM acetate buffer, pH 5.5, and fractions with MnP activity were collected, desalted, and concentrated.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) were conducted using a Multiphor II electrophoresis system (GE Healthcare UK Ltd., Buckinghamshire, England).

MnP activity staining

A staining solution containing 2 mM β-naphtol, 2 mM 3-amino-9-ethylcarbazole, 100 μM $MnSO_4$, 200 μM H_2O_2 and 20 % acetone in 80 mM acetate buffer (pH 4.5) was prepared to visualize MnP activity. The IEF gel was placed in the staining solution until the MnP bands were stained and washed with the solution containing 25 % ethanol and 8 % acetic acid. The IEF gel was then further rinsed with distilled water.

Determination of the N-terminal sequence

The purified MnP gave a single band on SDS-PAGE and further separated as 4 bands on IEF. The 4 MnP bands on IEF gel were brown and visible without staining. Each of the MnP bands on the IEF gel was cut out without staining before blotting to prevent cross-contamination. The cut-out gel slice was blotted to the PVDF protein sequencing membrane (Bio-Rad Laboratories, Inc. CA USA) and the N-terminal sequence was determined using the Edman method.

Isolation of genomic DNA

P. crassa WD1694 was grown in nitrogen-limited shake cultures as described previously [16–18]. The mycelia were collected by filtration, freeze-dried, and ground to powder by a Multi-beads shocker (Yasui Kikai, Osaka, Japan). The mycelia powder (100 mg) was completely dissolved in *N*-cetyl-*N,N,N*-trimethyl-ammonium bromide (CTAB, 700 μl, 65 °C), and extracted with chloroform:isoamylalcohol (24:1). After centrifuging for 14,000 rpm and 10 min, the supernatant was recovered, washed with chloroform:isoamylalcohol (24:1) and centrifuged. The supernatant had isopropanol added and the precipitate DNA was recovered and washed with ethanol.

Cloning of MnP genes

The first strands of genomic *P. crassa* mnp genes were obtained using the DNA sequences of *Phanerochaete chrysosporium mnp* genes as primers; FPA2, FPA2′, FPA3, FPA3′, FPB2, FPB2′, FPB3, and FPB3′ in Table 1 (GenBank accession Nos. M60672.1, S69963.1, and U70998). Four different sequences of the center part of *P. crassa* mnp genes (*P. crassa mnpA2, A3, B2*, and *B3*) were determined as the first strands. Subsequently, the 3′- and 5′-ends of these 4 sequences were determined respectively, by inverse PCR.

To determine the *P. crassa mnpA2* gene, primers FPA2 and FPA2′ were used to get the first strand of *P. crassa mnpA2*. Subsequently, inverse PCR was conducted to get the 3′-end of *P. crassa mnpA2* with primers a2p1 and a2p2, and the 5′-end of *P. crassa mnpA2* with primers PcrassaF2 and PcrassaR2. The remaining 3 genes, *P. crassa mnpA3, B2*, and *B3* were determined correspondingly. The primers used in these experiments are shown in Table 1.

The genome DNA from *P. crassa* WD1694 was digested with restriction enzymes and used for self-ligation with a DNA ligation kit (Toyobo) prior to inverse PCR. All PCR procedures were carried out with TaKaRa LA Taq (Takara Bio, Shiga, Japan) with a thermal cycler (Applied Biosystems, CA, USA). The thermal cycle parameters were as follows: a 1-min initial denaturation at 94 °C, 40 cycles of 30 s denaturation at 94 °C, 30 s of annealing at 55 °C, a 3 min extension at 68 °C, and a 10 min final extension at 72 °C. Specific PCR products were purified on 1 % agarose gel and extracted with a QIAquick Gel Extraction Kit (Quiagen, MD, USA). The amplified DNA samples were inserted into the pTA2 vector (Toyobo). Transformation was conducted with COMPENTENT high DH5α (Toyobo).

Results

We cloned and sequenced the genomic genes encoding MnP isozymes from the white-rot fungus *P. crassa* WD1694. The first fragments of the mnp genes of *P. crassa* WD1694 were obtained using *P. chrysosporium* mnp

Table 1 Primers used in this study

Primer name	Sequence	Target
FPA2	GTCTGACCTTCCACGTCGCTAT	The first strand of *P. crassa mnpA2*
FPA2′	GAACTGCGGCTCCAGTCAGA	The first strand of *P. crassa mnpA2*
FPA3	GCCTGACGTTCCACGACGCCAT	The first strand of *P. crassa mnpA3*
FPA3′	GAACTGAGACTCCAGTCTGA	The first strand of *P. crassa mnpA3*
FPB2	GTCTGACCTTCCACGACGCTAT	The first strand of *P. crassa mnpB2*
FPB2′	GAACTGCGACTCCAGTCTGA	The first strand of *P. crassa mnpB2*
FPB3	GCCTGACCTTCCACGACGCCAT	The first strand of *P. crassa mnpB3*
FPB3′	GAGCTGCGGCTCCAGTCCGA	The first strand of *P. crassa mnpB3*
PcrassaF2	GCATVGAGCCGTCGGCKCCTCCGCC	5′-end of *P. crassa mnpA2*
PcrassaR2	TCGGCAGCGGCRCSGACRYSGGC	5′-end of *P. crassa mnpA2*
a2p1	TTCCCCGGTACCCCTAACAACAC	3′-end of *P. crassa mnpA2*
a2p2	TCAACGCTGCTATTCAGCCTATTCG	3′-end of *P. crassa mnpA2*
A3-P1	CAACTGTAGAGCCGCTCTTCGTGGCTAG	5′-end of *P. crassa mnpA3*
A3-P2	AAGGCATCGGGCGGTCATAGAGCATGATC	5′-end of *P. crassa mnpA3*
A3-P1-2	TATCCGTCTCACCTTCCGTGAG	5′-end of *P. crassa mnpA3*
A3-P2-2	TGTCCTGAAGGTCCGATGCAAG	5′-end of *P. crassa mnpA3*
a3p1	CCCCGGCACAGCGAACAACACTGG	3′-end of *P. crassa mnpA3*
a3p2	CAGCGCCATTACCCTGCCTCCG	3′-end of *P. crassa mnpA3*
B2-P1	CATGCTCCTCTTCCCGACTGTTGAGCCTC	5′-end of *P. crassa mnpB2*
B2-P2	AAGGTGACGTTAGACAGGAATTGCTACCGC	5′-end of *P. crassa mnpB2*
b2-p1	ACCCAAGTCTTCCTCGAGGTGCTCCTGAAG	3′-end of *P. crassa mnpB2*
b2-p2	CGATGGAGTGCGAGGCAAGAAGAGAGACG	3′-end of *P. crassa mnpB2*
B3-P1	TTTTCCCGACTGTTGAGCCTCTGTTCCC	5′-end of *P. crassa mnpB3*
B3-P2	ATATCAGCCCCGTAAGACGAGCGGGCAAAC	5′-end of *P. crassa mnpB3*
b3-p1	GACACCCAGATCTTCCTCGAGGTA	3′-end of *P. crassa mnpB3*
b3-p2	GGGTCTGCGAGCCAAGAACATTAG	3′-end of *P. crassa mnpB3*

sequences as primers. Subsequently, inverse PCR was conducted repeatedly to get complete mnp genes from *P. crassa* WD1694.

Genomic clones encoding alleles of 4 MnP isozymes from *P. crassa* WD1694 were determined. The intron location and alignment for *P. crassa* mnp genes were determined by comparing each genomic sequence with the *P. chrysosporium* mnp genes [19–21]. All the intron splice junction sequences in the 4 *P. crassa* mnp genes conform to the GT-AG rule.

Figure 1 shows the intron alignments for the 4 *P. crassa* mnp genes sequenced in this study. The intron positions of *P. crassa mnpB2, B3, and P. crassa mnp A3* genes were the same as those reported for *P. chrysosporium mnp2* and *P. chrysosporium mnp3*, respectively [20, 21]. However, *P. crassa mnpA2* lacked two introns corresponding to the second and third introns of *P. chrysosporium mnp2*. The pattern of the intron number and position was used to classify the large family of LiP and MnP genes from *P. chrysosporium* [5]. Our result showed that *P. crassa mnpA2* represents another gene subfamily additional to the mnp subfamilies of *P. chrysosporium* previously reported.

In order to determine the MnP isozyme secreted in the *P. crassa* WD1694 culture, we produced and purified MnP from *P. crassa* WD1694. The purified MnP gave a single band at a molecular weight of 48,300 on SDS-PAGE (Fig. 2). However, it separated on IEF gel for 4 bands around pI 4.55, with very close pI (MnP 1-4: 4.61, 4.59, 4.52, and 4.50) (Fig. 3).

The N-terminal sequences of the 4 MnP bands were determined experimentally, and it was revealed that the first 13 amino acids of the N-terminal sequence of the 4 MnP bands were identical. The N-terminal sequences of the predicted translation product of *P. crassa* mnp genes and the experimentally determined first 13 amino acids of MnP proteins were compared in Table 2. The N-terminal sequences of *mnpA2, mnpA3*, and *mnpB2* differed from those of MnP proteins. However, *mnpB3* had the same N-terminal sequence as the MnP proteins. These results suggested that only the *mnpB3* gene among the 4 *P. crassa* mnp genes was the origin of *P. crassa* MnP shown as 4 bands on IEF.

The deduced amino acid sequence and the nucleotide sequence of *mnpB3* were shown in Fig. 4. The *mnpB3* gene

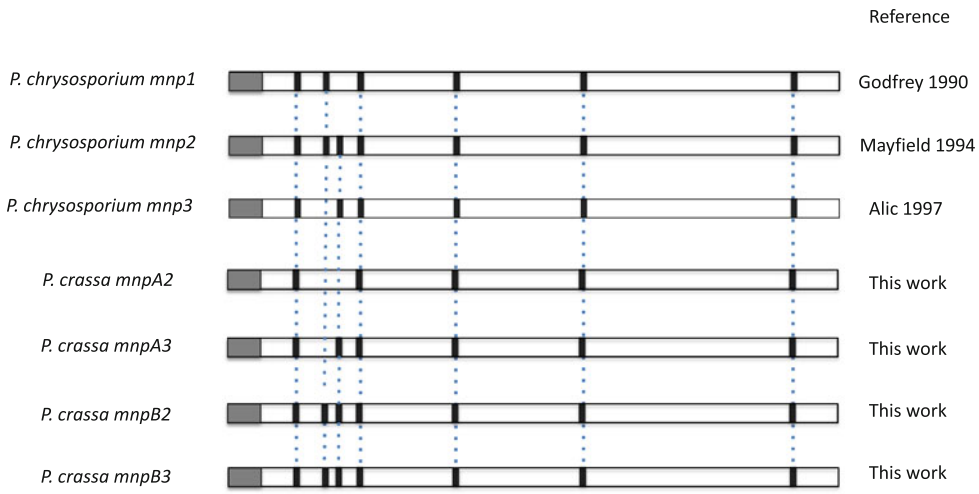

Fig. 1 The intron–exon structure of the genomic mnp genes from *P. crassa* WD1694 in comparison with *P. chrysosporium* mnp genes *Vertical bars* indicate intron positions. Identical intron positions among several genes are joined with a *dashed line*. The signal sequence is *shaded*

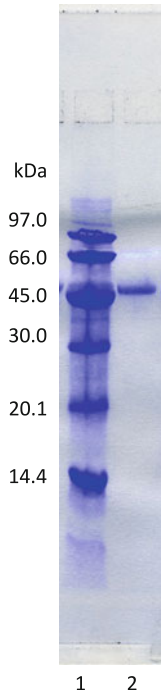

Fig. 2 SDS-PAGE of the purified MnP from *P. crassa* WD1694. *Lane1* molecular mass protein markers, *lane2* MnP from *P. crassa* WD1694

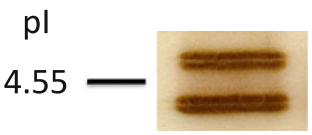

Fig. 3 Isoelectric focusing of purified MnP from *P. crassa* WD1694. Purified MnP was stained with MnP active staining. The *bar* indicates pI standard

encodes a mature protein of 384 amino acids preceded by a 24 amino acid signal peptide. The predicted molecular weight was 40,000, and the pI value was 4.35. The predicted pI value matched well with those of MnP proteins determined experimentally, but the predicted molecular weight was smaller than the experimentally determined MnP proteins.

Discussion

We cloned and sequenced the mnp gene from the *P. crassa* WD1694 genome comprehensively. Four mnp genes, *mnpA2, mnpA3, mnpB2*, and *mnpB3* were determined. The exon–intron structure of *P. crassa mnpA3, B2*, and *B3* was similar to those of *P. chrysosporium mnp2* and *mnp3*, respectively [20, 21]. *P. crassa mnpA2* had only 5 introns, which lacked two introns corresponding to the second and third introns of *P. chrysosporium mnp2*. The exon–intron structure was used to classify the large family of LiP and MnP genes from *P. chrysosporium* [5]. Recently, the exon–intron structure of mnp genes from *Formitiporia mediterranea*, *Physisporinus rivulosus*, and *Phlebia radiata* has been reported [22–24]. The exon–intron structure of *P. crassa mnpA2* differed from these reports, and represents another gene subfamily additional to the mnp subfamilies previously reported.

It has been reported that fungal class II ligninolytic peroxidases were divided into three families, LiP, MnP and VP [12]. Several groups reported the phylogenetic analysis of fungal ligninolytic peroxidases that also showed similar three groupings, mainly constructed with LiP, MnP and VP [13–15]. Our results showed that MnP of *P. crassa* WD1694 belongs to the classical MnP group, which oxidizes manganese (II) but not veratryl alcohol. The results correlate to the catalytic property of *P. crassa* WD1694 MnP in our previous reports [16–18].

Although *P. crassa mnpA2* had a different structure that lacked 2 introns, the intron position and length of exon from other regions were very similar to *P. crassa mnpA3* and *P. crassa mnpB2*. In general, MnP isozymes from same

Table 2 The nucleotide sequence and amino acid sequence of N-terminal of mnp genes and MnP protein

Name	Nucleotide sequence												
	N-terminal sequence												
P. chrysosporium mnp2	GCA	GTC	TGT	CCA	GAC	GGT	ACT	CGC	GTC	ACC	AAC	GCG	GCG
	A	V	C	P	D	G	T	R	V	T	N	A	A
P. chrysosporium mnp3	GCC	ACT	TGC	CCC	GAC	GGT	ACC	AAG	GTC	AAC	AAC	GCT	GCC
	A	T	C	P	D	G	T	L	V	N	N	A	A
P. crassa mnp A2	GCT	ACT	TGC	CCT	GAC	GGC	ACG	CAG	GTC	AAC	AAC	GAG	GCG
	A	T	C	P	D	G	T	Q	V	N	N	E	A
P. crassa mnp A3	GCT	ACG	TGC	CCT	GAT	GGT	ACG	CAG	GTT	AAC	AAC	GAA	GCT
	A	T	C	P	D	G	T	Q	V	N	N	E	A
P. crassa mnp B2	GCA	GTC	TGC	CCC	GAC	GGC	ACC	AGG	GTC	TCC	AAC	GCG	GCT
	A	V	C	P	D	G	T	R	V	S	N	A	A
P. crassa mnp B3	GCT	GTG	TGC	CCC	GAC	GGC	ACC	AGG	GTC	ACC	AAC	GAG	GCT
	A	V	C	P	D	G	T	R	V	T	N	E	A
P. crassa MnP proteins	A	V	C	P	D	G	T	R	V	T	N	E	A

origin belong to the same group of ligninolytic peroxidases [12–15]. *P. crassa mnpA2* should belong to the classic MnP group, like other *mnp genes* obtained from *P. crassa* WD1694.

The purified *P. crassa* WD1694 MnP gave a single band on SDS-PAGE, and was separated into 4 bands by isoelectric focusing. Generally, MnP isozymes of a white-rot fungus have the same molecular weight with different pI [25–29]. In these cases, each isozyme is encoded by a different gene [5]. In this study, we determined 4 different mnp gene sequences and anticipated that these genes should correspond to the 4 MnP bands. However, only *P. crassa mnpB3* had the same N-terminal sequence as the experimentally determined N-terminal sequence of MnP, while the other 3 genes, *mnpA2, A3*, and *B2* had different N-terminal sequences. These results suggested that the 4 MnP bands arose from a single gene.

P. crassa mnpB3 existed as an allele with different nucleotides at eight positions of coding region sequences, two of which were translated regions and the remaining six untranslated regions. However, both allele of *mnpB3* had the same predicted pI value, meaning the allele was not the reason for separation of the 4 MnP bands.

One possibility of multiple products from single gene is splicing variation. Recently, incomplete splicing of *mco* transcripts and incomplete processing of peroxidase transcripts from *P. chrysosporium* have been reported [30, 31]. Alternative splicing of introns in exocellobiohydrolase and in cytochrome P450 monooxygenase genes has also been reported for *P. chrysosporium* [32, 33]. Although there have been no reports of alternative splicing or incomplete processing of MnP, splicing variation should produce multiple products of different molecular weight. In our results, since the 4 MnP bands had the same molecular weight, it is unlikely that the 4 MnP bands arose from splicing variation.

It has been indicated that MnP was a glycoprotein modified with glycosylation or phosphorylation and that these posttranslational modifications could differ between MnP isozymes encoded by a single gene [5, 27, 34, 35]. The encoded mature protein of *mnpB3* had a molecular weight of 40,000, although the molecular weight determined for the purified MnP protein was 48,300. The gap between the encoded mature protein of *mnpB3* and the purified MnP protein of *P. crassa* WD1694 could be the difference between MnP proteins generated from the *mnpB3* gene. Additional analysis is required to determine whether the multiplicity of *P. crassa* WD1694 MnP arose due to posttranslational modification or for other reasons.

In this study, we did not detect MnP from other *P. crassa* mnp genes, *mnpA2, mnpA3*, and *mnpB2*. However, the expression of *P. crassa* MnP isozymes could be affected by culture conditions such as the concentration of manganese or nutrient nitrogen [29, 36, 37].

In conclusion, analysis of 4 genomic mnp genes from *P. crassa* WD1694 revealed that *P. crassa* WD1694 MnP belongs to the classical MnP type among the fungal class II peroxidases. The purified *P. crassa* WD1694 MnP consisted of 4 isozymes of equivalent molecular weight and N-terminal sequence, and different pI. Comparison of the N-terminal sequences between 4 MnP isozymes and deduced sequences from *P. crassa* mnp genes suggested that 4 MnP isozymes arose from a single gene. These results showed that the hyphal tip MnP reaction of *P. crassa* WD1694 was caused by the classical type MnP.

Fig. 4 The nucleotide and predicted amino acid sequence of *P. crassa* WD1694 *mnpB3* gene. The *underlined* amino acid sequence is the predicted signal peptide. Introns are indicated by *small letters*

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ATG GCT TTC GCG ACT CTC TTT GCT TTG GCT TCT CTC GCC GCG GTC GTC AGC GCC GCC CCC   60
MET Ala Phe Ala Thr Leu Phe Ala Leu Ala Ser Leu Ala Ala Val Val Ser Ala Ala Pro   20

GCC GCT GAG TCC GCT GTG TGC CCC GAC GGC ACC AGG GTC ACC AAC GAG GCT TGC TGT GCT  120
Ala Ala Glu Ser Ala Val Cys Pro Asp Gly Thr Arg Val Thr Asn Glu Ala Cys Cys Ala   40

TTC ATC CCT  g taagcaatag ccagctctaa taatttagcc gacgctcatc catcgtcctg cag CTC GCT 189
Phe Ile Pro                                                                Leu Ala  45

ACG GAT CTC CAG GAC AAC CTG TTC CAG GGT GAC TGT GGT GAA GAT  G gtatg ttacatatta   250
Thr Asp Leu Gln Asp Asn Leu Phe Gln Gly Asp Cys Gly Glu Asp  A                     60

ctcatcctgc aaaagtatta ttactaagtc tataccgcag CT CAC GAA GTC GTC CGT TTG ACT TTC C 317
                                             la His Glu Val Val Arg Leu Thr Phe H  69

gta tgctcagctt tctccgccac tctatggatt cgctcactta caccttttta ag AC GAC GCC ATT GCG 386
                                                          is Asp Ala Ile Ala   74

ATT TCT CGT AGC CAG GGC CCC AAG GC  gtgagtgt ttgcccgctc gtcttacggg gctgatatct    450
Ile Ser Arg Ser Gln Gly Pro Lys Al                                               82

gacatactct caatag C GGC GGA GGA GCC GAC GGC TCT ATG CTC CTT TTC CCG ACT GTT GAG  512
                  a Gly Gly Gly Ala Asp Gly Ser MET Leu Leu Phe Pro Thr Val Glu   98

CCT CTG TTC CCG CCC AAC AAC GGT ATC TCC GAC AGC GTC AAC AAC CTC CTC CAC TTC CTG 572
Pro Leu Phe Pro Pro Asn Asn Gly Ile Ser Asp Ser Val Asn Asn Leu Leu His Phe Leu 118

CCC CTC CAC CCG GTC AGC GCT GGA GAC CTC ATC CAG TTC GCT GGC GCC GTC GCT CTC TCG 632
Pro Leu His Pro Val Ser Ala Gly Asp Leu Ile Gln Phe Ala Gly Ala Val Ala Leu Ser 138

AAC TGC CCC  gtgagtggc cctaccaatg gcggttgatc ccagttgttg acatatttgc cacttgtcag   700
Asn Cys Pro                                                                    141

GGT GCT CCT CAG CTC GAG TTC TTG GCT GGT CGC CCC AAC AAG ACC ATC GCT GCC GTC CAG 760
Gly Ala Pro Gln Leu Glu Phe Leu Ala Gly Arg Pro Asn Lys Thr Ile Ala Ala Val Gln 161

GGC CTC ATT CCT GAG CCT CAG GAC AGC GTC ACC AAG ATT CTC GAG CGT TTC GAG GAC GCG 820
Gly Leu Ile Pro Glu Pro Gln Asp Ser Val Thr Lys Ile Leu Glu Arg Phe Glu Asp Ala 181

GGA GGC TTC ACG CCC TTC GAG GTC GTC TCT CTC CTC GCT TCG CAC TCC ATC GCT CGT GCC 880
Gly Gly Phe Thr Pro Phe Glu Val Val Ser Leu Leu Ala Ser His Ser Ile Ala Arg Ala 201

GAC AAG GTC GAC CCT ACC ATC GAC GCT GCG CCA TTC GAC TCG  gtacggat tggtctcctt    940
Asp Lys Val Asp Pro Thr Ile Asp Ala Ala Pro Phe Asp Ser                         215

ttggcgcata cacgcagagg ctaatgttct tggctcgcag ACC CCC TTC AGC TTC GAC ACC CAG ATC 1007
                                            Thr Pro Phe Ser Phe Asp Thr Gln Ile  224

TTC CTC GAG GTA CTC CTG AAG GGC ACT GGC TTC CCC GGA ACA GGC AAC AAC ACC GGC GAG 1067
Phe Leu Glu Val Leu Leu Lys Gly Thr Gly Phe Pro Gly Thr Gly Asn Asn Thr Gly Glu  244

GTC TCC TCT CCC CTT CCC CTC GGC AGC GGC GCC GAC ACC GGC GAG ATG CGT CTC CAG TCC 1127
Val Ser Ser Pro Leu Pro Leu Gly Ser Gly Ala Asp Thr Gly Glu MET Arg Leu Gln Ser  264

GAC TTC GAG CTC GCG CGT GAC GAG CGC ACC GCG TGC GCC TGG CAG AGC TTC GTG AAC GAG 1187
Asp Phe Glu Leu Ala Arg Asp Glu Arg Thr Ala Cys Ala Trp Gln Ser Phe Val Asn Glu  284

CAG GCG TTC ATG GCC GCG TCG TTC AAG GCC GCG ATG GCG AAG CTC GCG GTC CTC GGC CAC 1247
Gln Ala Phe MET Ala Ala Ser Phe Lys Ala Ala MET Ala Lys Leu Ala Val Leu Gly His  304

AAC CGC AAC GAC CTC ATC GAC TGC TCC GAC GTC GTC CCC AAG CCC AAG CCC GCC GTC AAC 1307
Asn Arg Asn Asp Leu Ile Asp Cys Ser Asp Val Val Pro Lys Pro Lys Pro Ala Val Asn  324

ACG CCC GCG ACG TTC CCC GCC ACC ACC GGC CCC AAG GAC CTC CAG CTC ACC TGC GAC GTG 1367
Thr Pro Ala Thr Phe Pro Ala Thr Thr Gly Pro Lys Asp Leu Gln Leu Thr Cys Asp Val  344

CTC CGC TTC CCG ACG CTC AAG ACC GCC C  gtaag cattctcgct gtgatgccct cgcgcatgcg   1430
Leu Arg Phe Pro Thr Leu Lys Thr Ala P                                          353

acgctaatca cttgtgttca cag CC GGC ACC GAG CAG GCC ATC ATC CCC CAC TGC TCC ACC GGC 1494
                         ro Gly Thr Glu Gln Ala Ile Ile Pro His Cys Ser Thr Gly  367

GGC ATG TCC TGC CCC GGC GTC GAC TTC GAC GGC CCC GCC CAG GAC AGC TCT TAA          1548
Gly MET Ser Cys Pro Gly Val Asp Phe Asp Gly Pro Ala Gln Asp Ser Ser              384

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