Short communication

An apple *atp9* pseudogene is maintained at high copy number in 'Golden Delicious' -type mitochondria but is present substoichiometrically in 'Delicious'-type mitochondria

Sumie Kato*, Kazuyoshi Kitazaki*, Akihito Wakatsuki, Yuji Kishima**, Tetsuo Mikami **

Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

* These authors contributed equally to this work

** Corresponding authors

Key words:

Apple, Cytoplasmic diversity, Mitochondrial *atp9* gene, Recombination, Substoichiometric sequences

Abstract

In this study, the mitochondrial *atp9* gene sequence of an apple cultivar 'Golden Delicious' was found to exist in one intact version and two truncated versions (termed *φatp9-1* and *φatp9-2*). Interestingly, the *φatp9-1* sequence is maintained at high copy number in the six 'Golden Delicious' -cytotype cultivars examined but present substoichiometrically in eight 'Delicious' -cytotype cultivars. Our data also suggest that *φatp9-1* originated in a homologous recombination event mediated by the short repeat in a common ancestral mitochondrial genome of 'Golden Delicious' and 'Delicious', and was preferentially amplified in an evolutionary lineage that gave rise to the 'Golden Delicious' -type genome. On the other hand, *φatp9-2* was revealed to be present in high abundance in all 14 cultivars examined.

1. Introduction

A fundamental characteristic of higher plant mitochondrial DNA (mtDNA) is its propensity to recombine across dispersed repetitive sequences (Knoop, 2004; Mackenzie, 2007). Extensive recombination frequently generates considerable variation in genomic organization. Such recombination events are also likely to be involved in the creation of mitochondrial chimeric genes and pseudogenes (Conklin and Hanson, 1994; Fauron et al., 2004). In apples (*Malus x domestica* Borkh.), mtDNA has been used to characterize the cytoplasmic diversity of a wide range of cultivars and landraces (Ishikawa et al., 1992; Kato et al., 1993). The use of mitochondrial *cox1* (cytochrome c oxidase subunit 1) and *atp9* (ATP synthase F1 subunit 9) gene probes detected restriction fragment length polymorphisms (RFLPs), which enabled classification of a large number of apple genotypes into four cytoplasmic groups: 'Golden Delicious' type, 'Delicious' type, 'McIntosh' type, and 'Dolgo Crab' type.

In order to understand the molecular basis of the changes in the mitochondrial genome leading to these diverse cytoplasmic types, it is necessary to analyze the genomic regions in each of the four mtDNA types that can be used to distinguish apple germplasm. Wakatsuki et al. (2011) have recently shown that in two apple cultivars, 'Golden Delicious' and 'Delicious,' the *cox1* reading frame exists as one full-length version (intact copy) and one truncated version (pseudocopy), and that the intact *cox1* and pseudocopy have an 1115 bp segment in common. They also suggested that recombination events may have occurred within the 1115 bp repeats to create the two distinct mitochondrial genome organizations characteristic of the 'Golden Delicious' and 'Delicious' cytotypes. In this paper, we present an analysis of the rearrangements involving the *atp9* loci of

79 the ‘Golden Delicious’ and ‘Delicious’ cytotype cultivars and rootstocks.
80
81 2. Materials and methods
82
83 *2.1. Plant material and nucleic acid preparation*
84
85 Leaf samples of apple cultivars and rootstocks (Table 1) were obtained
86 from the collections at the National Institute of Fruit Tree Science, National
87 Agriculture and Food Research Organization, Japan and the Field Center for
88 Northern Biosphere, Hokkaido University, Japan. The preparation of total
89 genomic DNA and total RNA from green leaves has been described previously
90 (Wakatsuki et al., 2011).
91
92 *2.2. Hybridization and sequence analysis*
93
94 Restriction enzyme digestion, agarose gel electrophoresis, Southern and
95 Northern blot analysis, DNA cloning and nucleotide sequencing were performed
96 using standard protocols (Sambrook et al., 1989; Wakatsuki et al., 2011). The
97 apple *atp9* gene copies were isolated from *Hin*dIII, *Bam*HI or *Eco*RI libraries of
98 total DNA by colony hybridization using the pea *atp9* sequence as the probe
99 (Morikami and Nakamura, 1987).
100
101 *2.3. PCR analysis*
102
103 PCR amplifications were performed with 100-150 ng of template DNA, 5
104 pmol of each forward and reverse primer, and GoTaq (Promega, Madison, WI).

105 The following PCR program was used: initial denaturation at 94˚C for 5 min, then
106 35 cycles of incubation at 94˚C for 30 s, 54-60˚C for 30 s and 72˚C for 2-3.5 min,
107 and a final extension at 72˚C for 5 min. All PCR experiments were repeated at
108 least three times. The oligonucleotide primers used for PCR are listed in Table 2.
109
110 3. Results and Discussion
111
112 *3.1. 'Golden Delicious' atp9 locus*
113
114 A previous study showed that hybridization of *Hin*dIII-digested apple
115 DNA with the pea *atp9* probe produced a 4.8 kb fragment in gels of the 'Golden
116 Delicious' and 'Delicious' cytotype cultivars, and an additional 9.2 kb fragment in
117 'Golden Delicious' cytotype cultivars (Kato et al., 1993). Kato et al. (1995)
118 subsequently showed that the 4.8 kb fragment from 'Delicious' contained an intact
119 *atp9* gene and the first and second exons of the *nad5* gene (*nad5* ex1 and *nad5*
120 ex2).
121 Here, we isolated the 4.8 kb *Hin*dIII fragment from a 'Golden Delicious'
122 DNA library. The restriction map of this clone was identical to that of the 4.8 kb
123 clone from 'Delicious' except for one *Eco*RI site (Fig. 1). Sequence analysis
124 indicated the presence of a 222 bp *atp9* ORF, which shared 100% sequence
125 identity with the 'Delicious' *atp9* (Figs. 1 and S1). The 'Golden Delicious' *atp9* was
126 found to be expressed as a 0.5 kb mRNA (Fig. S2). Hybridization analysis of the
127 4.8 kb 'Golden Delicious' fragment also showed the existence of *nad5* ex1 and *nad5*
128 ex2 homologous sequences in a 1.4 kb *Xho*I-*Eco*RI subfragment and a 1.4 kb
129 *Sac*I-*Sal*I subfragment, respectively (data not shown), confirming the
130 conservation of the *atp9*-*nad5* ex1-*nad5* ex2 linkage (Fig. 1).

131

132 *3.2. atp9 pseudocopies*

133

134 Our results suggest the presence of a second *atp9* copy in the 'Golden
135 Delicious' mitochondrial genome. The *atp9* probe was used to screen a *Bam*HI
136 library from 'Golden Delicious' and identified two clone families. Both families
137 contained *Bam*HI fragments (2.8 and 3.5 kb) corresponding to those detected
138 previously by Southern blot experiments (see Table 1 of Kato et al., 1993).
139 Restriction mapping of these clones indicated that the 4.8 kb *Hin*dIII fragment
140 overlapped with the 3.5 kb *Bam*HI fragment, but not with the 2.8 kb *Bam*HI
141 fragment. We therefore sequenced the 2.8 kb *Bam*HI fragment.

142 As shown in Figs. 1, S1 and S3, we found a truncated *atp9* sequence. The
143 truncated copy (termed *φatp9-1*) was virtually identical from nucleotide -116 to
144 +91 to the intact 'Golden Delicious' *atp9*. Upstream of nucleotide -116 and
145 downstream from nucleotide +91, the two sequences were completely different.
146 The 3' divergence resulted in a 62 codon extension of the *φatp9-1* ORF that was
147 not homologous to any known sequence. The 5' flanking region of *φatp9-1* included
148 a 375 bp *rps12* locus (Gualberto et al., 1988), which in turn was preceded by a
149 truncated *nad3* sequence (Gualberto et al., 1988).

150 We next asked whether the *φnad3* - *rps12* - *φatp9-1* gene cluster resulted
151 from genomic recombination of the intact *atp9* locus with a distant genomic region.
152 To investigate this question, an additional *nad3-rps12* cluster was isolated from a
153 'Golden Delicious' DNA library using the *φnad3* sequence as a probe. Sequence
154 analysis of the isolated clone not only indicated the existence of an apparently
155 intact *nad3* - *rps12* cluster (Figs. 1 and S1), but also revealed a very short
156 sequence of homology (8/9 bp repeat, boxed in Fig. 1) across which recombination

157 has presumably taken place to generate the *φatp9-1* arrangement. Over time,
158 sequence divergence could have occurred in the repeat.

159 During the RFLP analysis, we became aware that the *atp9* probe
160 reproducibly gave an additional weak hybridization signal (6.0 kb) in
161 *Eco*RI-digested DNAs of both 'Golden Delicious' and 'Delicious' (data not shown).
162 In order to determine whether a third *atp9* copy is present in the 'Golden
163 Delicious' mitochondrial genome, the 6.0 kb *Eco*RI fragment was cloned from
164 'Golden Delicious'. Restriction mapping and hybridization analysis of this clone
165 indicated that an *atp9* homologous sequence was located in a 0.45 kb
166 *Bgl*II-*Bam*HI subfragment (Fig. 1). Sequence analysis of the 0.45 kb subfragment
167 revealed a segment homologous to the 3' part (169 bp) of the *atp9* ORF and its 3'
168 flanking sequence (3 bp) (Figs. 1, S1 and S4). Significant similarity between this
169 truncated *atp9* (termed *φatp9-2*) and *atp9* abruptly disappeared upstream of
170 nucleotide + 54 and downstream from + 228 (Figs. 1 and S1).

171 The region surrounding the 0.45 kb *Bgl*II-*Bam*HI subfragment was
172 further sequenced. For this purpose, a 6.5 kb *Bam*HI fragment overlapping the
173 6.0 kb *Eco*RI fragment was also cloned and partially sequenced. The third exon of
174 *nad5* (*nad5* ex3) was shown to lie between 1245 and 1224 bp upstream of *φatp9-2*
175 (Figs. 1 and S4; Knoop et al., 1991). Furthermore, located downstream from the
176 *φatp9-2* stop codon were two ORFs with high similarity to the sequences of *rpl5*
177 and *rps14* genes in other higher plant mitochondria (Figs. 1 and S4; Brandt et al.,
178 1993; Ye et al., 1993).

179

180 *3.3. Distribution of atp9 pseudocopies in apple cultivars*

181

182 Next, we sought to determine whether the *atp9* pseudocopies are present

183 in diverse apple genotypes. Total genomic DNAs were prepared from six 'Golden
184 Delicious' cytotype cultivars and eight 'Delicious' cytotype cultivars and then used
185 for PCR amplification. PCR experiments were performed using the
186 oligonucleotide primers P1 and P2 that are specific to *φatp9-1*. The DNAs from all
187 14 genotypes generated a single product of the expected size (ca. 180 bp) (Fig. S5).
188 Some of the PCR products were sequenced to verify their identity. We did note,
189 however, that the amounts of amplification product differed among the cytotypes:
190 the *φatp9-1* sequence from six 'Golden Delicious' cytotype cultivars was always
191 more intensely amplified (Fig. S5). This indicates that *φatp9-1* exists at a higher
192 copy number within the 'Golden Delicious' type mitochondrial genome but is
193 maintained substoichiometrically within the 'Delicious' type mitochondrial
194 genome (Table 1, see Mackenzie (2007) for substoichiometric molecules). Taken
195 together, our observations suggest that *φatp9-1* originated in a homologous
196 recombination event mediated by the 8/9 bp repeat in the common ancestral
197 mitochondrial genome of 'Golden Delicious' and 'Delicious', and was preferentially
198 amplified in the lineage that led to the 'Golden Delicious' type mitochondrial
199 genome.

200 We carried our further PCR assays with two pairs of primers (P3/P4 and
201 P3/P5), which allow the detection of the *φatp9-2* sequence, using the DNAs from
202 the 14 apple genotypes as templates. All the plants examined yielded an intense
203 fragment of the predicted size (ca. 100 bp for P3/P4 and ca. 110 bp for P3/P5),
204 thereby revealing the presence of *φatp9-2* in high abundance irrespective of the
205 cytotype (Table 1, Fig. S5).

206

207 Acknowledgements

208 Leaf samples were kindly supplied by National Institute of Fruit Tree Science,

Tsukuba, Japan and Field Science Center for Northern Biosphere, Experiment Farm, Hokkaido University, Sapporo, Japan. This work was supported in part by Grants-in-Aids for Scientific Researches from Ministry of Education, Science, and Culture, Japan.

References

Brandt, P., Unseld, M., Eckert-Ossenkopp, U., Brennicke, A., 1993. An *rps14* pseudogene is transcribed and edited in *Arabidopsis* mitochondria. Curr. Genet. 24, 330-336.

Conklin, P. L., Hanson, M. R., 1994. Recombination of plant mitochondrial genomes. In: Paszkowski, J. (Ed.) Homologous recombination and gene silencing in plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 61-81.

Fauron, C., Allen, J., Clifton, S., Newton, K., 2004. Plant mitochondrial genomes. In: Daniell, H., Chase, C. D. (Eds.) Molecular biology and biotechnology of plant organelles. Springer, Dordrecht, Netherlands, pp. 151-177.

Gualberto, J. M., Wintz, H., Weil, J. H., Grienenberger, J. M., 1988. The genes coding for subunit 3 of NADH dehydrogenase and for ribosomal protein S12 are present in the wheat and maize mitochondrial genomes and are co-transcribed. Mol. Gen. Genet. 251, 118-127.

Ishikawa, S., Kato, S., Imakawa, S., Mikami, T., Shimamoto, Y., 1992. Organelle DNA polymorphism in apple cultivars and rootstocks. Theor. Apple. Genet. 83, 963-967.

Kato, S., Ishikawa, S., Imakawa, S., Komori, S., Mikami, T., Shimamoto, Y., 1993. Cytoplasmic relatedness of apple landraces and cultivars: a molecular analysis. Euphytica 66, 99-102.

Kato, S., Shimamoto, Y., Mikami, T., 1995. The apple mitochondrial *atp9* gene: RNA editing and co-transcription with exons a and b of the *nad5* gene. Physiol. Plant. 93, 572-575.

Knoop, V., 2004. The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. Curr. Genet. 46, 123-139.

Knoop, V., Schuster, W., Wissinger, B., Brennicke, A., 1991. Trans splicing integrates an exon of 22 nucleotides into the *nad5* mRNA in higher plant mitochondria. EMBO J. 10, 3483-3493.

Mackenzie, S. A., 2007. The unique biology of mitochondrial genome instability in plants. In: Logan, D. C. (Ed.), Plant mitochondria. Blackwell Publishing, Oxford, pp.36-49.

Morikami, A., Nakamura, K., 1987. The pea mitochondrial ATPase subunit 9 gene is located upstream of the ATPase α-subunit gene. Nucl. Acids Res. 15, 4692.

Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Molecular cloning: a laboratory manual. 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Wakatsuki, A., Kitazaki, K., Kato, S., Kishima, Y., Mikami, T., 2011. An intact mitochondrial *cox1* gene and a pseudogene with different genomic configurations are present in apple cultivars 'Golden Delicious' and 'Delicious': Evolutionary aspects. Sci. Hortic. 130, 49-53.

Ye, F., Bernhardt, J., Abel, W. O., 1993. Genes for ribosomal proteins S3, L16, L5 and S14 are clustered in the mitochondrial genome of *Brassica napus* L. Curr. Genet. 24, 323-329.

Figure captions

Fig. 1. Genomic organization of an intact *atp9* gene and two pseudocopies (*φatp9-1* and *φatp9-2*) from 'Golden Delicious'. The locations of *nad3*, *φnad3, rps12, nad5* ex1, *nad5* ex2, *nad5* ex3, *rpl5* and *φrps14* are also indicated. The homologous regions are shaded. The sequences of the homologous portions of 9.0-kb *Bam*HI clone, 2.8-kb *Bam*HI clone and 4.8-kb *Hin*dIII clone were aligned to reveal the 8/9-bp repeat boxed in the nucleotide sequences shown enlarged above for each of the three clones. Restriction sites are given for *Bam*HI(B), *Bgl*II(Bg), *Eco*RI(E), *Hin*dIII(H), *Nco*I(N), *Pst*I(P), *Pvu*II(Pv), *Sac*I(Sc), *Sal*I(Sl) and *Xho*I(X). An *Eco*RI site lost in the 'Golden Delicious' *atp9* locus relative to the 'Delicious' *atp9* locus is circled (see Fig. S1).

Supplementary Figure Captions

Fig. S1. Nucleotide sequence comparison of 'Delicious' *atp9* (1) (Kato et al. 1995), 'Golden Delicious' *atp9* (2), and two 'Golden Delicious' pseudocopies, *φatp9-1* (3) and *φatp9-2* (4). Numbering begins with the first nucleotide of the presumed start codon (+1). Start and stop codons are boxed. Identical bases are interconnected by asterisks. The 38-bp sequence shared by the four loci is shaded. Nucleotide substitutions in the 'Golden Delicious' *atp9* locus at positions -12 and -11 result in the loss of an *Eco*RI site (see text and Fig. 1). 'Delicious' *atp9* sequence is referred from accession number D37958 (nucleotide position 121-556). 'Golden Delicious' *atp9* sequence contains four nucleotide changes relative to 'Delicious' *atp9*: T to A T to A, T to G and C to A at nucleotide positions 80, 81, 170 and 171, which are located in the 5' non-coding region of *atp9*. Nucleotide sequence data for (3) and (4) are included in the sequences deposited as accession numbers AB674548 and AB674549, respectively.

Fig. S2. Transcription analysis of the 'Golden Delicious' *atp9* gene. The *atp9*-specific probe was generated by PCR and was allowed to hybridize to a Northern blot containing total RNA from 'Golden Delicious'. The 2.9-kb band probably corresponds to bicistronic transcripts covering *atp9*, *nad5* ex1 and *nad5* ex2, whereas the 0.5-kb band most likely represents the processed *atp9* mRNA.

Fig. S3. Nucleotide sequence comparison of the *nad3-rps12* gene cluster (1) and *φnad3-rps12-φatp9-1* gene cluster (2) in the 'Golden Delicious' mitochondrial genome. Amino-acid translations are given for *nad3* and *rps12*. Positions of the primers used are underlined. Nucleotide sequence data for (1) and (2) have been deposited as accession numbers AB674547 and AB674548, respectively .

Fig. S4. Nucleotide sequence of *φatp9-2* and flanking regions in the 'Golden Delicious' mitochondrial genome. Exon 3 of the *nad5* gene is located upstream of *φatp9-2*, whereas *rpl5* and *φrps14* are located downstream from *φatp9-2*. Amino-acid translations are given for *nad5* ex3 and *rpl5*. Positions of the primers used are underlined. Nucleotide sequence data have been deposited under accession number AB674549.

Fig. S5. PCR amplification of the *φatp9-1* and *φatp9-2* sequences. The PCR reaction mixture consisted of 100ng template DNA, 5pmol of each forward and reverse primer, and GoTaq (Promega, Madison, WI). The experiments were carried out using primers P1/P2 for the detection of *φatp9-1*, and P3/P4 and P3/P5 for *φatp9-2*. Total genomic DNA was prepared from 'Golden Delicious' (1), 'Delicious' (2), 'Cellini' (3), 'Geneva' (4), 'Hopa Crab' (5), 'Red Astrachan' (6), 'Yellow Transparent' (7), 'M9' (8), *M. prunifolia* (acc. 6109011-0001) (9), 'Fuji' (10), 'Jonathan' (11), 'Jonagold' (12), 'Tsugaru' (13) and 'Natsunobeni' (14).

Table 1

Apple cultivars and rootstocks analyzed for the presence of *atp9* pseudogene copies (see Fig.S5)

Cultivar/rootstock	Parentage	Relative abundance of	
		φatp9-1	*φatp9-2*
'Golden Delicious' -type cytoplasm			
Golden Delicious	Possibly Grimes Golden × Golden Reinette	High	High
Fuji	Ralls Janet × Delicious	High	High
Jonathan	Esopus Spitzenburg × Unknown	High	High
Jonagold	Golden Delicious × Jonathan	High	High
Tsugaru	Golden Delicious × Jonathan	High	High
Natsunobeni	Vista Bella×Unknown	High	High
'Delicious' -type cytoplasm			
Delicious	Possibly Yellow Bellflower × Unknown	Low	High
Cellini	U. K. cultivar	Low	High
Geneva	Canadian cultivar	Low	High
Hopa Crab	U. S. A. cultivar	Low	High
Red Astrachan	Russian cultivar	Low	High
Yellow Transparent	Russian cultivar	Low	High
M9	Jaune de Metz Paradise	Low	High
6109011-0001	*M. prunifolia*	Low	High

Table 2

List of primers used (see Figs. S3 and S4 for the positions of primers)

For amplification of *φatp9-1*	
P1	5'-AATGCTATCTTCGACAGTCTTGAGAGC-3'
P2	5'-GCACCTTCTAACATCTCGAGTTGATC-3'
For amplification of *φatp9-2*	
P3	5'-TCACGAAGAGGATTCCCGGACTG-3'
P4	5'-GATTTCGGGCCACGGAATGGATCA-3'
P5	5'-TGATTGTTTAGCCAATGATGGATTTCGGGC-3'

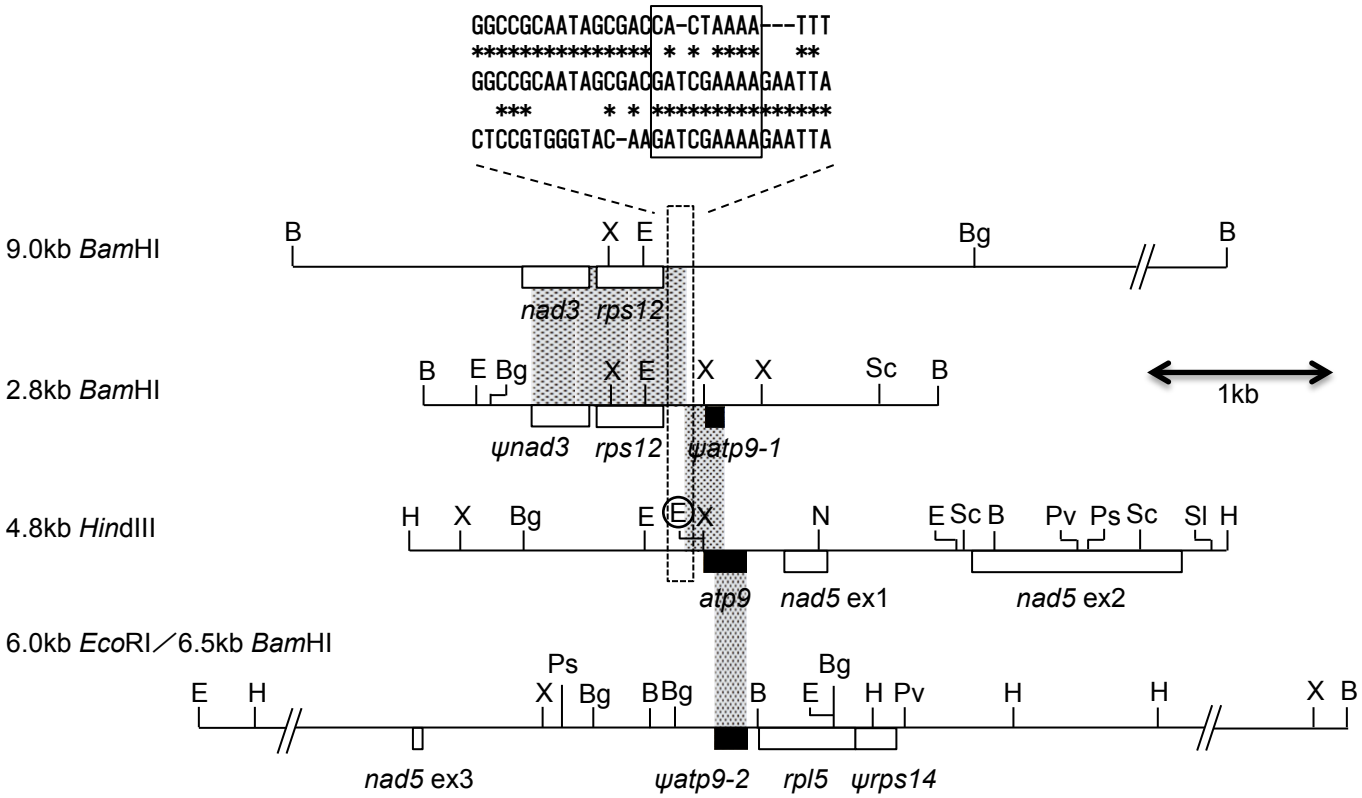
GGCCGCAATAGCGACCA-CTAAAA---TTT
GGCCGCAATAGCGACGATCGAAAAGAATTA
CTCCGTGGGTAC-AAGATCGAAAAGAATTA
9.0kb BamHI
2.8kb BamHI
4.8kb HindIII
6.0kb EcoRI／6.5kb BamHI
nad3
rps12
ψnad3
rps12
ψatp9-1
atp9
nad5 ex1
nad5 ex2
nad5 ex3
ψatp9-2
rpl5
ψrps14
1kb

Fig.1

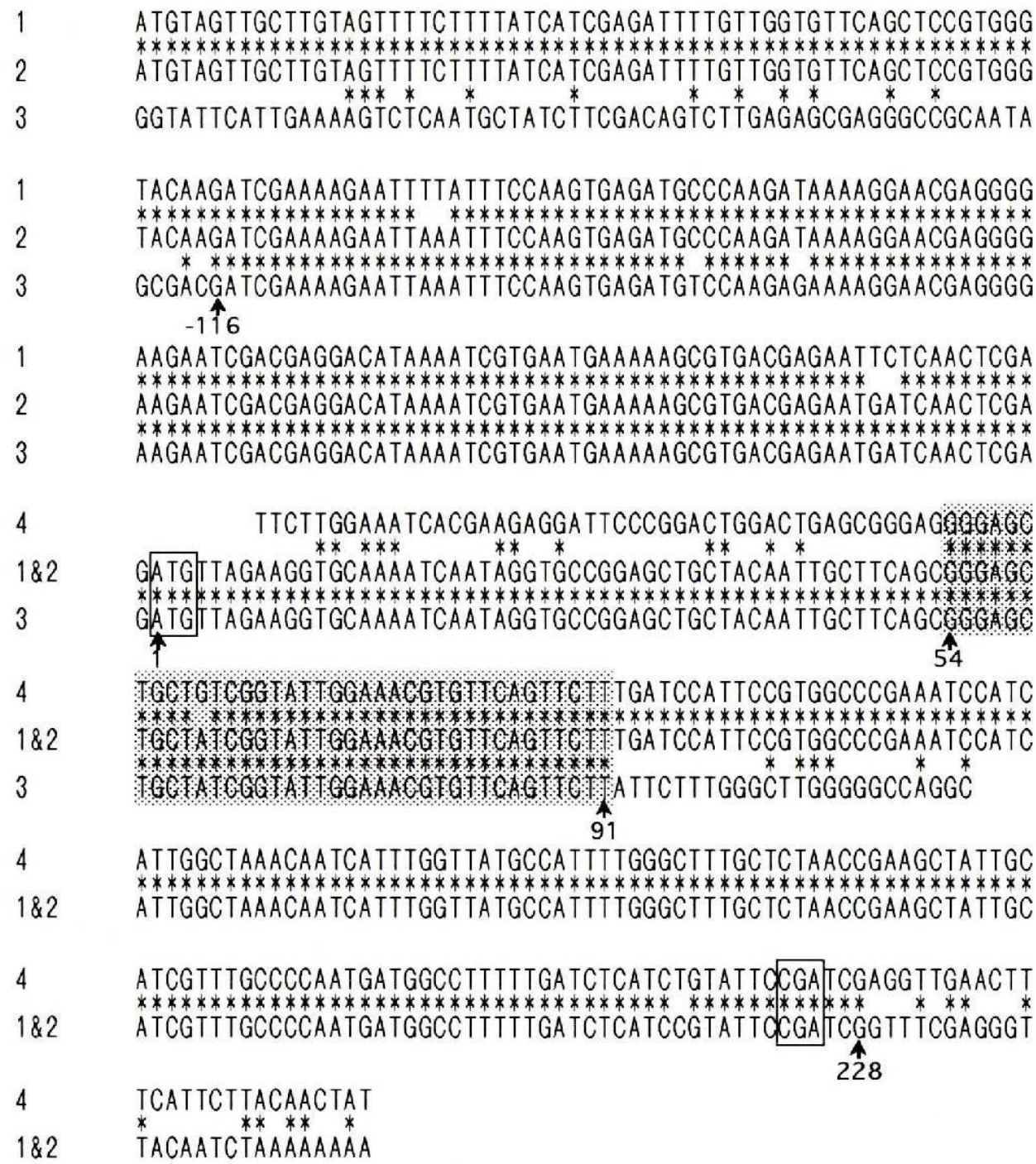

Fig.S1

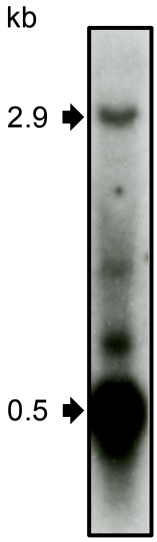

Fig.S2

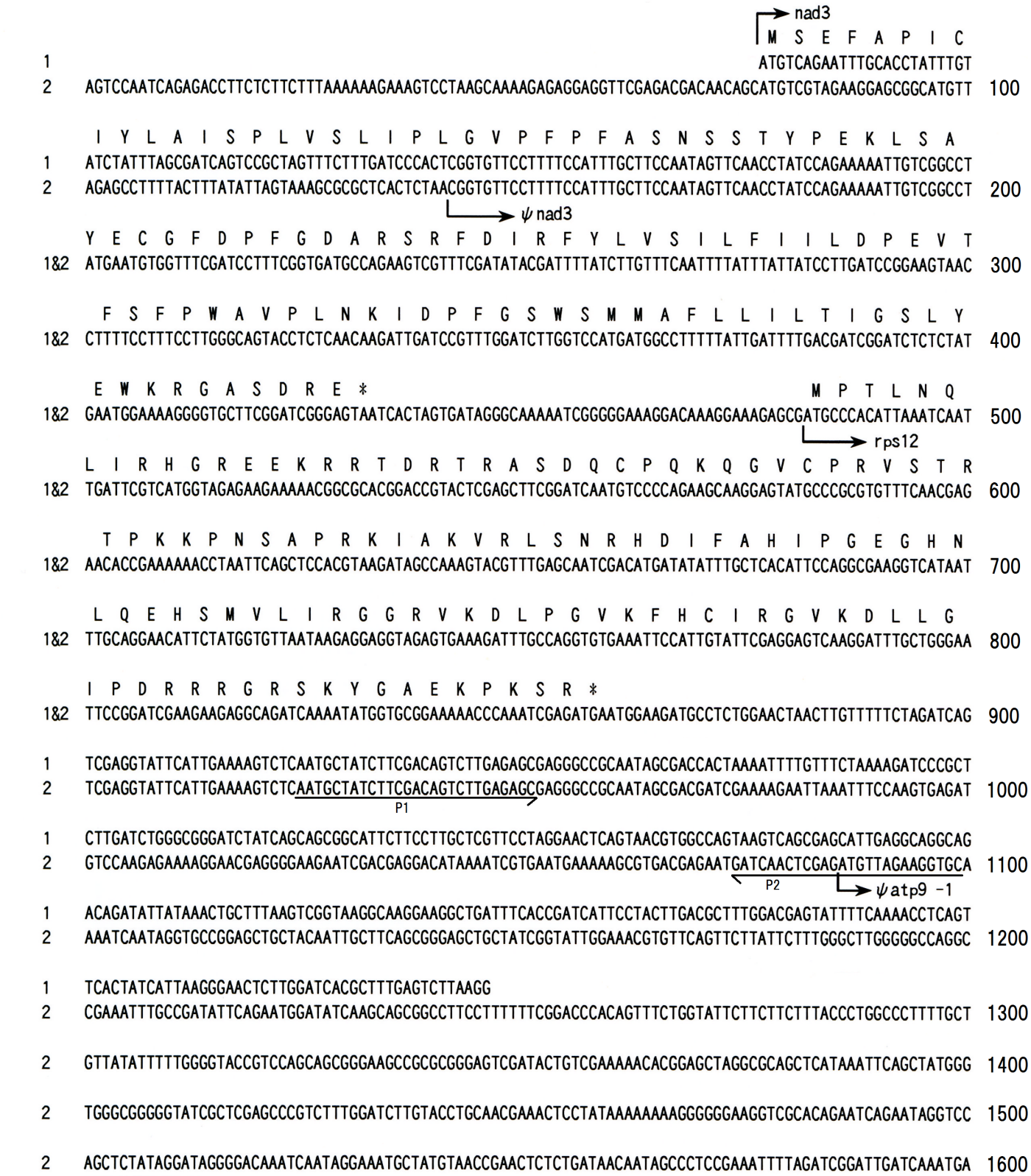
nad3
M S E F A P I C
ATGTCAGAATTTGCACCTATTTGT
AGTCCAATCAGAGACCTTCTCTTCTTTAAAAAAGAAAGTCCTAAGCAAAAGAGAGGAGGTTCGAGACGACAACAGCATGTCGTAGAAGGAGCGGCATGTT 100
I Y L A I S P L V S L I P L G V P F P F A S N S S T Y P E K L S A
ATCTATTTAGCGATCAGTCCGCTAGTTTCTTTGATCCCACTCGGTGTTCCTTTTCCATTTGCTTCCAATAGTTCAACCTATCCAGAAAAATTGTCGGCCT
AGAGCCTTTTACTTTATATTAGTAAAGCGCGCTCACTCTAACGGTGTTCCTTTTCCATTTGCTTCCAATAGTTCAACCTATCCAGAAAAATTGTCGGCCT 200
ψnad3
Y E C G F D P F G D A R S R F D I R F Y L V S I L F I I L D P E V T
ATGAATGTGGTTTCGATCCTTTCGGTGATGCCAGAAGTCGTTTCGATATACGATTTTATCTTGTTTCAATTTTATTTATTATCCTTGATCCGGAAGTAAC 300
F S F P W A V P L N K I D P F G S W S M M A F L L I L T I G S L Y
CTTTTCCTTTCCTTGGGCAGTACCTCTCAACAAGATTGATCCGTTTGGATCTTGGTCCATGATGGCCTTTTTATTGATTTTGACGATCGGATCTCTCTAT 400
E W K R G A S D R E * M P T L N Q
GAATGGAAAAGGGGTGCTTCGGATCGGGAGTAATCACTAGTGATAGGGCAAAAATCGGGGGAAAGGACAAAGGAAAGAGCGATGCCCACATTAAATCAAT 500
rps12
L I R H G R E E K R R T D R T R A S D Q C P Q K Q G V C P R V S T R
TGATTCGTCATGGTAGAGAAGAAAAACGGCGCACGGACCGTACTCGAGCTTCGGATCAATGTCCCCAGAAGCAAGGAGTATGCCCGCGTGTTTCAACGAG 600
T P K K P N S A P R K I A K V R L S N R H D I F A H I P G E G H N
AACACCGAAAAAACCTAATTCAGCTCCACGTAAGATAGCCAAAGTACGTTTGAGCAATCGACATGATATATTTGCTCACATTCCAGGCGAAGGTCATAAT 700
L Q E H S M V L I R G G R V K D L P G V K F H C I R G V K D L L G
TTGCAGGAACATTCTATGGTGTTAATAAGAGGAGGTAGAGTGAAAGATTTGCCAGGTGTGAAATTCCATTGTATTCGAGGAGTCAAGGATTTGCTGGGAA 800
I P D R R R G R S K Y G A E K P K S R *
TTCCGGATCGAAGAAGAGGCAGATCAAAATATGGTGCGGAAAAACCCAAATCGAGATGAATGGAAGATGCCTCTGGAACTAACTTGTTTTTCTAGATCAG 900
TCGAGGTATTCATTGAAAAGTCTCAATGCTATCTTCGACAGTCTTGAGAGCGAGGGCCGCAATAGCGACCACTAAAATTTTGTTTCTAAAAGATCCCGCT
TCGAGGTATTCATTGAAAAGTCTCAATGCTATCTTCGACAGTCTTGAGAGCGAGGGCCGCAATAGCGACGATCGAAAAGAATTAAATTTCCAAGTGAGAT 1000
P1
CTTGATCTGGGCGGGATCTATCAGCAGCGGCATTCTTCCTTGCTCGTTCCTAGGAACTCAGTAACGTGGCCAGTAAGTCAGCGAGCATTGAGGCAGGCAG
GTCCAAGAGAAAAGGAACGAGGGGAAGAATCGACGAGGACATAAAATCGTGAATGAAAAAGCGTGACGAGAATGATCAACTCGAGATGTTAGAAGGTGCA 1100
P2
ψatp9 -1
ACAGATATTATAAACTGCTTTAAGTCGGTAAGGCAAGGAAGGCTGATTTCACCGATCATTCCTACTTGACGCTTTGGACGAGTATTTTCAAAACCTCAGT
AAATCAATAGGTGCCGGAGCTGCTACAATTGCTTCAGCGGGAGCTGCTATCGGTATTGGAAACGTGTTCAGTTCTTATTCTTTGGGCTTGGGGGCCAGGC 1200
TCACTATCATTAAGGGAACTCTTGGATCACGCTTTGAGTCTTAAGG
CGAAATTTGCCGATATTCAGAATGGATATCAAGCAGCGGCCTTCCTTTTTTCGGACCCACAGTTTCTGGTATTCTTCTTCTTTACCCTGGCCCTTTTGCT 1300
GTTATATTTTTGGGGTACCGTCCAGCAGCGGGAAGCCGCGCGGGAGTCGATACTGTCGAAAAACACGGAGCTAGGCGCAGCTCATAAATTCAGCTATGGG 1400
TGGGCGGGGGTATCGCTCGAGCCCGTCTTTGGATCTTGTACCTGCAACGAAACTCCTATAAAAAAAAGGGGGGAAGGTCGCACAGAATCAGAATAGGTCC 1500
AGCTCTATAGGATAGGGGACAAATCAATAGGAAATGCTATGTAACCGAACTCTCTGATAACAATAGCCCTCCGAAATTTTAGATCGGATTGATCAAATGA 1600

Fig.S3

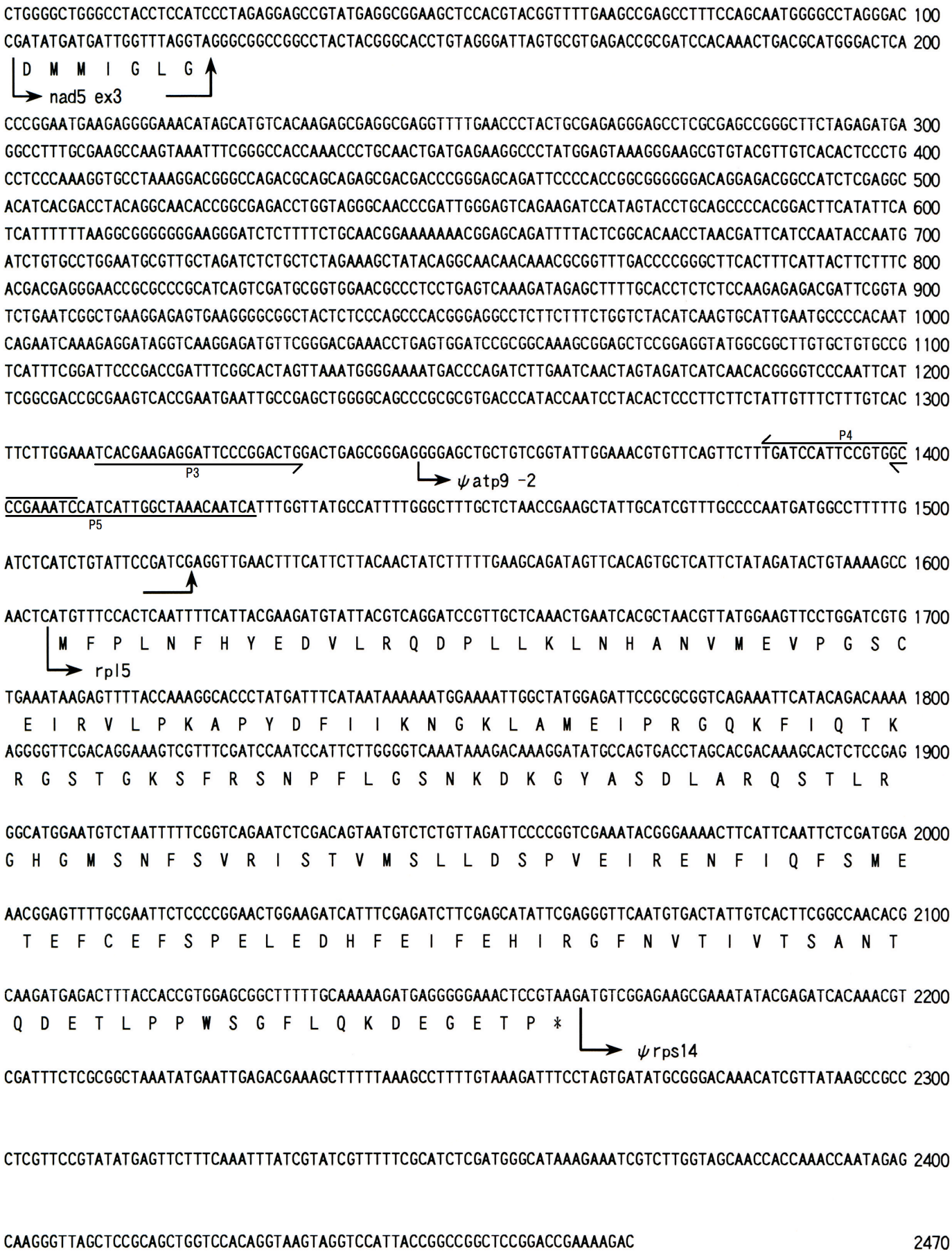
D M M I G L G
nad5 ex3
P3
P4
ψatp9 -2
P5
M F P L N F H Y E D V L R Q D P L L K L N H A N V M E V P G S C
rpl5
E I R V L P K A P Y D F I I K N G K L A M E I P R G Q K F I Q T K
R G S T G K S F R S N P F L G S N K D K G Y A S D L A R Q S T L R
G H G M S N F S V R I S T V M S L L D S P V E I R E N F I Q F S M E
T E F C E F S P E L E D H F E I F E H I R G F N V T I V T S A N T
Q D E T L P P W S G F L Q K D E G E T P *
ψrps14

Fig.S4

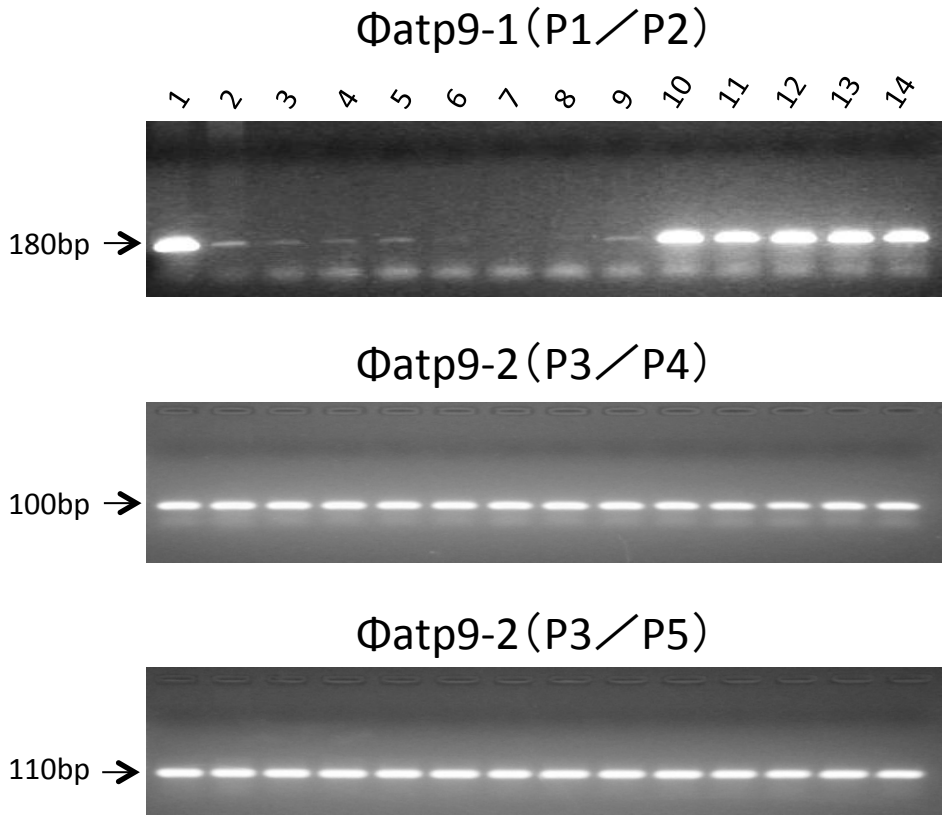
Φatp9-1（P1／P2）
1 2 3 4 5 6 7 8 9 10 11 12 13 14
180bp
Φatp9-2（P3／P4）
100bp
Φatp9-2（P3／P5）
110bp

Fig.S5