



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

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3 An apple *atp9* pseudogene is maintained at high copy number in ‘Golden  
4 Delicious’ -type mitochondria but is present substoichiometrically in  
5 ‘Delicious’-type mitochondria

6

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15 *Key words:*

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

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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.

53 1. Introduction

54

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

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



79 the ‘Golden Delicious’ and ‘Delicious’ cytotype cultivars and rootstocks.

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## 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*

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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 *Hind*III, *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*

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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 *Hind*III-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 *Hind*III 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-*Sa*II 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*

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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 *Hind*III 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  $\varphi atp9-1$ ) 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  $\varphi atp9-1$  ORF that was  
147 not homologous to any known sequence. The 5’ flanking region of  $\varphi atp9-1$  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  $\varphi nad3 - rps12 - \varphi atp9-1$  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  $\varphi nad3$  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  $\varphi atp9-1$  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 *EcoRI*-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 *EcoRI* 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 *BglIII-BamHI* 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  $\varphi atp9-2$ ) 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 *BglIII-BamHI* subfragment was  
172 further sequenced. For this purpose, a 6.5 kb *BamHI* fragment overlapping the  
173 6.0 kb *EcoRI* 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  $\varphi atp9-2$   
175 (Figs. 1 and S4; Knoop et al., 1991). Furthermore, located downstream from the  
176  $\varphi atp9-2$  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

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212 Culture, Japan.

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## 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 *Hind*III 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), *Bg*II(Bg), *Eco*RI(E), *Hind*III(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 *EcoRI* 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	
		<i>φatp9-1</i>	<i>φatp9-2</i>
‘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	<i>M. prunifolia</i>	Low	High

Table 2

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

---

For amplification of <i>φatp9-1</i>	
P1	5'-AATGCTATCTTCGACAGTCTTGAGAGC-3'
P2	5'-GCACCTTCTAACATCTCGAGTTGATC-3'
For amplification of <i>φatp9-2</i>	
P3	5'-TCACGAAGAGGATTCCCGGACTG-3'
P4	5'-GATTTTCGGGCCACGGAATGGATCA-3'
P5	5'-TGATTGTTTAGCCAATGATGGATTTTCGGGC-3'

---

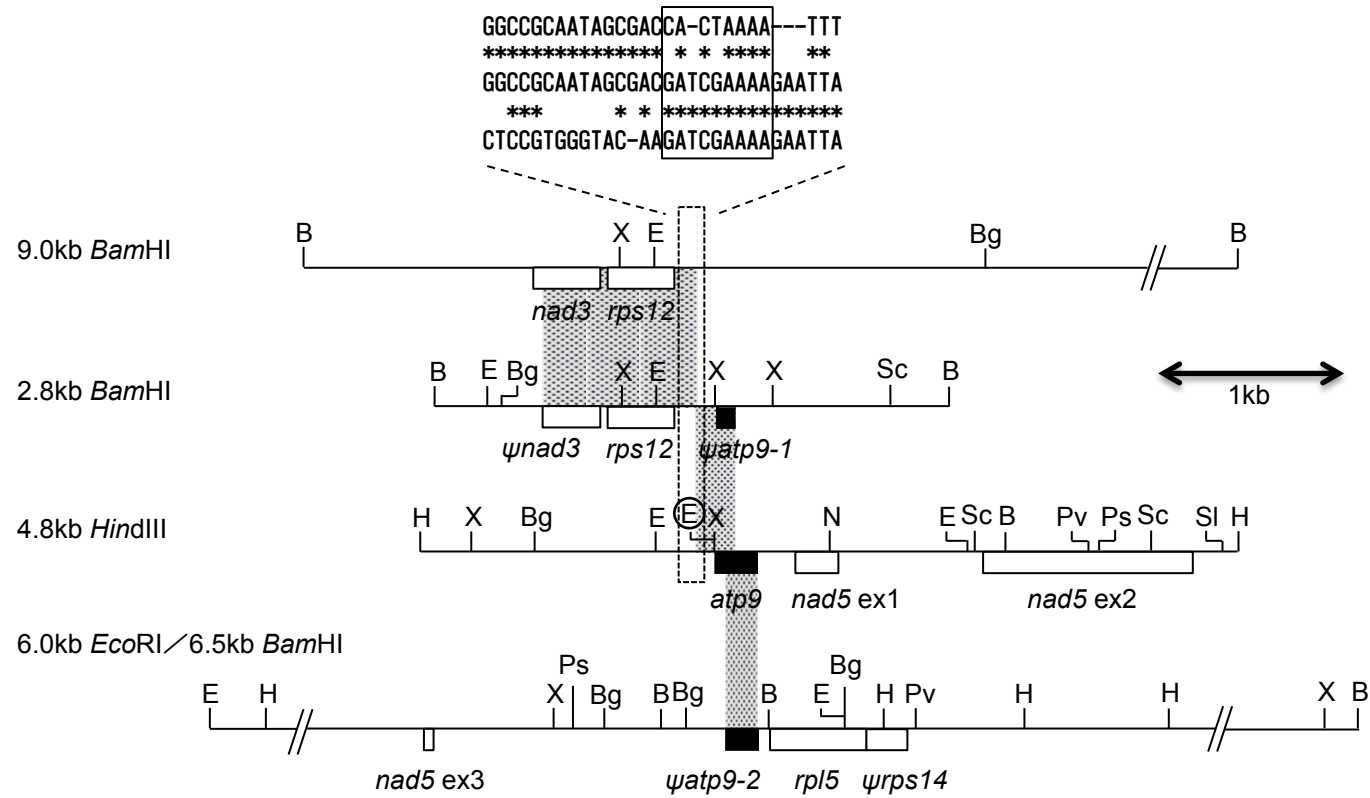


Fig.1

```

1   ATGTAGTTGCTTGTAGTTTTCTTTTATCATCGAGATTTTGTGGTGGTTCAGCTCCGTGGG
   *****
2   ATGTAGTTGCTTGTAGTTTTCTTTTATCATCGAGATTTTGTGGTGGTTCAGCTCCGTGGG
   *** * * * * *
3   GGTATTCATTGAAAAGTCTCAATGCTATCTTCGACAGTCTTGAGAGCGAGGGCCGCAATA

1   TACAAGATCGAAAAGAATTTTATTTCCAAGTGAGATGCCCAAGATAAAAGGAACGAGGGG
   *****
2   TACAAGATCGAAAAGAATTTTATTTCCAAGTGAGATGCCCAAGATAAAAGGAACGAGGGG
   * *****
3   GCGACGATCGAAAAGAATTTTATTTCCAAGTGAGATGTCCAAGAGAAAAGGAACGAGGGG
   -116
   ↑

1   AAGAATCGACGAGGACATAAAATCGTGAATGAAAAGCGTGACGAGAATTCTCAACTCGA
   *****
2   AAGAATCGACGAGGACATAAAATCGTGAATGAAAAGCGTGACGAGAATGATCAACTCGA
   *****
3   AAGAATCGACGAGGACATAAAATCGTGAATGAAAAGCGTGACGAGAATGATCAACTCGA

4   TTCTTGAAATCACGAAGAGGATCCCGGACTGGACTGAGCGGGAGGGGAGC
   ** ** * * * *
1&2  GATGTTAGAAGGTGCAAAATCAATAGGTGCCGGAGCTGCTACAATTGCTTCAGCGGGAGC
   *****
3   GATGTTAGAAGGTGCAAAATCAATAGGTGCCGGAGCTGCTACAATTGCTTCAGCGGGAGC
   *****
   ↑                               54
   ↑

4   TGCTGTCGGTATTGGAAACGTGTTTCAGTTCTTTGATCCATTCCGTGGCCCGAAATCCATC
   *****
1&2  TGCTATCGGTATTGGAAACGTGTTTCAGTTCTTTGATCCATTCCGTGGCCCGAAATCCATC
   *****
3   TGCTATCGGTATTGGAAACGTGTTTCAGTTCTTTATTCTTTGGGCTTGGGGCCAGGC
   *****
   ↑                               91

4   ATTGGCTAAACAATCATTGGTTATGCCATTTGGGCTTTGCTCTAACCGAAGCTATTGC
   *****
1&2  ATTGGCTAAACAATCATTGGTTATGCCATTTGGGCTTTGCTCTAACCGAAGCTATTGC

4   ATCGTTTGCCCAATGATGGCCTTTTTGATCTCATCTGTATTCGATTCGAGGTTGAACTT
   *****
1&2  ATCGTTTGCCCAATGATGGCCTTTTTGATCTCATCCGATTCGATTCGTTTCGAGGGT
   *****
   ↑                               228

4   TCATTCTTACAATAT
   * ** * *
1&2  TACAATCTAAAAAAA

```

Fig.S1



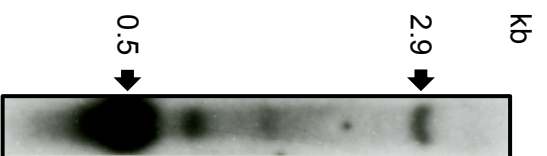




Fig.S2

 **nad3**  
**M S E F A P I C**  
**ATGTCAGAATTTGCACCTATTTGT**

1  
2 AGTCCAATCAGAGACCTTCTCTTTAAAAAAGAAAGTCTAAGCAAAAGAGAGGAGGTTTCGAGACGACAACAGCATGTCGTAGAAAGAGCGGCATGTT 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**

1 ATCTATTTAGCGATCAGTCCGCTAGTTTCTTTGATCCCACTCGGTGTTCTTTTCCATTTGCTTCCAATAGTTCAACCTATCCAGAAAAATTTGTCGGCCT  
2 AGAGCCTTTTACTTTATATTAGTAAAGCGCGCTCACTCTAACGGTGTTCCTTTTCCATTTGCTTCCAATAGTTCAACCTATCCAGAAAAATTTGTCGGCCT 200

 **ψ nad3**


1&2 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  
1&2 ATGAATGTGTTTCGATCCTTTTCGGTGATGCCAGAAGTCGTTTCGATATACGATTTTATCTTGTTCATTTTATTATTATCCTTGATCCGGAAGTAAC 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**

1&2 CTTTTCCTTTCCCTGGGCAGTACCTCTCAACAAGATTGATCCGTTTGGATCTGGTCCATGATGGCCTTTTTATTGATTTTGACGATCGGATCTCTCTAT 400

**E W K R G A S D R E \***
**M P T L N Q**

1&2 GAATGGAAAAGGGTGCTTCGGATCGGAGTAATCACTAGTGATAGGGCAAAAATCGGGGAAAGGACAAAGGAAAGAGCGATGCCACATTAATCAAT 500

 **rps12**

1&2 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  
1&2 TGATTCGTCATGGTAGAGAAGAAAACGGCGCACGGACCGTACTCGAGCTTCGGATCAATGTCCCAGAAAGCAAGGAGTATGCCCGGTGTTTCAACGAG 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**

1&2 AACACCGAAAAACCTAATTCAGCTCCACGTAAGATAGCCAAAGTACGTTTGAGCAATCGACATGATATATTTGCTCACATTCAGGCGAAGGTGATAAT 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**

1&2 TTGCAGGAACATTCTATGGTGTTAATAAGAGGAGGTAGAGTGAAAGATTTGCCAGGTGTGAAATTCATTGTATTTCGAGGAGTCAAGGATTTGCTGGGAA 800



**I P D R R R G R S K Y G A E K P K S R \***

1&2 TTCCGGATCGAAGAAGAGGAGCAGTCAAATATGGTTCGGAAAAACCCAAATCGAGATGAATGGAAGATGCCTCTGGAACCTAAGTTTCTAGATCAG 900

1 TCGAGGTATTCATTGAAAAGTCTCAATGCTATCTTCGACAGTCTTGAGAGCGAGGGCCGCAATAGCGACCACTAAAATTTTGTCTAAAAGATCCCGCT  
2 TCGAGGTATTCATTGAAAAGTCTCAATGCTATCTTCGACAGTCTTGAGAGCGAGGGCCGCAATAGCGACGATCGAAAAGAATTAATTTTCAAGTGAGAT 1000

 **P1**

1 CTTGATCTGGGCGGATCTATCAGCAGCGCATTCTTCTTGTCTGTTCTAGGAACTCAGTAACGTGGCCAGTAAGTCAGCGAGCATTGAGGCAGGCAG  
2 GTCCAAGAGAAAAGGAACGAGGGGAAGAATCGACGAGGACATAAAATCGTGAATGAAAAGCGTGACGAGAATGATCAACTCGAGATGTTAGAAGGTGCA 1100

 **P2**
 **ψ atp9 -1**

1 ACAGATATTATAAACTGCTTTAAGTCGTAAGGCAAGGAAGGCTGATTTACCGATCATTCTACTTGACGCTTTGGACGAGTATTTTCAAACCTCAGT  
2 AAATCAATAGGTGCCGGAGCTGCTACAATTGCTTCAGCGGAGCTGCTATCGGTATTGAAAACGTGTTTCAGTTCTTATTCTTTGGGCTTGGGGCCAGGC 1200

1 TCACTATCATTAAAGGAACTCTTGATCAGCCTTTGAGTCTTAAGG  
2 CGAAATTTGCCGATATTCAGAAATGGATATCAAGCAGCGCCTTCTTTTTTCGGACCCACAGTTTCTGGTATTCTTCTTTTACCCTGGCCCTTTTGCT 1300

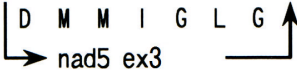
2 GTTATATTTTGGGGTACCGTCCAGCAGCGGGAAGCCGCGGGAGTCGATACTGTGAAAAACAGGAGCTAGGCCAGCTCATAAATTCAGCTATGGG 1400

2 TGGCGGGGTATCGCTCGAGCCGCTTTGGATCTGTACCTGCAACGAACTCCTATAAAAAAAGGGGGAAGGTGCGACAGAATCAGAATAGGTCC 1500

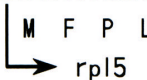
2 AGCTCTATAGGATAGGGACAAATCAATAGGAAATGCTATGTAACCGAACTCTGATAACAATAGCCCTCCGAAATTTTAGATCGGATTGATCAAATGA 1600

Fig.S3


CTGGGGCTGGGCTACCTCCATCCCTAGAGGAGCCGTATGAGGCGGAAGCTCCACGTACGGTTTTGAAGCCGAGCCTTCCAGCAATGGGGCTAGGGAC 100  
CGATATGATGATTGGTTAGGTAGGGCGGCCGGCCTACTACGGGCACCTGTAGGGATTAGTGCCTGAGACCGCATCCACAACTGACGCATGGGACTCA 200


  
nad5 ex3

CCC GGAATGAAGAGGGGAAACATAGCATGTCACAAGAGCGAGGCGAGGTTTTGAACCCTACTGCGAGAGGGAGCCTCGCGAGCCGGGCTTCTAGAGATGA 300  
GGCCTTTGCGAAGCCAAGTAAATTTGGGCCACCAAACCTGCAACTGATGAGAAGGCCCTATGGAGTAAAGGGAAGCGTGTACGTTGCACACTCCCTG 400  
CCTCCCAAAGTGCCTAAAGGACGGGCCAGACGACGAGAGCGACGCCGGGAGCAGATTCCCCACCGCGGGGGACAGGAGACGGCCATCTCGAGGC 500  
ACATCACGACCTACAGGCAACACCGGCGAGACCTGGTAGGGCAACCCGATTGGGAGTCAGAAGATCCATAGTACCTGCAGCCCCACGGACTTCATATTCA 600  
TCATTTTTAAGGCGGGGGGAAGGGATCTCTTTTCTGCAACGGAAAAAACGGAGCAGATTTTACTCGGCACAACCTAACGATTATCCAATACCAATG 700  
ATCTGTGCCTGGAATGCGTTGCTAGATCTCTGCTCTAGAAAGCTATACAGGCAACAACAACCGGTTTTGACCCCGGGCTTCACTTTTATTACTTCTTTC 800  
ACGACGAGGGAACCGCGCCGCATCAGTCGATGCGGTGGAACGCCCTCTGAGTCAAAGATAGAGCTTTTGCACCTCTCTCCAAGAGAGACGATTTCGGTA 900  
TCTGAATCGGCTGAAGGAGAGTGAAGGGCGGCTACTCTCCAGCCCACGGGAGGCCTTCTTTTCTGGTCTACATCAAGTGCATTGAATGCCCCACAAT 1000  
CAGAATCAAAGAGGATAGGTCAAGGAGATGTTGGGACGAAACCTGAGTGGATCCGCGGCAAAGCGGAGCTCCGGAGGTATGGCGGCTTGTGCTGTGCCG 1100  
TCATTTTCGGATTCCCACCGATTTTCGGCACTAGTTAAATGGGAAAATGACCCAGATCTTGAATCAACTAGTAGATCATCAACACGGGGTCCCAATTCAT 1200  
TCGGCGACCGCAAGTACCAGAAATGAATTGCCGAGCTGGGGCAGCCCGCGGTGACCCATACCAATCCTACTCCCTTCTTCTATTGTTTCTTTGTCCAC 1300  
TTCTTGAAATCACGAAGAGGATTCCC GGACTGGACTGAGCGGGAGGGGAGCTGCTGTCGGTATTGAAACGTGTTCA GTTCTTTGATCCATCCGTGGC 1400


  
rp15

ATCTCATCTGTATTCCGATCGAGTTGAACTTTCATTCTTACAACATCTTTTTGAAGCAGATAGTTCACAGTGTCTATTCTATAGATACTGTAAAAGCC 1600  
AACTCATGTTTCCACTCAATTTTTCATTACGAAGATGTATTACGTCAGGATCCGTTGCTCAAACCTGAATCACGCTAACGTTATGGAAGTTCCTGGATCGTG 1700  
TGAATAAGAGTTTTACCAAAGGCACCCTATGATTTATAATAAAAAATGGAAAATTGGCTATGGAGATTCCGCGGGTCAGAAATTCATACAGACAAAA 1800  
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  
AGGGTTTCGACAGGAAAGTCGTTTCGATCCAATCCATTCTTGGGGTCAAATAAGACAAAGGATATGCCAGTGACCTAGCACGACAAAGCACTCTCCGAG 1900  
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  
GGCATGGAATGTCTAATTTTTCGGTGAGAATCTCGACAGTAATGTCTCTGTTAGATTCCCCGGTGGAAATACGGGAAAACCTCATTCAATTCTCGATGGA 2000  
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  
AACGGAGTTTTGCGAATTCTCCCGGAACTGGAAGATCATTTTCGAGATCTTCGAGCATATTTCGAGGGTCAATGTGACTATTGTCACTTCGGCCAACAG 2100  
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  
CAAGATGAGACTTTACCACCGTGGAGCGGCTTTTTGCAAAAAGATGAGGGGAAACTCCGTAAGATGTCGGAGAAGCGAAATATACGAGATCACAAACGT 2200  
Q D E T L P P W S G F L Q K D E G E T P \*


  
psi rps14

CGATTTCTCGCGCTAAATATGAATTGAGACGAAAGCTTTTTAAAGCCTTTTGTAAAGATTTCTAGTGATATGCGGGACAAACATCGTTATAAGCCGCC 2300  
CTCGTTCCGTATATGAGTTCTTCAAATTTATCGTATCGTTTTTTCGCATCTCGATGGCATAAAGAAATCGTCTTGGTAGCAACCACCAAACCAATAGAG 2400  
CAAGGGTTAGCTCCGACGCTGGTCCACAGGTAAGTAGGTCCATTACCGGCCGGCTCCGGACCGAAAAGAC 2470

Fig.S4

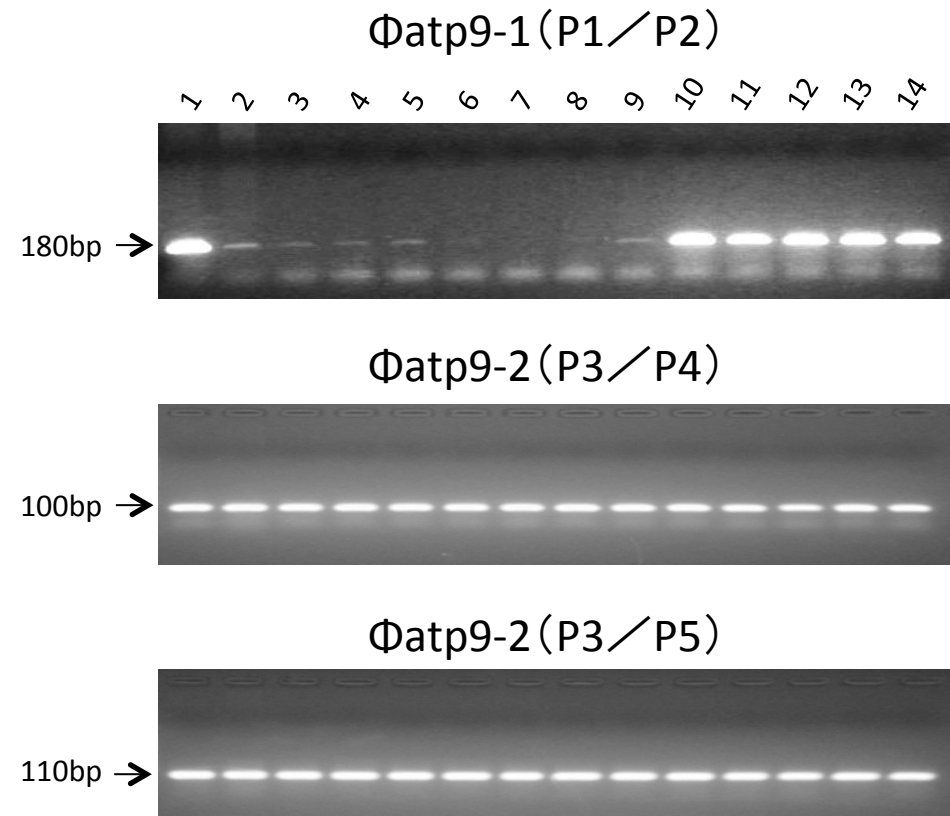


Fig.S5