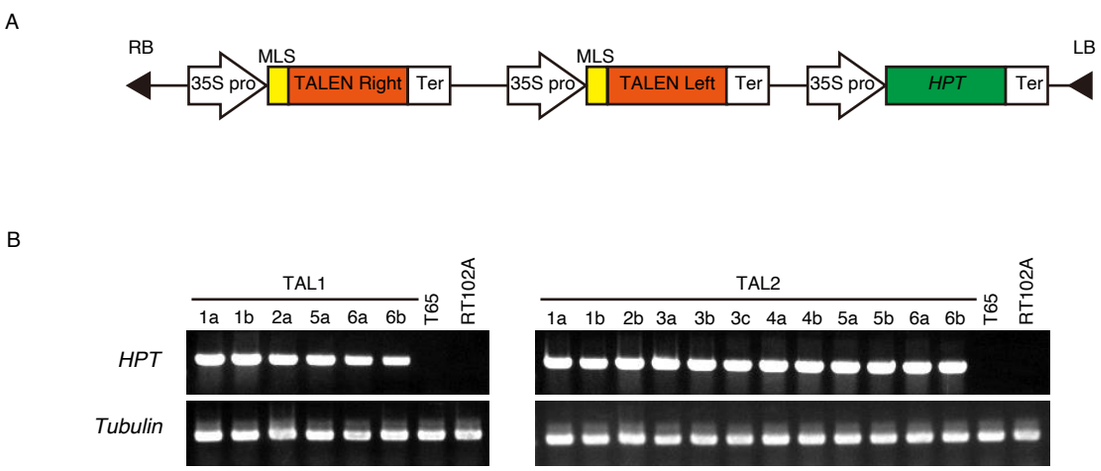
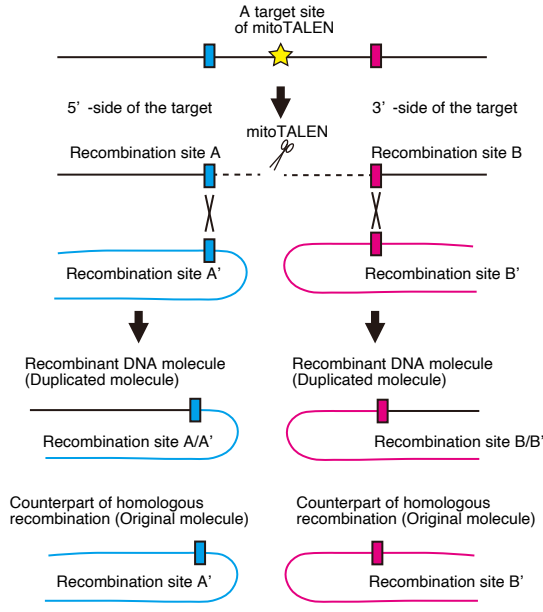
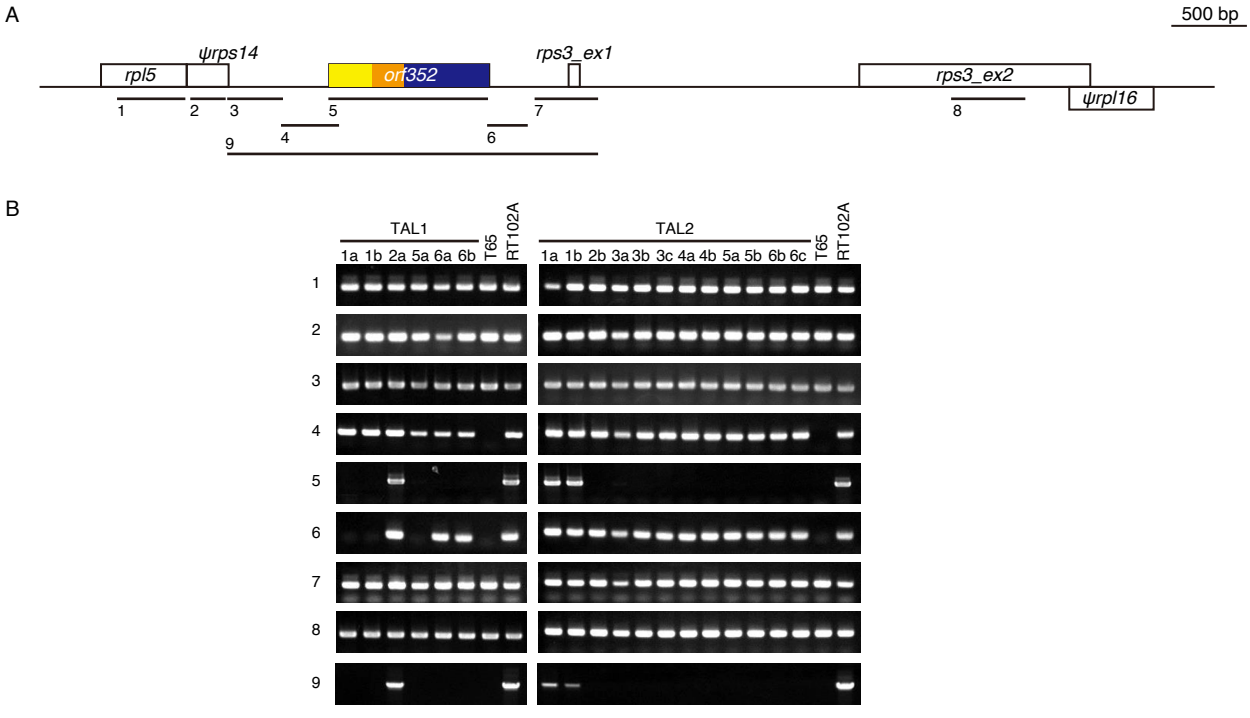


Supplemental Figure S1. Introduction and confirmation of the *orf352*-targeting mitoTALEN vectors, pTAL1 and pTAL2.

(A) Schematic diagram of the T-DNA region from the mitoTALEN vectors. 35S pro, cauliflower mosaic virus 35S promoter; MLS, mitochondrial localization signal; TALEN Right, right side domain of TALEN; Ter, transcriptional terminator of *Arabidopsis* heat shock protein (HSP18.2; encoded by *At5g59720*); TALEN Left, left side domain of TALEN; *HPT*, *Hygromycin Phosphotransferases gene*. Arrowheads indicate a right border (RB) and a left boarder (LB) of T-DNA region. **(B)** Confirmation of stable transformation with pTAL1 and pTAL2 by genomic PCR. Tubulin serves as a positive control for amplification. The data in (B) are representative of three independent experiments.



Supplemental Figure S2. Repair principle of the double-strand break introduced by mitoTALENs in the mitochondrial genome.
A double-strand break introduced by a mitoTALEN will be repaired by homologous recombination. At the 5′ (or 3′) side of a target site, the break is repaired between recombination site A (or B), which is near the target site, and recombination site A′ (or B′), which may be anywhere in the mitochondrial genome but is homologous to recombination site A (or B). As a consequence of repair by homologous recombination, the region between recombination sites A and B is deleted from the mitochondrial genome. Homologous recombination occurs non-reciprocally and is accompanied by replication, so finally, DNA molecules containing the site A/A′ and B/B′ will be duplicated.

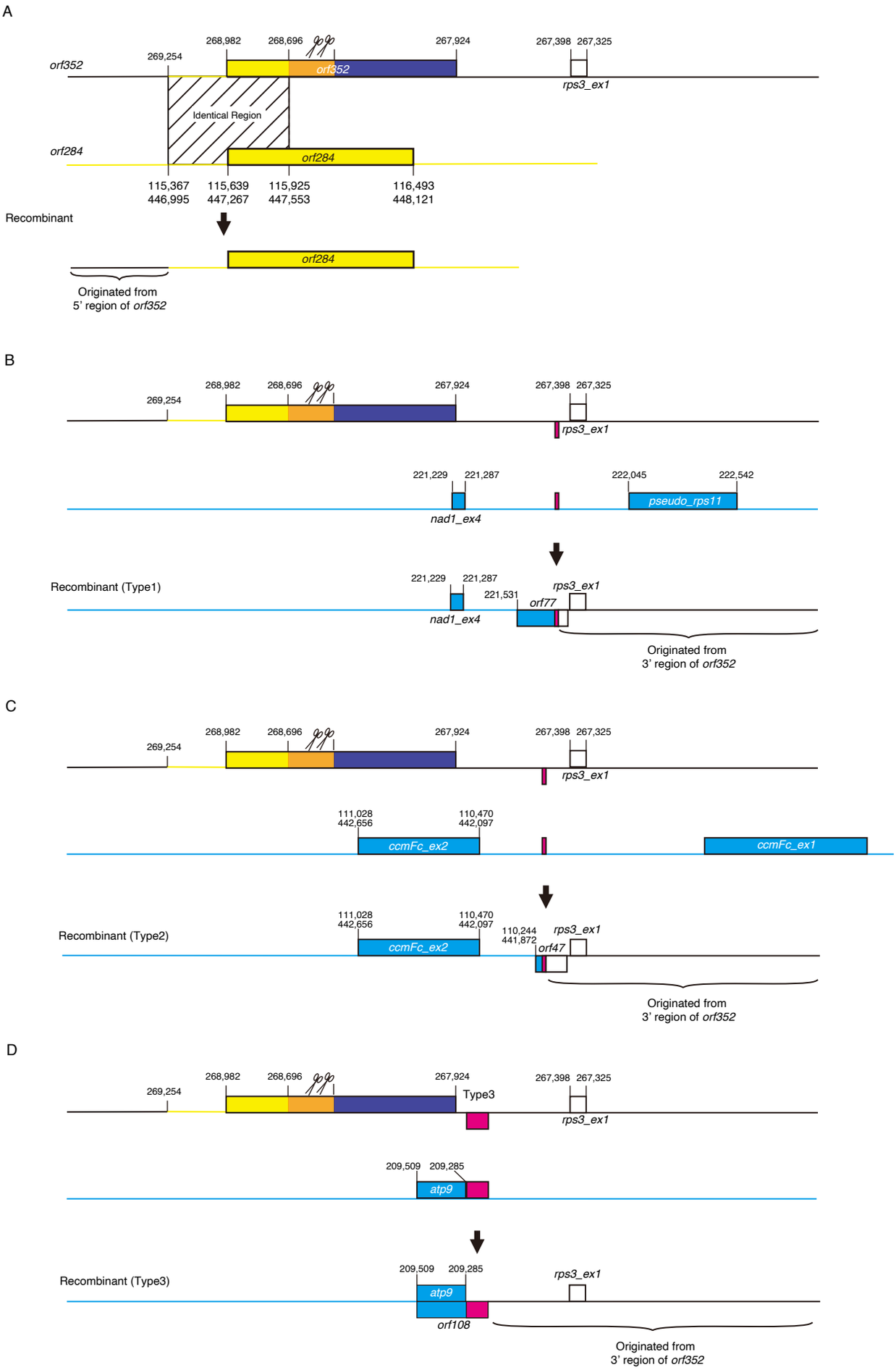


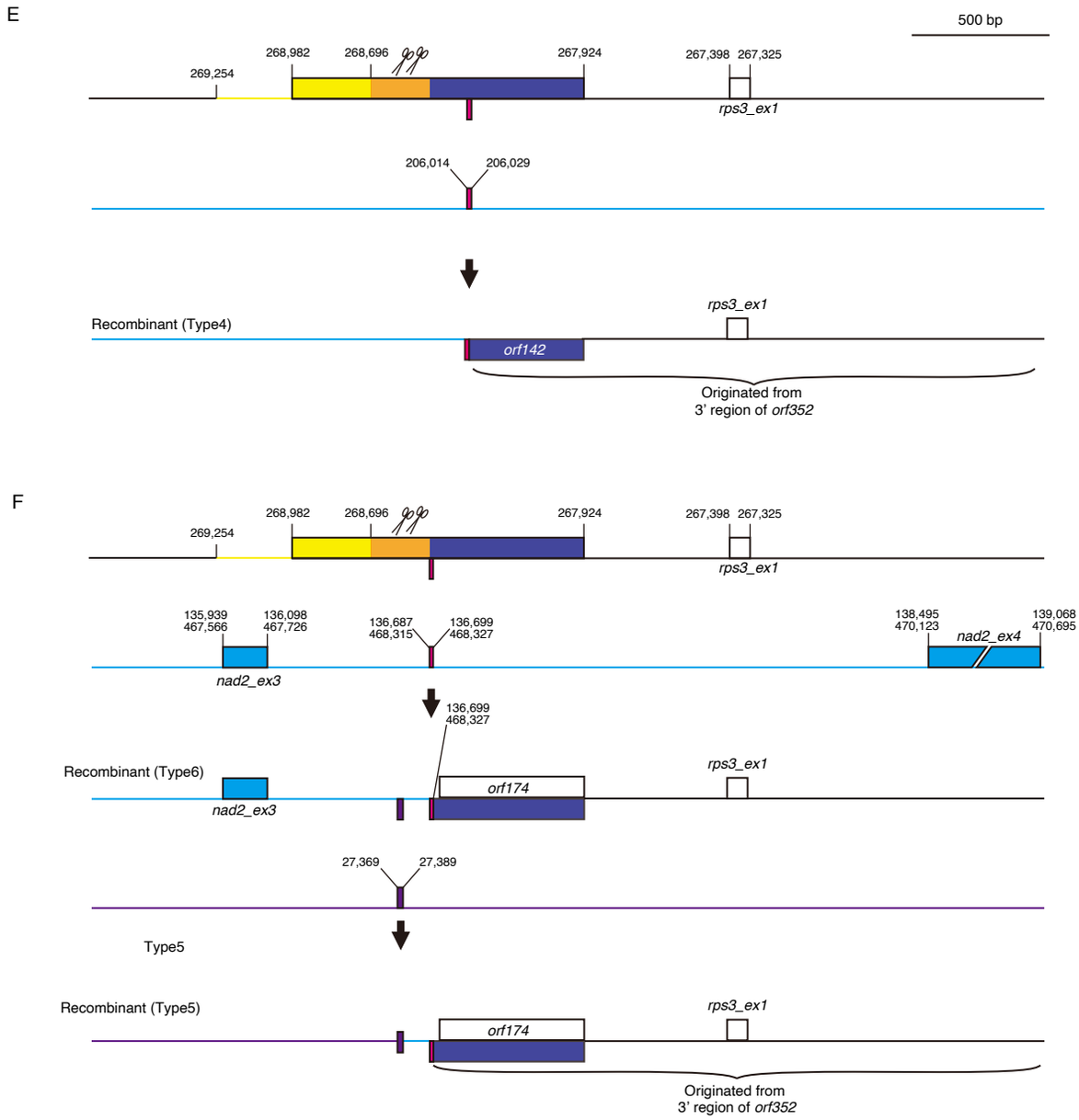
Supplemental Figure S3. Detection of the deleted region around the *orf352* after homologous recombination by PCR.

(A) Genomic structure around the *orf352* open reading frame. Yellow, region identical to *orf284*; navy blue, region homologous to *orf288*; *orf352*-specific sequences are shown in orange. *rpl5*, *ribosomal protein L5*; *ψrps14*, *pseudo ribosomal protein S14*; *rps3_ex1*, *exon 1* of *ribosomal protein S3*; *rps3_ex2*, *exon 2* of *rps3*; *ψrpl16*, *pseudo ribosomal protein L16*. Black lines, from 1–9, indicate PCR amplicons. **(B)** PCR analysis of each transgenic plant for the nine genomic regions indicated in (A). T65 is a fertile *japonica* rice, which lacks the sequence around *orf352*. RT102A serves as a positive control for the presence of *orf352*.

Primers are listed in Supplemental Table S1. The same images of region 5 are used in the Fig. 1C. The data in (B) are representative of three independent experiments.

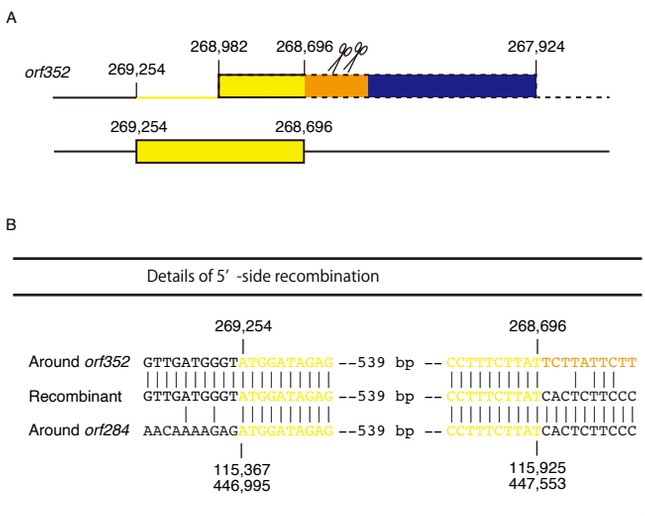
Supplemental Figure S4-1





Supplemental Figure S4. Schematic diagram of double-strand break repair in each type of transgenic plant.

(A) Genomic organization of double-strand break repair at the 5′ side of the target site in all *orf352*-edited plants. Numbers indicate nucleotide positions in the RT102-type mitochondrial genome, GenBank AP012528). Top, genomic structure around the *orf352* open reading frame. Yellow region identical to *orf284*; navy blue, region homologous to *orf288*; orange, *orf352*-specific sequences. Scissors indicate mitoTALENs (TAL1 and TAL2). Middle, genomic structure around the recombination site A′ (illustrated in Supplemental Figure S2). Bottom, genomic structure of new recombinants. Yellow line indicates region originating around the recombination site A' . **(B–F)** Genomic organization of double-strand break repair at the 3' side of the target site in Type 1 (B), Type 2 (C), Type 3 (D), Type 4 (E), and Type 5 or Type 6 (F) *orf352*-edited plants. Top, genomic structure around the *orf352* open reading frame. Yellow, region identical to *orf284*; navy blue, region homologous to *orf288*; orange, *orf352*-specific sequences. Scissors indicate mitoTALENs (TAL1 and TAL2). Magenta box indicates the recombination site B. Middle, genomic structure around the recombination site B′ (Supplemental Figure S2) indicates in blue. Bottom, genomic structure of new recombinant. Black line indicates the region originating around *orf352*. Blue line indicates the region originating around the recombination site B′. A new ORF is predicted at the recombination site for each recombinant type: *orf77* (Type 1); *orf47* (Type 2); *orf108* (Type 3); *orf142* (Type 4); *orf174* (Types 5 and 6). Type 5 recombination also harbors sequences originating around an additional recombination site (purple boxes). Purple line indicates the region around the additional recombination site. *rps3_ex1*, *exon 1* of *ribosomal protein S3*; *nad1_ex4*, *exon 4* of *NADH dehydrogenase subunit 1*; *rps11*, *ribosomal protein S11*; *ccmFc_ex2*, *exon 2* of *cytochrome c biogenesis Fc*; *nad2_ex3* and *ex4*, *exon 3* and *exon 4* of *NADH dehydrogenase subunit 2*, respectively.



Supplemental Figure S5. Double-strand breaks induced by mitoTALENs are repaired by identical homologous recombination at the 5′ side of the target site.
(A) Top, genomic structure around the *orf352* open reading frame. Yellow, region identical to *orf284*; navy blue, region homologous to *orf288*; orange, *orf352*-specific sequences. Scissors indicate mitoTALENs (TAL1 and TAL2). Bottom, position (in bp) of recombination site A across all *orf352*-edited plants. Yellow box indicates the recombination sites A. The dashed lines indicate the deleted region.
(B) Sequence comparisons between a recombinant sequence and its corresponding sequence in the RT102-type mitochondrial genome. The font colors correspond to the colors in (A).

Supplemental Table S1 Primer list used in this study

Primer name	Sequence (5' to 3')	Description
orf352_F2	ATGACGAGAGATAGAATGAG	For Figure 1B
orf352_R2	GGAGGCTGAGTTTTGATCCT	For Figure 1B
cox2_Fk	CAGTTCCGATGAACAGTCAC	For Figure 1B
cox2_Rk	TCTCGTTGTACCGAGATGGA	For Figure 1B
352A2_F	CTTGGTAGCAACCACCAAAC	P1 in Figure 3
orf352_R6	TGCTTTCTTGAAAGGAGGAC	P2 in Figure 3
orf284_Rz	TAGAGCCTGTGGGTCTTGAA	P3 in Figure 3
orf284_F	CTATCTGAGCCTTTACGAGC	P4 in Figure 3
orf284_R	GGAATTCGGTTCTTTCGAGC	P5 in Figure 3
HPT_ZF	GAGAGCCTGACCTATTGCAT	For Figure S1
HPT_ZR	TCGGCGAGTACTTCTACACA	For Figure S1
tubulin zF	TGGTCGGATTCGCCCCGCTG	For Figure S1
tubulin zR	TTACATGTCGTCAGCCTCCT	For Figure S1
rpl5_F	AGTACCAAAAGCTGCCTCTG	Region 1 in Supplemental Figure S3
rpl5_R	TTTCCCCCTCATCTTTTAGC	Region 1 in Supplemental Figure S3
rps14_F	CACAAACGTAGATTGCTCGC	Region 2 in Supplemental Figure S3
rps14_R	ATGCCCATCAAAGAACCTCG	Region 2 in Supplemental Figure S3
352A2_F	CTTGGTAGCAACCACCAAAC	Region 3 in Supplemental Figure S3
352A2_R	CCTCGGTGTCTCTATTTGCT	Region 3 in Supplemental Figure S3
352A1_F	CAAATAGAGACACCGAGGCC	Region 4 in Supplemental Figure S3
352A1_R	CGACTACTAAATGCTCGGCA	Region 4 in Supplemental Figure S3
352_F2	ATGACGAGAGATAGAATGAG	Region 5 in Supplemental Figure S3
352_R2	GGAGGCTGAGTTTTGATCCT	Region 5 in Supplemental Figure S3
352A3_F	AACTCAGCCTCCTAGACATG	Region 6 in Supplemental Figure S3
352A3_R	CTTAGGAACCGTACATGCAC	Region 6 in Supplemental Figure S3
352A4_F	TAGCGCGATCTCGTACTAAC	Region 7 in Supplemental Figure S3
352A4_R	GTTAGAAGAAGTCGTGTCCG	Region 7 in Supplemental Figure S3
rps3_F2	TTTCGACCCCGTCGTAGTTC	Region 8 in Supplemental Figure S3
rps3_R2	ACCCGTACGGAATTATCCTC	Region 8 in Supplemental Figure S3
FP1	GTAATACGACTCACTATAGGGCACGCGTGGTNTCGASTWTSGWGTT	For FPNI-PCR
FP2	GTAATACGACTCACTATAGGGCACGCGTGGTNGTCGASWGANAWGAA	For FPNI-PCR
FP3	GTAATACGACTCACTATAGGGCACGCGTGGTWGTGNAGWANCANAGA	For FPNI-PCR
FP4	GTAATACGACTCACTATAGGGCACGCGTGGTAGWGNAGWANCAWAGG	For FPNI-PCR
FP5	GTAATACGACTCACTATAGGGCACGCGTGGTNGTAWAASGTNTSCAA	For FPNI-PCR
FP6	GTAATACGACTCACTATAGGGCACGCGTGGTNGACGASWGANAWGAC	For FPNI-PCR
FP7	GTAATACGACTCACTATAGGGCACGCGTGGTNGACGASWGANAWGAA	For FPNI-PCR
FP8	GTAATACGACTCACTATAGGGCACGCGTGGTGTNCGASWCANAWGTT	For FPNI-PCR
FP9	GTAATACGACTCACTATAGGGCACGCGTGGTNCAGCTWSCTNTSCTT	For FPNI-PCR
FSP1	GTAATACGACTCACTATAGGGC	For FPNI-PCR
FSP2	ACTATAGGGCACGCGTGGT	For FPNI-PCR