A

B

C

D

mutant	Number of wild-type seeds	Number of mutant seeds	χ^2
mppr6-1_1	140	45	0,045
mppr6-1_2	138	43	0,128
mppr6-1_3	80	24	0,205
mppr6-1_4	110	38	0,036
appr6-1	61	19	0,067
appr6-2	93	29	0,098
appr6-3	66	21	0,035

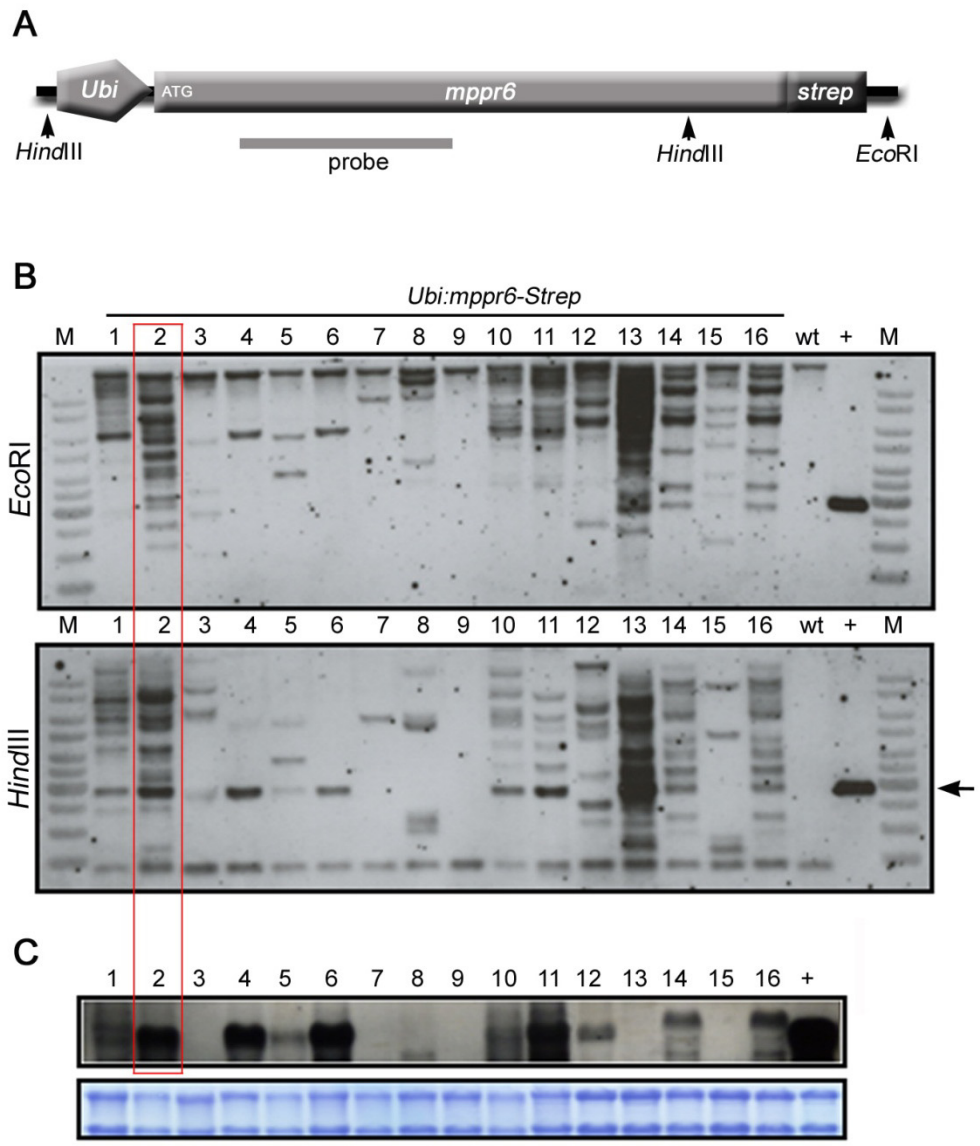
Supplemental Figure 1. Phenotype and Segregation of *mppr6*.

(A) Comparison of wild type (left) and *mppr6*/- (right) rescued plants.

(B) Close-up view of 4 months old *mppr6* mutant plant.

(C) Allelism test. Segregating *mppr6* ear resulting from crossing *mppr6-1* and *mppr6-2*.

(D) Genetic segregation ratios of *mppr6-1*, *appr6-1*, *appr6-2*, and *appr6-3*.

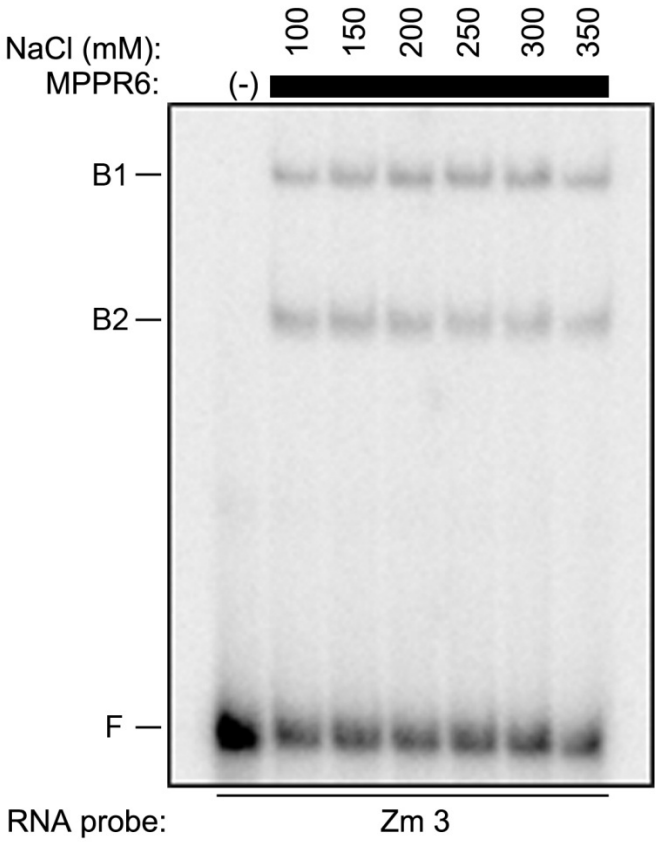


Supplemental Figure 2. Molecular Analysis of *mppr6-strep* Transgenic Plants.

(A) Diagram of the *Ubi:mppr6-strep* transgene. The recognition sites of the restriction enzymes and the region comprising the probe used in **(B)** are indicated.

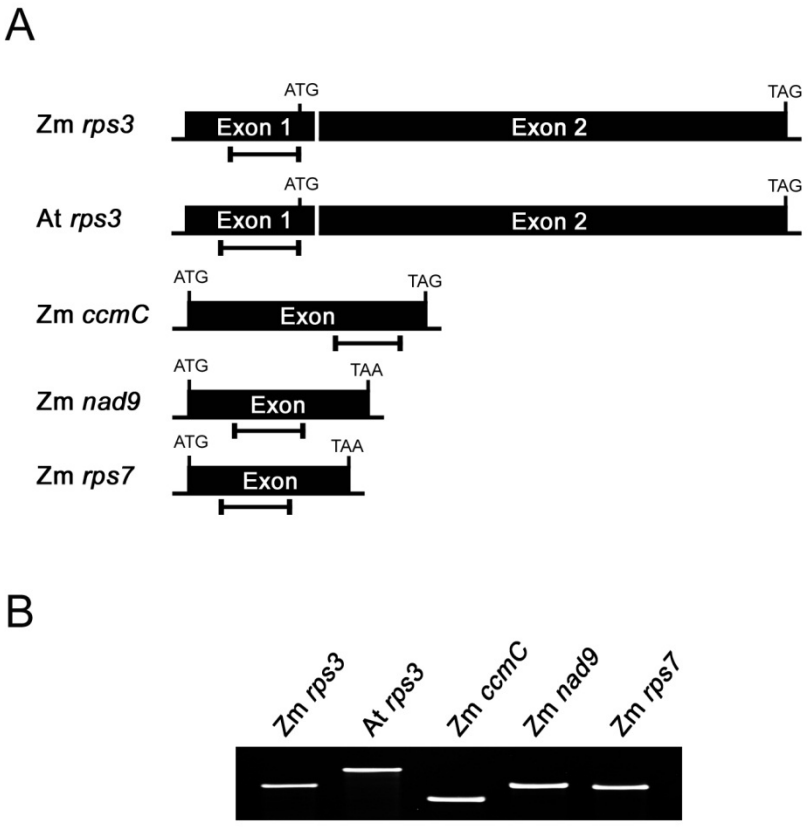
(B) DNA gel blot analysis of putative *Ubi:mppr6-strep* transgenic plants. The binding region of the DIG-labeled *mppr6*-specific probe is shown in **(A)**. As a positive control (+), the *Ubi:mppr6-strep* containing plasmid was digested with *Hind*III. The expected size of the *Hind*III band is indicated by arrow. M, molecular marker; wt, wild-type; +, positive control.

(C) RNA gel blot analysis of putative *Ubi:mppr6-Strep* transgenic plants. A transgenic *Arabidopsis 35S:mppr6* plant was used as positive control (+). The methylene blue staining of the ribosomal RNA is shown in the panel below as a loading control. The transgenic *PUbi:mppr6-strep* plant (2) used in the immunodetection and co-immunoprecipitation experiment is framed by a quadrangle.



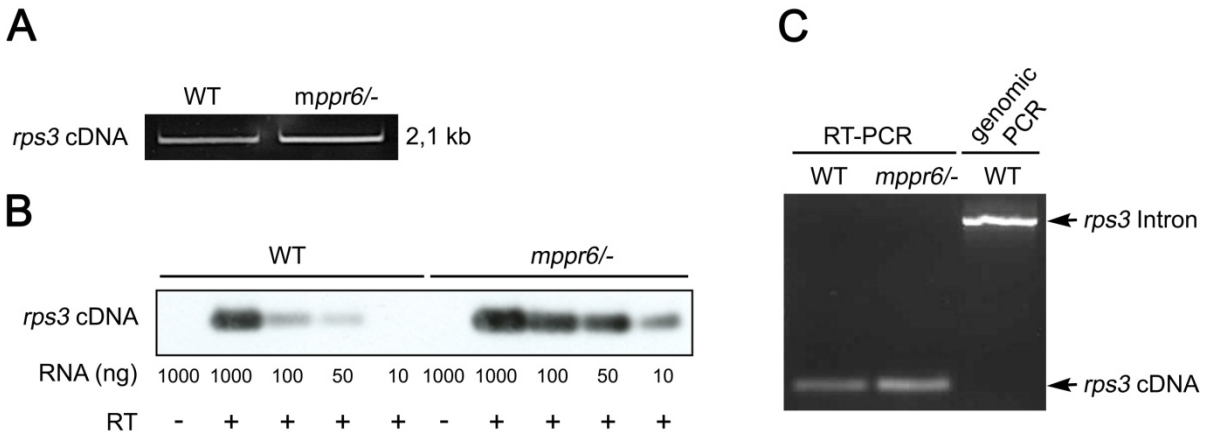
Supplemental Figure 3. MPPR6 Binds to 5' UTR of *rps3* RNA at High Salt Conditions.

Sequence specificity of MPPR6 binding was demonstrated by an EMSA experiment at high monovalent salt concentrations. The interaction was explored at a range of increasing NaCl concentrations (100 – 350 mM) using 250 nM recombinant MPPR6 and 100 pM of 5' UTR of *rps3* (Zm 3, 75 nt, see Fig. 10 **(A)**). Even at 350 mM of NaCl none of the shifted bands disappeared, indicating sequence specificity of binding. B1 and B2, bound RNA; F, free RNA.



Supplemental Figure 4. Unlabeled Competitor RNA.

(A) Diagrams of the exon organization of Zm *rps3*, At *rps3*, Zm *ccmC*, Zm *nad9*, and Zm *rps7*. The regions used as template for production of cold competitor RNA are indicated below (black lines). Start (ATG) and stop (TAA, TAG) codons are shown.
(B) Unlabeled RNA used in the competition experiments (see Fig. 10 **(C)**). 200 ng of unlabeled RNA was separated on 5% denaturing polyacrylamide gel and stained with ethidium bromide. RNA was generated by in vitro transcription using T7 polymerase and the PCR amplified regions of Zm *rps3* (primers: rps3_7fw/9rev), At *rps3* (primers: rps3at_2fw/rev), Zm *ccmC* (primers: ccmC_fw/rev), Zm *nad9* (primers: nda9_1fw/rev), and Zm *rps7* (primers: rps7_1fw/rev) indicated in **(A)**. After synthesis, RNA was treated with DNase I, gel-purified and quantified using absorption of light at 260 and 280 nm (A260/280).



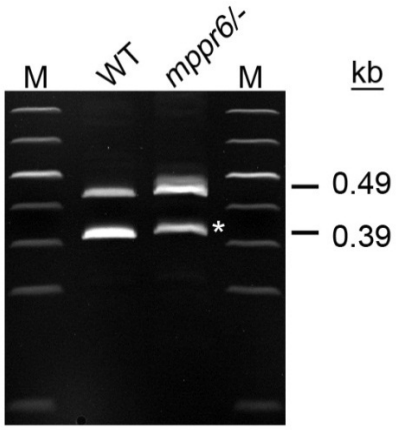
Supplemental Figure 5. Analyses of *rps3* Transcripts.

Total RNA extracted from 16 DAP wild-type and *mppr6* mutant embryos were treated with DNase I and used for cDNA synthesis utilizing hexanucleotides.

A) The complete ORF and parts of the UTRs of *rps3* in *mppr6* mutant and the wild type were amplified by RT-PCR. The forward primer was located 104 bp upstream of the ATG, the reverse primer 276 bp downstream of the stop codon (rps3_2fw and rps3_2rev).

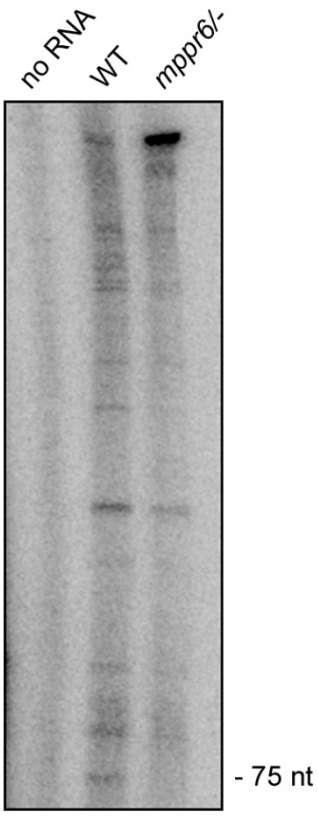
B) Abundance of *rps3* mRNA in the wild type and *mppr6* mutant. Different amounts of RNA were used for cDNA synthesis. RT-PCRs with primers flanking the intron (rps3_1fw and rps3_1rev) were carried out to amplify the *mppr6* mRNA. Low cycle conditions were chosen to yield PCR products that approximately mirror the input RNA amounts.

C) The *rps3* intron is spliced out in the *mppr6* mutant. RT-PCR was performed using primers flanking the intron (see **(B)**). Genomic PCR was carried out as a control for intron amplification (PCR profile as above). The intron and intron-free amplification products are indicated by arrows.



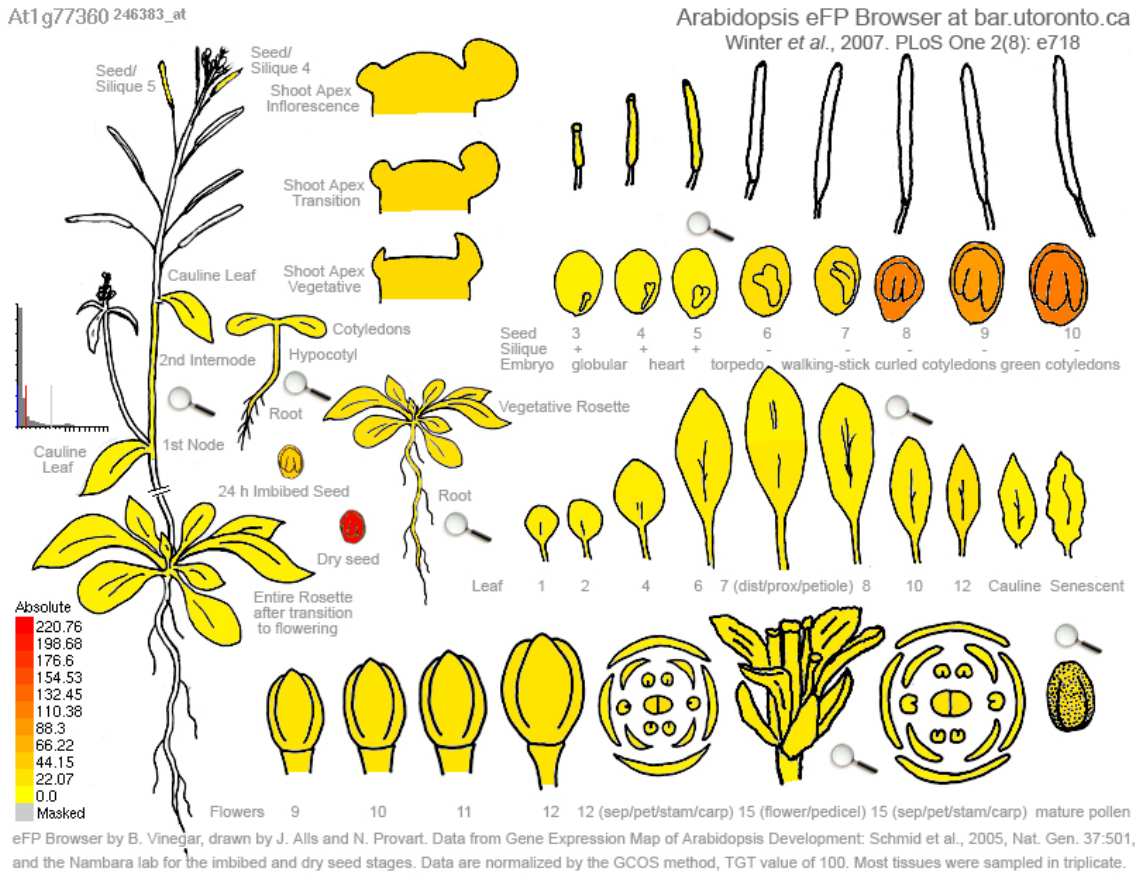
Supplemental Figure 6. *Rps3* cRT-PCR Products Separated on Polyacrylamide Gel.

In order to achieve a higher resolution, products of circular RT-PCR were additionally separated on a denaturing 5% polyacrylamide gel. The shift corresponding to the prolonged 5' *rps3* transcript ends in the *mppr6* mutant is marked by an asterisk.

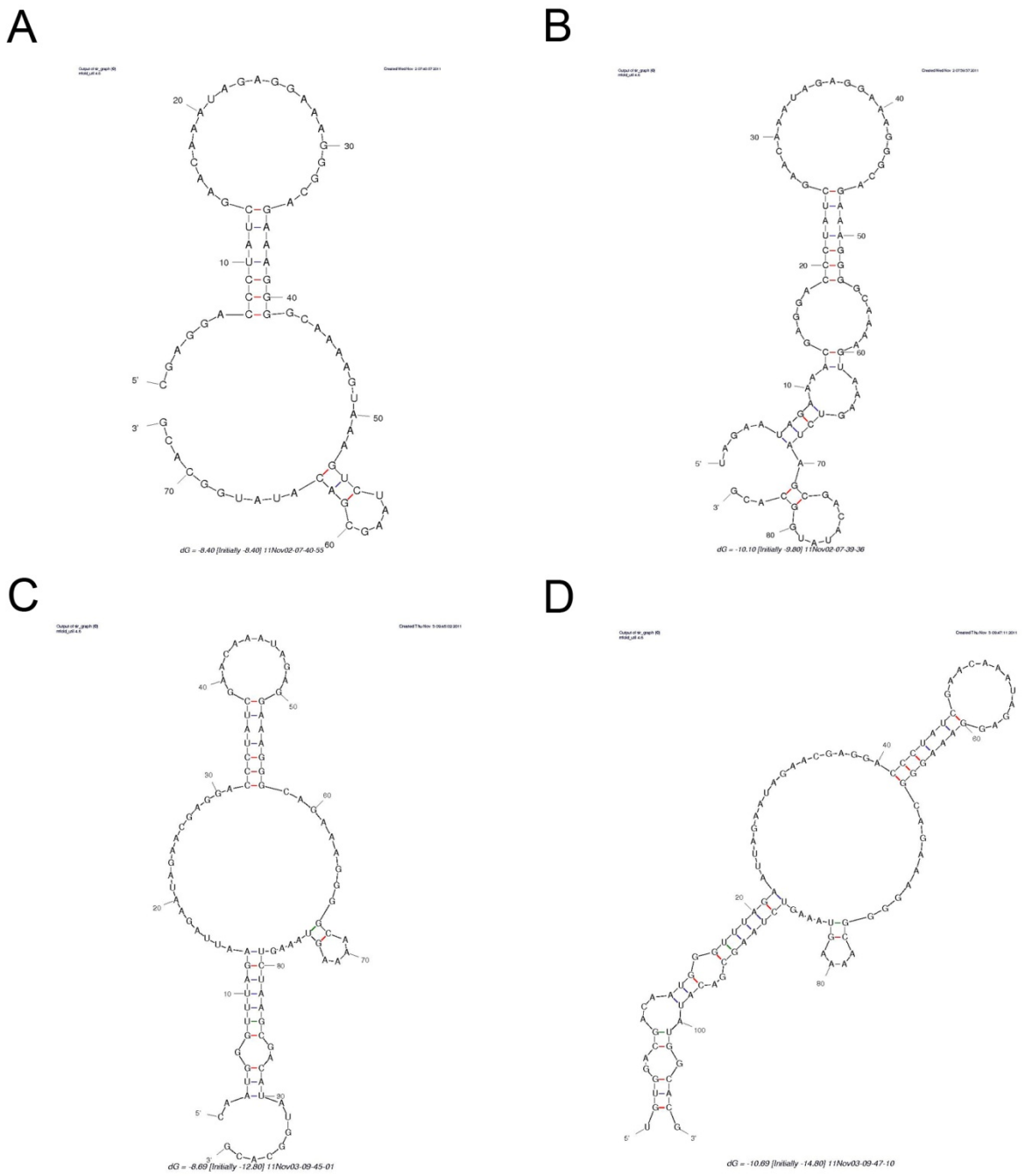


Supplemental Figure 7. Mapping of *rps3* 5' Termini by Primer Extension Assay.

Total RNA was extracted from callus tissue of wild type and *mppr6/-* mutants. The *rps3* transcript ends were analyzed with a primer (rps3_11) binding 6 nt downstream of the ATG. Primer extension products were resolved on a denaturing 10% polyacrylamide gel. For size determination, a 75 nt oligo corresponding to the length of the mature 5' *rps3* transcript ends (see cRT-PCR products in Figure 13) was run on a site lane (not shown).



Supplemental Figure 8. *APPR6* Expression Profile Determined by the *Arabidopsis* eFP Browser.



Supplemental Figure 9. Secondary Structure of *rps3* 5' UTRs Determined by M-fold.

(A) WT *rps3* 5' UTR (-65 to +8). dG = -8.40 (initially -8.40)
(B) *rps3* 5' UTR1 in *mppr6/-* (-75 to +8). dG = -10.10 (initially -9.80)
(C) *rps3* 5' UTR2 in *mppr6/-* (-90 to +8). dG = -8.69 (initially -12.80)
(D) *rps3* 5' UTR3 in *mppr6/-* (-99 to +8). dG = -10.69 (initially -14.80)

Supplemental Table 1. Names and Sequences of Used Primers.

Primer name	Sequence 5‘ → 3‘
mppr6_1fw	AGTGATATGCTTGCCGCTGGTTGC
mppr6_2rev	AATCCCATCCTTCTCCATATCCAAAAAT
mppr6_3fw	ATGGTAGGATCCATGGGTGGCTTCCACTTCCACCAC
mppr6_3rev	ATGGTAGAAGACAAGGATCCAGGTGCTTGAGGACGCTCTC
mppr6_4fw	GATGGGATTATGCCTGATGTTGTTGTGT
mppr6_4rev	GTCCGAGGCGTCCACCGAGATA
mppr6_5fw	GGTTAATATGGGATGGGTAGATGCTCA
mppr6_5rev	CGTGCTCGAGGGCGGATGC
mppr6_6fw	ATGGTAGGTCTCAAATGGGTGGCTTCCACTTCCACCAC
mppr6_6rev	ATGGTAGGTCTCAGCGCTATCAAACAAAGGTTCTTGAGCGAG
mppr6_7rev	TAATTCTAGATCAATCAAACAAAGGTTCTTGAGCGAG
mppr6_8fw	ATATATACTAGTGATGACGACGACAAGGAGCACGAGCTCGACCATAG C
mppr6_8rev	ATATATCTCGAGTTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTATCA AACAAAGGTTCTTGAGCGAG
mppr6_9rev	TAATGGATCCTCATTTTTCGAACTGCGGGTGGCTCCAGCTAGCATCAA ACAAAGGTTCTTGAGCGAG
F1 = mppr6_1fw	AGTGATATGCTTGCCGCTGGTTGC
F2	GATGAAGCTCTTGGCATTGTG
R1= mppr6_2rev	AATCCCATCCTTCTCCATATCCAAAAAT
R2	ATTTTGCCGATTTCGGAAC
R3	ATGGTAGGTCTCAGCGCTATCACACAACGGCTCATTGACCA
R4	CGAGGTGAACAAGATCTCTGG
rps3at_1fw	TAATTACGACTCACTATAGGGCTCAGACTTTCGAGAACAAATATTG
rps3at_1rev	CGTGCCATATTTTTTGACTTTATGG
rps3at_2fw	TAATACGACTCACTATAGGGAATGGTCGCAGGTTCAAGTC
rps3at_2rev	AATCGGATTTCCTTTTCGTG
rps3_1fw	TAGTTCAGATCCAAGTCGGTTCAGTG
rps3_1rev	CCGAGACGAAAGCCAAAGGTG
rps3_2fw	GAAACTGTGGACGACAATGG
rps3_2rev	CAACCCGTAGGATTTCCTTT
rps3_3fw	GGATACCATGACCGATCACC
rps3_3rev	CGGGCATACTCATAAAATGG
rps3_4fw	AAGCTCGACCAGCGATAAAA
rps3-rpl16_5fw	TGGATGGTGTGAGTTTGTCA
rps3_5rev	TGAACCGACTTGGATCTGAA
rps3-rpl16_6fw	CGCATAAACCATGTTCGTCA
rps3_6rev	CAAATAGAGGAAAGGGCAGA
rps3_7fw	TAATACGACTCACTATAGGGCACAACCGCGGTATGAGTTCT
rps3_7rev	TCGTCCACAGTTTCCTTGTC
rps3_8fw	TAATACGACTCACTATAGGGGACAAGGAAACTGTGGACGA
rps3_8rev	TGTTCGATAGGGTCCTCGTT
rps3_9fw	TAATACGACTCACTATAGGGAACGAGGACCCTATCGAACA
rps3_9rev	CGTGCCATATGTCGCTTAGAC
rps3_10fw	AAGGAAACTGTGGACGACAA
rps3_10rev	AGGTGGACGTATCGAACTGA
rps3_11	TCGTGCCATATGTCGCTTAG
rps3_80-mer as	AGCTTCCTCCCTTCGCTCCCCCCATCGTAGATGGTTCAGCCACACCCGG TGCCACGAAATGATTGAGAACTACGACGGGG

Supplemental Table 1. continued

18S rRNA_80-mer as	CGTTGTATCGAATTAAACCACATGCTCCACCGCTTGTGCAGGCCCCCGTCAATTCCTTTGAGTTTCGGTCTTGCGACCGT
26S rRNA_80-mer as	CCCTCTCGACTTTGGATCTTAGCACCCAATCAGTCTGTCTGTACTAAGGATGACGGCCTGTATTCGGAGTTTCCCTGGGG
ccmC_rev	GATTGGAATGGGCATAGGAA
nda9_1fw	TAATACGACTCACTATAGGGCGCAGATTTGAAGTTGTCCA
nda9_1rev	AATCCGGATGATTGATGGAA
nda9_2fw	GTCAGTCCATTTCCATCAGC
nda9_2rev	CTGTTCCCAAGGACTAGCAA
rps7_1fw	TAATACGACTCACTATAGGGCTCGAACTGAACGCGATGTA
rps7_1rev	GTTTGAAAGCTGCTCCAAGG
rps7_2fw	ATGGTTGACGCCGTAGATAA
rps7_2rev	ACGTGAAATTCCCCTCTTTC
emp4_1fw	ATTAGGATCCATGTGCATCTCAGTCCGCCACGGG
emp4_1rev	TAATGGATCCCTGCCGGAAAATTCTACTTCATCAGA
Muoligo1	TCYAAAMCASAGAAGCCAACGCCAASGCCTC
Muoligo2	CYCTCTTCKTCCATAATGGCAATTATCTC
OmuA	CTTCGTCCATAATGGCAATTATCTC
PPR_R03	ACAATGTCAGGCTGGCAACC
PPR_F03	GAAATGGGTGGCTTCCACTTC