Supporting Information

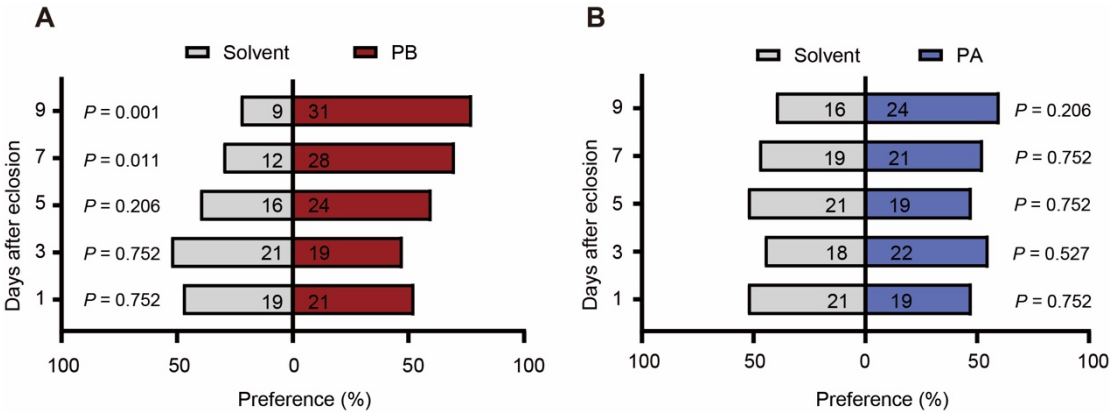

Figure S1. Sex pheromone components PA and PB elicit distinct sexual behavioral responses.

In a Y-tube olfactometer assay for male adults during 1-9 DAE between PB (0.1 ng applied to a filter paper in 10 μL dichloromethane) and the control solvent dichloromethane (10 μL) (**A**) or between PA (0.1 ng) and dichloromethane (10 μL) (**B**). Forty repeats were performed. Data are presented as mean ± s.e.m., and χ^2 test was used.

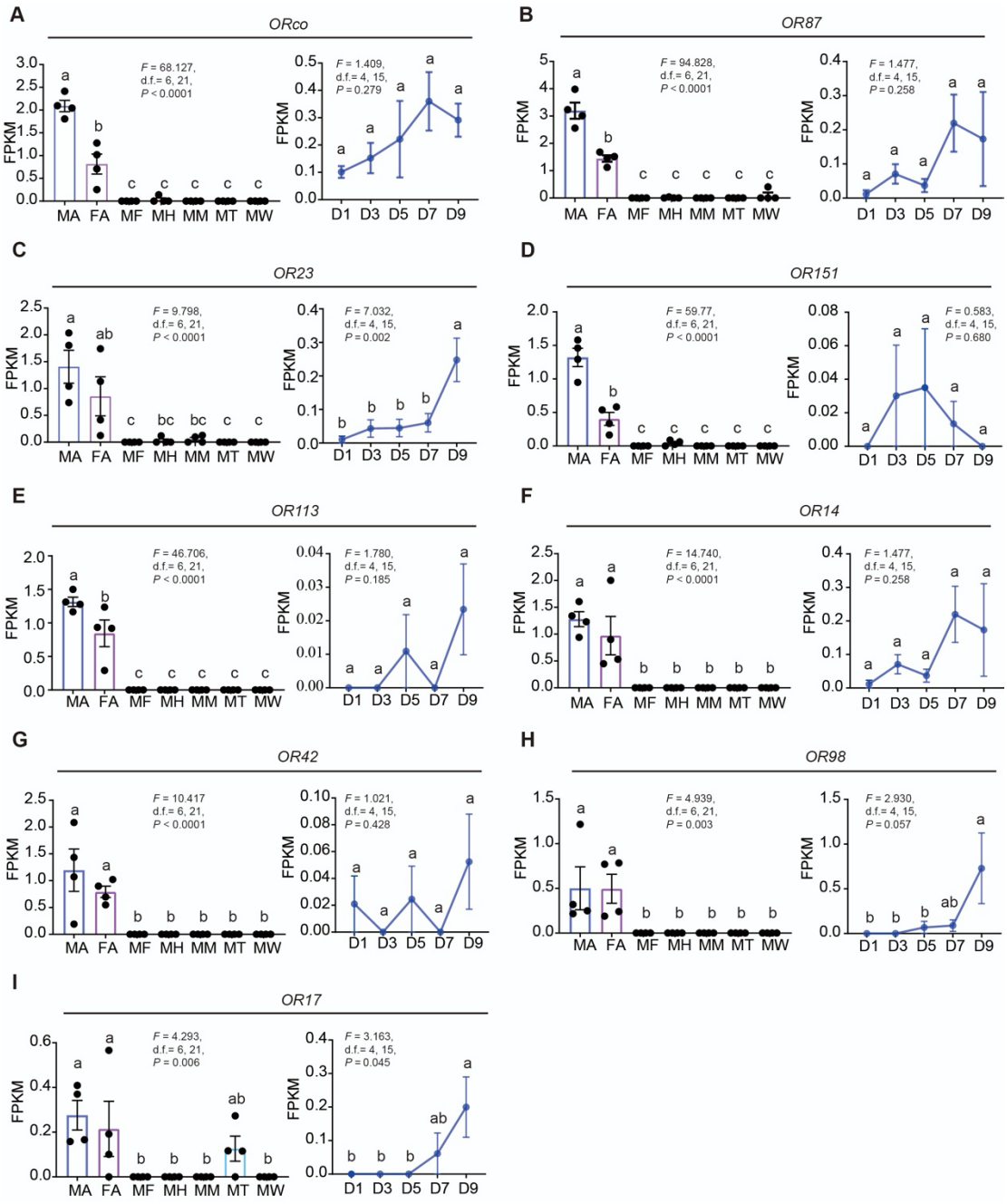

Figure S2. Expression levels of candidate *OR* genes from transcriptome sequencing.

(A-I) The FPKM value of *ORco* (**A**), *OR87* (**B**), *OR23* (**C**) *OR151* (**D**), *OR113* (**E**), *OR14* (**F**), *OR42* (**G**), *OR98* (**H**) and *OR17* (**I**) in different tissues (Left) and at different developmental stages in male antennae (Right) from transcriptome sequencing. n = 4 biological replicates. Data are mean ± s.e.m.

Different letters indicate statistically significant differences ($P < 0.05$; Tukey's HSD multiple-comparison tests following one-way ANOVA as indicated in each panel).

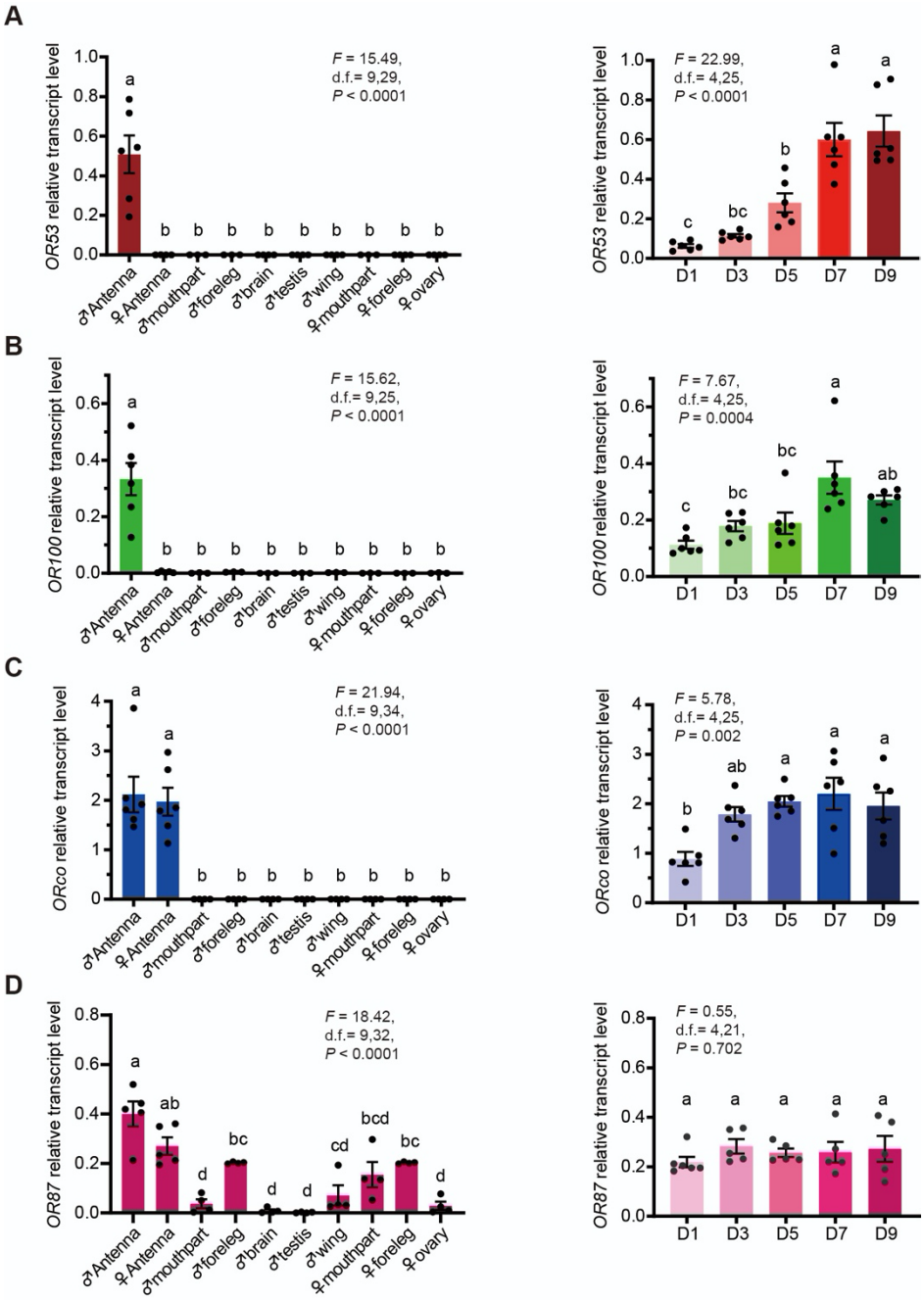

Figure S3. Relative expression of *OR53*, *OR100*, *ORco* and *OR87* in different tissues and at different developmental stages after eclosion of the American cockroach.

(A-D) Temporal and spatial expression of *OR53* **(A)**, *OR100* **(B)**, *ORco* **(C)**, and *OR87* **(D)**. (Left) Relative expression of mRNA of *OR53* in different tissues, n = 3-6 biological replicates. Data are mean ± s.e.m. (Right) Relative expression of mRNA of *OR53* at developmental stages after eclosion in males, n = 6 biological replicates. Data are mean ± s.e.m.

The *OR53*, *OR100*, *ORco* and *OR87* gene transcription levels were normalized to that of ribosomal protein 49 gene (*rp49*). Different letters indicate statistically significant differences ($P < 0.05$; Tukey's HSD multiple-comparison tests following one-way ANOVA as indicated in each panel).

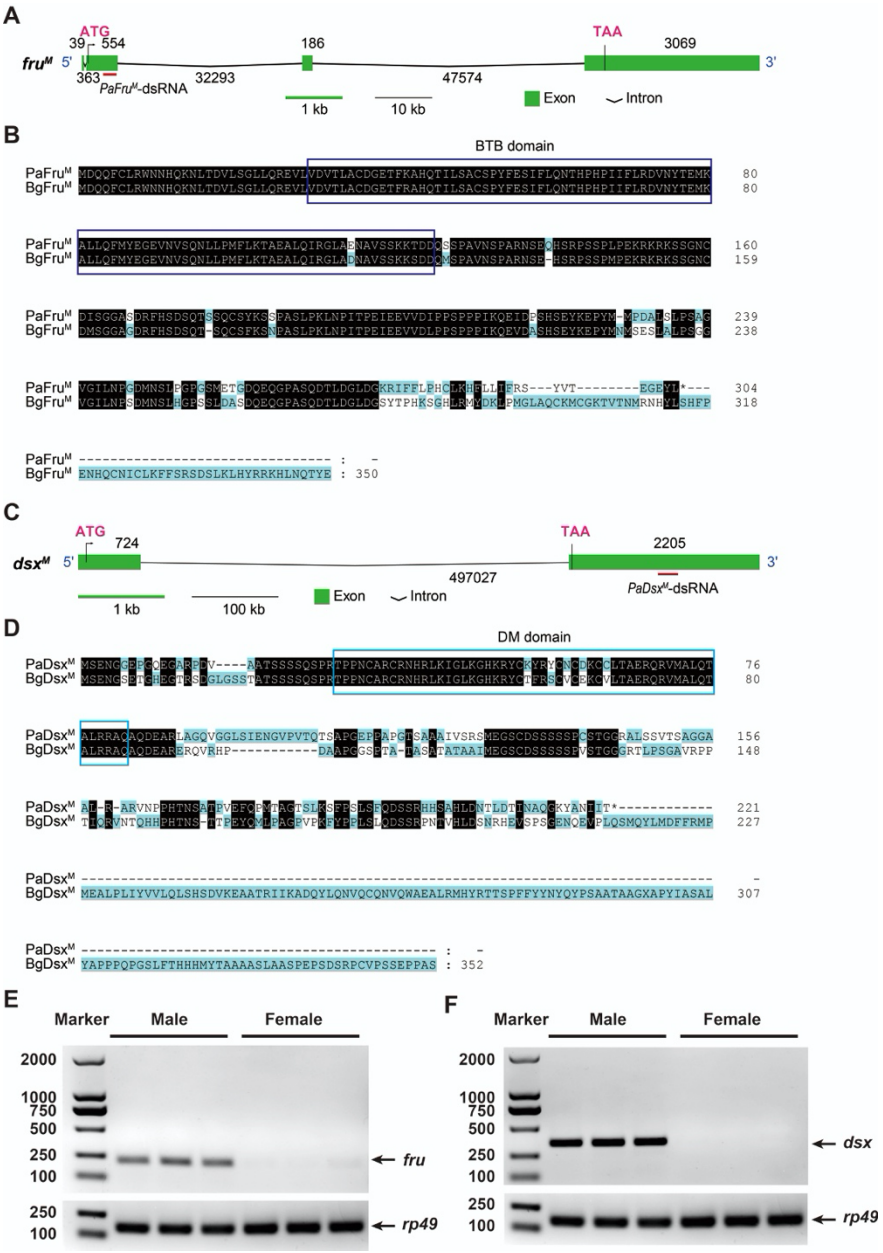

Figure S4. Identification of male-specific *fru* and *dsx* transcripts in *P. americana.*

(A and B) Genomic organization of *fru* identified from the full-length transcriptome of the male American cockroach antenna **(A)**. The numbers adjacent indicate the length of each exon or intron in base pairs (bp). Red lines indicate the location of fru^{M}-dsRNA. Alignment of Fru between the American cockroach and its closely related species, the German cockroach **(B)**. The blue box represents the conserved BTB domain of Fru proteins.

(C and D) Genomic organization of *dsx* (**C**) and alignment of Dsx (**D**). The blue box represents the conserved Doublesex DNA-binding motif (DM) domain.

(E and F) RT-PCR demonstrates the male-specific expression of *fru* (**E**) and *dsx* (**F**) in the antenna. PCR was performed on cDNA from adult male and female antennae using primers located in *fru* exon 2 as indicated in (**A**) and *dsx* exon 2 as identified in (**C**). *Rp49* served as an internal control.

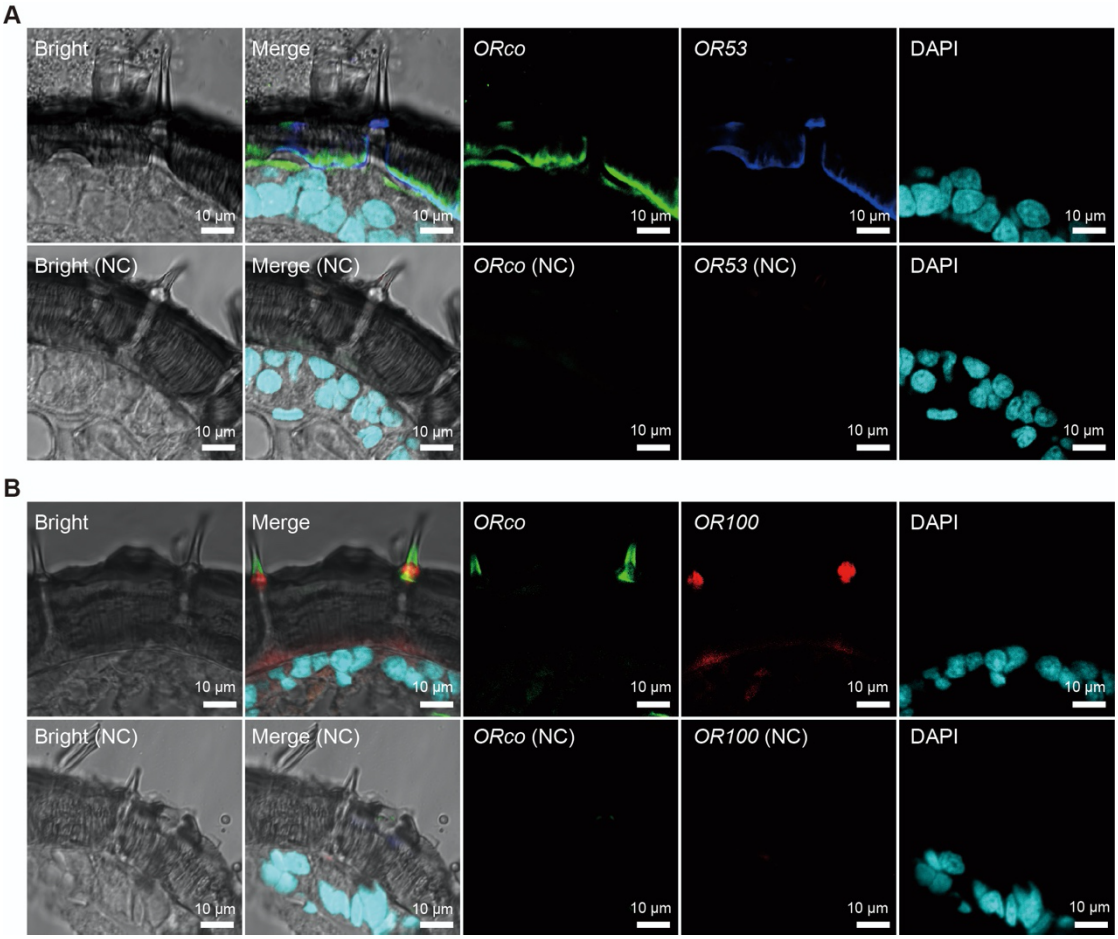

Figure S5. In situ hybridization of *OR53* and *OR100* expression.

(A) Localization of *OR53* in basiconic sensillia (highlighted in blue) on male antennae. Fluorescence in situ hybridization (FISH) analysis reveals co-localization of *OR53* and *ORco* in basiconic sensilla. 'Bright' indicates bright-field microscopy image. 'Merge' represents the overlay of signal channels for *OR53* and *ORco.* Cell nuclei were stained with DAPI.

(B) Localization of *OR100* in basiconic sensillia (highlighted in red) on male antennae. FISH analysis reveals co-localization of *OR100* and *ORco* in basiconic sensilla. 'Bright' indicates bright-field microscopy image. 'Merge' represents the overlay of signal channels for *OR100* and *ORco*. Cell nuclei were stained with DAPI.

Table S1. Primers used in this study.

Primer name	Primer sequence	Product length (bp)
For real-time quantitative PCR		
OR53-qPCR-F	CGGTGCTGCTAGCCCTATAC	194
OR53-qPCR-R	GTTTCTTGACACGGGGGCTA	
OR100-qPCR-F	TCATTACCATTGCGTGTACC	132
OR100-qPCR-R	CATCCTCTTCGTCCTCGTA	
ORco-qPCR-F	TTCCTCTTCTGCTTCTATGG	139
ORco-qPCR-R	CCTTCTGACACTGCTGAC	
OR87-qPCR-F	GTCATCTATTGCGGTTTT	141
OR87-qPCR-R	TCCACGATTTGATATTGCTA	
fru^M-qPCR-F	CAGATTCACAGACTTCATCG	102
fru^M-qPCR-R	GTATGTCCACAACCTCTTCT	
Dsx^M-qPCR-F	CCTGAAGTCTTTCCCATCAC	100
Dsx^M-qPCR-R	ATATTTACCTTGGGCGTTGA	
RP49-qPCR-F	CACTTCATCCGCCACCAGAG	152
RP49-qPCR-R	ATGTGCTTCGTCTTGCGGTT	
For dsRNA synthesis		
OR53-dsRNA-F	TCCAGTACATGAGATTATCGTTG	400
OR53-dsRNA-R	CATGCTTTCCCAGTTACTCCAGT	
OR53-dsRNA-T7-F	TAATACGACTCACTATAGGTCCAGTA CATGAGATTATCGTTG	419
OR53-dsRNA-T7-R	TAATACGACTCACTATAGGCATGCTT TCCCAGTTACTCCAGT	
OR100-dsRNA-F	GCTGACCAGTTCGTTTGGGAACA	301
OR100-dsRNA-R	CTACATACAACGTCATATAGCCA	
OR100-dsRNA-T7-F	TAATACGACTCACTATAGGGCTGAC CAGTTCGTTTGGGAACA	320
OR100-dsRNA-T7-R	TAATACGACTCACTATAGGCTACATA CAACGTCATATAGCCA	
ORco-dsRNA-F	CACCAAGCGGAAGATGTAAACG	401
ORco-dsRNA-R	GAAACGAAGTATCCTCCTCCTGAC	
ORco-dsRNA-T7-F	TAATACGACTCACTATAGGCACCAA GCGGAAGATGTAAACG	420
ORco-dsRNA-T7-R	TAATACGACTCACTATAGGGAAACG AAGTATCCTCCTCCTGAC	
fru^M-dsRNA-F	GTCAGAACTTGCTTCCTATG	216
fru^M-dsRNA-R	CACCTCCAGATATGTCACAA	
fru^M-dsRNA-T7-F	TAATACGACTCACTATAGGGTCAGAA CTTGCTTCCTATG	235
fru^M-dsRNA-T7-R	TAATACGACTCACTATAGGCACCTCC AGATATGTCACAA	

dsx^{M}-dsRNA-F	GGCTCTTGTAACTCCTGTAT	357
dsx^{M}-dsRNA-R	CTATAACCACGGTCTTGTCT	
dsx^{M}-dsRNA-T7-F	TAATACGACTCACTATAGGGGCTCTTGTAACTCCTGTAT	376
dsx^{M}-dsRNA-T7-R	TAATACGACTCACTATAGGCTATAACCACGGTCTTGTCT	
GFP-dsRNA-F	GTCACTACCTTCACCTATGG	340
GFP-dsRNA-R	CATCCTCAATGTTGTGTCTG	
GFP-dsRNA-T7-F	TAATACGACTCACTATAGGGTCACTACCTTCACCTATGG	359
GFP-dsRNA-T7-R	TAATACGACTCACTATAGGCATCCTCAATGTTGTGTCTG	
For gene cloning		
OR53-cds-F	ATGGCTATTGAAGACGACTCCAG	1218
OR53-cds-R	CTATTCTTCAAGTTCTTTGAAG	
OR100-partial-F	CCACGCTTCAAGTACATTG	669
OR100-partial-R	GCTTCCGTTCTCATCATCT	
OR100-5'RACE-outer-R	GCTACCCAGATACAGGCGGTGAT	>239
OR100-5'RACE-nest-R	ATGACTTCAGCAAACCAGCATAGAT	>152
OR100-3'RACE-outer-F	CGTGGTTCCCGTTTGATGTATCGT	>731
OR100-3'RACE-nest-F	GGAAGGAGATGATGAGAACGGAAG	>544
ORco-cds-F	ATGTACAAGGCACGGCTCCAC	1416
ORco-cds-R	CTAGTTGAGTTGCACCAGCAC	
OR87-partial-F	ATCGTGCGACTTCATCAGC	410
OR87-partial-R	GGACATGGACTCGTTTATCA	
fru^{M}-5utr-F	TAACGGTCGGTAATGTGATAGG	899
fru^{M}-partial-R	ATTCCATCAAGGCCATCCAA	
dsx^{M}-5utr-F	GAGTGTGTGAATGTCAAGTCGTC	1987
dsx^{M}-3utr-R	ACTGTCCACTTGTTCTGGTAAC	
For fluorescent in situ hybridization		
OR53-sense-F (T3)	AATTAACCCTCACTAAAGGGAGAGCTCAATCCACTGATATTGGGCCACTTCATAC	237
OR53-antisense-R (T7)	TAATACGACTCACTATAGGGAGAGGTCATCCACCCGTGTCGTGATGGTTTCTCCAAG	
OR100-sense-F (T3)	AATTAACCCTCACTAAAGGGAGAGCGTGTACCCAGTTACAAGAGGTGGGAATG	245
OR100-antisense-R (T7)	TAATACGACTCACTATAGGGAGAGAAAGTTGAACGTGCTCTCTATACCTTGCATG	
ORco-sense-F (T3)	AATTAACCCTCACTAAAGGGAGACTGGCTGTTCCTGGCTCTACTGCACGC	410

	CATG
ORco-antisense-R (T7)	TAATACGACTCACTATAGGGAGACC AGTACTTGATGGCTGACCGTACCAG AAG
For transgenic *Drosophila melanogaster* generation	
OR53-F：	tcttatcctttacttcaggcGGCCGCAAA**ATG**G CTATTGAAGACGACTCCAG
OR53-R：	GTTATTTTAAAAACGATTCATTCTAGA **CTA**TTCTTCAAGTTCTTTGAAG
OR100-F：	tcttatcctttacttcaggcGGCCGCcgcggatcc **ATG**GAAAACGCAC
OR100-R：	GTTATTTTAAAAACGATTCATTCTAGA **TTA**TTTTTCCTGGGCTTCATT