

Highly Efficient Temperature Inducible CRISPR-Cas9 Gene Targeting in *Drosophila suzukii*

Ying Yan^{1,*}, Yukino Kobayashi¹, Cong Huang^{2,3}, Bo Liu^{2,3}, Wanqiang Qian^{2,3}, Fanghao Wan^{2,3} and Marc F. Schetelig¹

¹ Department of Insect Biotechnology in Plant Protection, Institute for Insect Biotechnology, Justus-Liebig-University Giessen, Winchesterstraße 2, 35394 Giessen, Germany; Yukino.Kobayashi@ernaehrung.uni-giessen.de (Y.K.); Marc.Schetelig@agrar.uni-giessen.de (M.F.S.)

² Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China; huangcong16@163.com (C.H.); liubo03@caas.cn (B.L.); qianwanqiang@caas.cn (W.Q.); wanfanghao@caas.cn (F.W.)

³ Genome Analysis Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China; huangcong16@163.com (C.H.); liubo03@caas.cn (B.L.); qianwanqiang@caas.cn (W.Q.); wanfanghao@caas.cn (F.W.)

* Correspondence: Ying.Yan@agrar.uni-giessen.de

Supplemental tables S1–4 and figures S1–4.

Table S1. Primer sequences

Primer No.	Primer Name	Primer Sequence
P212	Bsp119I attP_F	ACTTTCGAAACTAGTACTGACGGAC
P213	Bsp119I attP_R	TCATTTCGAACTGTACTAGTCGCGCTC
MFS10	pBac 5' R	ACGACCGCGTGAGTCAAAATGACG
MFS17	EGFP 3' F	CAGAACACCCCCATCGGCGACGGC
P109	SV40 3' F	CAGCCATACCACATTTGTAGAGG
P106	EGFP RT-F	CAAAGACCCCAACGAGAAGC
P108	EGFP RT-R	GTCCATGCCGAGAGTGATCC
P1489	Cas9 RT-F	GCCACCGTGCGGAAAGTGCTGAGC
P1490	Cas9 RT-R	CGGTGGGGCTGTCTGAAGCCGCCG
P740	DsTBP RT-F	CCACGGTGAATCTGTGCT
P741	DsTBP RT-R	GGAGTCGTCCTCGCTCTT
P898	DsY ORF-F	ATGTTTCAGGACAAAGGATGGATCCTGGTGA
P899	DsY ORF-R	TTAACCCTGATGCTGGTGGTGCCACCAGCCAGA
P145	EGFP genotype_F	ACTTAATCGCCTTGCAGCACATCC
P55	EGFP genotype_R	TGTGATCGCGCTTCTCGTT
P1720	DsY genotype_F	CTGACTGGTTCGCCGGAGCTG
P1808	DsY genotype_R	CCAGCCACATTGAAGTCACCCCTC
P1172	sgRNA_EGFP2b-F	GAAATTAATACGACTCACTATAGGCTGAAGCACTGCACGCCGTGTTT TAGAGCTAGAAATAGC
P1686	sgRNA_Ds_Y_E2-F	GAAATTAATACGACTCACTATAGGTCGACCTGACCACCGATACGGTT TTAGAGCTAGAAATAGC
P369	sgRNA_UNI-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGC CTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

Table S2. Germline transmission of EGFP mutations under different heat-shock conditions

Injection mix (HS condition)^a	Founder G₀^b	G₁ mutants	Total No. of G₁	Inheritance from G₀ to G₁^c	Average inheritance	No. of detected mutations^d
V23 plasmid (Non-HS)	F18	42	74	42/74 (56.8%)	56.8%	1
EGFP-2b (Non-HS)	M2	154	315	154/315 (48.9%)	39.4%	2
	M6	82	313	82/313 (26.2%)		1
	M7	84	85	84/85 (98.8%)		1
	F4	13	143	13/143 (9.1%)		1
	F5	6	43	6/43 (14.0%)		1
EGFP-2b (HS-after 20 h)	M2	83	125	83/125 (66.4%)	78.3%	2
	M3	7	195	7/195 (3.6%)		1
	F2	8	8	8/8 (100%)		2
	F3	83	83	83/83 (100%)		2
	F5	25	25	25/25 (100%)		1
	F9	15	15	15/15 (100%)		2
EGFP-2b (HS-parents)	M2	193	203	193/203 (95.1%)	92.0%	2
	M4	169	180	169/180 (89.0%)		1

^a The composition of the injection mix and the heat-shock (HS) conditions are the same as in Table. 1

^b The G₀ flies that transmitted Cas9-derived mutant alleles to the G₁ generation.

^c The number of mutant G₁ offspring relative to the total number of G₁ offspring.

^d The number of verified mutations in the target EGFP region from 2-5 mutant (non-fluorescent) G₁ flies from each G₀ cross.

Table S3. Injection and crossing scheme to obtain *D. suzukii* yellow mutants (y^-)

Injection mix (HS condition) ^a	Injected embryos	Hatched larvae	Mosaic G ₀ adults ^b	G ₀ group crosses ^c	Ratio of y^- males in G ₁ ^d	Ratio of $y^{+/+}$ females in G ₁ ^d	G ₀ founders ^e
Ds_y_E2 (HS-after 20 h)	435	50	5.3% (2/38)	M ₁₋₅	2.6% (14/536)	1.9% (10/536)	2.6% (1/38)
				M ₆₋₁₀	0	0	
				M ₁₁₋₁₅	0	0	
				M ₁₆₋₂₀	0	0	
				M ₂₀₋₂₅	0	0	
				M ₂₆₋₂₇	0	0	
				F ₁₋₁₁	0	0	

^a The DsY_E2 is the synthesized gRNA (200 ng/μL) that targeting the second exon (E2) of *DsY* gene, and the heat-shock (HS) condition is the same as in Table. 1.

^b The number of mosaic individuals relative to the total number of G₀ adults.

^c The G₀ males or females were group crossed to wildtype (WT) partners (15 WT females were used for each G₀ male group and 11 WT males were used for the G₀ female group).

^d The number of *yellow* mutant flies relative to the total number of G₁ offspring.

^e The number of G₀ founder flies that predicted by the number of mutation events relative to the number of G₀ adults.

Table S4. Lethality tests for *DsY* males over multiple generations

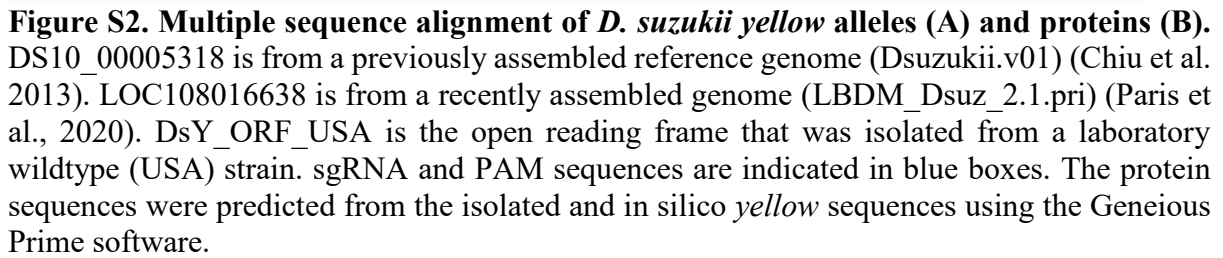
Flies	Generation (G) ^a	No. of newly emerged flies ^b	No. of flies after 1 day	No. of flies after 2 day	No. of flies after 10 day
<i>DsY^{-/+}</i> female	G2	7	7	7	7
<i>DsY⁻</i> male	G2	15	5	0	
	G3	15	2	0	
	G4	15	1	0	
	G5	15	3	0	
	G6	15	1	0	
	G7	15	1	0	
	G8	15	2	0	
	G9	15	1	0	
	G10	15	2	0	
	G11	15	3	0	
	G12	15	1	0	
	G13	15	1	0	
	G14	15	1	0	
	G15	15	2	0	
	G16	15	1	0	
	G17	15	1	0	
	G18	15	1	0	
	G19	15	3	0	
	G20	15	2	0	
<i>DsY^{-/+}</i> female	G20	15	15	14	13
WT male		15	15	15	11

^a The *yellow* mutant heterozygous females (*DsY^{-/+}*) were crossed with wildtype (WT) males in each generation for population maintenance due to the lethality in *yellow* mutant males (*DsY⁻*).

^b The newly emerged flies (<4 hours) were placed into fresh food vials, and the number (No.) of flies were counted every day until day 10 or all flies died.

<p>sgRNA-EGFP1</p> <p>Target Sequence: CTCGCCCTTGCTCACCATGG</p> <p>Protospacer Adjacent Motif (PAM): TGG</p> <p>Doench (2014) Activity Score: 0.027</p> <p>Zhang (2013) Specificity Score: 99.40%</p> <p># Off-target Sites: 3 (0 in CDS)</p> <p>Off-target #1 Score: 1.00%</p> <p>Sequence (#1): CTCGTCCTTGCCCGCCATGGTGG</p> <p>Off-target #2 Score: 1.00%</p> <p>Sequence (#2): CTCGTCCTTGCCCGCCATGGTGG</p> <p>Off-target #3 Score: 1.00%</p> <p>Sequence (#3): CTCGTCCTTGCCCGCCATGGTGG</p>	<p>sgRNA-EGFP2</p> <p>Target Sequence: GTCGCCACCATGGTGAGCAA</p> <p>Protospacer Adjacent Motif (PAM): GGG</p> <p>Doench (2014) Activity Score: 0.701</p> <p>Zhang (2013) Specificity Score: 100.00%</p> <p># Off-target Sites: 0 (0 in CDS)</p>
<p>sgRNA-EGFP2b</p> <p>Target Sequence: GCTGAAGCACTGCACGCCGT</p> <p>Protospacer Adjacent Motif (PAM): AGG</p> <p>Doench (2014) Activity Score: 0.329</p> <p>Zhang (2013) Specificity Score: 100.00%</p> <p># Off-target Sites: 0 (0 in CDS)</p>	<p>sgRNA-Ds_y_E2</p> <p>Target Sequence: TCGACCTGACCACCGATACG</p> <p>Protospacer Adjacent Motif (PAM): AGG</p> <p>Doench (2014) Activity Score: 0.684</p> <p>Zhang (2013) Specificity Score: 100.00%</p> <p># Off-target Sites: 0 (0 in CDS)</p>

Figure 1. Off-target analysis for the gRNAs. Assessment of potential off-target effects of each gRNA was performed using the “Find CRISPR sites” tool of the Geneious Software Package 11.0.6 (Kearse et al., 2012) based on the *D. suzukii* genome (Dsuzukii_v1_scaffolds.fa.gz) (Chiu et al., 2013). The activity of gRNA was predicted through its sequence features based on the model proposed by Doench et al. (2014), and scores are between 0 and 1, with a higher score denoting higher expected activity. The specificity of gRNA was scored based on the Zhang (2013) method by searching for CRISPR sites with 3' PAMs, and scores are between 0 and 100, with a higher score meaning better specificity and less off-target activity. The numbers of the potential off-target sequences in the target genome and coding region sequence (CDS) were indicated. Each off-target sequence was given a score based on how similar it was to the spacer sequence of gRNAs. A higher score (%) indicated a higher similarity to the original target site and a higher likelihood to cause off-target mutations.



Drosophila sechellia	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	75
Drosophila simulans	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	75
Drosophila melanogaster	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	75
Drosophila erecta	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	75
Drosophila yakuba	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	75
Drosophila takahashii	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	79
Drosophila suzukii	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	75
Drosophila biarmipes	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	76
Drosophila elegans	MFQDKGWILVT-LITLVTP---SWAAYKLQERYSNWQLDFAFPNTRLKQDALASGDYIPQNALPVGVEHFGNRLFVTV	75
Drosophila rhopalaoa	MFQDKGW6Lvt L6 L p sWAAYKLQERYsWnQLDFAFPN RLK QALASGDY6PQN LPVGEVHFgnRLFVTV	76
Drosophila sechellia	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	155
Drosophila simulans	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	155
Drosophila melanogaster	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	155
Drosophila erecta	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	155
Drosophila yakuba	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	155
Drosophila takahashii	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	159
Drosophila suzukii	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	155
Drosophila biarmipes	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	156
Drosophila elegans	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	155
Drosophila rhopalaoa	RWRDGIPTATLTYYINMDRSLTGSPELIYPYDWRSTAGDCANSITTAIRIKVDECGRWLVDLTGTVGIGNTTNNPCPYAVN	156
Drosophila sechellia	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	235
Drosophila simulans	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	235
Drosophila melanogaster	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	235
Drosophila erecta	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	235
Drosophila yakuba	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	235
Drosophila takahashii	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	239
Drosophila suzukii	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	235
Drosophila biarmipes	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	236
Drosophila elegans	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	235
Drosophila rhopalaoa	VFDLTTDTRIRRYELPGVDNTPNTFIANIAVDIGKNCDDAYAYFADELGYGLIAYSWEIKNKSWRFSAHSYFFPDPLRGDF	236
Drosophila sechellia	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	315
Drosophila simulans	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	315
Drosophila melanogaster	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	315
Drosophila erecta	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	315
Drosophila yakuba	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	315
Drosophila takahashii	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	319
Drosophila suzukii	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	315
Drosophila biarmipes	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	316
Drosophila elegans	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	315
Drosophila rhopalaoa	NVAGINFQWGEEGI FGMSLSPISRSDGYRTLYFSPLASHRQFAVSTRILRDETRTDSYHDFVALDERGPNSTHTSRVMSD	316
Drosophila sechellia	DGIELFNLIDQN-----AKPQTSWASSPPPP--SRTYLPAA--NSGNVVS	327
Drosophila simulans	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	395
Drosophila melanogaster	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	395
Drosophila erecta	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	395
Drosophila yakuba	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	395
Drosophila takahashii	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	399
Drosophila suzukii	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	395
Drosophila biarmipes	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	396
Drosophila elegans	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	395
Drosophila rhopalaoa	DGIELFNLIDQNAGVGCWHSMPYSPQFHGIVDRDDVGLVFPADVVIDENKNVWVLSDRMPVFLSLDLYSDTNFRITYAP	396
Drosophila sechellia	-----AKPQTSWASSPPPP--SRTYLPAA--NSGNVVS	355
Drosophila simulans	LATLIENTVCDLRNNAYGPPNTVSIKQ-AVLPMPGPLYTKQYRPVLPQKQTSWASSPPPP--SRTYLPAA--NSGNVVS	470
Drosophila melanogaster	LATLIENTVCDLRNNAYGPPNTVSIKQ-AVLPMPGPLYTKQYRPVLPQKQTSWASSPPPP--SRTYLPAA--NSGNVVS	470
Drosophila erecta	LATLIENTVCDLRNNAYGPPNTVSIKQ-AVLPMPGPLYTKQYRPVLPQKQTSWASSPPPP--SRTYLPAA--NSGNVVS	470
Drosophila yakuba	LATLIENTVCDLRNNAYGPPNTVSIKQ-TVLPVGPPLYTKQYRPVLPQKQTSWASSPPPP--SRTYLPAA--NSGNVVS	470
Drosophila takahashii	LATLIENTVCDLRNNAYGPPNTVSIKQAAALPIGPPPLYTKQYRPVLPQKQTSWASSPPPP--SRTYLPAGGNPNVVS	477
Drosophila suzukii	LATLIENTVCDLRNNAYGPPNAVSIKQ-PALPIGPPSYNKNYRPALPQKQTSWASSPPPP--SRTYLPAA--NAGNVVS	471
Drosophila biarmipes	LATLIENTVCDLRNNAYGPPNAVSIKQ-PALPIGPPSYNKNYRPALPQKQTSWASSPPPP--SRTYLPAA--SSGNVVS	470
Drosophila elegans	LATLIENTVCDLRNNAYGPPNTVSIKQ-AALPIGPPPLYTKQYRPVLPQKQTSWASSPPPP--SRTYLPAA--NPGNVVS	474
Drosophila rhopalaoa	LATLIENTVCDLRNNAYGPPNTVSIKQ-AALPIGPPPLYTKQYRPVLPQKQTSWASSPPPP--SRTYLPAA--NPGNVVS	471
Drosophila sechellia	latlientvcdlrnna gppn vsipkq lp gpp ytkqyr lpqKQTSW SSPPPP SRTYLPAA n GNVVS	
Drosophila simulans	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	426
Drosophila melanogaster	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	541
Drosophila erecta	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	541
Drosophila yakuba	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	541
Drosophila takahashii	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	547
Drosophila suzukii	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	541
Drosophila biarmipes	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	544
Drosophila elegans	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	540
Drosophila rhopalaoa	SISVSTNSVGPAGVEVPKAYIFNQHNGINYETSGPHLFPTHQPAQPGVQDGLKTYVNARQSGWWHHQHOG	541

Figure S3. Multiple sequence alignment of Yellow from ten *Drosophila* species. The alignment was performed by MAFFT v7. Aligned sequences were depicted with ESPrnt 3.0 server (<http://esprnt.ibcp.fr/ESPrnt/cgi-bin/ESPrnt.cgi>). The conserved Major Royal Jelly Protein (MRJP; pfam03022) domain is highlighted in yellow. Dsec: *Drosophila sechellia* (XP_002040222.1), Dsim: *Drosophila simulans* (XP_016037524.1), Dmel: *Drosophila melanogaster* (NP_476792.1), Dere: *Drosophila erecta* (XP_001982384.1), Dyak: *Drosophila yakuba* (XP_002099667.1), Dtak: *Drosophila takahashii* (XP_016995421.1), Dsuz: *Drosophila suzukii* (this study), Dbia: *Drosophila biarmipes* (XP_016948849.1), Dele: *Drosophila elegans* (XP_017114065.1), Drho: *Drosophila rhopalaoa* (XP_016982272.1).

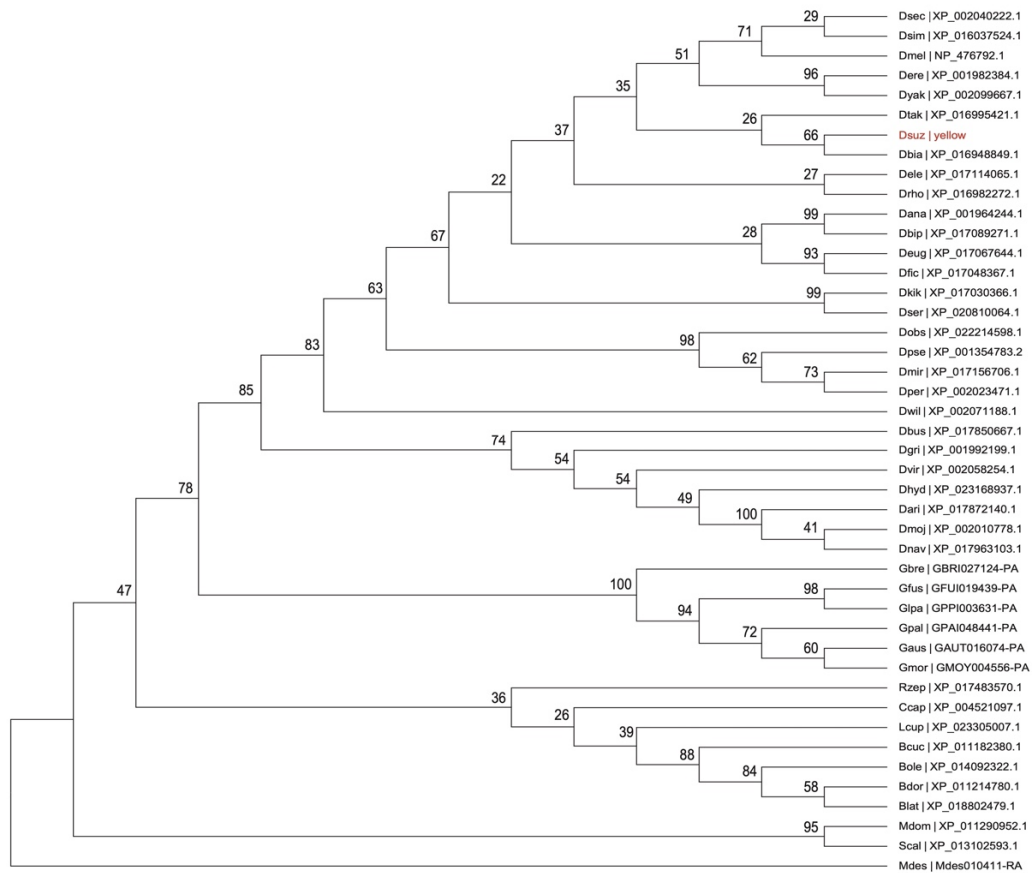


Figure S4. Phylogenetic tree of Yellow from 44 insect species. The neighbor-joining phylogenetic tree was constructed using the aligned sequences by the ClustalW algorithm in MEGA6 with the JTT substitution model. A bootstrap method of 1000 replicates was used to evaluate the branch strength of the model. The *D. suzukii* Yellow from this study is indicated in red, and the GenBank accession numbers for Yellow from other species are shown. Dsec: *Drosophila sechellia*, Dsim: *Drosophila simulans*, Dmel: *Drosophila melanogaster*, Dere: *Drosophila erecta*, Dyak: *Drosophila yakuba*, Dtak: *Drosophila takahashii*, Dsuz: *Drosophila suzukii*, Dbia: *Drosophila biarmipes*, Dele: *Drosophila elegans*, Drho: *Drosophila rhopalosa*, Dana: *Drosophila ananassae*, Dbip: *Drosophila bipectinata*, Deug: *Drosophila eugracilis*, Dfic: *Drosophila ficusphila*, Dkik: *Drosophila kikkawai*, Dser: *Drosophila serrata*, Dobs: *Drosophila obscura*, Dpse: *Drosophila pseudoobscura*, Dmir: *Drosophila miranda*, Dper: *Drosophila persimilis*, Dwill: *Drosophila willistoni*, Dbus: *Drosophila busckii*, Dgri: *Drosophila grimshawi*, Dvir: *Drosophila virilis*, Dhyd: *Drosophila hydei*, Dari: *Drosophila arizonae*, Dmoj: *Drosophila mojavensis*, Dnav: *Drosophila navojoa*, Gbre: *Glossina brevipalpis*, Gfus: *Glossina fuscipes*, Glpa: *Glossina palpalis*, Gpal: *Glossina pallidipes*, Gaus: *Glossina austeni*, Gmor: *Glossina morsitans*, Rzep: *Rhagoletis zephyria*, Ccap: *Ceratitis capitata*, Lcup: *Lucilia cuprina*, Bcuc: *Bactrocera cucurbitae*, Bole: *Bactrocera oleae*, Bdor: *Bactrocera dorsalis*, Blat: *Bactrocera latifrons*, Mdom: *Musca domestica*, Scal: *Stomoxys calcitrans*, Mdes: *Mayetiola destructor*.