Excision Validation Report

Report No.: RWG3016 **Date:** 2018.05.25
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Gene: *Taz / CG8766* **Case No.:** 17190

Project: Making a deletion allele of CG8766-RA by deleting the 8th-896th nt and knocking in a selection marker to facilitate genetic screening

Method: Excision of selection marker by *Cre*/loxP Rcombination

Progenitor: *SWG3944 17190B*
w[1118]; CG8766-RA CRISPR{Stop-RFP} / CyO
Bloomington 766
y[1] w[67c23] P{y[+mDint2]=Crey}1b; noc[Sco]/CyO

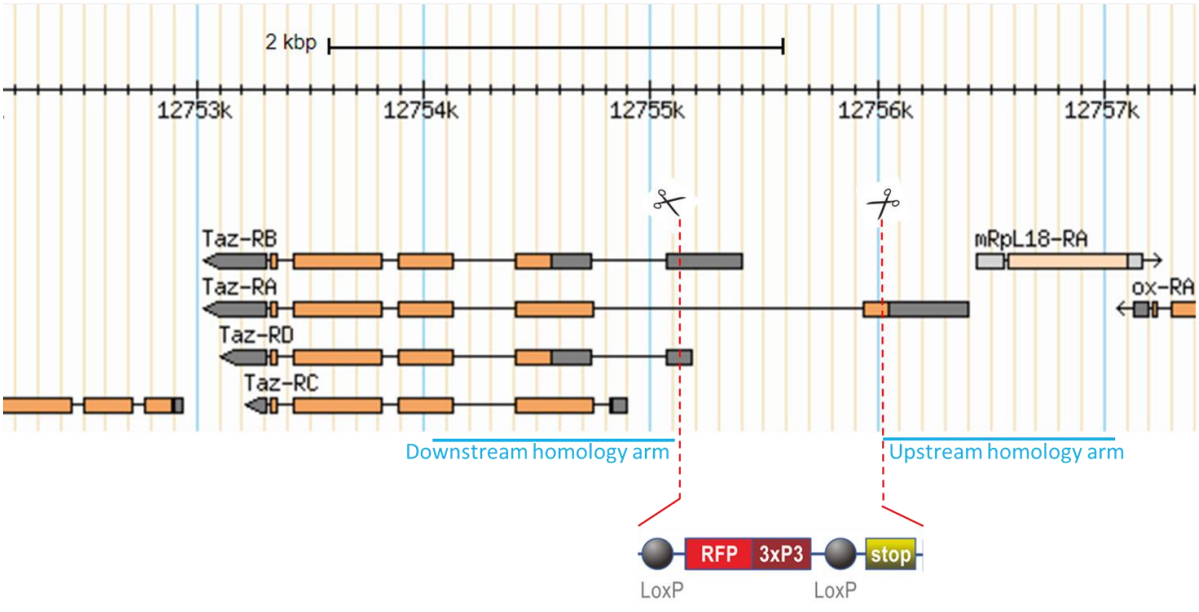
Alleles:

17190ex4 *w[1118]; CG8766-RA CRISPR{Stop} / CyO*
SWG4029 Excision is validated by genomic PCR and sequencing. Homozygous viable.

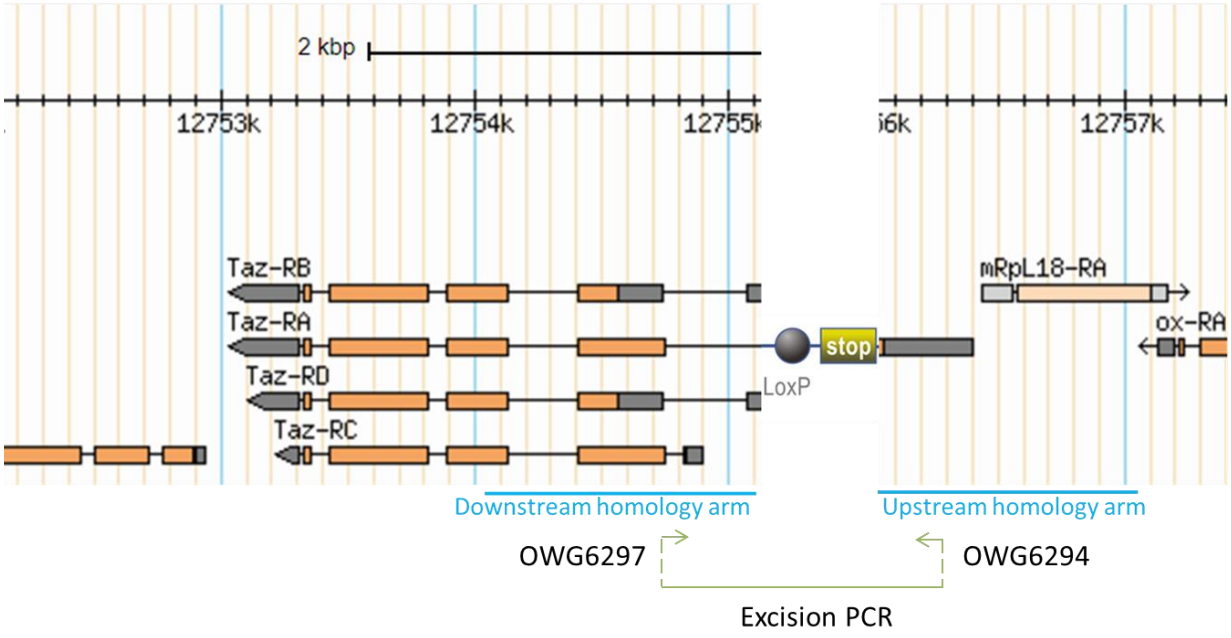
17190ex6 *w[1118]; CG8766-RA CRISPR{Stop} / CyO*
SWG4030 Excision is validated by genomic PCR. Homozygous viable.

Methods

Genome Editing Map:



↓ **Cre/loxP Excision**



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Strategy:

Using genomic PCR and sequencing methods to verify *Cre*/loxP recombination alleles of *w[1118]; CG8766-RA CRISPR{Stop-RFP} / CyO* fly by testing if the selection marker (3XP3-RFP) is precisely excised and leave one loxP between 3-frame stop codons and the first intron of *CG8766-RA*.

Excision PCR:

Forward primer (OWG6294) is designed at upstream homology arm and reverse primer (OWG6297) is designed at downstream homology arm as illustrated above. The PCR product will be 2515bp before excision and 721bp after excision. In w^{1118} control strain, the PCR product is 1538bp.

Primers:

OWG6294 5'- CACAATAACGCCCCCTTCAG

OWG6297 5'- TGGAAACGAGATAAGGGCGG

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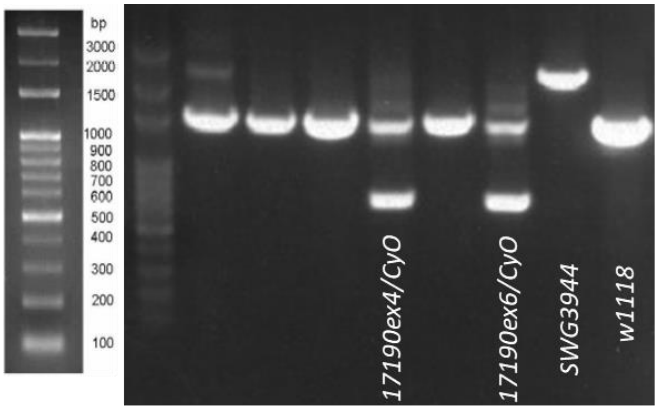
Genomic PCR

Gel:



Conclusion:

PCR bands at expected size were observed from heterozygous samples of *17190ex4* and *17190ex6* for Excision PCR (721bp), suggesting that selection marker is excised. A larger band (2515bp) was found in homozygous progenitor, *SWG3944*. Medium bands (1538bp) were also observed in injection strain control and heterozygous samples of *17190ex4* and *17190ex6*, suggesting that high specificity of the PCR reaction. PCR product of *17190ex4* was cut and sent for sequencing.

Methods:

Genomic DNA was obtained from single fly of each stock following single-fly DNA prep. Injection strain w^{1118} was used as a negative control. PCR was performed using KOD-FX (TOYOBO) on BioRad S1000 Thermal Cycler. 100bp DNA Ladder from GenePure was used as reference.

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Genomic Sequencing

Sequences:

>17190ex4_OWG6294

GGGTCCGTCACCTGCGCCTCCTGCCCGTACGCCCATGAGATTGCAGCTGAGGTGAAGAAGTGGCGGTTGGGT
GAAGAAGCAAGCAGTGCGCGCGTCCCAAATTGCAAAGTGATTTTTATTCGGCCCACAAAAGCCGGTTATGTA
AAGACCGTATAGGCCAAATATCTGCACACATATCGTACTCGTTAAATTAAGAACTTGTAGAAATGTTTATCTAGAT
AATGATTAGTGATTAATAAAGATCTATAACTTCGTATAATGTATGCTATACGAAGTTATGCTAGCTTCGCGCCGTC
CGGATGCGAAAGATTCTCAGGTGCCCAATGTAATACATCTCGAACCGAGTGAAGGTCGCCATAGATCTCTGAC
GACGTGAGTTTTTCCTGCATTATTATTCGCGCGAAAATGCAGTGCGGTGTCTCGCAGCAACAGATTTTCAGTG
CGCATGCTGCTAACCTGTGGCACAAAGGCGGCTTTTGCGGGGGTTGTTGGGTTAGTCGAACAGTTGACAAA
GGAATAATTCAAATAGAAATCAAAATGCGTAACACTGGGAGGCGTTTGGCTAGAGACAAGCACAAAAGGAA
ACTATTCTTGGGCCCGATAAGATTAAAGACTATCCATGGTCAGCAGTGGGATTTGTTGTGTGCACTTTGTGGCC
GTCGGTAATTCAATTTCCGCCCTTACTCCCCCTTTCCCAAAAAAA

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Blast Result:

Sequence read (>17190ex4_OWG6294, Sbjct) was aligned with 17190 excised donor sequence (Query) using Blast2. One loxP (shaded in light blue) were left precisely between 3-frame stop codons (shaded in pruple) and the first intron of *CG8766-RA* after excision in 17190ex4.

190ex46294_6294 sequence exported from 010_G03_190ex46294_6294.ab1
Sequence ID: Query_226659 **Length:** 703 **Number of Matches:** 1

Range 1: 14 to 686 Graphics ▼ Next Match

Score	Expect	Identities	Gaps	Strand
1210 bits(655)	0.0	668/674(99%)	1/674(0%)	Plus/Plus

```
Query  1103  GCGCCTCCTGCCCGTACGCCCATGAGATTGCTGCTGAGGTGAAGAAGTGGCGGTTGGGTG  1162
             ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  14    GCGCCTCCTGCCCGTACGCCCATGAGATTGCAGCTGAGGTGAAGAAGTGGCGGTTGGGTG  73

Query  1163  AAGAAGCAAGCAGTGCGCGCGTCCCAAATTGCAAAGTGATTTTTATTCGGCCCACAAAAG  1222
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  74    AAGAAGCAAGCAGTGCGCGCGTCCCAAATTGCAAAGTGATTTTTATTCGGCCCACAAAAG  133

Query  1223  CCGGTTATGTAAAGACCGTATAGGCCAAATATCTACACACATATCGTACTCGTTAAATTA  1282
              |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  134   CCGGTTATGTAAAGACCGTATAGGCCAAATATCTGCACACATATCGTACTCGTTAAATTA  193
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1st coding exon of *Taz/CG8766-RA* 3-frame stop codons loxP

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Query  1283  AGAACTTGTAGAAATGTTTATCTAGATAATGATTAGTGATTAATAAAGATCTATAACTTC  1342
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  194   AGAACTTGTAGAAATGTTTATCTAGATAATGATTAGTGATTAATAAAGATCTATAACTTC  253

Query  1343  GTATAATGTATGCTATACGAAGTTATGCTAGCTTCGCGCCGTCCGGATGCGAAAGATTCT  1402
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  254   GTATAATGTATGCTATACGAAGTTATGCTAGCTTCGCGCCGTCCGGATGCGAAAGATTCT  313
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1st intron of *Taz/CG8766-RA*

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Query  1403  CAGGTGCCCAATGTAATACATCTCGAACCGAGTGAAGGTCGCCATAGATCTCTGACGACG  1462
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  314   CAGGTGCCCAATGTAATACATCTCGAACCGAGTGAAGGTCGCCATAGATCTCTGACGACG  373

Query  1463  TGAGTTTTTCCTGCATTATTATTCGCGCGAAAATGCAGTGCGGTGTCTCGCAGCAACAGA  1522
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  374   TGAGTTTTTCCTGCATTATTATTCGCGCGAAAATGCAGTGCGGTGTCTCGCAGCAACAGA  433

Query  1523  TTTTCAGTGCGCATGCTGCTAACCTGTGGCACAAAGGCGGCTTTTGCGGGGGGTTGTTGGG  1582
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  434   TTTTCAGTGCGCATGCTGCTAACCTGTGGCACAAAGGCGGCTTTTGCGGGGGGTTGTTGGG  493

Query  1583  TTAGTCGAACAGTTGACAAAGGAATAATTCAAATAGAAATCAAAATGCGTAACACTGAGA  1642
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  494   TTAGTCGAACAGTTGACAAAGGAATAATTCAAATAGAAATCAAAATGCGTAACACTGGGA  553

Query  1643  GGCGTTTGGCTAGACACAAGCACAAAAGGAAACTATTCTTGGGCCCGATAAGATTAAAGA  1702
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  554   GGCGTTTGGCTAGAGACAAGCACAAAAGGAAACTATTCTTGGGCCCGATAAGATTAAAGA  613

Query  1703  CTATCCATGGTCAGCAGTGGGATTTGTTGTGTGCACTTTGTGGCCGTCGGTAATTAAATT  1762
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  614   CTATCCATGGTCAGCAGTGGGATTTGTTGTGTGCACTTTGTGGCCGTCGGTAATTCAATT  673

Query  1763  TCCGCCCTTATCTC  1776
              |||||||||| |||
Sbjct  674   TCCGCCCTTA-CTC  686
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Methods:

PCR bands of *17190ex4* were excised and submitted to Mission Biotech for gel extraction and sequencing. Sequence alignment shown here was using BLAST[1] (The Basic Local Alignment Search Tool) to find the regions of local similarity between sequences.

[1] BLAST is a registered trademark of the National Library of Medicine.

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