WELLGENETICS

Demo Case Report

Drosophila CRISPR Genome Editing Services

Gene: *CG**0**

Project Purpose: To make null mutation of *CG**0** to disrupt all isoforms

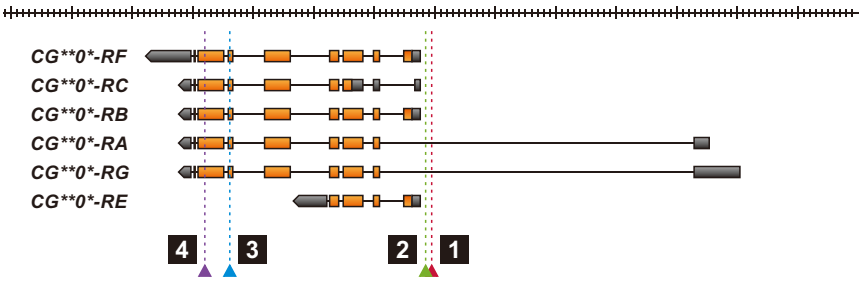
Method: CRISPR/Cas9-mediated genome editing by homology-dependent repair (HDR) using two guide RNAs and a dsDNA plasmid donor

Knock-in Cassette: attPX-RFP

Editing Strain: w^{1118}

Editing Region: There are 6 isoforms of *CG**0** found on FlyBase. Using *CG**0*-RF* as the reference, base 387 before ATG of *CG**0*-RF* to base 518 before stop codon of *CG**0*-RF* will be deleted and replaced by an inverted cassette with an *attPX* site, 3-frame stop codon and floxed *3xP3-RFP*.

Design Options:



● Strong Target Site ● Weak Target Site

▲ **Option 3:**
- ● 0 off target
- ● 60% GC
- ● 0 T at 17~20 bases

▲ **Option 1:**
- ● 0 off target
- ● 50% GC
- ● 1 T at 17~20 bases

▲ **Option 4:**
- ● 0 off target
- ● 35% GC
- ● 1 T at 17~20 bases

▲ **Option 2:**
- ● 0 off target
- ● 65% GC
- ● 1 T at 17~20 bases

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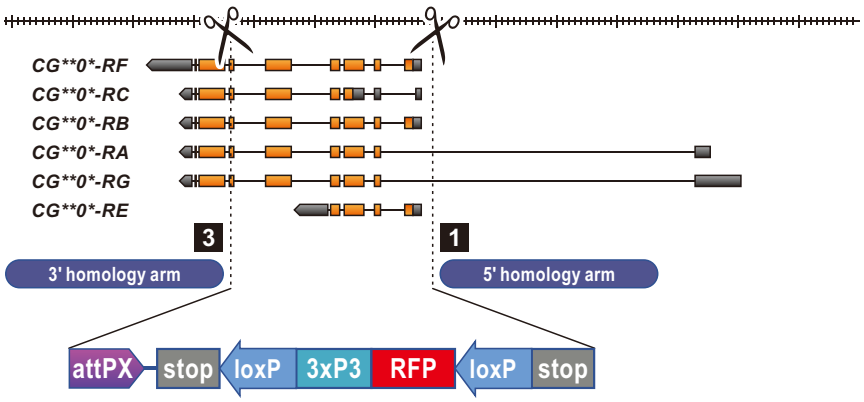
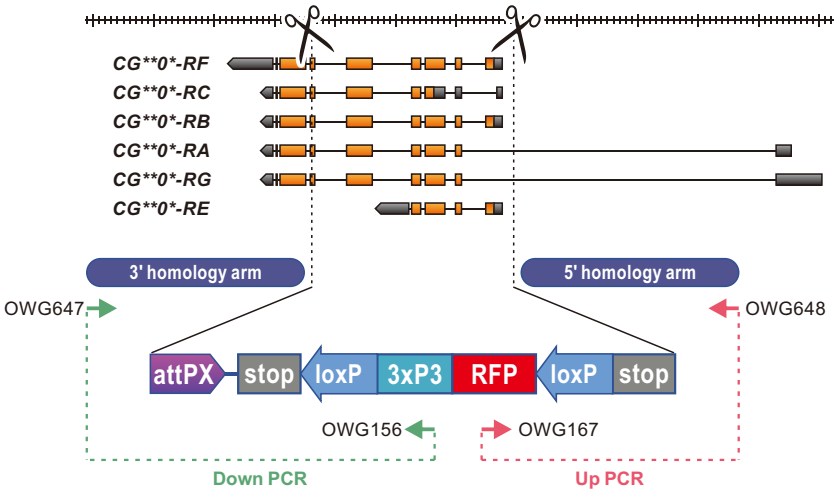
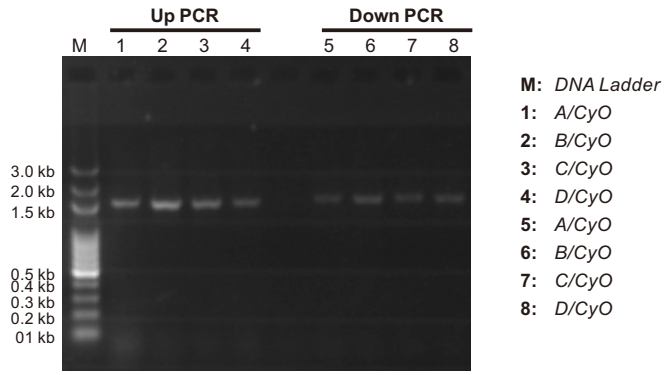
Client's Decision:



PCR Validation: PCR bands at expected sizes were observed from all samples for both upstream and downstream PCR reactions, suggesting inverted RFP cassette is inserted into *CG**0** gene locus at the correct orientation.





Final Results: Four CRISPR-edited fly of *CG**0** were screened by *3xP3-RFP* selection marker from 200 embryos-microinjection. These lines were validated at molecular level by genomic PCR and sequencing methods. Base 387 before ATG of *CG**0*-RF* to base 518 before stop codon of *CG**0*-RF* were deleted and replaced by the inverted *attPX-RFP* cassette.

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