

1 Supplementary information

efl1a1 promoter/enhancer region

mCherry coding region

HA tag region

T2A region

SV40 NLS region

Zinc finger DBD of GAGA transcription factor

SceGFP region

TaqI recognition site

I-SceI recognition site

efl1a1 3' UTR region

iGFP region

Scarlet target site region

scarlet gRNA spacer sequence

indel mutation region

scarlet gene exon region

*every line represents 100 bp nucleotide

5'-

cgccagtcgagcaagcttgatttaggtgacacAGCTTCAGCGACCTTGGGGAAAAATTTCAACTCACACAGCTATTGATTAAATTTAAAGGTAACGTTG
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 M V S K G E E D N M A I I K E F M R F K
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 V H M E G S V N G H E F E I E G E G E G R P Y E G T Q T A K L K V
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 T K G G P L P F A W D I L S P Q F M Y G S K A Y V K H P A D I P D Y
 CTTGAAGCTGTCCTTCCCCGAGGGCTCAAGTGGGAGCGCGTGATGAACCTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGAC
 L K L S F P E G F K W E R V M N F E D G G V V T V T Q D S S L Q D
 GCGGAGTTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATTGGGCTGGGAGGCCTCCTCCGAGC
 G E F I Y K V K L R G T N F P S D G P V M Q K K T M G W E A S S E
 GGATGTACCCCGAGGACGGCGCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAA
 R M Y P E D G A L K G E I K Q R L K L K D G G H Y D A E V K T T Y K
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 A K K P V Q L P G A Y N V N I K L D I T S H N E D Y T I V E Q Y E
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 C G D V E E N P G P P K K K R K V P K K H A A P P K K K R K V E D P
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 P A T C P I C Y A V I R Q S R N L R R H L E L R H F A K P G V D P
 CGGTGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTTCAGCGT
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 GTCGAGCTAGGGATAACAGGGTAATACCTACGGCAAGCTGACCCTGAAGTTTCATCTGCACCACCGGCAAGCTGCCCGTGCCTGGCCACCCTCGTGACC
 S S * G *
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 CAATAACTAATCCCAGTGAATTGTAATTTTCAAAAGTGGAGCAGGGAAATCTACTTTGCTCAATGCTTTGGCTTGCAGATGTCCTC

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6 **Supplementary Figure S1. The full sequence of integrated DR-GFP reporter in the**
 7 **genome.**

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Supplementary Figure S2. Distal-less (Dll) gRNA location. Illustration of exon 1 and 2 of *Dll* gene in *D. magna*. gRNA target (dash line) is located upstream of the homeodomain region (light grey box) of *Dll* gene.

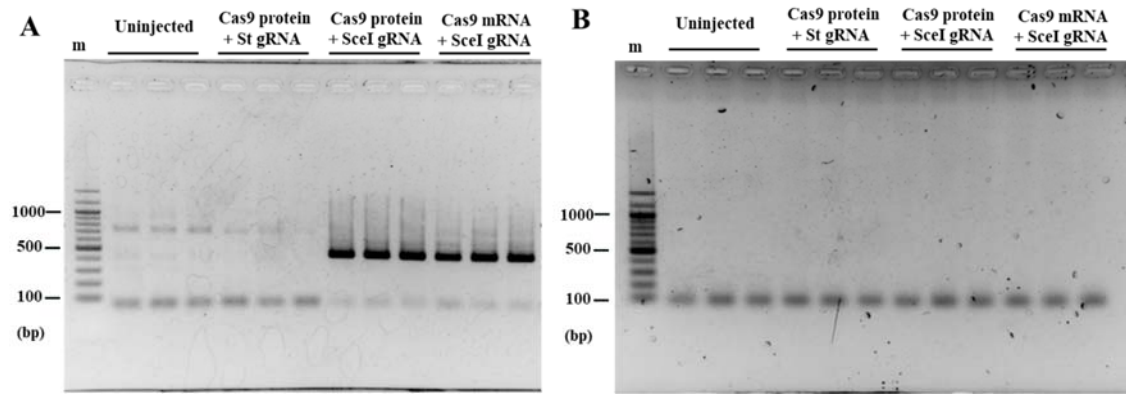


Figure S3. Gel electrophoresis of the qPCR result. (A) The first lane is the marker (m). Repaired *SceGFP* PCR product which was expected to be 449 bp was only observed in the Cas9 protein (lane 8-10) and mRNA (lane 10-12) co-injection with SceI gRNA. **(B)** The DNA marker was put in the first lane. All of the samples (lane 2-13) showed a band that was expected to be 67 bp that indicated the PCR product of ribosomal protein L32 gene as a reference gene.

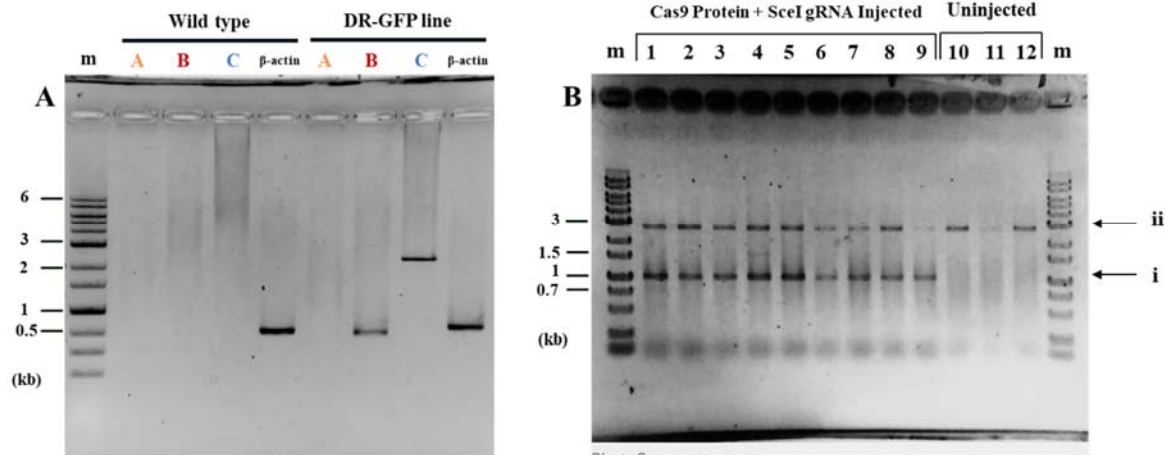


Figure S4. The full length of gels in Figure 2 and Figure 4. (A) PCR result for DR-GFP genotyping was visualized by gel electrophoresis. The first lane is the marker (m), followed by fragments A, B, C, and β -actin (beta-actin) for both wild type and DR-GFP line *Daphnia*. In the sample using DR-GFP line *Daphnia*, all fragments except the 5' junctions, Fragment A, were amplified (lane 6-9). In the wildtype sample only the β -actin band was amplified (lane 5). **(B)** PCR result for genotyping after DSB introduction was shown in the gel electrophoresis. The most left lane indicated the DNA marker (m) followed by amplified genome fragments (lanes 1-9) from Cas9 protein and SceI gRNA injected embryos. Uninjected DR-GFP (lane 10-12) was used as the negative control. The primer set amplified the repaired *SceGFP* region with the length 1,048 bp (i). The reverse primer was also attached to the *iGFP* region, which resulted in a 2,843 bp length PCR product (ii).

35 **Supplementary Table S1. Summary of Dll gRNA and Cas9 protein injection**
 36 **(co-injected with St gRNA)**

Injected (16h)		38
Hatch		50% (19/38)
Developed (48h)		68.4% (13/19)
White-eye		84.6% (11/13)
Truncated antennae	Strong	61.5% (8/13)
	Medium	15.4% (3/13)
	Mild	23.1% (2/13)

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