

**Additional file**

**COMPLEXITY OF DETECTING CRISPR/Cas9-MEDIATED HOMOLOGOUS  
RECOMBINATION IN ZEBRAFISH**

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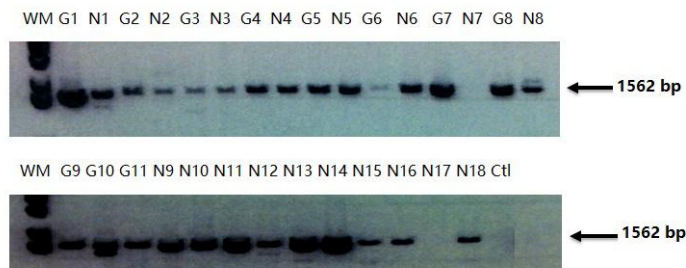
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**Supplementary Table 1.** The knock-in efficiency test of cas9 protein: gradient injection concentration of *gad* targeting vectors (*gad*-PAM-mutation plasmids).

	<b>total</b>	<b>death</b>	<b>deformed</b>	<b>normal</b>	<b>signal</b>
<b><i>gad</i> plasmid (50 ng/μl)</b>	75	13	4	58	0
<b>    Ctl</b>	50	8	0	42	0
<b><i>gad</i> plasmid (200 ng/μl)</b>	74	33	4	27	0
<b>    Ctl</b>	23	2	0	21	0
<b><i>gad</i> plasmid (600 ng/μl)</b>	75	29	28	28	0
<b>    Ctl</b>	50	13	6	31	0

**Supplementary Table 2. The knock-in efficiency test: gradient injection concentration of *gad* targeting vectors (*gad*-PAM-mutation plasmid).**

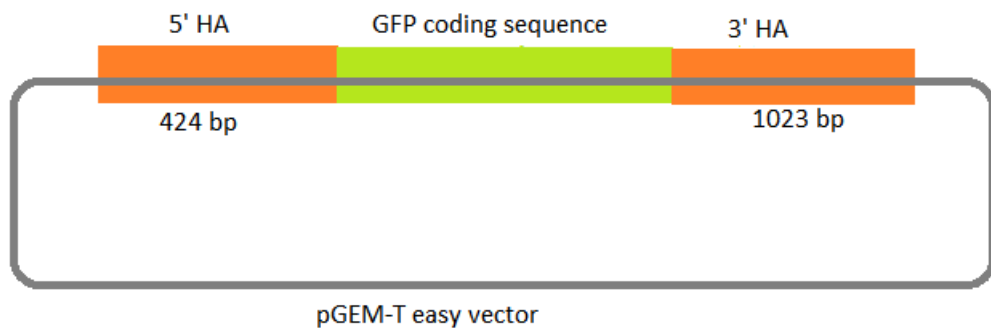
Cas9 protein/mRNA	<i>gad</i> sgRNA	<i>gad</i> -PAM-mutation plasmid
Cas9 protein (1100 ng/μl)	<i>gad</i> sgRNA-2 (30 ng/μl)	50 ng/μl
		200 ng/μl
		600 ng/μl
Cas9 mRNA (600 ng/μl)	<i>gad</i> sgRNA-2 (30 ng/μl)	50 ng/μl
		200 ng/μl
		600 ng/μl



**Supplementary Fig. 1** PCR-inducing artifacts from zebrafish embryos injected with the targeting construct together with the Cas9/sgRNA at 24 hpf. G1~11: the embryos with GFP<sup>+</sup> ; N1~18: the embryos with GFP<sup>-</sup> ; Ctl: control (wild type).



**Supplementary Fig. 2** PCR results from the embryos injected with only the targeting plasmids (50 ng/μl), for which two pools were employed in testing at each time point (2 hpf, 4 hpf, 6 hpf and 8 hpf). S: the embryos with GFP<sup>+</sup>.



**Supplementary Fig. 3** Construct of *gad* targeting plasmid.