



CRISPR Design Report

Report No.: RWG7110

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Gene: *BomT1/CG43202*

Case No.: 20141

Project: making a Gal4 reporter knockout of *BomT1* by deleting entire CDS and knocking in Gal4 and a floxed selection marker to facilitate genetic screening

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

Gene and Method

Gene: *BomT1/CG43202*

Location: 2R:18,381,393..18,381,844 [-]

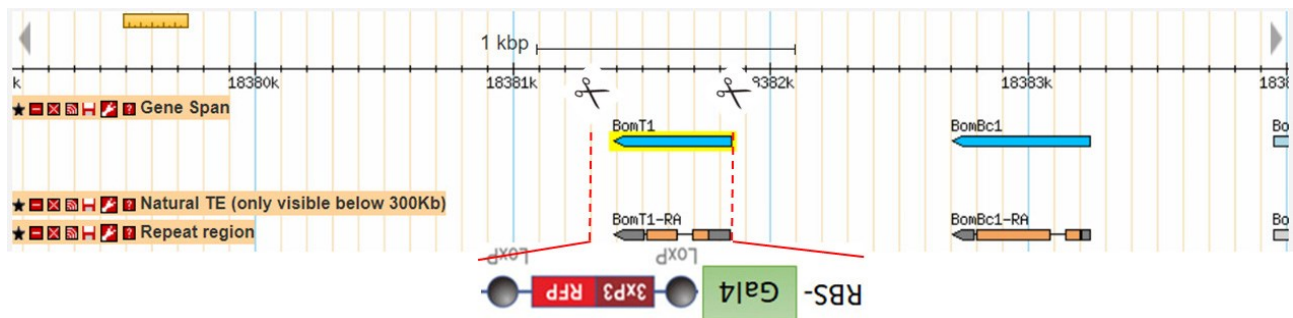
Method: CRISPR/Cas9-mediated genome editing by homology-dependent repair (HDR) using two guide RNAs and a dsDNA plasmid donor

Knock-in cassette: Gal4-3xP3-RFP



Injection strain: *w[1118]*

Genome Editing:



- (1) A 545-bp fragment of *BomT1* (-091 nt to +454 nt from ATG of *BomT1*) will be deleted and be replaced with cassette Gal4-3XP3-RFP.
- (2) Cassette Gal4-3xP3-RFP contains **ribosome binding sequence (RBS)**, Gal4, SV40 3'UTR and 3xP3-RFP as a selection marker.
- (3) The selection marker 3xP3-RFP contains loxP site, 3x Pax3 promoter, RFP, alpha-Tubulin 3'UTR and loxP site. It facilitates the genetic screening and can be flipped out by Cre recombinase.

CRISPR Target Sites

Guide RNA Quality Instruction

Guide RNA quality is shown in color dots for your references.

	Distance	Off target	GC Content	T #
● Strong site	<50bp	0	45%-70%	≤ 1
● Weak site	50-100bp	Potential off targets on untargeted chromosomes	40%, 75%	2
● Bad site	>100bp	Potential off targets on targeted chromosomes	< 40%, >75%	≥ 3

1. The distance from knockin site or deletion breakpoint to cutting site of Cas9.
2. Predicted off targets on other chromosomes are indicated.
3. GC content 45% to 70% of target sequence provides the best efficiency.
4. High thymine numbers at 17th to 20th nucleotide of the guide RNA target site may decrease the efficiency.

gRNA 1 (upstream)

CRISPR Target Site [PAM]: ATCTCGACGGTGAATAACAC[GGG]

CRISPR Target Strand in the Genome: plus

Cutting Site: -91 nt from ATG of *BomT1*

Distance: +0 nt from upstream breakpoint to cutting site of Cas9 ●

GC Content: 45% ●

T number at 17th to 20th Nucleotides: 0 ●

Off target: 0 ●

Guide RNA Primers:

Sense oligo 5'- CTTCGATCTCGACGGTGAATAACAC

Antisense oligo 5'- AAACGTGTTATTCACCGTCGAGATC

PAM mutation: not required

Upstream Homology Arm: 1,016bp, the -1,107 nt to -92 nt from ATG of *BomT1*

Forward Oligo 5'- TGGTTACAGTGGTCTGTCTGC

Reverse Oligo 5'- CACGGGAGTAACAGAAAATACCC

Downstream Homology Arm: same as below

gRNA 2 (downstream)



CRISPR Target Site [PAM]: AGTACACTGCTGAAGCAGTC[AGG]

CRISPR Target Strand in the Genome: plus

Cutting Site: +203 nt from Stop Codon of *BomT1*

Distance: +0 nt from downstream breakpoint to cutting site of Cas9 ●

GC Content: 50% ●

T number at 17th to 20th Nucleotides: 1 ●

Off target: 0 ●

Guide RNA Primers:

Sense oligo 5'- CTTCGAGTACACTGCTGAAGCAGTC

Antisense oligo 5'- AAACGACTGCTTCAGCAGTGTACTC

PAM mutation: not required

Upstream Homology Arm: same as above

Downstream Homology Arm: 1,026bp, the +204 nt to +1,229 nt from Stop Codon of *BomT1*

Forward Oligo: 5'- TGCTTCAGCAGTGTACTAAATTAA

Reverse Oligo: 5'- TGTGTGCAATACGTTTTACTGG

Donor Design

Annotation

NNNNN: Homology Arm / Coding Region

NNNNN: Homology Arm / UTR

nnnnn: Homology Arm/ Intron

nnnnn: Homology Arm/ Intergenic Region

nnnnn: gRNA target sequence

nnnnnn: RE site

NNNNN: Gal4

nnnnn: SV40 3'UTR

nnnnn: LoxP

nnnnn: 3xP3 Promoter

NNNNN: RFP

nnnnn: alpha Tubulin 3'UTR

Sequence

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