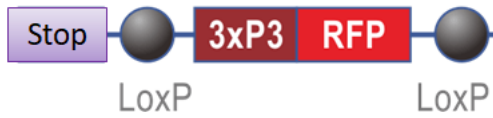


Gene: *TpnT*/ CG7107

Location: X (12A7-12A7)

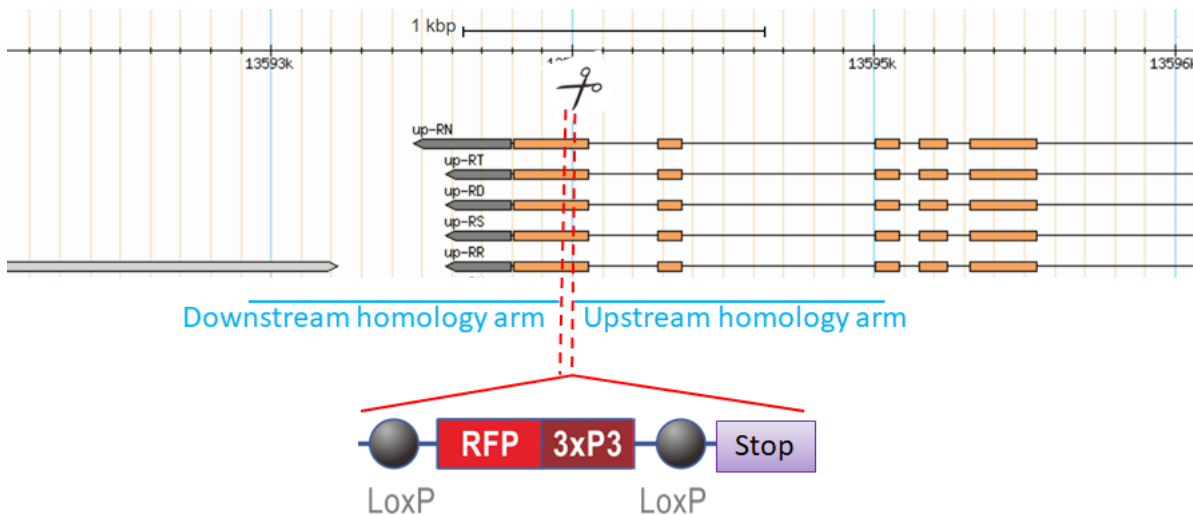
Method: CRISPR/Cas9-mediated genome editing by homology-dependent repair (HDR) using one guide RNA and a dsDNA plasmid donor

Knock-in cassette: Stop-RFP



Injection strain: *w*¹¹¹⁸

Genome Editing:



- (1) Introduce 3 frame stops at the requested last 70th amino acid of TpnT.
- (2) 3XP3-RFP facilitates the genetic screening and can be excised by Cre recombinase. Only BglIII-loxP-NheI (6+34+6bp) will be left after excision.
- (3) Cre/loxP excision is included in this service.
- (4) Selection marker need to be excised to keep the regulation.
- (5) A small deletion (<50bp) will be introduced into genome.

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

NNNNN: Stop

nnnnn: LoxP

nnnnn: 3xP3 Promoter

NNNNN: RFP

nnnnn: alpha Tubulin 3'UTR

Sequence

TGTGGACACCCGCTCTTATGACGACAAGAAGAAGCTCTTCGAGGGTgtaagtattcgggttcctcactaaagctaacta
tacttgggtatTTTTTGGGTTgcctctttgtaaaactgtatcaatattacgcacatttagGGCTATAATACGGTCTATGCGGAAACC
TTAGAAAAGACCTGGCAAGAAAGACAGGAAAGATCACTCAGCGCACAAAATgtaagcgtccgctgaagcattcac
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tgtactgtgctcataaaatcacacacaaaacacgaacacaactgttactacacacag**GGCTGGGATGAGATCTCCAAGGAC**
TCGAACGAGAAGATCTGGAACGAGAAGAAGGAGCAATACACCGGCCGTCAAAATgtatgtactttagatcagat
gcgagttatgtagctagggtaaatggggatcaactgtaccctttcaaagggacgacaacaacagaacaacattcattaatttgttcattgtc
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TAG

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