Seydoux lab Cas9 preparation test: Tag gtbp-1 with eGFP or mCherry using dpy-10 co-CRISPR

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Abstract

This is a positive control experiment (to test protocol in your hands and/or activity of your home-made Cas9): tag *gtbp-1* with *eGFP* or *mCherry* using *dpy-10* co-CRISPR.. The protocol is from:

Paix A, Folkmann A, Rasoloson D, and Seydoux G (2015) High Efficiency, Homology-Directed Genome Editing in Caenorhabditis elegans Using CRISPR/Cas9 Ribonucleoprotein Complexes. Genetics genetics.115.179382; Early online July 17, 2015. doi:10.1534/genetics.115.179382

Please see the full manuscript for detail.

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Guidelines

Main protocol: Direct delivery CRISPR-HDR editing protocol for *C. elegans*

Reagents for gtbp-1 eGFP and mCherry tagging

crRNA gtbp-1 Ct (#728 in Table S3): CCACGAGGUGGUAUGCGCAG + GUUUUAGAGCUAUGCUGUUUUG

Primers pairs to generate template (from eGFP containing-plasmid pAP682-1):

Forward primer (5’ to 3’, lower case indicating the homology arm sequence) for eGFP insertion: ggttcgggtggtgctccacgaggtggtatgcgcGTGAGTAAAGGAGAAGAAC

Reverse primer (5’ to 3’, lower case indicating the homology arm sequence) for eGFP insertion: cttctaattttgtcccgcattttggaaaccgctCTTGTACAGCTCGTCCATGCC

Primers pairs to generate template (from mCherry containing-plasmid pAP582-1):

Forward primer (5’ to 3’, lower case indicating the homology arm sequence) for mCherry insertion: ggttcgggtggtgctccacgaggtggtatgcgcGTCTCAAAGGGTGAAGAAGATAAC

Reverse primer (5’ to 3’ lower case indicating the homology arm sequence) for mCherry insertion: cttctaattttgtcccgcattttggaaaccgctCTTATACAATTCATCCATGCC

PCR condition: use annealing temperature of 63 °C, elongation step of 45s, pAP682-1 (eGFP) or

pAP582-1
(mCherry) as a plasmid template. Do 8 PCR reactions and pool them (400µl total), purify on minelute column (see Repair template synthesis).

Protocol

Step 1.
Prepare the repair PCR template as indicated in **Reagents for gtbp-1 eGFP and mCherry tagging (**see guidelines) and Repair template synthesis.

Step 2.
Make the injection mix as indicated in injection mixes, part I. Use the crRNA gtbp-1 Ct (#728 in Table S3).

Step 3.
Inject 15-20 young adult N2 worms and recover as described in Worm recovery and handling.

Step 4.
Pool the recovered worms (P0s) on one plate and incubate for 22-23h at 20 °C (day 1).

DURATION
22:00:00

Step 5.
Clone the P0s to individual OP50 plates and incubate at 20 °C for 4-5 days (day 2).

Step 6.
When the F1s reach the adult stage, check for Rollers.

NOTES
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At least 3 P0s should give a high number of Rollers (>15)
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Note that we do not count/examine Dumpy F1s since these are homozygous edits at the *dpy-10* locus.

Step 7.
From those “jackpot broods”, screen the Rollers for fluorescent protein expression as described in Screening for edit of interest.

NOTES
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50% or more of the Rollers (at day 2) should be positive for fluorescence.