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Step by step guide to tag endogenous genes with split-wrmScarlet and/or split-sfGFP in *C. elegans* V.2

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Jerome Goudeau

Here is a step by step protocol describing our strategy to label endogenous proteins with split-sfGFP and/or split-wrmScarlet in C. elegans. See our manuscript "Split-wrmScarlet and split-sfGFP: tools for faster, easier fluorescent labeling of endogenous proteins in Caenorhabditis elegans " for more details.

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Jerome F Goudeau, Cynthia Kenyon, Maria Ingaramo 2022. Step by step guide to tag endogenous genes with split-wrmScarlet and/or split-sfGFP in C. elegans. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b34vqqw6 Jerome Goudeau

Typos have been identified and were fixed in this version.

split-sfGFP, split GFP, split-wrmScarlet, split wrmScarlet, CRISPR, Cas9, C. elegans, fluorescent tag

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Here is a step by step protocol describing our strategy to label endogenous proteins with split-sfGFP and/or split-wrmScarlet in *C. elegans*. See our manuscript "Split-wrmScarlet and split-sfGFP: tools for faster, easier fluorescent labeling of endogenous proteins in *Caenorhabditis elegans*" for more details.

Microinjection practices and equipment, see wormbook chapter:
http://www.wormbook.org/chapters/www_transformationmicroinjection/transformationmicroinjection.html

Evans, T. C., ed. Transformation and microinjection (April 6, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook. doi/10.1895/wormbook.1.108.1, http://www.wormbook.org.

If you are not already, get familiar with CRISPR/Cas genome editing, and general guidelines.

Kohler S, Dernburg A. (2016) *C. elegans* injection: Ribonucleoprotein delivery using the Alt-R CRISPR-Cas9 System. [Online] Coralville, Integrated DNA Technologies. [December, 2017.]

Farboud B, Severson AF, Meyer BJ (2019). Strategies for Efficient Genome Editing Using CRISPR-Cas9.. Genetics. https://doi.org/10.1534/genetics.118.301775

Alexandre Paix, Andrew Folkmann, Dominique Rasoloson and Geraldine Seydoux. Direct delivery CRISPR-HDR editing protocol for C. elegans. http://dx.doi.org/10.17504/protocols.io.dri54d

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

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Select a *C. elegans* strain expressing the split-fluorescent protein$_{1-10}$ of your choice

1 **Select a *C. elegans* strain expressing wrmScarlet$_{1-10}$ and/or sfGFP$_{1-10}$ in your tissue of interest**

Somatic split-sfGFP$_{1-10}$:
CF4587 *muIs253[(Peft-3::sfGFP$_{1-10}$::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III*

Somatic split-wrmScarlet$_{1-10}$:
CF4582 *muIs252[Peft-3::wrmScarlet$_{1-10}$::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III*

Dual Somatic split-sfGFP$_{1-10}$ and split-wrmScarlet$_{1-10}$:
CF4588 *muIs253[Peft-3::sfGFP$_{1-10}$::unc-54 3'UTR, Cbr-unc-119(+)], muIs252[Peft-3::wrmScarlet$_{1-10}$::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III*

Muscle-specific split-wrmScarlet$_{1-10}$:
CF4610 *muIs257[Pmyo-3::wrmScarlet$_{1-10}$::unc-54 3'UTR] I*

Select a guide sequence and order a crRNA and tracrRNA

2 Download the DNA sequence of your gene and transcript of interest from Wormbase.
Identify the desired insertion or knock-in site in the genomic DNA.

3 Using ~50 nucleotides flanking Identify DNA sequence(s) followed by a PAM site (5' NGG 3') in your target gene using a CRISPR/Cas9 target online predictor, such as CCTop.

When using CCTop, use the default parameters and adjust the following two criteria:
PAM type: NGG (Streptococcus pyogenes)
Species: *C. elegans*

Insert the DNA sequence in the query section, then submit.
Select the crRNA target site with a high score, the closest to your editing site, with no off-targets.

4 Order the crRNA corresponding to your selected guide sequence from IDT.
Note: Do not include the PAM in the query
Typically, we order 10 nmol of each Alt-R® CRISPR-Cas9 crRNA that we ressuspend in 14.5 µL TE.

5 Order the universal 67 mer tracrRNA from IDT under the section "CRISPR-Cas9 tracrRNA".
https://www.idtdna.com/pages/products/crispr-genome-editing/alt-r-crispr-cas9-system

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Typically, we order 20 nmol.

Design and order a single-stranded donor oligonucleotides (ssODN) of 200 mers

6 Design the sequence of a single-stranded donor oligonucleotides (ssODN) with the Fluorescent Protein$_{11}$ of choice, (ie. wrmScarlet$_{11}$ or sfGFP$_{11}$), a linker and homology arms.

Sequences:
wrmScarlet$_{11}$:
5' **TACACCGTCGTCGAGCAATACGAGAAGTCCGTCGCCCGTCACTGCACCGGAGGA** 3'

sfGFP$_{11}$:
5' **CGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATTACA** 3'

Linker:
5' **GGAGGAGGATCC** 3'

The linker should be inserted at the 5' end or 3' end of the FP$_{11}$ depending on the site chosen to insert it in your gene of interest, for example:

- When tagging the N-terminal of your gene of interest, the final ssODN sequence should look like this:
Left homology arm :: endogenous START codon (ATG) :: Fluorescent Protein$_{11}$ :: Linker :: Right homology arm

- When tagging the C-terminal of a gene of interest, the final ssODN sequence should look like this:
Left homology arm :: Linker:: Fluorescent Protein$_{11}$ :: endogenous STOP codon :: Right homology arm

Note:
If C-terminus tagging is required, we suggest the alternative wrmScarlet11(MDELYK) sequence:

Protein sequence: YTVVEQYEKSVARHCTGGMDELYK

Nucleotide sequence:
5'
TACACCGTCGTCGAGCAATACGAGAAGTCCGTCGCCCGTCACTGCACCGGAGGAATGGAT
GAGTTATACAAG 3'

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Ensure that the linker and the Fluorescent Protein$_{11}$ sequences are in frame with the coding sequence of your gene of interest

Linker length and flexibility may need to be optimized depending on the protein structure and function

The final ssODN can be up to 200 mers, allowing each homology arms to be 67 nucleotides when tagging a gene with wrmScarlet$_{11}$ and the suggested linker

Order the ssODN from IDT in the section "Ultramer DNA Oligos".
We typically order up to 4 nmol at 100 µM in IDTE buffer

Design oligos to amplify the insertion site

7 Design and order oligos that will be used to perform the genotyping and sequencing of the insertion site.

- 18-24 bases in length
- Melting temperature (Tm) between 50 and 60ºC
- GC content ranging from 45 to 55%
- G or C on the 3' end
- Design primers 200 nt upstream and downstream from the sequence of interest

Prepare and inject CRISPR/Cas9 ribonucleoprotein mix

8 Assemble CRISPR/Cas9 ribonucleoproteins complex and ssODN into injection mix:

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A	B
Nuclease-free Water (Final volume = 10 µL)	5.25 µL
HEPES pH 7.5 (117 mM) + KCl (1.53M)	0.75 µL
tracrRNA (8 µg/µL)	1 µL
crRNA designed at step #4 (8 µg/µL)	1 µL
ssODN (100 µM) (designed at step #6)	0.5 µL
Purified Cas9 Nuclease (10 µg/µL)	1.5 µL

Protocol without co-CRISPR

Alternatively:

A	B
Nuclease-free Water (Final volume = 10 µL)	3.75 µL
HEPES pH 7.5 (117 mM) + KCl (1.53M)	0.75 µL
tracrRNA (8 µg/µL)	1.5 µL
crRNA designed at step #4 (8 µg/µL)	1 µL
crRNA dpy-10 (8 µg/µL)	0.5 µL
ssODN with FP11 - designed at step #6 - (100 µM)	0.5 µL
ssODN dpy-10(cn64) (100 µM)	0.5 µL
Purified Cas9 Nuclease (10 µg/µL)	1.5 µL

Protocol with co-CRISPR *dpy-10(cn64)*

Pipet up and down a few times without introducting air in the mix.

Incubate **37 °C** **15 min**

Quick spin **13000 rpm**

Load 0.5 to 1 µL of the mix in injection needles and inject 10 to 20 day-1 adult worms successfully, ideally in both gonad arms.

Single injected worms in a drop of M9 buffer on NGM plates seeded with OP50, and place them in a **25 °C** incubator **Overnight** .

Step F - Screen for successful FP_{11} integrants

9 Starting 3 days post injections, screen daily until identifying fluorescent progeny in the F_1 and/or F_2 of singled injected worms.

Perform PCR genotyping and sequencing using regular worm protocols with the primers designed in step C.

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