**SUPPLEMENTARY TEXT**

**Supplementary Materials and Methods**
**Mammalian cell culture.** HEK293T cells (ATCC # CRL-3216) were cultured in high-glucose DMEM supplemented with 10% FBS, 1 mM glutamine and 100 µg/mL penicillin/streptomycin (Gibco). An split-wrmScarlet1-10 cDNA codon-optimized for mammalian expression was fused to the C-terminus of eGFP and cloned into a pCDH lentiviral expression vector (SFFV GFP-mScarlet1-10). Lentivirus was prepared using standard protocols (Kamiyama *et al.* 2016) and used to infect HEK293T cells. A polyclonal population of GFP-split-wrmScarlet1-10 positive cells was isolated by FACS (using GFP fluorescence) and served as parental cell line for further experiments. For CLTA-N CRISPR engineering, *S. pyogenes* Cas9/sgRNA ribonucleoprotein complexes were prepared as in (Leonetti *et al.* 2016), mixed with HDR donor templates and electroporated into of GFP-split-wrmScarlet1-10 cells by nucleofection.

**CLTA-N split-wrmScarlet11 donor library.** A cDNA pool of degenerate split-wrmScarlet11 sequences was generated by oligonucleotide synthesis (GenScript) and homology arms for HDR-mediated insertion at CLTA N-terminus were appended by PCR (Supplementary Material – Table-S1 for full sequences). Library diversity was verified by Illumina MiSeq deep-sequencing.

**Supplementary Results**

**Split-wrmScarlet screening in mammalian cells**

We tested the applicability of the split-wrmScarlet1-10 system for mammalian cell engineering but were surprisingly unsuccessful at detecting fluorescence. We designed a human codon-optimized split-wrmScarlet1-10 cDNA and expressed it as a C-terminal GFP fusion in HEK293T cells by lentiviral transduction. Expression of GFP verified the successful expression of the fusion protein (Figure S10A). However, subsequent expression of split-wrmScarlet11 fragments did not give rise to detectable red fluorescence despite numerous attempts. We reasoned that the split-wrmScarlet11 amino-acid sequence might be sub-optimal for complementation in human cells and synthesized a library of degenerate split-wrmScarlet11 sequences covering any possible single and double amino-acid mutants. Using an established assay for CRISPR-based knock-in of sequences at the CLTA N-terminus (a highly expressed gene in HEK293T cells (Leonetti *et al.* 2016), neither our original split-wrmScarlet11 sequence nor its mutant library enabled detectable complementation (Figure S10B, left panels). By contrast, a control experiment using the sfGFP1-10/sfGFP11 system showed a high level of knock-in and complementation in HEK293T (Figure S10B, right panels). It is possible that split-wrmScarlet1-10 is expressed in a non-functional form in human cells, or that its binding to split-wrmScarlet11 is occluded by competing interactions (with cellular chaperones, for example). In addition, we did not attempt complementation in primary non-transformed cell lines, like WI-38 cells, whose different proteostasis network and chaperones could aid split-wrmScarlet folding. At this point, more experiments would be required to establish the portability of split-wrmScarlet to mammalian systems.

**Experiments to investigate whether split-wrmScarlet11 functions as a degron in *C. elegans***

After finalizing our experiments, a paper that shows that C-terminal gly-gly sequences might function as degrons in mammalian cells was brought to our attention (Koren, *et al.* 2018). Since we were unable to obtain non-sterile positive clones of TOMM-20::split-wrmScarlet11 that did not have a mutation on the last glycine, and we were also unable to obtain EAT-6 homozygotes, we were concerned that our split-wrmScarlet11 might be recognized as a degron. To investigate this, we first labeled HIS-3, EAT-6, and TOMM-20 with the 24 a.a. split-wrmScarlet11(MDELYK), which adds the sequence MDELYK to the C-terminus of split-wrmScarlet11. These worms were fertile, and at least as bright as those labeled with split-wrmScarlet11 (Figure S6). However, increased fluorescence could be due to increased molecular brightness rather than increased abundance. To address this, we compared the abundance of nuclear HIS-3, HIS-3::split-wrmScarlet11, and HIS-3::split-wrmScarlet11(MDELYK) by western blot, and were unable to detect a significant change in abundance (Figure S11). We also could not detect differences in abundance in *S. cerevisiae*, using a p416-GPD plasmid expressing a mTagBFP-mScarlet fusion or the same fusion truncated so that it ends with gly-gly (Figure S12). However, because HIS-3 is a nuclear protein, and expression in yeast was done from an overexpressing plasmid, we cannot exclude that a protein ending with two glycines might be recognized as a degron in other cellular compartments, or at different expression levels, nor that there is no DesCEND degron pathway in yeast and worms. For these reasons, we recommend using the 24 a.a. split-wrmScarlet11(MDELYK) when labeling proteins at their C-termini.

**Supplementary Results – Literature cited**

Kamiyama, D. *et al.* Versatile protein tagging in cells with split fluorescent protein. *Nature Communications* **7,** 11046–9 (2016).

Leonetti, M. D., Sekine, S., Kamiyama, D., Weissman, J. S. & Huang, B. A scalable strategy for high-throughput GFP tagging of endogenous human proteins. *Proc. Natl. Acad. Sci. U.S.A.* **113,** E3501–8 (2016).

**SUPPLEMENTARY PROTOCOL**

**Step by step guide to tag endogenous genes with split-wrmScarlet and/or split-sfGFP in *C. elegans* V.1.** [doi.org/10.17504/protocols.io.bamkic4w](http://doi.org/10.17504/protocols.io.bamkic4w)

MATERIALS

Microinjection practices and equipment, see WormBook chapter:

[www.wormbook.org/chapters/www\_transformationmicroinjection/transformationmicroinjection.html](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.org/10.1895/wormbook.1.108.1](http://doi.org/10.1895/wormbook.1.108.1), http://www.wormbook.org.

BEFORE STARTING

If you are not already, become familiar with CRISPR/Cas genome editing, and general guidelines (Paix, *et al*. 2015; Kohler & Dernburg 2016; Farboud *et al.* 2019).

**1**. Select a *C. elegans* strain expressing split-wrmScarlet1-10 and/or sfGFP1-10 in your tissue of interest:

Somatic split-sfGFP1-10:

[CF4587](https://cgc.umn.edu/strain/CF4587) *muIs253[(Peft-3::sfGFP1-10::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III*

Somatic split-wrmScarlet1-10:

[CF4582](https://cgc.umn.edu/strain/CF4582) *muIs252[Peft-3::split-wrmScarlet1-10::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III*

Dual Somatic split-sfGFP1-10 and split-wrmScarlet1-10:

[CF4588](https://cgc.umn.edu/strain/CF4588) *muIs253[Peft-3::sfGFP1-10::unc-54 3'UTR, Cbr-unc-119(+)], muIs252[Peft-3::split-wrmScarlet1-10::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III*

Muscle-specific split-wrmScarlet1-10:

[CF4610](https://cgc.umn.edu/strain/CF4610) *muIs257[Pmyo-3::split-wrmScarlet1-10::unc-54 3'UTR] I*

Germline-specific split-wrmScarlet1-10:

[DUP237](https://cgc.umn.edu/strain/DUP237) *glh-1(sam140[glh-1::T2A::split-wrmScarlet1-10]) I*

Germline-specific sGFP21-10:

[DUP223](https://cgc.umn.edu/strain/DUP223) *glh-1(sam129[glh-1::T2A::sGFP21-10]) I*

**2**. Select a guide sequence and order a crRNA and tracrRNA.

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 (Stemmer *et al.* 2015; Labuhn, M. *et al.* 2018).

If 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 resuspend in 14.5 μL TE.

**5**. Order the universal 67 mer tracrRNA from IDT under the section "CRISPR-Cas9 tracrRNA" ([www.idtdna.com/pages/products/crispr-genome-editing/alt-r-crispr-cas9-system](https://www.idtdna.com/pages/products/crispr-genome-editing/alt-r-crispr-cas9-system)). Typically, we order 20 nmol.

**6**. Design and order single-stranded donor oligonucleotides (ssODN) of 200-mers.

Design the sequence of a single-stranded donor oligonucleotides (ssODN) with the Fluorescent Protein11 of choice, (*i.e.* split-wrmScarlet11 or sfGFP11), a linker and homology arms.

Sequences:

Split-wrmScarlet11 for N-terminus labeling:

5' TACACCGTCGTCGAGCAATACGAGAAGTCCGTCGCCCGTCACTGCACCGGAGGA 3'

Split-wrmScarlet11 for C-terminus labeling:

5’ TACACCGTCGTCGAGCAATACGAGAAGTCCGTCGCCCGTCACTGCACCGGAGGAATGGATGAGTTATACAAG

3’

sfGFP11:

5' CGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATTACA 3'

Linker:

5' GGAGGAGGATCC 3'

The linker should be inserted at the 5' end or 3' end of the FP11 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 Protein11 :: Linker :: Right homology arm

- When tagging the N-terminal of a gene of interest, the final ssODN sequence should look like this:

Left homology arm :: Linker:: Fluorescent Protein11 :: endogenous STOP codon :: Right homology arm

Ensure that the linker and the Fluorescent Protein11 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 split-wrmScarlet11 and the suggested linker at the N-terminus or 58 for tagging at the C-terminus.

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

**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

**8**. Prepare and inject CRISPR/Cas9 ribonucleoprotein mix

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

Protocol without co-CRISPR:

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

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

Pipet up and down a few times without introducing air into 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.

**9**. Screen for successful FP11 integrants

Starting 3 days post injections, screen daily until identifying fluorescent progeny in the F1 and/or F2 of singled injected worms. Perform PCR genotyping and sequencing using regular worm protocols with the primers designed in step **7**.

**Supplementary Protocol – Literature cited**

Farboud, B., Severson, A. F. & Meyer, B. J. Strategies for Efficient Genome Editing Using CRISPR-Cas9. *Genetics* **211,** 431–457 (2019).

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

Labuhn, M. *et al.* Refined sgRNA efficacy prediction improves large- and small-scale CRISPR-Cas9 applications. *Nucleic Acids Res* **46,** 1375–1385 (2018).

Paix *et al.* Direct delivery CRISPR-HDR editing protocol for *C. elegans*. http://dx.doi.org/10.17504/protocols.io.dri54d.

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