SapTrap Assembly of Caenorhabditis elegans MosSCI Transgene Vectors

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ABSTRACT The Mos1-mediated Single-Copy Insertion (MosSCI) method is widely used to establish stable* Caenorhabditis elegans transgenic strains. Cloning MosSCI targeting plasmids can be cumbersome because it requires assembling multiple genetic elements including a promoter, a 3′UTR and gene fragments. Recently, Schwartz and Jorgensen developed the SapTrap method for the one-step assembly of plasmids containing components of the CRISPR/Cas9 system for C. elegans. Here, we report on the adaptation of the SapTrap method for the efficient and modular assembly of a promoter, 3′UTR and either 2 or 3 gene fragments in a MosSCI targeting vector in a single reaction. We generated a toolkit that includes several fluorescent tags, components of the ePDZ/LOV optogenetic system and regulatory elements that control gene expression in the C. elegans germline. As a proof of principle, we generated a collection of strains that fluorescently label the endoplasmic reticulum and mitochondria in the hermaphrodite germline and that enable the light-stimulated recruitment of mitochondria to centrosomes in the one-cell worm embryo. The method described here offers a flexible and efficient method for assembly of custom MosSCI targeting vectors.

The rich toolbox of techniques available to manipulate gene expression in C. elegans is a major attraction of this model organism. Several approaches have been developed to introduce transgenes and to induce efficient CRISPR/Cas9 mediated gene editing (Nance and Frøkjær-Jensen 2019). The Mos1-mediated Single-Copy Insertion (MosSCI) method has been widely adopted to introduce transgenes in C. elegans because single-copy transgenes are integrated at defined chromosomal positions, thereby mitigating potential concerns of transgene integration at random positions (Frøkjær-Jensen et al. 2008; Frøkjær-Jensen et al. 2012; Frøkjær-Jensen et al. 2014). MosSCI transgene integration results from homologous recombination between a MosSCI targeting vector containing the transgene construct and one of the safe-harbor integration sites that have been engineered at defined positions in the genome.

Transgenes typically include multiple genetic elements including a promoter, one or more gene fragments and a 3′UTR. A number of strategies can be used to assemble these elements together including traditional restriction enzyme cloning, Gateway cloning (Hartley et al. 2000), in vivo recombineering (Philip et al. 2019) or Gibson cloning (Gibson et al. 2009). Each of these strategies has both advantages and disadvantages. For example, Gateway cloning allows the efficient modular “mix and match” cloning of large collections of promoter, ORF and 3′UTR cassettes (Brasch et al. 2004; Dupuy et al. 2004; Mangone et al. 2010; Zeiser et al. 2011). However, Gateway cloning can be expensive due to the required use of proprietary enzyme mixes and leaves ~25 base pair att recombination site “scars” at each cassette junction. In contrast, Gibson cloning allows the efficient, “scar-free” assembly of
multiple gene fragments but does not allow the “mix and match” cloning of existing cassettes, making this approach laborious if many constructs are needed.

Schwartz and Jorgensen recently developed the SapTrap method for efficient, modular and single step assembly of CRISPR/Cas9 vectors for *C. elegans* (Schwartz and Jorgensen 2016). The SapTrap method is based on the Golden Gate cloning technique (Engler et al. 2008) and takes advantage of the SapI type II restriction enzyme, which cuts DNA at defined positions adjacent to its recognition sequence to generate three-base 5’ overhangs. By designing SapI restriction fragments with complementary overhangs, multiple fragments can be assembled together in a defined order in a single digestion and ligation reaction. In this study, we report on the adaptation of the SapTrap system for the efficient, inexpensive, modular, and “scar-free” assembly of transgenes in a MosSCI targeting vector. We have developed a toolkit for expression of transgenes in the *C. elegans* germline, including a collection of cassettes containing tags for fluorescence imaging and for the ePDZ/LOV optogenetic system (Strickland et al. 2012; Fielmich et al. 2018).

As a proof of principle, we have used this system to generate a collection of mitochondrial and endoplasmic reticulum reporter strains and a strain in which light induces the transport of mitochondria to centrosomes in the one-cell worm embryo.

### MATERIALS AND METHODS

#### C. elegans

*C. elegans* hermaphrodite strains were maintained at either 20° or 25° on Nematode Growth Medium (NGM) plates containing 3 g/L NaCl, 2.5 g/L peptone and 17 g/L agar supplemented with 1 mM CaCl₂, 1 mM MgSO₄, 1 mM KPO₄ and 5 mg/L cholesterol with *E. coli* OP50 as a source of food. All strains used in this study are listed in Table 1.

#### Cloning

To generate the expression vector pXF87, the two SapI restriction sites in pCFJ350 (Frøkjær-Jensen et al. 2012) were mutated using Q5 Site-Directed Mutagenesis (New England Biolabs (NEB)) with the oligo pairs XF30F/XF30R and XF31F/XF31R. In addition, the annealed oligos Eg717 and Eg718 were cloned between the XhoI and SpeI sites of pCFJ350 (Frøkjær-Jensen et al. 2012) and the intron-free MitoTracker Deep Red FM dye (Cell Signaling Technology, Cat #8778S).

To assemble HSP-3 (aa 1-19) into the second cassette of the expression vector pJF13, 10 μM of oligos XF17F and XF17R were gradually cooled from 95° to 25° in a BioRad T1000 thermocycler. Annealed oligos were phosphorylated by T4 polynucleotide kinase (NEB) for two hours at 37°, followed by an enzyme inactivation step at 65° for 20 min. The donor plasmids and primers are listed in Tables 2 and 3, respectively.

#### Assembly reaction

Assembly reactions (total final volume of 50 μL) included 1 nM of pXP87 and of each donor position plasmid, 400 units of T4 DNA ligase (NEB), 10 units of SapI enzyme (NEB), 1x NEB CutSmart buffer and 1 mM ATP. For assemblies including annealed oligos, phosphorylated annealed oligos were used at a final concentration of 3 nM in the assembly reaction. Reactions were incubated for 22-24 hr at 25°, and transformed into Stellar Competent cells (Clontech). Four to six plasmid clones were first screened by restriction digest with Xhol and SpeI. Plasmids with the correct restriction digest pattern were sequenced across each cassette boundary. MosSCI targeting vector assembly reactions are listed in Table 4. Note that because the background of unassembled vectors in our assembly reactions was typically low, our protocol omits the counterselection restriction enzyme step described in the original SapTrap protocol (Schwartz and Jorgensen 2016).

#### Transgenesis

Double-stranded breaks at Mos1 landing sites were generated using CRISPR/Cas9. With the exception of strains EGD623, EGD629, EGD631 and EGD633, injection mixes contained 50 ng/μL of each of the following vectors: an assembled MosSCI targeting vector, pXW7.01 and pXW7.02 sgRNA/Cas9 vectors (gifts from Katya Veronina, University of Montana), which direct Cas9 to generate double-stranded breaks at the *tfl5605* universal MosSCI insertion site. For strains EGD623, EGD629, EGD631 and EGD633, injection mixes contained 0.25 μg/μL Cas9 protein, 0.1 μg/μL tracrRNA, 0.028 μg/μL crRNA BH0278 (GGGCUUCUGAUCCUUUUUGGGUUUUGAGGCUGAUGCUGUUUG), 0.028 μg/μL crRNA BH0279 (GUCCCAUGCAAAAGGGUUUAGGCUUGCUGUUUG) (Dharmacon) and 0.1 μg/μL assembled MosSCI targeting plasmid. The universal MosSCI strains EGR078 or EGR079 (Frøkjær-Jensen et al. 2014) were injected, singled and incubated for 10 days at 20°. ~10 worms from plates containing non-Unc animals were transferred to new plates. Plates that stably gave rise to non-unc progeny were visually screened for fluorescent transgene expression.

#### HaloTag staining

20 to 30 L4 worms were stained in 25 μL S medium containing concentrated OP50 bacteria and 2.5 μM of either JF613 HaloTag ligand or JF629 HaloTag ligand (Grimm et al. 2015) in a darkened 96-well plate shaking at 150 rpm for 19 hr at 23°. Water was placed in the neighboring wells to help prevent evaporation. Animals were recovered on NGM plates for up to two hours before imaging.

#### MitoTracker deep red staining

L4 worms were fed overnight on an NGM plate that had been seeded with 100 μL concentrated OP50 bacteria mixed with 1 μL of 1 mM MitoTracker Deep Red FM dye (Cell Signaling Technology, Cat #8778S).

#### Imaging

With the exceptions of the TOMM-20::Dendra2 strain and optogenetic strains (Figure 4), all images were collected on a spinning-disk

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**Figure 4**: MitoTracker deep red staining of mitochondria in L4 worms fed on a NGM plate for up to two hours before imaging. (A) A representative single confocal optical section of a wild-type worm stained with 0.5 μM MitoTracker Deep Red FM dye showing MitoTracker Deep Red FM staining in the cytoplasm and nuclei. (B) Top: A representative single confocal optical section of a wild-type worm stained with 0.5 μM MitoTracker Deep Red FM dye. Bottom: A representative single confocal optical section of a wild-type worm stained with 0.5 μM MitoTracker Deep Red FM dye. (C) A representative single confocal optical section of a wild-type worm stained with 0.5 μM MitoTracker Deep Red FM dye showing MitoTracker Deep Red FM staining in the cytoplasm and nuclei. (D) A representative single confocal optical section of a wild-type worm stained with 0.5 μM MitoTracker Deep Red FM dye showing MitoTracker Deep Red FM staining in the cytoplasm and nuclei.
Table 1 Strains used in this study

| Strain  | Genotype                              | Construction                          | Reference                              |
|---------|---------------------------------------|---------------------------------------|----------------------------------------|
| EG8078  | oxTi185; unc-119(ed3) I                | Injected pRF13 into EG8078            | Frøkjær-Jensen et al. (2014)           |
| EG8079  | oxTi179; unc-119(ed3) II               | Injected pXF17 into EG8079            | This study                             |
| EGD329  | egxSi126 [Mex-5p::hsp-3(aa 1-19)::halotag::dendra2::pie-1 3'-UTR + unc119(+)] I; unc-119(ed3) III | Injected pSM16 into EG8079 | This study                             |
| EGD412  | egxSi136 [Mex-5p::tomm-20::halotag::pie-1 3'-UTR + unc119(+)] II; unc-119(ed3) III | Injected pXF253 into EG8078 | This study                             |
| EGD496  | egxSi117 [Mex-5p::npp-20::gfp::pie-1 3'-UTR + unc119(+)] I; unc-119(ed3) III | Injected pXF255 into EG8079 | This study                             |
| EGD497  | egxSi118 [Mex-5p::npp-20::halotag::pie-1 3'-UTR + unc119(+)] II; unc-119(ed3) III | Injected pXF266 into EG8079 | This study                             |
| EGD549  | egxSi144 [Mex-5p::cox-4::halotag::pie-1 3'-UTR + unc119(+)] II; unc-119(ed3) III | Injected pXF13 into EG8079 | This study                             |
| EGD565  | egxSi145 [Mex-5p::hsp-3(aa 1-19)::halotag::dendra2::pie-1 3'-UTR + unc119(+)] III; unc-119(ed3) III | Crossed EGD412 and JJ2586 | This study                             |
| EGD623  | egxSi152 [Mex-5p::tomm-20::gfp::pie-1 3'-UTR + unc119(+)] II; unc-119(ed3) III | Injected pSM17 into EG8079 | This study                             |
| EGD629  | egxSi155 [Mex-5p::tomm-20::mkate2::pie-1 3'-UTR + unc119(+)] II; unc-119(ed3) III | Injected pSM20 into EG8079 | This study                             |
| EGD631  | egxSi157 [Mex-5p::tomm-20::dendra2::pie-1 3'-UTR + unc119(+)] II; unc-119(ed3) III | Injected pSM22 into EG8079 | This study                             |
| EGD633  | egxSi159 [Mex-5p::tomm-20::mscarlet::pie-1 3'-UTR + unc119(+)] II; unc-119(ed3) III | Crossed EGD412 and JJ2586 | This study                             |
| EGD615  | cox-4[2x476]::eGFP::3XFLAG]; egxSi136 [Mex-5p::tomm-20::halotag::pie-1 3'-UTR + unc119(+)] I; unc-119(ed3) III | Crossed EGD412 and JJ2586 | This study                             |
| JJ2586  | cox-4[2x476]::eGFP::3XFLAG]; egxSi136 [Mex-5p::tomm-20::halotag::pie-1 3'-UTR + unc119(+)] I; unc-119(ed3) III | Crossed EGD412 and JJ2586 | This study                             |
| TBD307  | dhc-1:ne255::epdz::mcherry::dhc-1 I;_utdSi51[5p::tomm-20(aa 1-55); halotag::lov::tbb-2 3'-UTR + unc119(+)] II | Injected pSDH68 into EG8079 | Raideas et al. 2018                   |
| SV2095  | dhc-1:ne255::epdz::mcherry::dhc-1 I; wuts571[1p::gfp::tbb-2 + unc119(+)] V | Crossed EGD412 and JJ2586 | This study                             |

microscope built on a Nikon Eclipse Ti base and equipped with an Andor CSU-W1 two camera spinning disk module, Zyla sCMOS cameras, an Andor ILE laser module and a Nikon 100X Plan Apo 1.45 NA oil immersion objective (Micro Video Instruments, Avon, MA).

TOMM-20::Dendra2 was imaged on a Marianas spinning disk microscope (Intelligent Imaging Innovations) built around a Zeiss Axio Observer Z.1 equipped with a Photometrics Evolve EMCCD camera, 50 mW 488 and 561 nm solid state lasers, a CSU-X1 spinning disk (Yokogawa, Tokyo Japan) and a Zeiss 100X Plan-Apochromat objective. Photoconversion was performed by 5 sec illumination with a 405 epi-fluorescent light source.

To stimulate the relocalization of mitochondria (Figure 4), embryos were illuminated with a 50 mW 640 nm solid-state laser used to excite MitoTracker DeepRed (20% laser power, 100 msec exposure, camera gain of 1) and a 50 mW 488 nm solid-state laser used to stimulate the interaction between ePDZ and LOV domains (80% laser power and 100 msec exposure). A Plan-Apochromat 100x/1.4 NA oil immersion DIC objective (Zeiss) was used and Z-stacks (one micrometer step size, 11 steps) were collected at 60-second intervals. The images displayed in Figure 4 are maximum intensity projections of three Z planes from the cell midplane.

Data availability
With the exception of EGD633, the C. elegans strains generated in this study have been deposited at the Caenorhabditis Genetics Center (CGC; https://cgc.umn.edu). The plasmids listed in Figures 1 and 3 have been deposited at Addgene (http://www.addgene.org). Other donor plasmids, assembled expression plasmids and EGD633 are available upon request. Supplemental materials describing the sequence of tag donor cassettes are available through the GSA gshare portal: https://doi.org/10.25387/g3.9978611.

RESULTS
Adaptation of the SapTrap system for cloning MosSCI targeting vectors
To adapt the SapTrap approach (Schwartz and Jorgensen 2016) for the assembly of MosSCI targeting vectors, we started by making two changes to the universal MosSCI targeting vector pCFJ350 (Frøkjær-Jensen et al. 2012), which targets transgenes for insertion at the commonly used ttTi5605 site (Frøkjær-Jensen et al. 2008). First, we introduced single base pair changes to disrupt the two SapI restriction sites located in the “Left” and “Right” homology arms of pCFJ350. Second, we inserted two SapI sites into the multiple cloning site that were oriented such that they were removed from the vector backbone by digestion with SapI. The resulting MosSCI targeting vector was named pXF87 (Figure 1A). Although pXF87 is compatible with the standard Mos1-mediated transgenesis protocol, the transgenic strains described in this study were isolated using CRISPR/Cas9 to generate double-stranded breaks in MosSCI integration sites (described in the Methods section).
We next cloned a series of plasmids that contain donor cassettes flanked by SapI restriction sites (Figure 1B). Following digestion with SapI, the cassettes are liberated from the vector backbone and are flanked by 5′ overhangs that direct their order of assembly in pXF87 (Figure 1C). A four-insert cassette system was designed with a promoter in cassette 1, gene fragments in cassettes 2 and 3 (typically a gene and a target) and a 3′ UTR in cassette 4 (Figure 1B). To minimize the inclusion of extraneous sequences, the junction between the first and second cassettes is the translation start (ATG), between second and third cassettes is glycine (GGT) and between the third and fourth cassettes is the ochre translation stop codon (TAA) (Figure 1C). Donor cassettes encoding tags (such as fluorescent proteins) include short flexible linkers at the protein fusion site (the carboxy terminus of cassette 2 and the amino terminus of cassette 3) (Supplemental Figure S1-S7). The currently available promoter, tag and 3′ UTR donor cassette plasmids are listed in Figure 1E and Table 2.

The *C. elegans* germline is a notoriously difficult tissue in which to achieve stable transgene expression due to silencing of multi-copy extra-chromosomal arrays (Kelly et al. 1997), single-copy insertions generated by MosSCI (e.g., Shirayama et al. 2012; Frokjaer-Jensen et al. 2016)) or endogenous genes tagged using CRISPR/Cas9 gene editing (e.g., Fielmich et al. 2018)). Each of our tag donor cassettes encoding gene tags incorporates at least one modification that buffers against silencing, including the inclusion of PATC introns in HaloTag and ceGFP (Frokjaer-Jensen et al. 2016), the elimination of piRNA binding sites in mScarlet, mKate2 and Dendra2 (Seth et al. 2018; Zhang et al. 2018) and the use of sequence motifs found in native germline genes in epPDZ and the LOV domain (Fielmich et al. 2018).

Similar to the SapTrap method developed by Schwartz and Jorgensen (Schwartz and Jorgensen 2016), MosSCI targeting vectors were assembled in a single tube by incubating pXF87, four donor cassette plasmids, SapI enzyme, ATP and T4 DNA ligase at 25° for 22-24 hr (Figure 1D and Materials and Methods). This reaction was then transformed into *E. coli* and plasmid cloned were screened by restriction enzyme digestion followed by sequencing. We assembled nine vectors using the 4-cassette system and 32 of 46 (69.6%) of the plasmids screened had the correct restriction digest pattern (Table 4). Of the vectors with the correct restriction digest pattern, 22 of 23 were correct based on Sanger sequencing analysis of the cassette junctions. Therefore, the SapTrap method provides an efficient method for the assembly of MosSCI targeting vectors.

### A collection of fluorescent ER and mitochondria strains

We used SapTrap-assembled MosSCI targeting vectors to generate a collection of transgenic strains for analysis of endoplasmic reticulum and mitochondrial dynamics. We first targeted *GFP*, mKate2, mScarlet, Dendra2 and HaloTag to the cytoplasmic face of the mitochondrial outer membrane by fusing them to the carboxy terminus of TOMM-20. The expression of these transgenes was controlled by the *mex-5* promoter and by the *pie-1* 3′UTR, which results in germline expression that increases around the bend of the adult hermaphrodite gonad (Merritt et al. 2008) (Figure 2A). Strains expressing TOMM-20 fused to HaloTag were labeled with the fluorescent JF646 HaloTag ligand (Grimm et al. 2015) by feeding hermaphrodites bacteria mixed with the ligand. Each TOMM-20 fusion protein exhibited the expected tubular localization pattern in the early embryo (Figure 2B-I). We confirmed that TOMM-20::HaloTag colocalized to the same organelle as the mitochondrial matrix protein COX-4::GFP (Raiders et al. 2018) (Figure 2C). We additionally generated strains in which the HaloTag was targeted to the mitochondrial matrix (COX-4::HaloTag) (Figure 2J) and the lumen of the endoplasmic reticulum (HSP-3(aa 1-19)::HaloTag:HDEL) (Figure 2K) (Lee et al. 2016). We fused both GFP and HaloTag to NPP-20, the worm homolog of SEC13, which is both a component of the COPII coat that concentrates to ER exit sites (ERES) (D′Arcangelo et al. 2013) and a component of nuclear pore complexes (Siniosoglu et al. 1996) (Figure 2L, M).

### Five-cassette system

One of the advantages of the SapTrap approach is that it can be easily expanded to include additional insert fragments to create more complex transgenes. To establish a five-cassette system, we used the cassettes 1, 2 and 4 from the four-cassette system and replaced cassette 3 with cassettes 3A and 3B (Figure 3A and 3B). We used this approach to generate an optogenetic system to control the localization of mitochondria in the early embryo based on the light induced interaction between the epPDZ and LOV domains (Strickland et al. 2012; Fielmich et al. 2018).

We assembled a MosSCI targeting vector that directed expression of TOMM-20::HaloTag:LOV, which targets the LOV domain to...
### Table 3 Primers used in this study

| Name          | Description | Sequence (SAP1 site and Overhang) | Corresponding plasmid |
|---------------|-------------|-----------------------------------|-----------------------|
| XF32F         | mex-5       | GCA GCC CTT CAC TGG GTG ATC ATC GTA CTT TCT TCT GTG ACAA ATG AAT ATT TTT AATA TTA | pXF121                 |
| XF32R         | mex-5       | GCA GCC CTT CAC TGG GTG ATC ATC GTA CTT TCT TCT GTG ACAA ATG AAT ATT TTT AATA TTA | pXF121                 |
| JF5F          | 10XX02 (F)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pJF7                   |
| JF5R          | 10XX02 (R)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pJF5                   |
| JF1F          | gfp (F)     | GCA GCC CTT CAC ATG GCC GGC CGT CTT CTT GCT GAG ACT CGTC CAT | pJF5                   |
| JF1R          | gfp (R)     | GCA GCC CTT CAC ATG GCC GGC CGT CTT CTT GCT GAG ACT CGTC CAT | pJF5                   |
| XF2F          | gfp (F)     | GCA GCC CTT CAC ATG GCC GGC CGT CTT CTT GCT GAG ACT CGTC CAT | pJF6                   |
| XF2R          | gfp (R)     | GCA GCC CTT CAC ATG GCC GGC CGT CTT CTT GCT GAG ACT CGTC CAT | pJF6                   |
| XF17F         | hsp-3 (1-19aa) (F) | ATG AAG AC TCT TTT TTT TTT TTT GCT GCG GCT GAG ATG AAT ATT TTT AATA TTA | pJF7                   |
| XF17R         | hsp-3 (1-19aa) (R) | ATG AAG AC TCT TTT TTT TTT TTT GCT GCG GCT GAG ATG AAT ATT TTT AATA TTA | pJF5                   |
| spe-11        | spe-11      | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSDH60                 |
| (SAPC1) F     |             |                                   |                       |
| spe-11        | spe-11      | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSDH61                 |
| (SAPC1) R     |             |                                   |                       |
| XF24F         | halotag (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSF89                  |
| XF24R         | halotag (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSF222                 |
| XF63F         | mkate2 (F)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF88                  |
| XF63R         | mkate2 (R)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF130                 |
| ePDZ          | epdz (F)    | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF130                 |
| ePDZ          | epdz (R)    | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF130                 |
| XF79F         | cox-4 (F)   | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF262                 |
| XF79R         | cox-4 (R)   | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF250                 |
| XF76F         | npp-20 (F)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF250                 |
| XF76R         | npp-20 (R)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF250                 |
| XF23F         | halotag (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF250                 |
| XF23R         | halotag (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF250                 |
| XF53F         | mkate2 (F)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF130                 |
| XF53R         | mkate2 (R)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF130                 |
| XF22F         | halotag: hdel (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF90                  |
| XF22R         | halotag: hdel (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF90                  |
| SiM8F         | mscarlet (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM10                   |
| SiM8R         | mscarlet (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM12                   |
| SiM10F        | dendra2 (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM21                   |
| SiM10R        | dendra2 (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM21                   |
| SiM11F        | dendra2 (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM23                   |
| SiM11R        | dendra2 (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM23                   |
| SiM2F         | mkate2 (F)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM01                   |
| SiM2R         | mkate2 (R)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM01                   |
| SiM3F         | mkate2 (F)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM02                   |
| SiM3R         | mkate2 (R)  | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM02                   |
| SiM6F         | mscarlet (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM08                   |
| SiM6R         | mscarlet (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSM08                   |
| mScarlet      | mscarlet (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSDH57                  |
| mScarlet      | mscarlet (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSDH57                  |
| XF84F         | lov (F)     | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF76                   |
| XF84R         | lov (R)     | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF76                   |
| XF12F         | pie-1 3’ UTR (F) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF85                   |
| XF12R         | pie-1 3’ UTR (R) | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pXF85                   |
| tbb2 3’ UTR   | (SAPC5)F    | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSDH54                  |
| tbb2 3’ UTR   | (SAPC5)R    | GCA GCC CTT CAC ATG GCC AGA CAAT ATG CTT CTG GAT CTA GTC | pSDH54                  |

(continued)
the mitochondrial outer membrane. 11 of 15 assembled plasmids had the corrected restriction digest pattern and 2 of 2 of these plasmids were correct by Sanger sequence analysis of the cassette junctions. A TOMM-20::HaloTag::LOV strain was crossed with a strain in which the dynein heavy chain DHC-1 was fused to ePDZ (Fielmich et al. 2018). Whereas mitochondria in wild-type embryos are dispersed

### Table 3, continued

| Name (SAP C3/F) | Description | Sequence (SAP 1 site and Overhang) | Corresponding plasmid |
|-----------------|-------------|------------------------------------|-----------------------|
| Halo halotag (F) | GCCGCTCTTCGGTCCGGAGATCTCGAGGTTGG | pSDH51 |
| Halo halotag (R) | GCCGCTCTTCGGTCCGGAGATCTCGAGGTTGG | pSDH52 |
| ePDZ epdz (F)   | GCCGCTCTTCAACGGGAGATCTCGAGGTTGG | pSDH6 |
| ePDZ epdz (R)   | GCCGCTCTTCAACGGGAGATCTCGAGGTTGG | pSDH6 |
| unc-54 (SAPC5)F | GCCGCTTTGCTAAAGGCTTCCGATCGGCTG | pSDH66 |
| unc-54 (SAPC5)R | GCCGCTTTGCTAAAGGCTTCCGATCGGCTG | pSDH66 |
| Eg717           | TCGAGTGCGAAGAGCCATGGATCTCAGGCTCTCGTAA | pXF87 |
| Eg718           | CTAGTACGAAGAGGGAGATCTCGAGGTTGG | pXF87 |
| XF30F           | GATTATGGGCACTTCTTTATCC | pXF87 |
| XF30R           | CGACAGAAGACTTCTTTATCC | pXF87 |
| XF31F           | AATGGCGAAGTCAAGGCGAGG | pXF87 |
| XF31R           | GTTCCTGAAATAATGAATCTGGATMG | pXF87 |

Note: For the expression plasmid pJF13 annealed oligos were used to generate HSP-3(aa 1-19) in cassette 2. Additional oligo sequences used to generate pSDH50: TOMM-20 short forward. GCGGCTCTTCGGTCCGGAGATCTCGAGGTTGGCCAGGTAGACCATCGAGGCTG.

### Table 4 MosSCI targeting vectors used in this study

| Name | Comments | Assembly |
|------|----------|----------|
| pXF87 | MosSCI backbone | Derived from pCFJ350 |

Donor vectors used for assembly

| Donor vectors used for assembly | Cassette 1 | Cassette 2 | Cassette 3 | Cassette 4 |
|---------------------------------|-----------|-----------|-----------|-----------|
| pXF121                           | pXF17/F/R | pXF90     | pXF85     |
| pJF17                           | pXF121    | pJF7      | pXF88     |
| pXF253                          | pXF250    | pJF6      | pXF85     |
| pXF255                          | pXF250    | pJF6      | pXF85     |
| pSM20                          | pXF250    | pJF7      | pXF130    |
| pSM22                          | pXF250    | pJF7      | pXF130    |
| pSM17                          | pXF250    | pJF7      | pXF130    |
| pSM16                          | pXF250    | pJF7      | pXF130    |

Assembly efficiency

| Digestion | Sequencing |
|-----------|------------|
| 4/5       | 2/2        |
| 4/5       | 1/2        |
| 4/6       | 2/2        |
| 5/6       | 2/2        |
| 1/4       | 1/1        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |

Note: For the expression plasmid pJF13 annealed oligos were used to generate HSP-3(aa 1-19) in cassette 2.

### Table 3 MosSCI targeting vectors used in this study

| Name | Comments | Assembly |
|------|----------|----------|
| pXF87 | MosSCI backbone | Derived from pCFJ350 |

Donor vectors used for assembly

| Donor vectors used for assembly | Cassette 1 | Cassette 2 | Cassette 3 | Cassette 4 |
|---------------------------------|-----------|-----------|-----------|-----------|
| pXF121                           | pXF17/F/R | pXF90     | pXF85     |
| pJF17                           | pXF121    | pJF7      | pXF88     |
| pXF253                          | pXF250    | pJF6      | pXF85     |
| pXF255                          | pXF250    | pJF6      | pXF85     |
| pXF266                          | pXF266    | pJF6      | pXF85     |
| pSM20                           | pXF250    | pJF7      | pXF130    |
| pSM22                           | pXF250    | pJF7      | pXF130    |
| pSM17                           | pXF250    | pJF7      | pXF130    |
| pSM16                           | pXF250    | pJF7      | pXF130    |

Assembly efficiency

| Digestion | Sequencing |
|-----------|------------|
| 4/5       | 2/2        |
| 4/5       | 1/2        |
| 4/6       | 2/2        |
| 5/6       | 2/2        |
| 1/4       | 1/1        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |

Note: For the expression plasmid pJF13 annealed oligos were used to generate HSP-3(aa 1-19) in cassette 2.

### Table 4 MOSCI targeting vectors used in this study

| Name | Comments | Assembly |
|------|----------|----------|
| pXF87 | MosSCI backbone | Derived from pCFJ350 |

Donor vectors used for assembly

| Donor vectors used for assembly | Cassette 1 | Cassette 2 | Cassette 3 | Cassette 4 |
|---------------------------------|-----------|-----------|-----------|-----------|
| pXF121                           | pXF17/F/R | pXF90     | pXF85     |
| pJF17                           | pXF121    | pJF7      | pXF88     |
| pXF253                          | pXF250    | pJF6      | pXF85     |
| pXF255                          | pXF250    | pJF6      | pXF85     |
| pXF266                          | pXF266    | pJF6      | pXF85     |
| pSM20                           | pXF250    | pJF7      | pXF130    |
| pSM22                           | pXF250    | pJF7      | pXF130    |
| pSM17                           | pXF250    | pJF7      | pXF130    |
| pSM16                           | pXF250    | pJF7      | pXF130    |

Assembly efficiency

| Digestion | Sequencing |
|-----------|------------|
| 4/5       | 2/2        |
| 4/5       | 1/2        |
| 4/6       | 2/2        |
| 5/6       | 2/2        |
| 1/4       | 1/1        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |
| 4/5       | 2/2        |

Note: For the expression plasmid pJF13 annealed oligos were used to generate HSP-3(aa 1-19) in cassette 2.

Additional oligo sequences used to generate pSDH50:

**TOMM-20 short forward.** GCGGCTCTTCGGTCCGGAGATCTCGAGGTTGGCCAGGTAGACCATCGAGGCTG.

**TOMM-20 short reverse** GCGGCTCTTCGGTCCGGAGATCTCGAGGTTGGCCAGGTAGACCATCGAGGCTG.
through the cytoplasm (Figure 4A), upon the recruitment of ePDZ:mCherry::DHC-1 to mitochondria by stimulation with 488 nm light, mitochondria were transported onto centrosomes, leaving the peripheral cytoplasm largely devoid of mitochondria (Figure 4B).

**DISCUSSION**

The SapTrap system described here provides an efficient and simple method for the assembly of MosSCI targeting vectors. This approach is similar to the Gateway assembly system (ThermoFisher Scientific) in that once donor cassette plasmids are cloned, they can be assembled in any modular combination. The Gateway system has been widely used to generate MosSCI transgenes and is attractive because there are large collections of promoter, ORF, and 3’UTR donor plasmids available (Brasch et al. 2004; Dupuy et al. 2004; Mangone et al. 2010; Zeiser et al. 2011). However, the Gateway system has disadvantages, including i) ~25 bp att recombination sites present between each cassette after assembly, ii) the cost of proprietary enzyme mixes, and iii) the difficulty in assembling more than four cassettes together. In contrast, the SapTrap system i) uses three-base pair junctions, two of which are designed to encode the translation start and STOP codons, ii) is relatively inexpensive, and iii) can efficiently assemble at least 5 cassettes. In principle, the number of cassettes could be increased if desired. The most significant consideration in generating new donor cassette plasmids for SapTrap assembly is that internal SapI sites cannot be present within the donor cassette sequence. Gibson cloning also allows the “scar-free” cloning of transgene vectors, but the specific cloning strategies must be designed for each unique vector. While we have focused on generating transgenes expressed in the hermaphrodite germline, the MosSCI
Figure 2 Images of transgenic strains. A. Images of TOMM-20::HaloTag labeled with JF646 HaloTag ligand in the adult gonad (outlined with curved dotted line), including an inset of the region in the stippled box. B. Images of embryos expressing TOMM-20::HaloTag labeled with JF646 HaloTag ligand at the 1-cell, 4 cell and ~100 cell stages. C. Images of a 4 cell embryo expressing TOMM-20::HaloTag labeled with JF646 HaloTag ligand (magenta) and COX-4::GFP (green) (Raiders et al., 2018). D – F. Images of embryos expressing the indicated transgenes at the 4-cell stage. G – I. Images of a 4 cell embryo expressing TOMM-20::Dendra2 before and after photoconversion (PC). Dendra2 switches from green to red fluorescence upon photoconversion. J – M. Images of embryos expressing the indicated transgenes at the 1-cell, 4 cell and ~100 cell stages.
targeting vector pXF87, the gene tag donor cassettes and cloning approach described here should be readily adaptable to expressing transgenes in other tissues.

The advantages of tagging and fluorescently labeling proteins with the HaloTag include increased brightness and photostability (especially compared to red fluorescent proteins) and excellent optical pairing with

Figure 3  SapTrap assembly of MosSCI targeting vectors using the five-cassette system. A. Schematic of pXF87 and the donor cassettes following SapI digestion. The dotted lines indicate the overhangs that anneal during ligation. B. Summary of available promoter, gene tag and 3'UTR donor cassette plasmids for the five-cassette system.

targeting vector pXF87, the gene tag donor cassettes and cloning approach described here should be readily adaptable to expressing transgenes in other tissues.

Figure 4 Optogenetic control of mitochondrial distribution in the 1-cell embryo. A. Control embryo stained with Mitotracker DeepRed and imaged with 488 nm and 640 nm illumination (640 nm channel shown). B. 1-cell epdz::mcherry::dhc-1; tomm-20::halotag::lov embryo stained with Mitotracker DeepRed and imaged with 488 nm and 640 nm illumination (640 nm channel shown). The 488 nm illumination was used to stimulate the interaction between the ePDZ and LOV domains.
green fluorescent proteins for 2-color imaging. Additionally, HaloTag labeling offers the flexibility to label a single strain with either JF549 HaloTag ligand or JF646 HaloTag ligand (Grimm et al. 2015). The disadvantages of HaloTag labeling include the need to introduce the fluorescent ligand (for example, using small scale liquid culture) and the cost of the ligand. Additionally, care should be taken to optimize labeling procedures for each protein to maximize labeling efficiency and minimize background from free ligand. In practice, we find that HaloTag labeling is particularly useful when photobleaching of conventional fluorescent proteins is limiting and/or when imaging in far red is advantageous.

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