

1 **SapTrap assembly of *C. elegans* MosSCI transgene vectors**

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24 **Abstract**

25 The Mos1-mediated Single-Copy Insertion (MosSCI) method is widely used to establish stable
26 *Caenorhabditis elegans* transgenic strains. Cloning MosSCI targeting plasmids can be
27 cumbersome because it requires assembling multiple genetic elements including a promoter, a
28 3'UTR and gene fragments. Recently, Schwartz and Jorgensen developed the SapTrap method
29 for the one-step assembly of plasmids containing components of the CRISPR/Cas9 system for *C.*
30 *elegans* (Schwartz and Jorgensen 2016 Genetics, 202:1277-1288). Here, we report on the
31 adaptation of the SapTrap method for the efficient and modular assembly of a promoter, 3'UTR
32 and either 2 or 3 gene fragments in a MosSCI targeting vector in a single reaction. We generated
33 a toolkit that includes several fluorescent tags, components of the ePDZ/LOV optogenetic
34 system and regulatory elements that control gene expression in the *C. elegans* germline. As a
35 proof of principle, we generated a collection of strains that fluorescently label the endoplasmic
36 reticulum and mitochondria in the hermaphrodite germline and that enable the light-stimulated
37 recruitment of mitochondria to centrosomes in the one-cell worm embryo. The method described
38 here offers a flexible and efficient method for assembly of custom MosSCI targeting vectors.

39 **Introduction**

40 The rich toolbox of techniques available to manipulate gene expression in *C. elegans* is a
41 major attraction of this model organism. Several approaches have been developed to introduce
42 transgenes and to induce efficient CRISPR/Cas9 mediated gene editing (Nance and Frøkjær-
43 Jensen, 2019). The Mos1-mediated Single-Copy Insertion (MosSCI) method has been widely
44 adopted to introduce transgenes in *C. elegans* because single-copy transgenes are integrated at
45 defined chromosomal positions, thereby mitigating potential concerns of transgene integration at
46 random positions (Frøkjær-Jensen et al., 2012; Frøkjær-Jensen et al., 2008; Frøkjær-Jensen et al.,
47 2014). MosSCI transgene integration results from homologous recombination between a MosSCI
48 targeting vector containing the transgene construct and one of the safe-harbor integration sites
49 that have been engineered at defined positions in the genome.

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

62 Schwartz and Jorgensen recently developed the SapTrap method for efficient, modular
63 and single step assembly of CRISPR/Cas9 vectors for *C. elegans* (Schwartz and Jorgensen,
64 2016). The SapTrap method is based on the Golden Gate cloning technique (Engler et al., 2008)
65 and takes advantage of the SapI type II restriction enzyme, which cuts DNA at defined positions
66 adjacent to its recognition sequence to generate three-base 5' overhangs. By designing SapI
67 restriction fragments with complementary overhangs, multiple fragments can be assembled
68 together in a defined order in a single digestion and ligation reaction. In this study, we report on
69 the adaptation of the SapTrap system for the efficient, inexpensive, modular, and “scar-free”
70 assembly of transgenes in a MosSCI targeting vector. We have developed a toolkit for
71 expression of transgenes in the *C. elegans* germline, including a collection of cassettes
72 containing tags for fluorescence imaging and for the ePDZ/LOV optogenetic system (Fielmich et
73 al., 2018; Strickland et al., 2012). As a proof of principle, we have used this system to generate a
74 collection of mitochondrial and endoplasmic reticulum reporter strains and a strain in which light
75 induces the transport of mitochondria to centrosomes in the one-cell worm embryo.

76

77 **Results and Discussion**

78 *Adaptation of the SapTrap system for cloning MosSCI targeting vectors*

79 To adapt the SapTrap approach (Schwartz and Jorgensen, 2016) for the assembly of
80 MosSCI targeting vectors, we started by making two changes to the universal MosSCI targeting
81 vector pCFJ350 (Frøkjær-Jensen et al., 2012), which targets transgenes for insertion at the
82 commonly used *tTi5605* site (Frøkjær-Jensen et al., 2008). First, we introduced single base pair
83 changes to disrupt the two SapI restriction sites located in the “Left” and “Right” homology arms
84 of pCFJ350. Second, we inserted two SapI sites into the multiple cloning site that were oriented

85 such that they are removed from the vector backbone by digestion with SapI. The resulting
86 MosSCI targeting vector was named pXF87 (Figure 1A).

87
88 We next cloned a series of plasmids that contain donor cassettes flanked by SapI
89 restrictions sites (Figure 1B). Following digestion with SapI, the cassettes are liberated from the
90 vector backbone and are flanked by 5' overhangs that direct their order of assembly in pXF87
91 (Figure 1C). A four-insert cassette system was designed with a promoter in cassette 1, gene
92 fragments in cassettes 2 and 3 (typically a gene and a tag) and a 3'UTR in cassette 4. To
93 minimize the inclusion of extraneous sequences, the junctions between the first and second
94 cassette is the translation start (ATG), between second and third cassettes is glycine (GGT) and
95 between the third and fourth cassettes is the ochre translation stop codon (TAA) (Figure 1C).
96 Donor cassettes encoding tags (such as fluorescent proteins) include short flexible linkers at the
97 protein fusion site (the carboxy terminus of cassette 2 and the amino terminus of cassette 3)
98 (Supplemental Figure S1- S7). The currently available promoter, tag and 3'UTR donor cassette
99 plasmids are listed in Figure 1E and Table 1.

100
101 The *C. elegans* germline is a notoriously difficult tissue in which to achieve stable
102 transgene expression due to silencing of multi-copy extra-chromosomal arrays (Kelly et al.,
103 1997), single-copy insertions generated by MosSCI (*e.g.*, (Frøkjær-Jensen et al., 2016;
104 Shirayama et al., 2012)) or endogenous genes tagged using CRISPR/Cas9 gene editing (*e.g.*,
105 (Fielmich et al., 2018)). Each of our tag donor cassettes encoding gene tags incorporates at least
106 one modification that buffers against silencing, including the inclusion of PATC introns in
107 HaloTag and ceGFP (Frøkjær-Jensen et al., 2016), the elimination of piRNA binding sites in

108 mScarlet, mKate2 and Dendra2 (Seth et al., 2018; Zhang et al., 2018) and the use of sequence
109 motifs found in native germline genes in ePDZ and the LOV domain (Fielmich et al., 2018).

110

111 Similar to the SapTrap method developed by Schwartz and Jorgensen (Schwartz and
112 Jorgensen, 2016), MosSCI targeting vectors were assembled in a single tube by incubating
113 pXF87, four donor cassette plasmids, SapI enzyme, ATP and T4 DNA ligase at 25°C for 22 - 24
114 hours (Figure 1D and Materials and Methods). This reaction was then transformed into *E. coli*
115 and plasmid clones were screened by restriction enzyme digestion followed by sequencing. We
116 assembled nine vectors using the 4-cassette system and 32 of 46 (69.6%) of the plasmids
117 screened had the correct restriction digest pattern (Table 2). Of the vectors with the correct
118 restriction digest pattern, 22 of 23 were correct based on Sanger sequencing analysis. Therefore,
119 the SapTrap method provides an efficient method for the assembly of MosSCI targeting vectors.

120

121 *A collection of fluorescent ER and mitochondria strains*

122 We used SapTrap-assembled MosSCI targeting vectors to generate a collection of
123 transgenic strains for analysis of endoplasmic reticulum and mitochondrial dynamics. We first
124 targeted GFP, mKate2, mScarlet, Dendra2 and HaloTag to the cytoplasmic face of the
125 mitochondrial outer membrane by fusing them to the carboxy terminus of TOMM-20. The
126 expression of these transgenes was controlled by the *mex-5* promoter and by the *pie-1* 3'UTR,
127 which results in germline expression that increases around the bend of the adult hermaphrodite
128 gonad (Merritt et al., 2008) (Figure 2A). Strains expressing TOMM-20 fused to HaloTag were
129 labeled with the fluorescent JF₆₄₆ HaloTag ligand (Grimm et al., 2015) by feeding
130 hermaphrodites bacteria mixed with the ligand. Each TOMM-20 fusion protein exhibited the

131 expected tubular localization pattern in the early embryo (Figure 2B-I). We confirmed that
132 TOMM-20::HaloTag colocalized to the same organelle as the mitochondrial matrix protein
133 COX-4::GFP (Raiders et al., 2018) (Figure 2C). We additionally generated strains in which the
134 HaloTag was targeted to the mitochondrial matrix (COX-4::HaloTag) (Figure 2J) and the lumen
135 of the endoplasmic reticulum (HSP-70(aa1-19)::HaloTag::HDEL) (Figure 2K). We fused both
136 GFP and HaloTag to NPP-20, the worm homologue of SEC13, which is both a component of the
137 COPII coat that concentrates to ER exit sites (ERES) (D'Arcangelo et al., 2013) and a component
138 of nuclear pore complexes (Siniosoglou et al., 1996) (Figure 2L, M).

139

140 **Five-cassette system**

141 One of the advantages of the SapTrap approach is that it can be easily expanded to
142 include additional insert fragments to create more complex transgenes. To establish a five-
143 cassette system, we used the cassettes 1, 2 and 4 from the four-cassette system and replaced
144 cassette 3 with cassettes 3A and 3B (Figure 3A). We used this approach to generate an
145 optogenetic system to control the localization of mitochondria in the early embryo based on the
146 light induced interaction between the ePDZ and LOV domains (Fielmich et al., 2018; Strickland
147 et al., 2012). We assembled a MosSCI targeting vector that directed expression of TOMM-
148 20::HaloTag::LOV, which targets the LOV domain to the mitochondrial outer membrane. 11 of
149 15 assembled plasmids had the corrected restriction digest pattern and 2 of 2 of these plasmids
150 were correct by sequence analysis. A TOMM-20::HaloTag::LOV strain was crossed with a strain
151 in which the dynein heavy chain DHC-1 was fused to ePDZ (Fielmich et al., 2018). Whereas
152 mitochondria in wild-type embryos are dispersed through the cytoplasm (Figure 4A), upon the
153 recruitment of ePDZ::mCherry::DHC-1 to mitochondria by stimulation with 488 nm light,

154 mitochondria were transported on to centrosomes, leaving the peripheral cytoplasm largely
155 devoid of mitochondria (Figure 4B).

156

157 The SapTrap system described here provides an efficient and simple method for the
158 assembly of MosSCI targeting vectors. This approach is similar to the Gateway assembly system
159 (ThermoFisher Scientific) in that once donor cassette plasmids are cloned, they can be assembled
160 in any modular combination. The Gateway system has been widely used to generate MosSCI
161 transgenes and is attractive because there are large collections of promoter, ORF, and 3'UTR
162 donor plasmids available (Brasch et al., 2004; Dupuy et al., 2004; Mangone et al., 2010; Zeiser et
163 al., 2011). However, the Gateway system has disadvantages, including i) ~25 bp *att*
164 recombination sites present between each cassette after assembly, ii) the cost of proprietary
165 enzyme mixes, and iii) the difficulty in assembling more than four cassettes together. In contrast,
166 the SapTrap system i) uses three-base pair junctions, two of which are designed to encode the
167 translation start and STOP codons, ii) is relatively inexpensive, and iii) can efficiently assemble
168 at least 5 cassettes. In principle, the number of cassettes could be increased if desired. The most
169 significant consideration in generating new donor cassette plasmids for SapTrap assembly is that
170 internal SapI sites cannot be present within the donor cassette sequence. Gibson cloning also
171 allows the “scar-free” cloning of transgene vectors, but the specific cloning strategies must be
172 designed for each unique vector. While we have focused on generating transgenes expressed in
173 the hermaphrodite germline, the MosSCI targeting vector pXF87, the gene tag donor cassettes
174 and cloning approach described here should be readily adaptable to expressing transgenes in
175 other tissues.

176

177 The advantages of tagging and fluorescently labeling proteins with the HaloTag include
178 increased brightness and photostability (especially compared to red fluorescent proteins) and
179 excellent optical pairing with green fluorescent proteins for 2-color imaging. Additionally,
180 HaloTag labeling offers the flexibility to label a single strain with either JF₅₄₉ HaloTag ligand or
181 JF₆₄₆ HaloTag ligand (Grimm et al., 2015). The disadvantages of HaloTag labeling include the
182 need to introduce the fluorescent ligand (for example, using small scale liquid culture) and the
183 cost of the ligand. Additionally, care should be taken to optimize labeling procedures for each
184 protein to maximize labeling efficiency and minimize background from free ligand. In practice,
185 we find that HaloTag labeling is particularly useful when photobleaching of conventional
186 fluorescent proteins is limiting and/or when imaging in far red is advantageous.

187

188

189 **Materials and Methods**

190 *C. elegans*

191 *C. elegans* hermaphrodite strains were maintained at either 20°C or 25°C on Nematode Growth
192 Medium (NGM) plates containing 3 g/L NaCl, 2.5 g/L peptone and 17 g/L agar supplemented
193 with 1 mM CaCl₂, 1 mM MgSO₄, 1 mM KPO₄ and 5 mg/L Cholesterol with *E. coli* OP50 as a
194 source of food. All strains used in this study are listed in Table 4.

195

196 *Cloning*

197 To generate the expression vector pXF87, the two SapI restriction sites in pCFJ350
198 (Frøkjær-Jensen et al., 2012) were mutated using Q5 Site-Directed Mutagenesis (New England

199 Biolabs) with the oligo pairs XF30F/XF30R and XF31F/XF31R. In addition, the annealed oligos
200 Eg717 and Eg718 were cloned between the XhoI and SpeI sites of pCFJ350.

201 HaloTag and ceGFP containing PATC-rich endogenous introns were generated in several
202 steps. First, genes were designed *in silico* to minimize germline silencing and increase
203 expression by codon adaptation (Redemann et al., 2011), removal of homology to piRNAs
204 (Batista et al., 2008), and inclusion of a short endogenous intron from *rpl-18* and four synthetic
205 introns (Okkema et al., 1993) using the freely available gene editor ApE (M. Wayne Davis,
206 unpublished). Second, the synthetic genes were synthesized as gBlocks (IDT), cloned into a
207 plasmid, and sequence verified. Third, PATC-rich introns from a gene that is resistant to
208 germline silencing, *smu-1* (Spike et al., 2001), were introduced into the synthetic genes by
209 Golden Gate cloning as described previously (Frøkjær-Jensen et al., 2016). Finally, correct
210 splicing and expression was verified by expression of the synthetic genes with and without
211 PATC-rich introns using an *eft-3* promoter and *tbb-2* 3'UTR.

212 Donor cassette plasmids numbered pXF, pJF and pSM were generated by cloning PCR
213 products into the pCR BluntII vector backbone using the Zero Blunt™ Topo™ system (Thermo
214 Fisher Scientific). pSDH donor cassette plasmids were cloned by ligating PCR products into
215 pSDH76, a derivative of pCR BluntII containing two XcmI sites that generate T-overhangs
216 following digestion with XcmI. PCR primers for each plasmid are listed in Table 3. pXF87 and
217 all donor plasmids were sequence verified.

218 To assemble HSP-70 (aa1-19) into the first cassette position of the expression vector
219 pXF108, 10mM of oligos XF17F and XF17R were gradually cooled from 95°C to 25°C in a
220 BioRad T1000 thermocycler. Annealed oligos were phosphorylated by T4 polynucleotide kinase

221 (NEB) for two hours at 37°C followed by 65°C for 20 minutes. The donor and primers plasmids
222 are listed in Tables 1 and 3, respectively.

223

224 *Assembly reaction*

225 Assembly reactions 50 μ L included 1 nM of pXF87 and each donor cassette plasmid, 400
226 units of T4 DNA ligase (NEB), 10 units of SapI enzyme (NEB), 1X NEB CutSmart buffer and 1
227 mM ATP. For assemblies including annealed oligos, phosphorylated annealed oligos were used
228 at a final concentration of 3 nM in the assembly reaction. Reactions were incubated for 22-24
229 hours at 25°C, transformed into Stellar Competent cells (Clontech). Four to six plasmid clones
230 were first screened by restriction digest with XhoI and SpeI. Plasmids with the correct restriction
231 digest pattern were sequenced across each cassette boundary. MosSCI targeting vector assembly
232 reactions are listed in Table 2. Note that because the background of unassembled vectors in our
233 assembly reactions was typically low, our protocol omits the counterselection restriction enzyme
234 step described in the original SapTrap protocol (Schwartz and Jorgensen, 2016).

235

236 *Transgenesis*

237 Double strand breaks at Mos1 landing sites were generated using CRISPR/Cas9. With
238 the exception of strains EGD615, EGD629, EGD631 and EGD633, injection mixes contained 50
239 ng/ μ L assembled MosSCI targeting vectors and pXW7.01 and pXW7.02 sgRNA/Cas9
240 vectors (gifts from Katya Voronina, University of Montana), which generate double strand
241 breaks at the *ttTi5605* universal MosSCI insertion site. For strains EGD615, EGD629, EGD631
242 and EGD633, injection mixes contained 0.25 μ g/ μ L Cas9 protein, 0.1 μ g/ μ L tracrRNA, 0.028
243 μ g/ μ L crRNAs BH0278

244 (GCGUCUUCGTACCUUUUUGGGUUUUAGAGCUAUGCUGUUUUG) and BH0279
245 (GUCCCAUCGAAGCGAAUAGGGUUUUAGAGCUAUGCUGUUUUG) (Dharmacon) and
246 0.1 µg/µL assembled MosSCI plasmids. The universal MosSCI strains EG8078 or
247 EG8079 (Frøkjær-Jensen et al., 2014) were injected, singled and incubated for 10 days at
248 20°C. ~10 worms from plates containing non-Unc animals were transferred to new plates. Plates
249 that stably gave rise to non-*unc* progeny were visually screened for fluorescent transgene
250 expression.

251

252 *HaloTag staining*

253 20 to 30 L4 worms were stained in 25 µL S media containing concentrated OP50 bacteria
254 and 2.5 µM of either JF₅₄₉ HaloTag ligand or JF₆₄₆ HaloTag ligand (Grimm et al., 2015) in a
255 darkened 96 well plate shaking at 150 rpm for 19 hours at 23°C. Water was placed in the
256 neighboring wells to help prevent evaporation. Animals were recovered on NGM plates for up to
257 two hours before imaging.

258

259 *MitoTracker Deep Red staining*

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

263

264 *Imaging*

265 With the exceptions of the TOMM-20::Dendra2 strain and optogenetic strains (Figure 4),
266 all images were collected on a spinning-disk microscope built on a Nikon Eclipse Ti base and

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

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

275 To stimulate the relocalization of mitochondria (Figure 4), embryos were illuminated
276 with a 50 mW 640 nm solid-state laser used to excite MitoTracker DeepRed (20% laser power,
277 100 msec exposure, camera gain of 1) and a 50 mW 488 nm solid-state laser used to stimulate
278 the interaction between ePDZ and LOV domains (80% laser power and 100 msec exposure). A
279 Plan-Apochromat 100x/1.4 NA oil immersion DIC objective (Zeiss) was used and Z-stacks (one
280 micron step size, 11 steps) were collected at 60-second intervals. The images displayed in Figure
281 4 are maximum intensity projections of three Z planes from the cell midplane.

282

283 *Data and reagent availability*

284 Strains and reagents are available upon request. Supplemental materials describing the sequence
285 of tag donor cassettes are available through the GSA FigShare portal.

286

287

288

289

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299

300

301

302 **Figure Legends**

303 **Figure 1.** SapTrap assembly of MosSCI targeting vectors using the four-cassette system. **A.** The
304 MosSCI targeting vector pXF87 was derived from pCFJ350 by mutating two SapI restriction
305 sites (indicated by arrowheads in the “Left” (L) and “Right” (R) homology arms) and introducing
306 two SapI sites (blue text) between the XhoI and SpeI sites (green text). SapI cleavage sites are in
307 red text. The SapI recognition sites are oriented such that upon digestion they are removed from
308 the vector backbone. The *cbr-unc-119* gene is used as a positive selection marker to facilitate the
309 identification of transgenic animals. **B.** Design of the donor cassette vectors used for the 4-
310 cassette cloning strategy. **C.** The curved dotted lines indicate the overhangs that anneal during
311 the ligation reaction. **D.** Overview of the assembly protocol. For a detailed protocol, see the
312 Materials and Methods section. **E.** Summary of available promoter, gene tag and 3’UTR donor
313 cassette plasmids.

314
315 **Figure 2.** Images of transgenic strains. **A.** Images of TOMM-20::HaloTag labeled with JF₆₄₆
316 HaloTag ligand in the adult gonad (outlined with curved dotted line), including an inset of the
317 region in the stippled box. **B.** Images of embryos expressing TOMM-20::HaloTag labeled with
318 JF₆₄₆ HaloTag ligand at the 1-cell, 4 cell and ~100 cell stages. **C.** Images of a 4 cell embryo
319 expressing TOMM-20::HaloTag labeled with JF₆₄₆ HaloTag ligand (magenta) and COX-4::GFP
320 (green) (Raiders et al., 2018). **D – F.** Images of embryos expressing the indicated transgenes at
321 the 4-cell stage. **G – I.** Images of a 4 cell embryo expressing TOMM-20::Dendra2 before and
322 after photoconversion (PC). Dendra2 switches from green to red fluorescence upon
323 photoconversion. **J – M.** Images of embryos expressing the indicated transgenes at the 1-cell, 4
324 cell and ~100 cell stages.

325

326 **Figure 3.** SapTrap assembly of MosSCI targeting vectors using the five-cassette system. **A.**

327 Schematic of pXF87 and the donor cassettes following SapI digestion. The dotted lines indicate

328 the overhangs that anneal during ligation. **B.** Summary of available promoter, gene tag and

329 3'UTR donor cassette plasmids for the five-cassette system.

330

331 **Figure 4.** Optogenetic control of mitochondrial distribution in the 1-cell embryo. **A.** Control

332 embryo stained with Mitotracker DeepRed and imaged with 488 nm and 640 nm illumination

333 (640 nm channel shown). **B.** 1-cell *epdz::mcherry::dhc-1; tomm-20::halotag::lov* embryo

334 stained with Mitotracker DeepRed and imaged with 488 nm and 640 nm illumination (640 nm

335 channel shown). The 488 nm illumination was used to stimulate the interaction between the

336 ePDZ and LOV domains.

337

338

339 **Literature Cited**

340 Batista, P. J., J. G. Ruby, J. M. Claycomb, R. Chiang, N. Fahlgren, *et al.*, 2008 PRG-1 and 21U-
341 RNAs interact to form the piRNA complex required for fertility in *C. elegans*. *Mol. Cell* 31: 67-78.

342
343 Brasch, M. A., J. L. Hartley, and M. Vidal, 2004 ORFeome cloning and systems biology:
344 standardized mass production of the parts from the parts-list. *Genome Res.* 14:2001-2009.

345
346 D'Arcangelo, J. G., K. R. Stahmer, and E. A. Miller, 2013 Vesicle-mediated export from the ER:
347 COPII coat function and regulation. *Biochim. Biophys. Acta* 1833:2464-2472.

348
349 Dupuy, D., Q. R. Li, B. Deplancke, M. Boxem, T. Hao, *et al.*, 2004 A first version of the
350 *Caenorhabditis elegans* Promoterome. *Genome Res.* 14: 2169-2175.

351
352 Engler, C., R. Kandzia, and S. Marillonnet, 2008 A one pot, one step, precision cloning method
353 with high throughput capability. *PLoS One* 3: e3647.

354
355 Fielmich, L. E., R. Schmidt, D. J. Dickinson, B. Goldstein, A. Akhmanova, *et al.*, 2018 Optogenetic
356 dissection of mitotic spindle positioning in vivo. *Elife* 7: e38198.

357
358 Frøkjær-Jensen, C., M. W. Davis, M. Ailion, and E. M. Jørgensen, 2012 Improved Mos1-
359 mediated transgenesis in *C. elegans*. *Nat. Methods* 9: 117-118.

360

361 Frøkjaer-Jensen, C., M. W. Davis, C. E. Hopkins, B. J. Newman, J. M. Thummel, *et al.*, 2008
362 Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nat. Genet.* 40: 1375-1383.
363
364 Frøkjaer-Jensen, C., M. W. Davis, M. Sarov, J. Taylor, S. Flibotte, *et al.*, 2014 Random and
365 targeted transgene insertion in *Caenorhabditis elegans* using a modified Mos1 transposon. *Nat.*
366 *Methods* 11: 529-534.
367
368 Frøkjaer-Jensen, C., N. Jain, L. Hansen, M. W. Davis, Y. Li, *et al.*, 2016 An Abundant Class of Non-
369 coding DNA Can Prevent Stochastic Gene Silencing in the *C. elegans* Germline. *Cell* 166: 343-
370 357.
371
372 Gibson, D. G., L. Young, R. Y. Chuang, J. C. Venter, C. A. Hutchison 3rd, *et al.*, 2009 Enzymatic
373 assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* 6: 343-345.
374
375 Grimm, J. B., B. P. English, J. Chen, J. P. Slaughter, Z. Zhang, *et al.*, 2015 A general method to
376 improve fluorophores for live-cell and single-molecule microscopy. *Nat. Methods* 12: 244-250.
377
378 Hartley, J. L., G. F. Temple, and M. A. Brasch, 2000 DNA cloning using in vitro site-specific
379 recombination. *Genome Res.* 10: 1788-1795.
380 Kelly, W. G., S. Xu, M. K. Montgomery, and A. Fire, 1997 Distinct requirements for somatic and
381 germline expression of a generally expressed *Caenorhabditis elegans* gene. *Genetics* 146: 227-
382 238.

383

384 Mangone, M., A. P. Manoharan, D. Thierry-Mieg, J. Thierry-Mieg, T. Han, *et al.*, 2010 The
385 landscape of *C. elegans* 3'UTRs. *Science* 329: 432-435.

386

387 Merritt, C., D. Rasoloson, D. Ko, and G. Seydoux, 2008 3' UTRs are the primary regulators of
388 gene expression in the *C. elegans* germline. *Curr. Biol.* 18: 1476-1482.

389

390 Nance, J., and C. Frøkjær-Jensen. (2019). The *Caenorhabditis elegans* Transgenic Toolbox.
391 *Genetics* 212, 959-990.

392

393 Okkema, P. G., S. W. Harrison, V. Plunger, A. Aryana, and A. Fire, 1993 Sequence requirements
394 for myosin gene expression and regulation in *Caenorhabditis elegans*. *Genetics* 135: 385-404.

395

396 Philip, N. S., F. Escobedo, L. L. Bahr, B. J. Berry, and A. P. Wojtovich, 2019 Mos1 Element-
397 Mediated CRISPR Integration of Transgenes in *Caenorhabditis elegans*. *G3* 9: 2629-2635.

398

399 Raiders, S.A., M. D. Eastwood, M. Bacher, and J. R. Priess, 2018 Binucleate germ cells in
400 *Caenorhabditis elegans* are removed by physiological apoptosis. *PLoS Genet.* 14: e1007417.

401

402 Redemann, S., S. Schloissnig, S. Ernst, A. Pozniakowsky, S. Ayloo, *et al.*, 2011 Codon adaptation-
403 based control of protein expression in *C. elegans*. *Nat. Methods* 8: 250-252.

404

405 Schwartz, M. L., and E. M. Jorgensen 2016 SapTrap, a Toolkit for High-Throughput CRISPR/Cas9
406 Gene Modification in *Caenorhabditis elegans*. Genetics 202: 1277-1288.

407

408 Seth, M., M. Shirayama, W. Tang, E. Z. Shen, S. Tu, *et al.*, 2018 The Coding Regions of Germline
409 mRNAs Confer Sensitivity to Argonaute Regulation in *C. elegans*. Cell Rep. 22: 2254-2264.

410

411 Shirayama, M., M. Seth, H. C. Lee, W. Gu, T. Ishidate, *et al.*, 2012 piRNAs initiate an epigenetic
412 memory of nonself RNA in the *C. elegans* germline. Cell 150: 65-77.

413

414 Siniosoglou, S., C. Wimmer, M. Rieger, V. Doye, H. Tekotte, *et al.*, 1996 A novel complex of
415 nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear
416 pores. Cell 84: 265-275.

417

418 Spike, C. A., J. E. Shaw, and R. K. Herman, 2001 Analysis of *smu-1*, a gene that regulates the
419 alternative splicing of *unc-52* pre-mRNA in *Caenorhabditis elegans*. Mol. Cell Biol. 21: 4985-
420 4995.

421

422 Strickland, D., Y. Lin, E. Wagner, C. M. Hope, J. Zayner, *et al.*, 2012 TULIPs: tunable, light-
423 controlled interacting protein tags for cell biology. Nat Methods 9: 379-384.

424

425 Zeiser, E., C. Frøkjaer-Jensen, E. Jorgensen, and J. Ahringer, 2011 MosSCI and gateway
426 compatible plasmid toolkit for constitutive and inducible expression of transgenes in the C.
427 elegans germline. PLoS One 6: e20082.
428
429 Zhang, D., S. Tu, M. Stubna, W. S. Wu, W. C. Huang *et al.*, 2018 The piRNA targeting rules and
430 the resistance to piRNA silencing in endogenous genes. Science 359: 587-592.

Table 1. Donor cassette plasmids used in this study.

Name	Description
Cassette 1 for 4-cassette or 5 cassette system (5'-TGG 3'-TAC)	
pXF121	<i>mex-5</i> promoter
pSDH60	<i>spe-11</i> promoter
Cassette 2 for 4-cassette or 5-cassette system (5'-ATG-3' 3'-CCA-5')	
Tags	
pXF89	<i>halotag</i> (no STOP codon, PATC introns)
pJF5	<i>gfp</i> (no STOP codon, PATC introns)
pXF222	<i>mkate2</i> (no STOP codon)
pSDH61	<i>epdz</i> (no STOP codon)
pSM10	<i>mscarlet</i> (no STOP codon)
pSM12	<i>dendra2</i> (no STOP codon)
Genes	
pJF7	<i>tomm-20</i> (no STOP codon)
pSDH50	<i>tomm-20</i> (aa1-55) (no STOP codon)
pXF262	<i>cox-4</i> (no STOP codon)
pXF250	<i>npp-20</i> (no STOP codon)
Cassette 3 for 4-cassette system (5'-GGT-3' 3'-ATT-5')	
Tags	
pXF88	<i>halotag</i> (includes STOP codon, PATC introns)
pJF6	<i>gfp</i> (includes STOP codon, PATC introns)
pXF130	<i>mkate2</i> (includes STOP codon)
pSM08	<i>mscarlet</i> (includes STOP codon)
pSM03	<i>dendra2</i> (includes STOP codon)
ORFs	
pXF90	<i>halotag::HDEL</i> (includes STOP codon, PATC introns)
Cassette 3A for 5-cassette system (5'-GGT-3' 3'-TGC-5')	
Tags	
pSDH51	<i>halotag</i> (no STOP codon, PATC introns)
pSM04	<i>mkate2</i> (no STOP codon)
pSDH57	<i>mscarlet</i> (no STOP codon)
Cassette 3B for 5-cassette system (5'-ACG-3' 3'-ATT-5')	
Tags	
pXF276	<i>lov</i> domain (includes STOP codon)
pSDH52	<i>epdz</i> (includes STOP codon)
pSM05	<i>mkate2</i> (includes STOP codon)
Cassette 4 for 4-cassette or 5-cassette system (5'-TAA-3' 3'-CAT-5')	
pXF85	<i>pie-1</i> 3'UTR
pSDH54	<i>tbb-2</i> 3'UTR
pSDH66	<i>unc-54</i> 3'UTR

Note: For the expression plasmid pXF108, the annealed oligos were used to generate HSP-70 in cassette 2.

Table 2. MosSCI targeting vectors used in this study.

Name	Comments	Assembly				Assembly efficiency		
pXF87	MosSCI backbone	Derived from pCFJ350						
		Donor vectors used for assembly				Assembly efficiency		
		Cassettes				Digestion	Sequencing	
		1	2	3	4			
pJF13	ER lumen, Halotag	pXF121	XF17F/R*	pXF90	pXF85	4/5	2/2	
pJF17	Mitochondrial OM, Halotag	pXF121	pJF7	pXF88	pXF85	4/5	1/2	
pXF253	ERES + nuclear pores (NPP-20), GFP	pXF121	pXF 250	pJF6	pXF85	4/6	2/2	
pXF255	ERES + nuclear pores (NPP-20), Halotag	pXF121	pXF 250	pXF88	pXF85	5/6	2/2	
pXF266	Mitochondrial matrix, Halotag	pXF121	pXF 262	pXF88	pXF85	1/4	1/1	
pSM20	Mitochondrial OM, mKate2	pXF121	pJF7	pXF130	pXF85	4/5	2/2	
pSM22	Mitochondrial OM, mScarlet	pXF121	pJF7	pSM08	pXF85	4/5	2/2	
pSM17	Mitochondrial OM, Dendra2	pXF121	pJF7	pSM03	pXF85	4/5	2/2	
pSM16	Mitochondrial OM, GFP	pXF121	pJF7	pJF6	pXF85	2/5	2/2	
		1	2	3A	3B	4		
pSDH68	Mitochondrial OM, Halotag, LOV	pXF121	pSDH50	pSDH51	PCR fragment	pSDH54	11/15	2/2

* Annealed oligos.

Table 3. Primers used in this study.

Name	Description	Sequence (SAP1 site and Overhang)	Corresponding plasmid
XF32F	<i>mex-5 promoter</i> (F)	GCAGCTCTTCGTTGGATATCAGTITTTAAAAAATA	pXF121
XF32R	<i>mex-5 promoter</i> (R)	GCAGCTCTTCGATTCTCTGTCTGAAACA	
JF5F	<i>tomm-20</i> (F)	GCAGCTCTTCGATGTCGGACACAATTCTTGG	pJF7
JF5R	<i>tomm-20</i> (R)	GCAGCTCTTCGACCCTCCAAGTCGTCGGTGTCT	
JF1F	<i>gfp</i> (F)	GCAGCTCTTCGATGTCGAAGTAACACTTAGTTT	pJF5
JF1R	<i>gfp</i> (R)	GCAGCTCTTCGACCGCCGCTTCCCTGTAGAGCTCGTCCAT	
JF2F	<i>gfp</i> (F)	GCAGCTCTTCGGGTGGAAGCGGCTCAAGAACAATTAGTTT	pJF6
JF2R	<i>gfp</i> (R)	GCAGCTCTTCGTTACTTGTAGAGCTCGTCCAT	
XF17F	<i>hsp-70 (1-19aa)</i> (F)	ATGAAGACCTTATCTTGTGGGCTTGATCGCCCTATCCGCCGTCAGTGTCTACTGC	
XF17R	<i>hsp-70 (1-19aa)</i> (R)	ACCGCAGTAGACACTGACGGCGGATAGGGCGATCAAGCCCAACAAGAATAAGGTCTT	
spe-11(SAP C1) F	<i>spe-11 promoter</i> (F)	GCAGCTCTTCGTTGGTCCGACAGAACAATTTTCCGT	pSDH60
spe-11(SAP C1) R	<i>spe-11 promoter</i> (R)	GCAGCTCTTCGATTATTTACTAGTCGGTTTGGCA	
XF24F	<i>halotag</i> (F)	GCAGCTCTTCGATGGCCGAGGTAACACTTAGTTTGT	pXF89
XF24R	<i>halotag</i> (R)	GCAGCTCTTCGACCGCCGCTTCTCCGGAGATCTCGAGGGT	
XF63F	<i>mkate2</i> (F)	GCAGCTCTTCGATGGTCTCCGAGCTCATAAAGAAAACA	pXF222
XF63R	<i>mkate2</i> (R)	GCAGCTCTTCGACCACTCCACCTCCACGGTGTCCGAGCTTGG	
ePDZ (SAP C2) F	<i>epdz</i> (F)	GCAGCTCTTCGATGCCAGAGCTCGGATTTCTCAT	pSDH61
ePDZ (SAP C2) R	<i>epdz</i> (R)	GCAGCTCTTCGACAGCTCCCGTCCGACGGGTGGATCAC	
XF79F	<i>cox-4</i> (F)	GCAGCTCTTCGATGATGCTGCCACGTTT	pXF262
XF79R	<i>cox-4</i> (R)	GCAGCTCTTCGACCTTCCACTTCTTGTTCGTAATC	
XF76F	<i>npp-20</i> (F)	GCAGCTCTTCGATGACCACGGTCCGCCAG	pXF250
XF76R	<i>npp-20</i> (R)	GCAGCTCTTCGACCTTCTGTAGCTCCCGAGCT	
XF23F	<i>halotag</i> (F)	GCAGCTCTTCGGGTGGAAGCGCCGAGGTAACACTTAGTTTGT	pXF88
XF23R	<i>halotag</i> (R)	GCAGCTCTTCGTTATCCGGAGATCTCGAGGGT	
XF53F	<i>mkate2</i> (F)	GCAGCTCTTCGGGTGGAGGTGGAGGTGTCTCCGAGCTCATAAAGAAAACA	pXF130
XF53R	<i>mkate2</i> (R)	GCAGCTCTTCGTTAACGGTGTCCGAGCTTGG	
XF22F	<i>halotag::hdel</i> (F)	GCAGCTCTTCGGGTGGAAGCGCCGAGGTAACACTTAGTTTGT	pXF90
XF22R	<i>halotag::hdel</i> (R)	GCAGCTCTTCGTTAGAGTTCGTATGTCGGAGATCTCGAGGGT	
SIM8F	<i>mscarlet</i> (F)	GCAGCTCTTCGATGGTCTCCAAGGGCGAGGCA	pSM10
SIM8R	<i>mscarlet</i> (R)	GCAGCTCTTCGACCACTCCACTTCCCTTGTACAGCTCGTCCATTCCT	
SIM10F	<i>dendra2</i> (F)	GCAGCTCTTCGATGAACCTTATTAAGGAAGATATG	pSM12
SIM10R	<i>dendra2</i> (R)	GCAGCTCTTCGACCGCCGCTTCCCACTTACTTGACTTGGTAG	
SIM1F	<i>dendra2</i> (F)	GCAGCTCTTCGGGTGGAAGCGGCAACCTTATTAAGGAAGATATG	pSM03
SIM1R	<i>dendra2</i> (R)	GCAGCTCTTCGTTACCATACTTGACTTGGTAG	
SIM2F	<i>mkate2</i> (F)	GCAGCTCTTCGGGTGGAGGTGGAGGTGTCTCCGAGCTCATAAAGAAAACA	pSM04
SIM2R	<i>mkate2</i> (R)	GCAGCTCTTCGCGTACCTCCACCTCCACGGTGTCCGAGCTTGG	
SIM3F	<i>mkate2</i> (F)	GCAGCTCTTCGACGGGAGGTGGAGGTGTCTCCGAGCTCATAAAGAAAACA	pSM05
SIM3R	<i>mkate2</i> (R)	GCAGCTCTTCGTTAACGGTGTCCGAGCTTGG	
SIM6F	<i>mscarlet</i> (F)	GCAGCTCTTCGGGTGGAGGTGGAGGTGTCTCCAAGGGCGAGGCA	pSM08
SIM6R	<i>mscarlet</i> (R)	GCAGCTCTTCGTTACTTGTACAGCTCGTCCATTCCT	
mScarlet (SAPC3)F	<i>mscarlet</i> (F)	GCAGCTCTTCGGGTGTCTCCAAGGGCGAGGAGTCAT	pSDH57
mScarlet (SAPC3)R	<i>mscarlet</i> (R)	GCAGCTCTTCGCGTGGCCGGGCTTTTGCAGCGG	
XF84F	<i>lov</i> (F)	GCAGCTCTTCGACGCCCTGCTTGTGTCT	pXF276
XF84R	<i>lov</i> (R)	GCAGCTCTTCGTTAGACCCAAGTGTCCAGCGG	
XF12F	<i>pie-1 3'UTR</i> (F)	GCAGCTCTTCGTAATTTTGCCTGATTTTCCAT	pXF85
XF12R	<i>pie-1 3'UTR</i> (R)	GCAGCTCTTCGTACATCATCGTTCACATTTTCC	
tb2 3'UTR (SAPC5)F	<i>tb2 3'UTR</i> (F)	GCAGCTCTTCGTAATAAGCAAGATCCTTCAAGCATTC	pSDH54
tb2 3'UTR (SAPC5)R	<i>tb2 3'UTR</i> (R)	GCAGCTCTTCGTACGACTTTTCTTGTGGCGCAC	
Halo (SAP C3)F	<i>halotag</i> (F)	GCAGCTCTTCGGGTGGAAGC	pSDH51
Halo (SAP C3)R	<i>halotag</i> (R)	GCAGCTCTTCGCGTTCGGAGATCTCGAGGGTGG	
ePDZ (SAP C4)F	<i>epdz</i> (F)	GCAGCTCTTCGACGGGAGGTTCGGAGGATCTGGC	pSDH52
ePDZ (SAP C4)R	<i>epdz</i> (R)	GCAGCTCTTCGTTACGTCGCGACGGGTGGAT	
unc-54 (SAPC5)F	<i>unc-54 3'UTR</i> (F)	GCAGCTCTTCGTAAGAGCTCCGCATCGGCCGCTG	pSDH66
unc-54 (SAPC5)R	<i>unc-54 3'UTR</i> (R)	GCAGCTCTTCGTACAAACAGTTATGTTTGGTATATTGGGA	
Eg717	Replace pCFJ350 MCS (F)	TCGAGTGGCGAAGAGCCATGGATCCCATATGGAATTCTGCAGGCCGTCTTCGGTAA	pXF87
Eg718	Replace pCFJ350 MCS (R)	CTAGTTACCGAAGAGCAGGCCGTGAGAATCCATATGGGATCCATGGGCTCTTCGCCAC	
XF30F	Mutate Sap1 site in pCFJ350	GATTATGGGCACCTTCTTTATCC	pXF87
XF30R	Mutate Sap1 site in pCFJ350	CGACAAGCAACTTTTCTATAC	
XF31F	Mutate Sap1 site in pCFJ350	AATGGCGAAGGCAAGCAGAG	pXF87
XF31R	Mutate Sap1 site in pCFJ350	GTTTCTGAAAAATAATGTAACCTGAAATTG	

Note: For the expression plasmid pJF13 annealed oligos were used to generate HSP-70(aa1-19) in cassette 2.

Additional oligo sequences used to generate pSDH50:

TOMM-20 short forward.

GCAGCTCTTCGATGTCGGACACAATTCTTGGTTTCA AcaaatcaaacgctgtttggctgctgtaattgctgagccgttctcctgctactgattactctgatcataagagaatcaacgctcagactacaaggacaagattagcgaagtcagtttttaacaacatattctctcgattttatctaaacaactattttcttcagagagaCTGTCCACAGGCTGGAGCAggagctgtgcaggcgtgagccggagccGGTCAAGAGCTgc

TOMM-20 short reverse

GCAGCTCTTCGACCggctccggctccagcgccctgcaccagctccTGCTCCAGCCTGGGCACGtctctctgaaagaaaataagttgttttagataaaaatccgaaggaaatattgtttaaactgacttgcctaatct
gtcctttagtctggagcgttgattctctatgatcgaagtaaatgcagtagccgaggaagcggctccagcaattccagcagccaaaacgacgttgattgTTGAAACCAAGAATTGTGTCCGACATCGAAGAGCt
gc

Table 4. Strains used in this study

Strain	Genotype	Construction	Reference:
EG8078	<i>oxTi185 I; unc-119(ed3) III</i>		Frokjaer-Jensen et al (2014)
EG8079	<i>oxTi179 II; unc-119(ed3) III</i>		Frokjaer-Jensen et al (2014)
EGD329	<i>egxSi126 [unc-119(+), pmex-5::hsp-70(aa1-19)::halotag::HDEL::pie-1 3'UTR] I; unc-119(ed3) III</i>	Injected pJF13 into EG8078	This study
EGD412	<i>egxSi136 [unc-119(+); pmex-5::tomm-20::halotag::pie-1 3'UTR] II; unc-119(ed3) III</i>	Injected pJF17 into EG8079	This study
EGD496	<i>egxSi117 [unc-119 (+); pmex-5::npp-20::gfp::pie-1 3'UTR] I; unc-119(ed3) III</i>	Injected pXF253 into EG8078	This study
EGD497	<i>egxSi118 [unc-119 (+); pmex-5::npp-20::halotag::pie-1 3'UTR] II; unc-119 (ed3) III</i>	Injected pXF255 into EG8079	This study
EGD549	<i>egxSi144 [unc-119 (+); pmex-5::cox-4::halotag::pie-1 3'UTR] II; unc-119 (ed3) III</i>	Injected pXF266 into EG8079	This study
EGD565	<i>egxSi145 [unc-119 (+); pmex-5::hsp-70(aa1-19)::halotag::HDEL::pie-1 3'UTR] II; unc-119 (ed3) III</i>	Injected pJF13 into EG8079	This study
EGD623	<i>egxSi152 [unc-119(+); pmex-5::tomm -20::gfp::pie-1 3'UTR] II; unc-119(ed3) III</i>	Injected pSM16 into EG8079	This study
EGD629	<i>egxSi155 [unc-119(+); pmex-5::tomm -20::mKate2::pie-1 3'UTR] II; unc-119(ed3) III</i>	Injected pSM20 into EG8079	This study
EGD631	<i>egxSi155 [unc-119(+); pmex-5::tomm -20::Dendra2::pie-1 3'UTR] II; unc-119(ed3) III</i>	Injected pSM17 into EG8079	This study
EGD633	<i>egxSi159 [unc-119(+); pmex-5::tomm -20::mScarlet::pie-1 3'UTR] II; unc-119(ed3) III</i>	Injected pSM22 into EG8079	This study
EGD615	<i>cox-4(zu476[cox-4::eGFP::3XFLAG] I; egxSi136 [unc-119(+); pmex-5::tomm -20::halotag::pie-1 3'UTR] II; unc-119(ed3?) III</i>	Crossed EGD412 and JJ2586	This study
JJ2586	<i>cox-4(zu476[cox-4::eGFP::3XFLAG] I</i>		Raiders et al, 2018
TBD307	<i>dhc-1(he255[epdz::mcherry::dhc-1] I; utdSi51(Pmex-5::tomm -20(aa1-55)::halotag::lov::tbb-2 3'UTR) II</i>	Injected pSDH68 into EG8079. Crossed to SV2095.	This study
SV2095	<i>dhc-1(he255[epdz::mcherry::dhc-1] I; ruls57[Ppie-1::gfp::tbb-2 + unc119(+)] V</i>		Fielmich et al, 2018

Figure 1

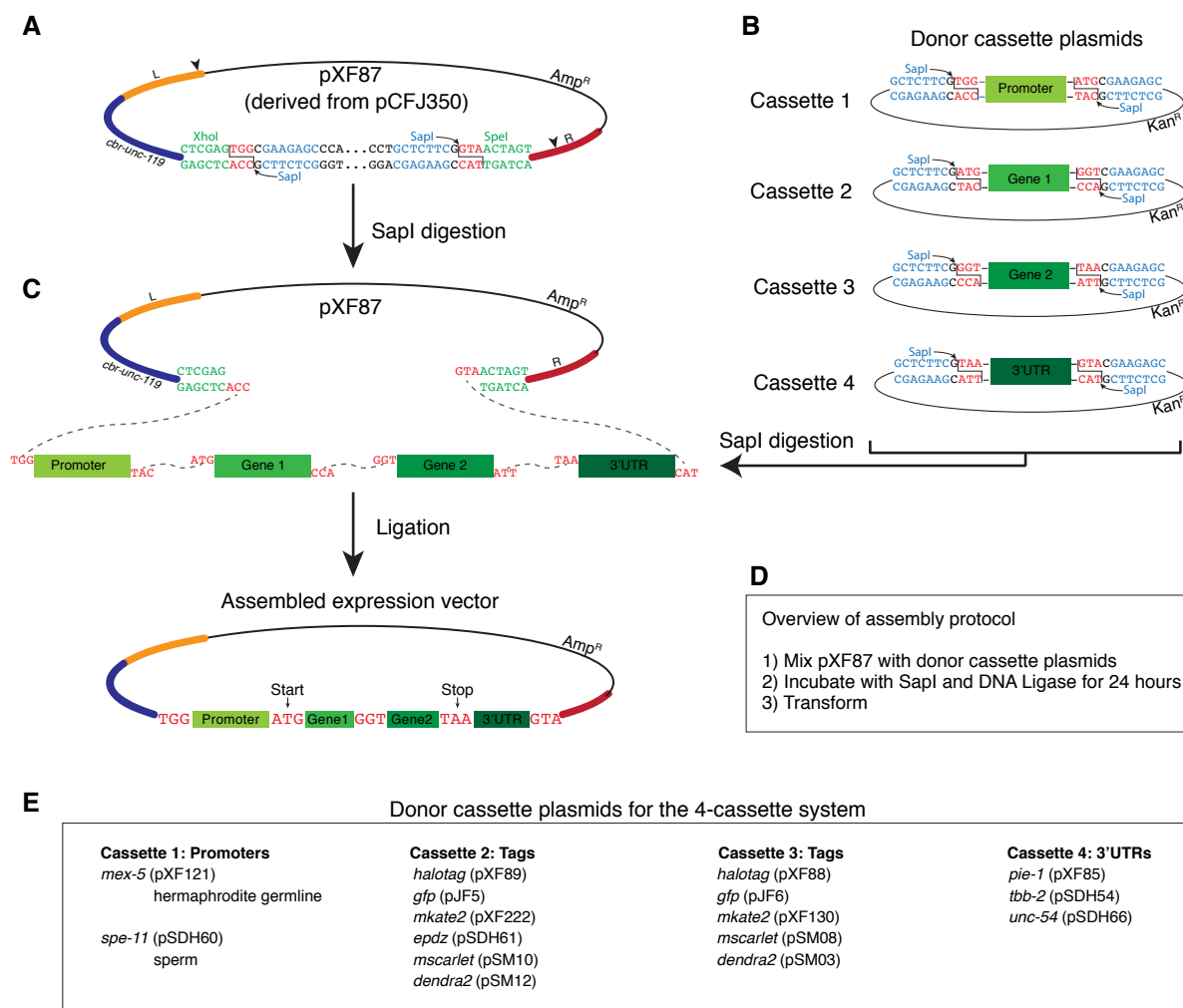


Figure 2

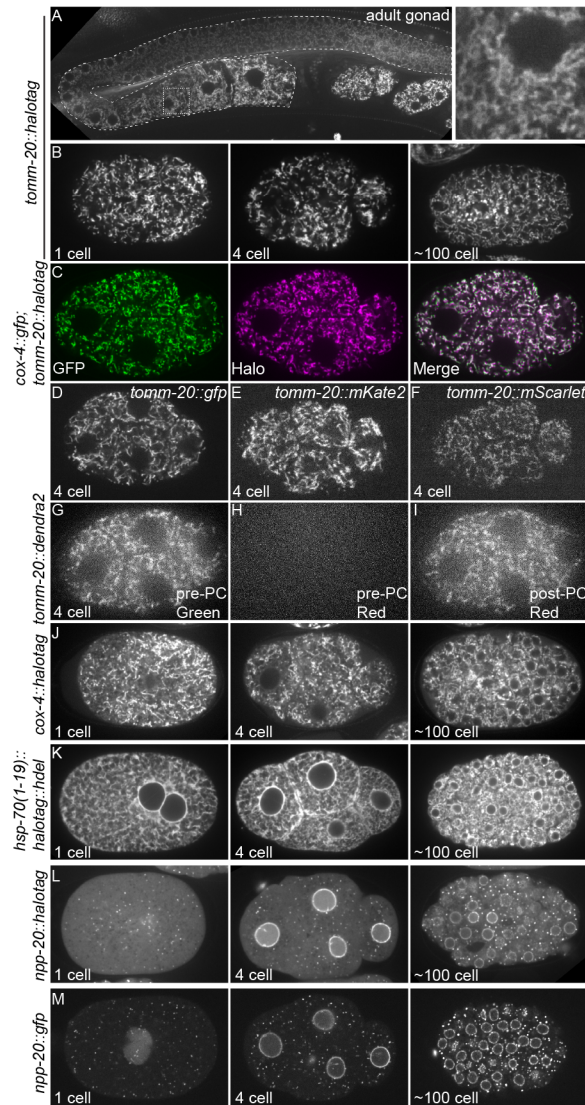


Figure 3

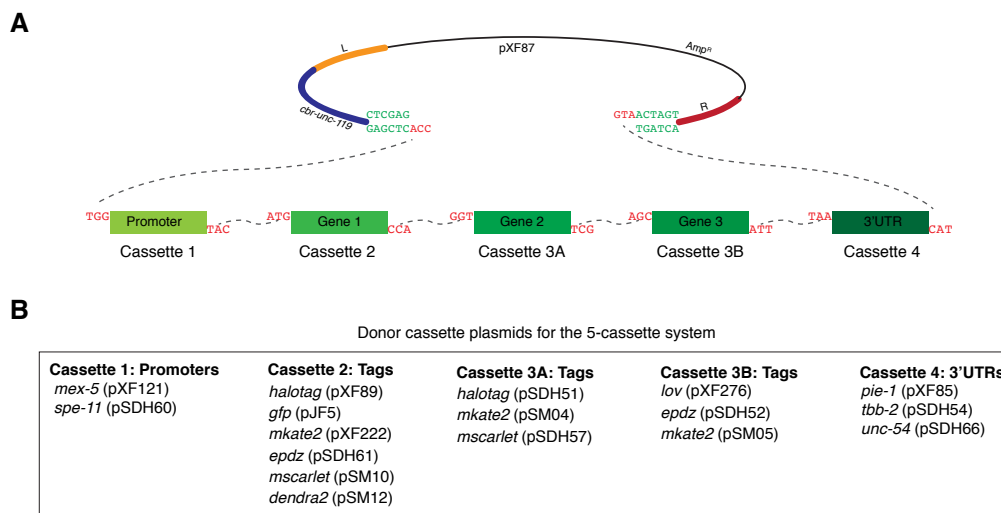
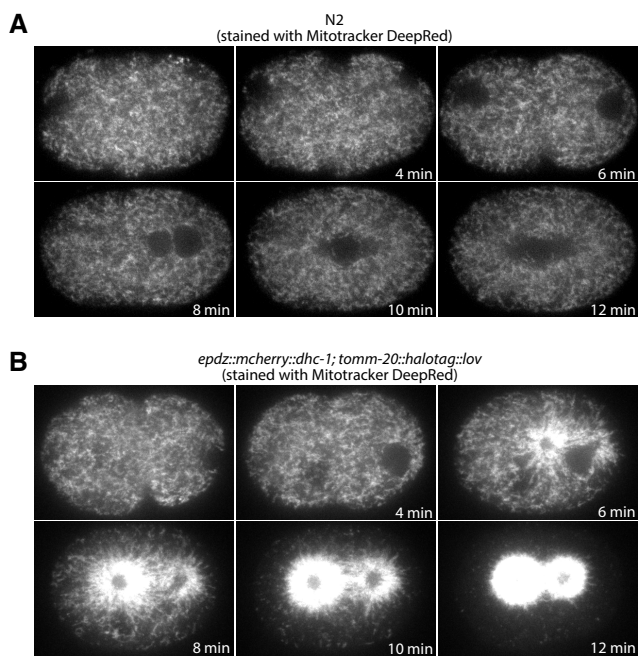


Figure 4



Halotag

pXF89: (Cassette 2) GCTCTTCGATG_Halotag_GGAAGCGGCGGTGAAGAGC
SapI M G S G G SapI

pXF88: (cassette 3) GCTCTTCGGGTGGAAGCGGC_Halotag_TACGAAGAGC
SapI G G S G * SapI

pSDH51: (cassette 3a) GCTCTTCGGGTGGAAGCGGC_Halotag_ACGCGAAGAGC
SapI G G S G T SapI

GCCGAGgtaaacacttagtttttgttgctctgtttaaaaattaat
A E
tttaaagATCGGAACCGGATTCCCATTCGACCCACACTACGTCGAGGTCCTCGGAGAGCGTATGC
I G T G F P F D P H Y V E V L G E R M
ACTACGTCGAGCTCGGACCAGTGCAGGAAACCCAGTCTCTTCTCCACGGAAACCCCACTC
H Y V D V G P R D G T P V L F L H G N P T S
CTCCTACGTCGGCGTAACATCATCCCACAGTCGCCCAACCCACCGTTGCATCGCCCGAGgt
S Y V W R N I I P H V A P T H R C I A P
aagttttcttatgggaaagaaggaacccagattttacttgaaaaatgaaatttttcgcg
gattttcaccacaaaattgttgaatattcattatttcacgctgtaaaacaaaaaaaaaaaaatc
aaaaactacgttgaaatcggtttttaagcgaatttcttcagaattgccagattttaacccca
aatttgcagtttttaataaaaatttcaaccttttcggtcaaattgtagattttctgaaatt
tagtcaaaaaacaatttctcgtaaaattttcaaatagattttcagACCTCATCGGAATGGGA
D L I G M G
AAGTCCGACAAGCCAGACCTCGGATACTTCTTCGACGACCACGTCGTCCTCATGGAGCCTTCA
K S D K P D L G Y F F D D H V R F M D A F
TCGAGGCCCTCGACTCGAGGAGTCTCCTCGTCATCCAGACTGGGGATCGGCTCTCGGATT
I E A L G L E E V V L V I H D W G S A L G F
CCACTGGGCCAAGCGTAACCCAGAGCGTGTCAAGGAATCGCCTTCATGGAGTTCATCCGTCCA
H W A K R N P E R V K G I A F M E F I R P
ATCCCAACCTGGGACGAGTGGCCAGgtaagttatgtctacctgcctgcctaccgcctaaat
I P T W D E W P
ttgtgaagtttcttcaaaaaatccagaaaaaaacaattttcatacagattttttcccttaaaa
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gttttgagaaatacacaaattttttaaatgtaattttcaaattttcaactagaaaatt
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caataactacaaattttaacattttcagAGTTCGCCCGTGAGACATTCAGCCTTCCGTACCAC
E F A R E T F Q A F R T T
CGACGTCGGACGTAAGTTCATCATCGACCAAAACGTCCTCATCGAGGGAACCCCTCCCAATGGGA
D V G R K L I I D Q N V F I E G T L P M G
GTCGTCGTCCTCACTACCGAGGTCGAGATGGACCCTACCGTGAGCCATTCCTCAACCCAGTGC
V V R P L T E V E M D H Y R E P F L N P V
ACCGTGAGCCACTTGGCGTTTCCCAAACGAGCTCCCAATCGCCGAGAGCCAGCCAACATCGT
D R E P L W R F P N E L P I A G E P A N I V
CGCCCTCGTCGAGGAGTACATGGACTGGCTCCACCAATCCCAAGTCCCAAGCTCCTCTCTG
A L V E E Y M D W L H Q S P V P K L L F W
GGAACCCAGgtaagttcttttttgaaaagtgcagtttgtagtctaattttcattttttct
G T P
Ttttaaaaaacgcatcaattttaaatattttgggacaaaaatccgaaaactgtactaatttgtg
gtttgtaaaaatataaaaaaacgcaaaaaatgtttcaaaaatgottagaataaaaaataaaa
tttctaaaaaattgacaaaataaacatttaaaaaatcaaaaagtttgaaaaatgcaagttt
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acagtttttaaatgttaaatgcaaaaaaaagcaaaaaaaatgtaaaatgtaacaga
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gagaaacaaatcaaattgtatattacgaaaaacgataactaaaattcgaaaatggtggttttt
gcgtaaaaaatacgggttccgtaattttcagGAGTCTCATCCACCAGCCGAGGCCGCCGCT
G V L I P P A E A A R L
CGCCAAGTCCCTCCCAAACGCAAGGCCGTCGACATCGGACCAGGACTCAACCTCCTCCAAGAG
A K S L P N C K A V D I G P G L N L L Q E
GACAACCCAGgtaagttcatagattttgaaaaaaagttaagaactgaaaaatggaataaaaaat
D N P
Atttaagagcatttttaaatgtaaaatatacaaaaaagcgccctaagaatgtttcaaaaacagt
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aaaatgtttctaaaaatataaaaaatggttaaatgtgtaaaagttaaaatataaaaaataaaa
ttatgaaaaagttatggttcatactttttctaaatgttggtaaaaaatataaaatccagctt
tttatcacattatgtgttctaaaaatctttccccaattttcgggaccggtttccagtatatt
ccatttttcaaaaagatttttaactgaaattcatggttttcaatgtaaaaaatcaataaaaaag
aatttttcagACCTCATCGGTTCCGAGATCGCCCGTTGGCTCTCCACCCTCGAGATCTCCGGA
D L I G S E I A R W L S T L E I S G

Figure S1. Sequence of HaloTag donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

ceGFP with PATC introns

pJF5: (cassette 2) GCTCTTCGATG_ceGFP_GGAAGCGCGGT_CGAAGAGC
SapI M G S G G SapI

pJF6: (cassette 3) GCTCTTCGGTGGAAAGCGGC_ceGFP_TAAACGAAGAGC
SapI G G S G * SapI

TCCAAGgtaacacttagtttttgttgcctgttttaaaataat
S K
tttaagGGAGGAGCTGTTCCACGGAGTCCGTCCTCCGTCGAGCTCGACGGGGACGTC
G E E L F T G V V P I L V E L D G D V
ACGGACACAAGTTCTCCGTGTCGGAGAGGGAGAGGATGCCACCTACGGAAAGCTCACCT
N G H K F S V S G E G E G D A T Y G K L T L
CAAGTTCATCTGCACCACCGAAAGCTCCAGTCCCATGGCCAACCTCGTACCACCTTCTGC
K F I C T T T G K L P V P W P T L V T T F C
TACGGAGTCCAATGCTTCTCCGTTACCCAGgtaagttttctgttttatttactgtttctgag
Y G V Q C F S R Y P
Aattgggatttttagctgaaaaattggattttaagatgaaaaattgaaattttcaactaaaaat
tcaatttttagctgaaaaattgaaatttttagctaaagatgggatttttagtgaaaaatcga
gatttctagctaaaaagatgatttttagctgaaagattggaattttaagcagaaaaattgta
tttttagctgataaatcaattttaagcggaaaaattgaaatttttagctaaaaattccattt
tcaactaaaaatcgtgatttttagctttaaaaattggatttttagctgaaaaatcggattttta
gctgaaaaattgaaatttttagttaaagtgagatttttaacagaaaaagtgagatttttagt
taaaaaataatttttagctaaaaatacaattttcaactaaagatcgtgatttttagcttta
aaaaattggatttttagctaaaaatcggatttttagctgaaaaattgaaatttttttagttaa
gtgggattttaagatgaaaaagtgagatttttagctgaaaaagtgagatttttagctaaaaata
caattttcaactgaaaaattgggatttttagctttaaaaattggatttttagctttaaaaattg
gttttttagctgaaaaagtgagatttttagttaaagattgaaatttttagctgaaaaattggaa
tttttaagtaaaaattgggattttaagatgaaaaagtgagatttttagctgaaaaagtgagat
tttagctaaaaatacaattttcaactgaaaaattgggatttttagctgaaaaagtgagacgaa
agacgaaaaaaaatcgattttttacaaaaattagatttttagACCACATGAAGCGTCACGA
D H M K R H D
CTTCTTCAAGTCCGCCATGCCAGAGGATACGTCCAAGAGCGTACCATCTTCTCAAGGACGAC
F F K S A M P E G Y V Q E R T I F F K D D
GGAAACTACAAGgtaagttatgtacgagattttctcggcgaaaaattggacttttttgcctaaaa
G N Y K
Actgaatttttagctgttttttgcactgaaaaatgctgaaaaattggttttttgcgtaaaattca
cggaaaaagctgaaaaatattgcagaaatcgacgaaaaatgcttttttgcgtaaaattcagcc
agaaaaattgttcagaaatcgcaattttcctgcaaaaatcgacgttttttgcgcaaaaattggtagat
ttgaaaccgaaaaattgtgaaaaattggacttttttgcctaaaaacagactttttgagctttttt
acactgaaaaattgggaaaaattggattttccagcagaaaaattcgaaattttcaatttttggcg
gaaatttgacgaaaaatatgaaaaaacccgatttttgcctgaaaaagttcaaaagccgaaaa
attggccaaaaatctccaaaaatcgacttttttgcggttttttgcgtaaaaaattgaaatttttt
tgaaaaattgagcaaaaatcgccgaaaaaccgctaaaaaaaactgcccgaattggcaattttgag
cttaaaaaaacttgaaaaatgctcaaaattacccaattttcaccggaaattgagctaaaaatcgt
cgaaaaagactgaaattttcaatttttagctgaaaaatctgcctattttcggctaaaaattgcttta
aaaaatcaaaattcaaaatttttggctaaaaataactcagattgoccttttttttccaaaaa
tgacaaaaaaaatcaatcagaaaaatcgactttttttacaaaaattggctcaaaaacgctct
aaattctccctaatttttgcctgtaaaattgtgcaaaaaatcaccgaaaaatgacgatttttagg
ctgaaaaatctcaaaaaagctcaaaattcccaacttttcaccgcaaaaaagcagaaaaagctc
caattttatcgattaaactttttagACCCTGCGAGGTCAAGTTCGAGGGAGACCCCTCGTC
T R A E V K F E G D T L V
AACCGTATCGAGCTCAAGGGAATCGACTTCAAGGAGGACGGTAAACATCCTGGGACACAAGCTGG
N R I E L K G I D F K E D G N I I L G H K L
AGTACAACCTACAACCTCCACAACGTCTACATCATGGCCGACAAGCAAAGAACGGAATCAAGGT
E Y N Y N S H N V Y I M A D K Q K N G I K V
CAACTTCAAGgtaagttcttttttgcctgatttttgagtgaaaaattgaggaattcaca
N F K
aatggccaaaagaatcctcaaaattggccgaaaaattctgaaaaatgacctaaaaatcaccgaaaa
acggccaaaaatctgaaaaatcgccgaaaaatctcaaaattttgaggaattcccgctaaaaat
taataaaaaatcaccgaaaaaaggccaaaaattacctaataaagccgcaaaaatcgctaaaaag
caaaaatctgaaaaatggcgaatttcaattttttcacacacaaaaaatccctgaattttctta
tttttactcgaaaaatctgtgattttacggggtaaaaaatcagtttttagtgctatttcacaag
aaaaacacagcattttcgcaaaaaactcggatttttaagccaaaaattgaggttttttagtttt
ttacgtgaaaaactgaaatttttgcacaaaaaacacttttttttagactaaaaatcaattttc
atcaaaaatcactaaattttgcctgaaaaaatatagatttttccactttttcactgaaaaaaaac
tggattttcactgaaaaatcaagttgttttttagacaaaaattttatttttccgctgaaaaatta
tcgtttttcagctcaattttcagctaaaaatccctgaaattctggattttcagccaaaaaacctg
aaattttcacaacaccccggttaaaaaatgagtttagactgaaaaatccgattttttgagccaa
aaatttgatttttaactgaaaaaaattggattttcaacaaaaaatcagtttttcagtgatttt
tagagtaaaaaatcactgatttttaacacaaaaatccagtttttgcggtattttcactgaaaaatc
ccgatttttccccaaaaaatccatttttccgactaaaaatcccccaagtaattttcagATCCGT
I R
CACAACTCGAGGACGGATCGGTCCAACCTCGCCGACCCTACCAACAAAAACCCCAATCGGTG
H N I E D G S N V Q L A D H Y Q Q N T P I G
ACGGACAGTCTCTCCAGACAACCCTACCTCTCCACCAATCCGCCCTCTCAAGGACCC
D G P V L L P D N H Y L S T Q S A L S K D P
AAACGAGAAGCGTGACCACATGGTCTCCCTCGAGTTCGTACCCGCGGAAATCACCACGGA
N E K R D H M V L L E F V T A A G I T H G
ATGGACGAGCTCTACAAG
M D E L Y K

Figure S2. Sequence of ceGFP donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

mKate2

pXF222 (Cassette 2) GCTCTTCGATG_mKate2_GGAGGTGGAGGTGGT CGAAGAGC
 SapI M G G G G G SapI

pXF130 (Cassette 3) GCTCTTCGGTGGAGGTGGAGGT_mKate2_TAACGAAGAGC
 SapI G G G G G * SapI

pSM04 (Cassette 3a) GCTCTTCGGTGGAGGTGGAGGT_mKate2_AGCCGAAGAGC
 SapI G G G G G S SapI

pSM05 (Cassette 3b) GCTCTTCGAGCGGAGGTGGAGGT_mKate2_TAACGAAGAGC
 SapI S G G G G * SapI

GTCTCCGAGCTCATTAAAGAAAACATGCATATGAAGCTCTACATGGAGGGAAC
 V S E L I K E N M H M K L Y M E G T
 CGTCAACAACCACCCTCAAGTGCACCTCCGAGGGAGAGGGAAAGCCATACGAGGGAACCCAA
 V N N H H F K C T S E G E G K P Y E G T Q
 ACCATGCGTATCAAGGCCGTCGAGGGAGGACCCTCCATTGCGCTTCGACATCCTCGCCACCT
 T M R I K A V E G G P L P F A F D I L A T
 CCTTCATGTACGGATCCAAGgtaagtttaaacatataataactaactaaccctgattatthaa
 S F M Y G S K
 ttttcagACCTTCATCAACCACACCCAAGGAATCCCAGACTTCTTCAAGCAATCCTCCAGAG
 T F I N H T Q G I P D F F K Q S F P E
 GGATTCACCTGGGAGCGTGTACACACCTACGAGGACGGAGGAGTCTCACCGCCACCCAAGACA
 G F T W E R V T T Y E D G G V L T A T Q D
 CCTCCCTCCAAGACGGATGCCTCATCTACAACGTCAAGgtaagtttaaacagttcggtaactaac
 T S L Q D G C L I Y N V K
 taaccatacatatthaaattttcagATCCGTGGAGTCAACTTCCCATCCAACGGACCAGTCATG
 I R G V N F P S N G P V M
 CAAAAGAAGACCCTCGGATGGGAGGCCCTCCACCAGACCCTTACCCAGCCGACGGAGGACTTG
 Q K K T L G W E A S T E T L Y P A D G G L
 AGGGACGTGCCGACATGGCCCTCAAGCTCGTCCGAGGAGGACACCTCATCTGCAACCTCAAGgt
 E G R A D M A L K L V G G G H L I C N L K
 aagtttaaacatgattttactaactaactaatctgattthaaattttcagACCACCTACCGTTCC
 T T Y R S
 AAGAAGCCAGCCAAGAACCTCAAGATGCCAGGAGTCTACTACGTCGACCGTCGTCTTGAGCGTA
 K K P A K N L K M P G V Y Y V D R R L E R
 TCAAGGAGGCCGACAAGGAGACCTACGTCGAGCAACACGAGGTCGCCGTCGCCCGTTACTGCGA
 I K E A D K E T Y V E Q H E V A V A R Y C D
 CCTCCCATCCAAGCTCGGACACCGT
 L P S K L G H R

Figure S3. Sequence of mKate2 donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

mScarlet

pSM10 (Cassette 2) GCTCTTCGATG_mKate2_GGAGGTGGAGGTGGTCGAAGAGC
SapI M G G G G G SapI

pSM08 (Cassette 3) GCTCTTCGGGTGGAGGTGGAGGT_mScarlet_TAACGAAGAGC
SapI G G G G G * SapI

pSDH57 (Cassette3a)

GCTCTTCGGGT_mScarlet_TTGGCTGAAGCGGCAGCTAAAGAAGCTGCCGCGAAGGAAGCAG
SapI G L A E A A A K E A A A K E A
CAGCCAAGGAGGCCGAGCGAAGGAAGCCGCTGCAAAAAGCCCGGCCACGCGAAGAGC
A A K E A A A K E A A A K A A A S SapI

GTCTCCAAGGGCGAGGCAGTCATCAAGGAGTTCATGCGTTT
V S K G E A V I K E F M R F
CAAGGTGCACATGGAGGGATCCATGAACGGACACGAGTTCGAGATCGAGGGCGAGGGAGAGGGA
K V H M E G S M N G H E F E I E G E G E G
CGCCCATACGAGGGAACCCAGACCCGCAAGCTCAAGGTGACCAAGgtaagtttaaacatatata
R P Y E G T Q T A K L K V T K
tactaactaacacctgattattttaaatcttcagGGTGGACCCTGCCATTTCTCTGGGACATCCT
G G P L P F S W D I L
CTCCCCACAATTCATGTACGGCTCCCGTGCCTTACCAAGCACCCAGCCGACATCCCCGACTAC
S P Q F M Y G S R A F T K H P A D I P D Y
TACAAGCAATCCTTCCCCGAGGGATTCAGTGGGAGCGCGTGTGAACCTTCGAGGACGGAGGAG
Y K Q S F P E G F K W E R V M N F E D G G
CCGTGACCGTCACCCAAGACACCTCCCTGGAGGACGGAACCCCTCATCTACAAGGTGAAGTCCG
A V T V T Q D T S L E D G T L I Y K V K L R
TGGAACCAACTTCCACCTGACGGCCAGTCATGCAGAAGAAGACCATGGGATGGGAAGCCTCC
G T N F P P D G P V M Q K K T M G W E A S
ACCGAGCGTTTGTACCCAGAGGACGGAGTGTCAAGgtaagtttaaacagttcggtaactaacta
T E R L Y P E D G V L K
accatacatattttaaatcttcagGGAGACATTAAGATGGCCCTCCGTCTGAAGGACGGAGGACG
G D I K M A L R L K D G G R
TTACCTGGCGGACTTCAAGACCACCTACAAGCCAAGAAGCCAGTCCAGATGCCAGGAGCCTAC
Y L A D F K T T Y K A K K P V Q M P G A Y
AACGTCGACCGCAAGCTCGACATCACCTCCCAACGAGGACTACACCGTCGTGGAGCAATACG
N V D R K L D I T S H N E D Y T V V E Q Y
AACGTTCCGAGGGACGCCACTCCACCGGAGGAATGGACGAGCTGTACAAG
E R S E G R H S T G G M D E L Y K

Figure S4. Sequence of mScarlet donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

Dendra2

pSM12 (Cassette 2) GCTCTTCGATG_Dendra2_GGAAGCGGCGGTCTGAAGAGC
SapI M G S G G SapI

pSM03 (Cassette 3) GCTCTTCGGGTGGAAGCGGC_Dendra2_TAACGAAGAGC
SapI G G S G * SapI

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AACCTTATTAAGGAAGATATGAGAGTCAAAGTGCATATGGAAGG
N L I K E D M R V K V H M E G
AAACGTCAACGGTCATGCATTTGTTATGGAAGTGAAGGTAAAGGAAAGCCATACGAAGGAACT
N V N G H A F V I E G E G K G K P Y E G T
CAAACGTAACTTGACTGTCAAAGAAGGAGCACCCTACCATTTAGTTACgtaagtttaaca
Q T A N L T V K E G A P L P F S Y
tatatataactaactaaccctgattattttaaatTTTcagGATATTCTCACTACTGCCGTCCATTA
D I L T T A V H Y
CGGAAACAGAGTTTTTACTAAATACCCAGAAGATATTCCTGATTACTTCAAGCAATCGTTTCCA
G N R V F T K Y P E D I P D Y F K Q S F P
GAAGGATACTCGTGGAAAGAAGTATGACTTTTTCGAAGATAAAGGTATTTGCACTATTgtaagtt
E G Y S W E R T M T F E D K G I C T I
taaacagttcggtaactaactaaccatacatatTTTaaatTTTcagAGAAGTGATATTAGTCTCGA
R S D I S L E
AGGTGATTGCTTCTTCCAAAATGTGAGATTTAAAGGTACTAACTTTCTCCTAACGGACCAGTT
G D C F F Q N V R F K G T N F P P N G P V
ATGCAAAAGAAGACTCTTAAGTGGGAACCATCGACTGAAAACTACATGTTAGAGATGGACTAC
M Q K K T L K W E P S T E K L H V R D G L
TTGTTGGAgtaagtttaacttggacttactaactaacggattatTTTaaatTTTcagAACAT
L V G N I
TAACATGGCACTACTACTAGAAGGTGGAGGTCACTACCTTTGCGATTTTAAAACCACTTACAAA
N M A L L L E G G G H Y L C D F K T T Y K
GCAAAGAAGTTCGTCCTCAACTTCCAGATGCACACTTTGTTGATCACAGAATTGAAATAC TAGGAA
A K K V V Q L P D A H F V D H R I E I L G
ACGATTCCGATTACAACAAGTTAAGCTATACGAACACGCAGTTGCAAGATACAGTCTCTACC
N D S D Y N K V K L Y E H A V A R Y S P L P
AAGTCAAGTATGG
S Q V W
```

Figure S5. Sequence of Dendra2 donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

ePDZ

PSDH61 (Cassette 2) GCTCTTCGATG_ePDZ_GGAGCTGGTCTGAAGAGC
SapI M G A G SapI

pSDH52 (Cassette 3b)

GCTCTTCGAGCGGAGGTTCCGGAGGATCTGGCGGATCT_ePDZ_TAACGAAGAGC
SapI S G G S G G S G G S * SapI

CCAGAGCTCGGATTCTCGATCTCCGGAGGTGTCGGAGGCCGTGGAAATCCATTCCGTCCTGACG
P E L G F S I S G G V G G R G N P F R P D
ATGATGGAATTTTTGTTACTCGGGTCCAACCAGAAGACCAGCTAGCAAACCTTCTTCAACCTGG
D D G I F V T R V Q P E G P A S K L L Q P G
AGACAAGgtaagttaattaatttcacgagagatcgtgcaattttctcattcatgaagacttttc
D K
agATCATCCAAGCCAACGGTTACTCTTTTCATTAATATTGAGCACGGTCAGGCTGTCAGCCTTCT
I I Q A N G Y S F I N I E H G Q A V S L L
CAAGACCTTCCAGAACACAGTCGAGCTCATCATCGTCCGAGAGGTAGGAAACGGAGCTAAGCAG
K T F Q N T V E L I I V R E V G N G A K Q
GAGATCCGTGTCCGCGTCGAAAAGGACGGAGGATCCGGAGgtaagttataatctcctacaaa
E I R V R V E K D G G S G
tttaaaactgacttacttttttctaatttttcagGAGTTTCCAGTGTTCACCAACCTTGAGGT
G V S S V P T N L E V
CGTTGCTGCCACACCAACAAGCCTTCTCATCTCCTGGGATGCTTACCGTGAACCTTCCAGTCTCC
V A A T P T S L L I S W D A Y R E L P V S
TACTATAGGATCACCTACGGAGAGACCGGAGGAAATCTCCAGTCCAAGAATTCACGGTCCCAG
Y Y R I T Y G E T G G N S P V Q E F T V P
gtaagttataatctcctacaaaagtaatttgagaggtacaatattttcagGAAGC
G S
AAGTCGACCGCCACAATTTCCGGATTGAAGCCAGGAGTCGACTACACCATCACTGTCTATGCTC
K S T A T I S G L K P G V D Y T I T V Y A
ATTACAATATCATTACTACTCATCACCAATCTCCATCAATTATAGAACGAGTAGATTGGAGCT
H Y N Y H Y Y S S P I S I N Y R T S R L E L
CAAGCTCCGTATTTTGCAATCGACAGTGCCACGCGCCCGTGATCCACCCGTCGCGACG
K L R I L Q S T V P R A R D P P V A T

Figure S6. Sequence of ePDZ donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

LOV

pXF276 (Cassette 3b) GCTCTTCGAGC_LOV_TAACGAAGAGC
SapI S * SapI

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CCTCGTCTTGCTGCTGCACTTGAGCGCATTGAGAAGAATTTTGTAATCACCGATCCCCGACTTC
P R L A A A L E R I E K N F V I T D P R L
CGGATAACCCAATCATTTTCGCTTCTGATTCGTTCCCTTCAGCTCACCGAGTATTCACGTGAAGA
P D N P I I F A S D S F L Q L T E Y S R E E
GgtaagtttaattaattaagttccaaatcaagatacgttcttttcgccatacattttcagATCC
I
TTGGAAGAAATTGCCGATTCTCCAGGGACCAGAACTGACAGAGCTACCGTCAGAAAGATCCG
L G R N C R F L Q G P E T D R A T V R K I R
TGACGCCATCGACAATCAGACTGAGGTTACCGTCCAGCTCATCAACTACACCAAGTCCGGAAAG
D A I D N Q T E V T V Q L I N Y T K S G K
gtaagttataattttcgcatttggtgtatcgtatcaatgtttaactgaatttttcagAAGTTCT
K F
GGAATCTTTTCCATCTTCAACCAATGCGTGATCAGAAGGGAGATGTTCAATACTTCATTGGAGT
W N L F H L Q P M R D Q K G D V Q Y F I G V
TCAACTCGATGGAACCGAACATGTTTCGTGACGCTGCCGAGCGTGAAGCTGTTATGCTCGCCAAG
Q L D G T E H V R D A A E R E A V M L A K
AAGACTGCTGAAGAAATTGATAAAGCCGTCGACACTTGGGTC
K T A E E I D K A V D T W V
```

Figure S7. Sequence of LOV donor cassette. Flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.