Improved *Mos1*-mediated transgenesis in *C. elegans*

To the Editor: The ability to add or delete genes to the genome of genetic model organisms is essential. Previously, we had developed methods based on the *Mos1* transposon[1] to make targeted transgene insertions (*Mos1*-mediated single-copy transgene insertions; MosSCI[2]) and targeted deletions (*Mos1*-mediated deletions; MosDEL[3]) in *Caenorhabditis elegans*, the latter published in *Nature Methods*. Here we present new reagents that improve the efficiency, facilitate the selection for transgenic strains and expand the set of MosSCI insertion sites (**Supplementary Table 1**).

In our system, the Mos1 transposase is expressed from a helper plasmid injected together with template DNA. Increased transposase expression would be expected to improve both insertions and targeted gene deletions. We tested several promoters driving transposase expression for their effect on MosSCI and MosDEL efficiency (**Fig. 1a** and **Supplementary Fig. 1**). Relative to the *glh-2* promoter, the most effective promoter (*eft-3;* also known as *eef-1A.1*) resulted in a more than sixfold improvement in transgene insertion efficiency (from 8% to 54% of worms) and gene deletion efficiency (from 3% (n = 66 worms)[2] to 20% (n = 30 worms); **Fig. 1b**).

An effective, inducible negative selection marker would facilitate identification of transgenic strains. We developed a negative selection marker (*Phsp-16.41::peel-1*) based on the toxin PEEL-1 (ref. 4). Worms carrying the *peel-1* plasmid were killed by a 2-h heat-shock at 34 °C with ~10% false positives (2/19 transgenic worms) (**Fig. 1c** and **Supplementary Fig. 2**). A positive selection marker is critical for identifying transgenic worms with insertions or deletions, and we have used *unc-119* selection extensively. Recently, antibiotic selection markers have been developed for nematode transgenesis[5,6]. We generated targeted *dpy-13* deletions at comparable frequencies using either the neomycin-resistance gene or *unc-119* selection (24%, 12/51 worms, **Fig. 1b**). We discuss the recommended use of selection markers in **Supplementary Methods**.

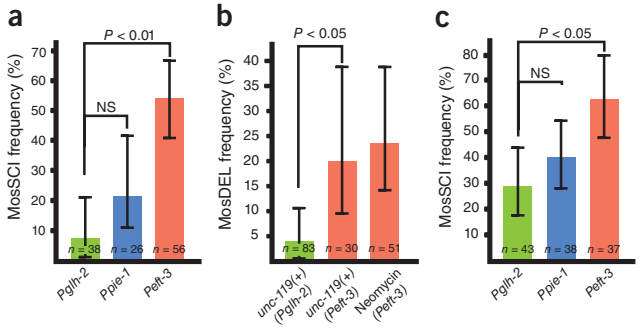

Figure 1 | Improvements to *Mos1*-based genome manipulation. (**a**) Insertion frequency with low total DNA concentration (32.5 ng μl^{-1}). A plasmid expressing Mos1 transposase from the indicated promoters was injected together with a 4.4-kb transgene into *unc-119* worms. Insertion frequency into the *ttTi5605* locus is plotted. (**b**) Frequency of a 5-kb targeted deletion of *dpy-13*. *Pglh-2* data are from ref. 2 using the indicated selection markers. (**c**) Insertion frequency with higher total DNA concentrations (~100 ng μl^{-1}) and in the presence of the negative selection marker *peel-1*. Error bars, 95% confidence intervals; significance was determined with Fischer's exact test.

Multiple insertion sites are important for generating complex genotypes. We expanded the number of MosSCI insertion sites from two to six (**Supplementary Fig. 3**) with a full set of outcrossed strains containing the *Mos1* insertion and targeting vectors (three-way Gateway-compatible or multiple cloning site–compatible) based on *unc-119* selection and for one site, *unc-18* selection (**Table 1**). All sites readily enabled generation of MosSCI inserts and expression in somatic tissue. Three of the insertion sites (*ttTi4348* I, *ttTi5605* II and *cxTi10816* IV) led to robust expression in the germline from a ubiquitous promoter (**Supplementary Fig. 4**). Because MosSCI reagents are important for expression in

Table 1 | **MosSCI site characteristics**

Selection	Locus	Genetic position[a]	Insertion strain[b]	Gateway vector[c]	Multiple cloning site vector	Germline expression[d]	Insertion frequency[e] (percentage)	Balancer strain
***unc-119*[f]**	*ttTi4348*	I:–5.32	EG6701	pCFJ210	pCFJ352	Yes	3/12 (25%)	EG6173
	ttTi4391	I:7.93	EG6702	pCFJ604	pCFJ353	No	4/14 (29%)	EG6171
	ttTi5605	II:0.77	EG6699	pCFJ150	pCFJ350	Yes	6/14 (43%)	EG6070
	cxTi10816	IV:1.41	EG6703	pCFJ212	pCFJ356	Yes	2/10 (20%)	EG6401
	cxTi10882	IV:–0.05	EG6700	pCFJ201	pCFJ351	Variable	4/14 (29%)	EG5568
	ttTi14024	X:22.84	EG6705	pCFJ606	pCFJ355	Limited	3/14 (21%)	EG6109
***unc-18*[f]**	*ttTi4348*	I:–5.32	EG6032	pCFJ448	pCFJ676	Yes	ND	EG6173

[a]Linkage group: genetic map position (cM). [b]4× outcrossed, distributed with extrachromosomal *unc-119* rescue to facilitate handling and maintenance. [c]pDESTR4-R3, three-way Gateway-compatible vector. [d]Based on germline expression of *Pdpy-30::GFP::H2B* transgene (*GFP::H2B* encodes the GFP–histone H2B fusion). [e]Insertion frequency of *Pdpy-30::GFP::H2B* transgene. ND, not determined. [f]*unc-119* is necessary for nervous system development and *unc-18* is necessary for neurotransmission. Both mutants are viable but severely uncoordinated and can be rescued by extrachromosomal arrays.

the germline, we generated an expression vector to express GFP-histone with the inserted transgene for confirmation of expression (**Supplementary Fig. 5**). All strains are available from the *Caenorhabditis* Genetics Center, and plasmids (targeting, transposase and negative selection vectors) are available from Addgene (**Supplementary Table 1**).

Note: Supplementary information is available on the Nature Methods website.

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The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemethods/.

Christian Frøkjær-Jensen[1,2], M Wayne Davis[1], Michael Ailion[1,3] & Erik M Jorgensen[1]

[1]Howard Hughes Medical Institute, Department of Biology, University of Utah, Salt Lake City, Utah, USA. [2]Department of Biomedical Sciences and Danish National Research Foundation Centre for Cardiac Arrhythmia, University of Copenhagen, Copenhagen, Denmark. [3]Present address: Department of Biochemistry, University of Washington, Seattle, Washington, USA.
e-mail: jorgensen@biology.utah.edu

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Generating transgenic nematodes by bombardment and antibiotic selection

To the Editor: In an extension of methods we[1] and others[2] have previously described in *Nature Methods*, we report here single- or dual-antibiotic selection to isolate transgenic nematodes after microparticle bombardment. The protocol makes it straightforward to generate integrated transgenes in diverse *Caenorhabditis* strains and species.

Microparticle bombardment[3,4] is widely used to generate transgenic *C. elegans* but requires specialized strains, large populations of worms and a slow selection procedure. To overcome some of these shortcomings and to facilitate the generation of transgenic strains in non-model nematode species, we developed a bombardment protocol that uses antibiotic selection[1,2] (**Supplementary Fig. 1**). Selection after bombardment can be effective using single antibiotics, but 'dual selection' using a combination of puromycin and G418 is efficient, cost-effective and more robust across species (**Supplementary Figs. 2** and **3**). To facilitate dual selection, we constructed plasmid vectors that express both antibiotic-resistance genes from a single operon (**Supplementary Fig. 4**).

After bombardment, worms are plated onto a limited supply of food sufficient to allow egg laying but resulting in the arrest of progeny as starved first-stage (L1) larvae (**Supplementary Methods** and **Supplementary Fig. 1**). On the next day, worms are washed off the plates, and adults are removed by gravity sedimentation, a key step for high selection efficiency. Selection is then performed for 4 d in small volumes of liquid nematode growth medium supplemented with 0.1% (vol/vol) Triton X-100, 0.5 mg ml^{-1} puromycin and 0.5 mg ml^{-1} G418, surviving worms are plated on nematode growth medium, and transgenic worms expressing a fluorescent marker are picked after 2–3 d of growth and checked for integration by selfing.

Table 1 | Summary of bombardments in *Caenorhabditis* species and strains

Caenorhabditis species	Strain	Total bombardments	Bombardments yielding a transmitting strain	Bombardments yielding an integrated strain (percentage)
C. elegans	N2	32	24	18 (56%)
C. elegans	CB4856	2	1	1 (50%)
C. briggsae	AF16	11	9	6 (55%)
C. briggsae	HK104	1	1	1 (100%)
C. remanei	PB4641	6	4	2 (33%)
C. brenneri	PB2801	4	2	1 (25%)

Using this protocol and the standard laboratory (Bristol N2) strain of *C. elegans*, we obtained transgenic worms from >70% of bombardments, and integrated transgenes with stable expression resulted from >50% of bombardments (**Table 1**, **Supplementary Fig. 5** and **Supplementary Table 1**). We recovered both high-copy-number and single-copy transgenes, with just over half of the strains that we tested carrying fewer than ten copies of the transgene (**Supplementary Fig. 6**). Selection was also efficient in other species, with transgenic worms obtained from 83% of bombardments in *C. briggsae*, and integrated lines from 58% of bombardments (**Table 1**, **Supplementary Fig. 7** and **Supplementary Table 1**). We also obtained transgenic worms from 60% of bombardments in the gonochoristic *Caenorhabditis* species *C. remanei* and *C. brenneri*, with 30% of bombardments in these species resulting in an integrated line (**Table 1** and **Supplementary Table 1**).

Compared to other approaches for generating integrated transgenes[3,5,6], the combination of bombardment and antibiotic selection is rapid and straightforward. The protocol can be used in diverse genetic backgrounds, which should facilitate research on non-model nematode species. Details of primers, strain genotypes and vectors are available in **Supplementary Tables 2–4** and **Supplementary Figures 8** and **9**. Vectors are available from Addgene (**Supplementary Table 4**).

Note: Supplementary information is available on the Nature Methods website.

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Jennifer I Semple[1], Laura Biondini[1] & Ben Lehner[1,2]