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

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To the Editor:

The ability to add or delete genes to the genome of genetic model organisms is essential. Previously, we 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 reported in your pages. Here, we present new reagents that improve the efficiency, facilitate the selection for transgenic strains and expand the set of MosSCI insertion sites.

The Mos1 transposase is expressed from a helper plasmid co-injected with template DNA. Increased transposase expression would be expected to improve both single copy 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*) resulted in a more than 6-fold improvement in transgene insertion efficiency (from 8% to 54% of injected animals) and gene deletion efficiency (from 3%, n=66 injected animals[2] to 20%, n=30 injected animals, Fig. 1b).

An effective, inducible negative selection marker would facilitate identification of transgenic strains. We developed a negative selection marker (*Phsp16-2::peel-1*) based on the toxin *peel-1*[4]. Array animals carrying the *peel-1* plasmid are killed by a two-hour heat-shock at 34°C with approximately 10% false positives (2/19 transgenic animals) (Fig. 1c and Supplementary Fig. 2). A positive selection marker is critical for identifying transgenic animals with insertions or deletions and we have used *unc-119(+)* extensively. Recently, antibiotic selection markers have been developed for nematode transgenesis[5,6]. Targeted *dpy-13* deletions were generated with Neomycin/G418 selection at frequencies comparable to *unc-119* selection (24%, 12/51 injected animals, Fig. 1b), see Supplementary Methods for a discussion of the recommended use of selection markers.

Multiple insertion sites are important for generating complex genotypes. We have 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 or multiple cloning site compatible) based on *unc-119* selection and for one site, *unc-18* selection (Table 1). All sites readily generate MosSCI inserts and express in somatic tissue. Three of the insertion sites (*ttTi4348* I, *ttTi5605* II and *cxTi10816* IV) express robustly in the germline from a ubiquitous promoter (Supplementary Fig. 4). Because

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MosSCI reagents are important for expression in the germline, we generated an expression vector that coexpresses GFP-histone for confirmation of expression (Supplementary Fig. 5). All strains are available from the *Caenorhabditis elegans* Genetics Center (CGC) and targeting plasmids (targeting, transposase and negative selection vectors) from Addgene.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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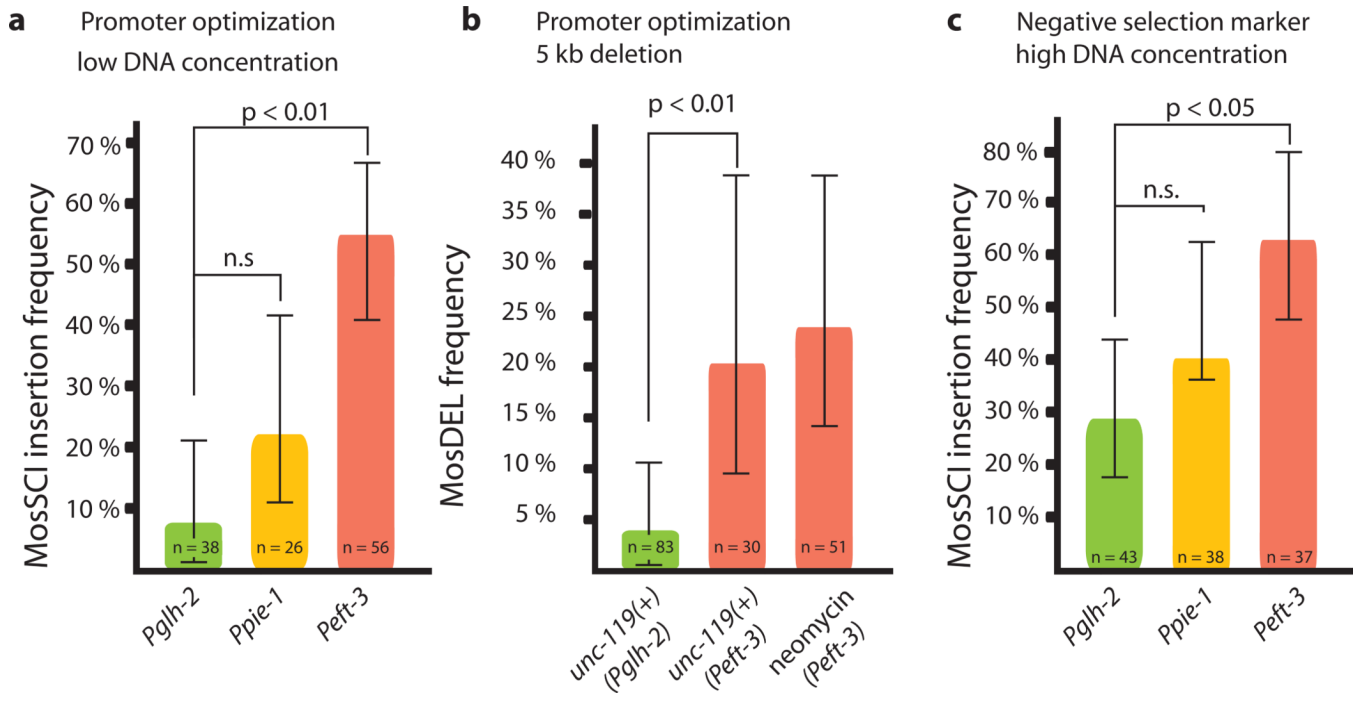

Figure 1.

Improvements to *Mos1*-based genome manipulation. (**a**) A plasmid expressing transposase under the indicated promoters was coinjected with low DNA concentration (32.5 ng/ul) of a 4.4 kb transgene. The plot shows insertion frequency into the *ttTi5605* locus. (**b**) The plot shows the frequency of a 5 kb targeted deletion of *dpy-13*. *Pglh-2* data from Frøkjær-Jensen et al (2010)[2]) using the indicated selection markers (see Supplementary Methods for discussion). (**c**) Insertion frequency with higher total DNA concentrations (~100 ng/ul) and in the presence of the negative selection marker *peel-1*. Error bars, 95 % confidence intervals, significance was determined with Fischer's exact test

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Table 1

MosSCI insertion sites.

	Locus	Genetic position	Insertion strain[1]	Gateway vector[2]	MCS vector	Germline expression[3]	Insertion frequency[4]	Balancer strain
unc-119	ttTi4348	I: −5.32	EG6701	pCFJ210	pCFJ352	yes	25% (3/12)	EG6173
	ttTi4391	I: 7.93	EG6702	pCFJ604	pCFJ353	no	29% (4/21)	EG6171
	ttTi5605	II: 0.77	EG6699	pCFJ150	pCFJ350	yes	43% (6/14)	EG6070
	cxTi10816	IV: 1.41	EG6703	pCFJ212	pCFJ356	yes	20% (2/10)	EG6401
	cxTi10882	IV: −0.05	EG6700	pCFJ201	pCFJ351	variable	29% (4/14)	EG5568
	ttTi14024	X:22.84	EG6705	pCFJ606	pCFJ355	no	21% (3/14)	EG6109
unc-18	ttTi4348	I: −5.32	EG6032	pCFJ448	pCFJ676	yes	N.D.	EG6173

1) 4× outcrossed strain, distributed with extrachromosomal *unc-119* rescue to facilitate handling and maintenance

2) pDESTR4-R3, three-way Gateway Compatible vector

3) Based on germline expression of *Pdpy-30*::GFP transgene

4) Insertion frequency of *Pdpy-30*::GFP::H2B transgene

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