

# Mos1-mediated insertional mutagenesis in *Caenorhabditis elegans*

Thomas Boulin<sup>1,2</sup> & Jean-Louis Bessereau<sup>1,2</sup>

<sup>1</sup>ENS, 46 rue d'Ulm, F-75005 Paris, France. <sup>2</sup>INSERM, U789, 46 rue d'Ulm, F-75005 Paris, France. Correspondence should be addressed to J.-L.B. (jlbesse@biologie.ens.fr.).

Published online 17 May 2007; doi:10.1038/nprot.2007.192

**We describe a protocol for mutating genes in the nematode *Caenorhabditis elegans* using the *Mos1* transposon of *Drosophila mauritiana*. Mutated genes containing a *Mos1* insertion are molecularly tagged by this heterologous transposable element. *Mos1* insertions can therefore be identified in as little as 3 weeks using only basic molecular biology techniques. Mutagenic efficiency of *Mos1* is tenfold lower than classical chemical mutagens. However, the ease and speed with which mutagenic insertions can be mapped compares favorably with the vast amount of work involved in classical genetic mapping. Therefore, *Mos1* could be the tool of choice when screening procedures are efficient. In addition, *Mos1* mutagenesis can greatly simplify the mapping of mutations that exhibit low penetrance, subtle or synthetic phenotypes. The recent development of targeted engineering of *C. elegans* loci carrying *Mos1* insertions further increases the attractiveness of *Mos1*-mediated mutagenesis.**

## INTRODUCTION

Forward genetic screens have been used with great success in the nematode *C. elegans* to identify the genes involved in a variety of biological processes such as programmed cell death<sup>1,2</sup> or RNA interference (RNAi)<sup>3,4</sup> (for review, see ref. 5). Chemical mutagens such as ethyl methanesulfonate (EMS) are most commonly used to generate random mutations in the *C. elegans* genome because they are easy to use, efficient and create a wide range of genetic lesions. For example, a loss-of-function mutation can be obtained for a given gene by screening on average 2,000–4,000 chromosomes mutated by EMS under standard conditions<sup>5</sup>. However, the relative ease with which mutants can be isolated often contrasts with the difficulty of identifying the mutated gene when no further information is available. Genetic mapping, although simplified and rendered more systematic by the advent of single-nucleotide polymorphism (SNP) mapping<sup>6</sup>, is still a tedious and time-consuming task. This is especially true when performing modifier screens (e.g., enhancers, suppressors or synthetic screens), which require manipulation of both the initial mutation and the modifier mutation over the course of the mapping experiments. When phenotypes are subtle or of low penetrance, following the relevant mutation reliably can be difficult, especially if the SNP-containing strain modifies the phenotypic expression of that mutation<sup>7</sup>.

One way to circumvent the need for genetic mapping is to use genome-wide RNAi screening strategies<sup>8</sup>. In *C. elegans*, systemic downregulation of a specific gene can be achieved by feeding animals with bacteria that express double-stranded RNA corresponding to that gene. Libraries that contain most open reading frames of the *C. elegans* genome are now available. Such screens have been spectacularly successful in the identification of the genes with roles during early embryogenesis<sup>9</sup>, for example. However, RNAi screens also have intrinsic limits. First, 15% (ref. 8) to 40% (ref. 10) of the predicted *C. elegans* genes are still absent from RNAi-feeding libraries. Second, RNAi does not downregulate all genes with the same efficiency. Some genes are not sensitive to RNAi or show “hypomorphic” behaviors when compared to actual genetic mutants. Third, RNAi by feeding is very inefficient at targeting genes expressed in neurons<sup>11</sup>. Mutant strains that are more sensitive to RNAi than wild type have been isolated and have

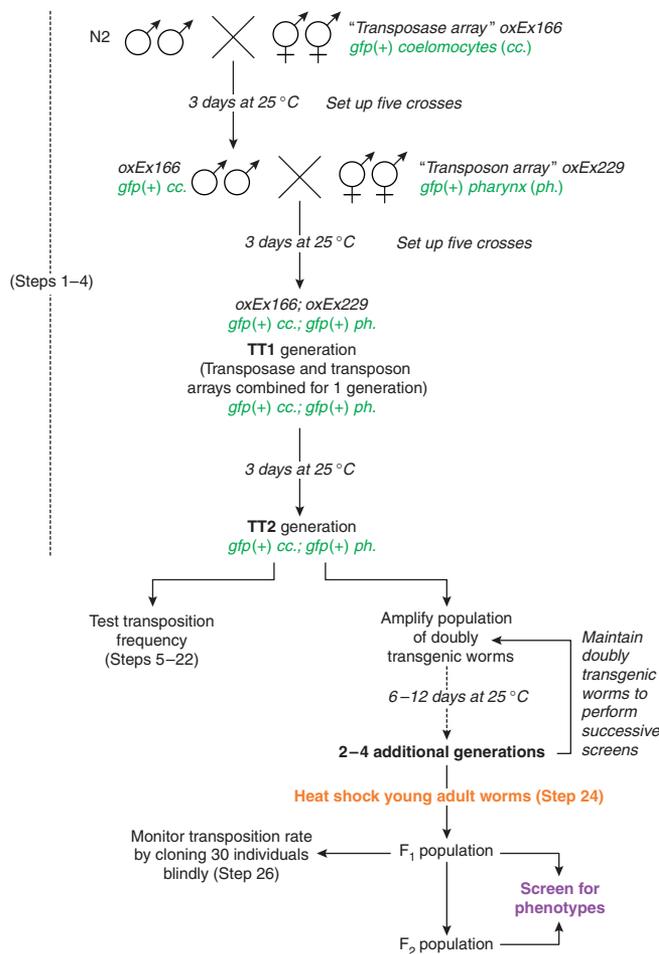
been used successfully to RNAi genes in neurons<sup>12–14</sup>. However, these strains display intrinsic mutant phenotypes that can interfere with the process of interest. Therefore, there exists the need for random forward genetic screens.

We describe here a protocol for implementing a mutagenesis technique based on the mobilization of the *Drosophila mauritiana* transposon *Mos1* (see ref. 15) in the germ line of *C. elegans*<sup>16,17</sup>. This technique has been used successfully in forward genetic screens to identify genes of interest<sup>18–21</sup>. This system has three main advantages.

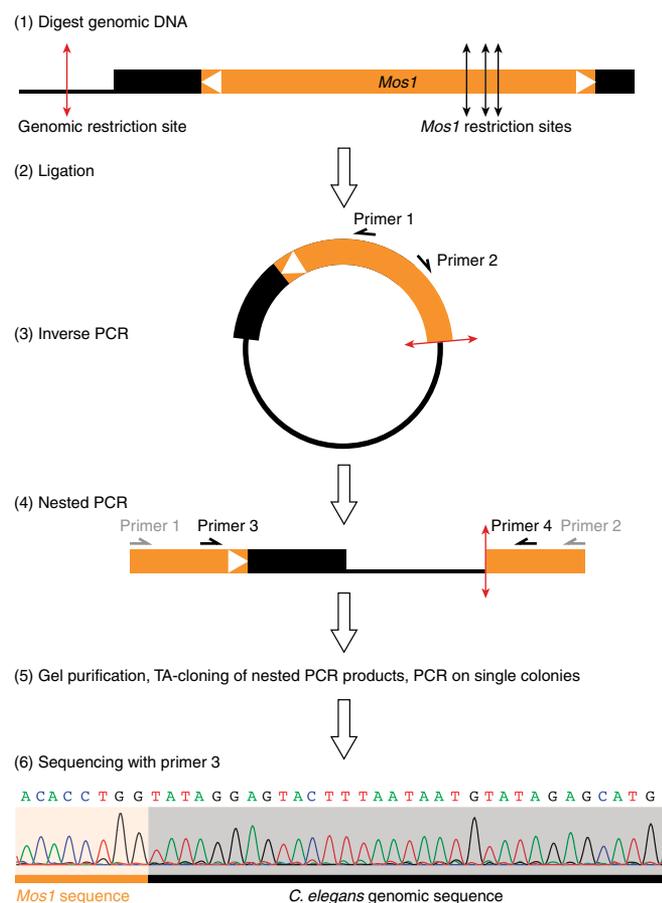
- Mutated genes are molecularly tagged by the insertion of a *Mos1* transposon. By identifying genomic sequences flanking these transposons, they can be mapped with single-nucleotide resolution in as little as 3 weeks, hence circumventing the need for genetic mapping.
- *Mos1* mutagenesis is safe for the experimenter, in contrast to EMS, which is highly mutagenic in all metazoans, including *Homo sapiens*.
- We recently developed a technique called *MosTIC* to engineer custom *C. elegans* alleles of genes of interest by homologous recombination<sup>22</sup>. This technique relies on the remobilization of a *Mos1* transposon from the targeted locus. The remobilization of the transposon induces a double-strand break, which can be repaired by copying engineered sequences from a transgene into the genome. Therefore, *Mos1*-tagged alleles recovered in a screen represent valuable entry points to further manipulate genes of interest.

*Mos1* is a member of the *Tc1/mariner* family of transposons. The *Mos1* sequence is composed of a single open reading frame encoding a transposase, flanked by short terminal inverted repeats. All *Tc1/mariner* elements transpose via a conserved “cut-and-paste” mechanism<sup>23,24</sup>. The transposase binds to the inverted repeats and catalyzes the excision and subsequent insertion of the element from one genomic locus into the other. The general strategy of *Mos1*-mediated mutagenesis is depicted in **Figure 1**. It uses a two-component system to mobilize *Mos1* in the *C. elegans* germ line. The first component, called the “transposon array”, is an

**Figure 1** | Setting up a *Mos1* screen. Set up crosses between *oxEx166* hermaphrodites and N2 males at 25 °C (Steps 1–4). Three days later, select male cross-progeny carrying the “transposon array” *oxEx166* and cross them to hermaphrodites carrying the “transposase array” *oxEx229*. Three days later, select doubly transgenic *oxEx166; oxEx229* hermaphrodites. Pick pools of three doubly transgenic worms. Record the number of generations when both arrays are maintained together in a strain, starting with TT1 for the doubly transgenic worms picked from the second cross. At the TT2 generation, test the transposition rate (Steps 5–22). Amplify the population of doubly transgenic worms for another two to four generations until you are ready to begin a screen (Steps 23–26). As a control, test the transposition rate during the screen by setting aside 30 F<sub>1</sub>s (Step 26). While screening, maintain the doubly transgenic population to perform successive heat shocks and screens. This figure has been adapted with permission from Bessereau<sup>29</sup>.



extrachromosomal transgene carrying multiple copies of *Mos1*. The second is another transgene, the “transposase array”, in which the expression of the *Mos1* transposase gene is under the regulation of a heat shock-inducible promoter. When worms carrying both transgenes are subjected to heat shock, the transposase is expressed and catalyzes the transposition of *Mos1* from the “transposon array” into chromosomal loci. The F<sub>1</sub> or F<sub>2</sub> progeny of these heat-shocked worms can then be screened for phenotypes of interest. As *Mos1* transposons are normally absent from *C. elegans*, *Mos1* insertions represent molecular tags that are easy to identify using inverse PCR<sup>25</sup> (Fig. 2), a DNA amplification method that is often used to identify flanking sequences to a known stretch of DNA. We have adapted this technique to identify *Mos1* insertions in the *C. elegans* genome (see Fig. 2). The average number of insertions per line is 2.5 but can be up to 10 (ref. 17 and unpublished results). When



multiple insertions are present, the mutagenic insertion can be identified either by serial outcrossing of the mutant strain in order to eliminate non-mutagenic insertions or by testing for linkage between a genetic marker (genetic mutant or SNP) and the mutation (Fig. 3). In ideal cases, it can take as little as 3 weeks to molecularly identify the mutated gene in a given mutant.

However, there are a number of points to consider before choosing *Mos1*-mediated mutagenesis for a specific screen.

- *Mos1* mutagenesis is not as straightforward as exposing worms to chemical mutagens such as EMS and requires careful monitoring of the different steps to ensure that mutagenesis will be successful.
- *Mos1* mutagenesis is about ten times less efficient than EMS<sup>17</sup> and generates mostly strong loss-of-function or null alleles as mutations are caused by the insertion of the 1.3 kb *Mos1* sequence into the target gene. It is therefore highly recommended to perform a small-scale pilot screen using EMS to verify that mutants of interest are generated at a frequency

**Figure 2** | Principle of inverse PCR. (1) genomic DNA is first digested, then (2) DNA fragments are ligated onto themselves. (3) Using primers 1 and 2 in *Mos1*, a first PCR serves to amplify genomic sequences flanking the transposon insertion. (4) A nested PCR with primers 3 and 4 enriches for specific products. (5) Fragments are gel-purified and TA-cloned. Individual transformant colonies are screened by PCR. (6) The precise insertion point can be determined by sequencing PCR products using primer 3. This figure has been adapted with permission from Bessereau<sup>29</sup>.

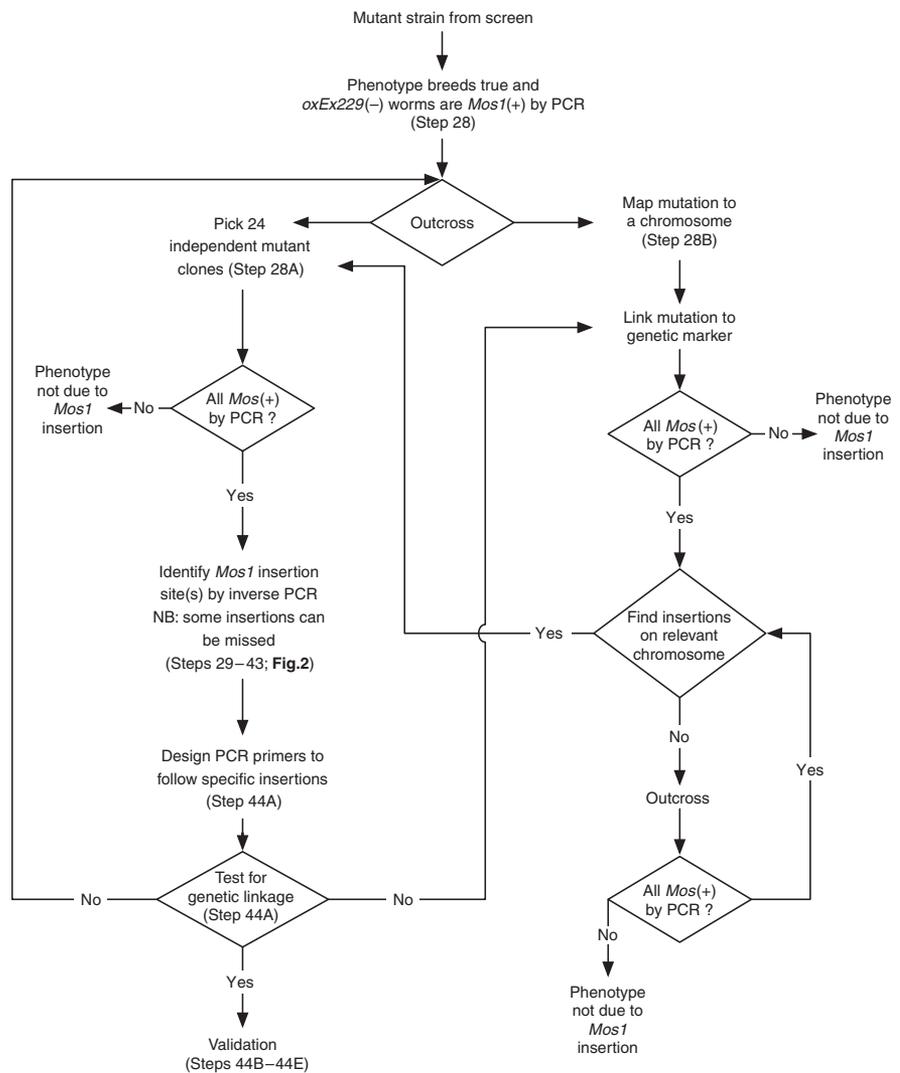


compatible with standard loss-of-function mutations before performing a *Mos1*-based screen. The *Mos1* screen can then be scaled appropriately to maximize the chances of success.

- Like for any mutagen, it is unlikely that all genes are equally mutable by *Mos1*. The only absolute requirement for *Mos1* insertion is the presence of a TA dinucleotide in the target sequence. The analysis of 914 independent *Mos1* insertions did not reveal significant hotspots for *Mos1* insertions except for a region of chromosome I (see ref. 26). Conversely, one could imagine cold-spots, that is, regions of the genome from which *Mos1* would be excluded. However, such cold-spots cannot be defined conclusively before more insertions are identified.
- Transposons can cause mutations by “hit-and-run” mechanisms<sup>27,28</sup>. In this case, the transposon is re-excised from the genome, leaving a molecular scar behind and the resulting mutation is no longer tagged by the transposon. Out of 72 mutants, which we isolated using *Mos1* mutagenesis screens, five mutations were not attributable to *Mos1* insertions. However, we cannot be certain that these mutants have been caused by hit-and-run events because they were recovered at frequencies compatible with spontaneous mutations (unpublished results).

In conclusion, choosing *Mos1*-mediated mutagenesis will depend on the trade-off between time spent screening for mutants and time spent mapping and rescuing a mutation caused by a chemical mutagen. The recent development of *MosTIC*<sup>22</sup>, a genome-engineering technique based on the re-excision of *Mos1* from the locus to manipulate, is an additional parameter to support the choice of *Mos1* as a mutagen. Theoretically, all *Mos1* alleles can serve as

reagents for the *MosTIC* technique, opening new avenues to analyze gene function in a near-physiological context.



**Figure 3 |** Identification of *Mos1*-mutated genes. Confirm that a candidate mutant carries *Mos1* insertions by PCR (Steps 19 and 20). Outcross the candidate mutant and either (i, Step 28B) map the mutation to a chromosome and proceed with inverse PCR (Steps 29–43), or (ii, Step 28A) verify that all outcrossed lines carry *Mos1* insertions (Steps 19 and 20), perform inverse PCR (Steps 29–43) and test for genetic linkage between candidate *Mos1* insertions and the phenotype (Step 44A). Validate candidate mutants by additional means (Steps 44B–E). This figure has been adapted with permission from Bessereau<sup>29</sup>.

**MATERIALS**

**REAGENTS**

- *C. elegans* strains (see **Table 1** and REAGENT SETUP) can be obtained, at no expense, from the *Caenorhabditis* Genetics Center (<http://www.cbs.umn.edu/CGC/>) or from the Bessereau Lab (<http://www.biologie.ens.fr/bcsgnce/>)
- Oligonucleotides, stored at –20 °C as 25 μM stocks (see **Table 2**). The position of each oligonucleotide on the *Mos1* sequence is shown in **Figure 4**
- *Mos1* sequence: NCBI accession number X78906 (<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=1335619>)
- dNTPs 10 mM (Fermentas, #R0182)
- *Taq* DNA polymerase (Invitrogen, 10342-020)
- T4 DNA ligase (Fermentas, #EL0015)
- TA-cloning kit pGEM-T Easy Vector System I (Promega, A1360)
- Transformation-competent *Escherichia coli* bacteria
- QIAquick gel extraction kit (QIAGEN, 28704)

- QIAquick PCR purification kit (QIAGEN, 28704)
- DNEasy DNA extraction kit (QIAGEN, 69504)
- 1 kb Plus DNA Ladder (Invitrogen, 10787-026)
- Worm lysis solution (see REAGENT SETUP)
- Restriction enzymes: *Class A*: *Hae*III (R0108S), *Hpa*II (R0171S) and *Sau*3AI (R0169S); *class B*: *Acl*I (R0137S), *Hha*I (R0139S) and *Mse*I (R0525S); NEB
- Agarose gels for molecular biology
- LB agar plates with appropriate antibiotic (according to TA-cloning kit instructions)
- Nystatin solution (Sigma, N3503, <http://www.sigmaaldrich.com>; see REAGENT SETUP)
- Nematode growth media (NGM) plates (see REAGENT SETUP)

**EQUIPMENT**

- Dedicated Pipetman to load PCR samples onto gels (Gilson) **▲ CRITICAL**  
This minimizes stock reagent contamination by *Mos1* PCR products.



**TABLE 1** | *C. elegans* strains used for *Mos1*-mediated mutagenesis.

Strain	Genotype	Comments
N2	Wild-type strain	N2 is the wild-type reference strain. It does not contain <i>Mos1</i> transposons and is used in many genetic manipulations
EG2762	<i>oxEx166[hsp::MosTransposase; unc-122::gfp; lin-15(+)]</i> The <i>oxEx166</i> transgene will be referred to as “transposase array” throughout the manuscript	This strain contains an extrachromosomal transgene driving the expression of the <i>Mos1</i> transposase under the control of a heat-shock promoter. The EG2762 strain segregates a mixture of non-transgenic and transgenic animals. Transgenic animals are identified based on expression of GFP in coelomocytes
EG1470	<i>oxEx229[Mos1; myo-2::gfp]</i> The <i>oxEx229</i> transgene will be referred to as “transposon array” throughout the manuscript	This strain contains an extrachromosomal transgene with multiple wild-type copies of <i>Mos1</i> . The EG1470 strain segregates a mixture of non-transgenic and transgenic animals. Transgenic animals are identified based on GFP expression in the pharynx

- Parafilm M\* Laboratory Wrapping Film (Fisher, 13-374-10, <http://www.fishersci.com>)
- Dissecting scope equipped with epifluorescence to visualize GFP in *C. elegans*
- Heating waterbath
- Thermal cycler (MJ Research PTC-200)
- 15, 20 and 25 °C incubators

**REAGENT SETUP**

***C. elegans* care and handling** Instructions for maintaining *C. elegans* strains can be found at [http://www.wormbook.org/chapters/www\\_strainmaintain/strainmaintain.html](http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html). Strains are grown at 20 °C on NGM plates unless otherwise noted. Propagate the “transposase array” strain (EG2762 *oxEx116*) and the “transposon array” (EG1470 *oxEx229*) strain at 25 °C in order to diminish the likelihood of transgene silencing in the germ line. Freeze multiple vials of these two strains when you receive them to have multiple backup stocks, which can be thawed if the transgenes become silenced. Instructions on how to freeze *C. elegans* strains can be found at [http://www.wormbook.org/chapters/www\\_strainmaintain/strainmaintain.html](http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html). The strain carrying the “transposase array” can sometimes give rise to “abnormal” progeny (e.g., worms showing uncoordinated, sterile or protruding vulva phenotypes). These phenotypes are likely caused by leaky expression

of the transposase in somatic cells and never inherited in the progeny. Strains containing the “transposon array” and the “transposase array” are maintained separately to prevent spontaneous insertion of *Mos1* transposons and to avoid a decrease of transposition efficacy when the two transgenes are maintained together over many generations. Therefore, the first step of *Mos1*-mediated mutagenesis is to combine both arrays into a single strain (Figs. 1 and 5 and Steps 1–3).

**Worm lysis buffer** 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl<sub>2</sub>, 0.45% (v/v) Tween 20, 0.45% (v/v) NP-40 and 0.01% (w/v) gelatin. Worm lysis buffer can be stored at 4 or –20 °C.

**Worm lysis solution** Add 1 mg ml<sup>-1</sup> proteinase K to worm lysis buffer. Worm lysis solution is used to prepare crude worm lysates (Step 15) and should be prepared fresh.

**Nystatin solution** Dissolve 4 g of nystatin in 200 ml ethanol and 200 ml ammonium acetate. Heat to 50 °C until the solution is homogeneous and filter-sterilize using a 0.22 μm filter.

**NGM plates** 3 g NaCl, 16 g agar, 2.5 g peptone, 975 ml H<sub>2</sub>O, 1 ml cholesterol (5 mg ml<sup>-1</sup> in ethanol), 2.5 ml nystatin, 1 ml of 1 M CaCl<sub>2</sub>, 1 ml of 1 M MgSO<sub>4</sub> and 25 ml KH<sub>2</sub>PO<sub>4</sub> pH 6; the last five ingredients should be filter-sterilized and added after autoclaving using sterile techniques.

**PROCEDURE**

**Generating doubly transgenic worms ● TIMING 12 days**

**1** | Cross 12 N2 males with four hermaphrodites carrying the “transposase array” (*oxEx166*—labeled by *gfp* expression in coelomocytes) (see Figs. 1 and 5 and REAGENTS). Grow the worms at 25 °C for 3 days. Only a fraction of the male progeny produced by these crosses will carry the “transposase array” since the transgene is not integrated into the genome. To obtain a sufficient number of transgenic males for Step 2, set up at least five crosses.

**2** | From the crosses in Step 1, select male cross progeny carrying the “transposase array” and set up at least 5 crosses between 12 transgenic males and 4 L4 hermaphrodites carrying the “transposon array” (*oxEx229*—labeled by *gfp* expression in the pharynx) (see Figs. 1 and 5 and REAGENTS). Grow the worms at 25 °C for 3 days.

**3** | From the crosses in Step 2, select doubly transgenic worms carrying both the “transposon array” and the “transposase array” (see Figs. 1 and 5 and REAGENTS). Label these worms T1 to indicate that this is the first generation in which the “transposase array” and the “transposon array” are combined. To amplify the population of doubly transgenic worms, pick pools of three doubly transgenic hermaphrodites to fresh plates and let them self-fertilize at 25 °C; pick five or more pools.

**▲ CRITICAL STEP** If brood size of doubly transgenic worms is very low at 25 °C (less than 50 progeny per animal), temperature can be decreased down to 20 °C without significant loss of transposition efficiency in the later steps.

**TABLE 2** | Oligonucleotides used in this protocol.

Oligonucleotide name	Sequence	Used in
oJL102	5'-CAACCTTGACTGTGCAACCACCATAG-3'	Steps 18, 19, 34
oJL103	5'-TCTGCGAGTTGTTTTGCGTTGAG-3'	Steps 18–20, 32
oJL104	5'-ACAAGAGCGAACGCAGACAGT-3'	Step 20
oJL114	5'-AAAGATTCAGAAGGTTCGGTAGATGGG-3'	Step 32
oJL115	5'-GCTCAATTCGCGCCAACTATG-3'	Step 34
oJL116	5'-GAACGAGAGGCAGATGGAGAGG-3'	Step 34
iPCR1a	5'-GACCTTGTAAGTGTCAACCTTGACTG-3'	Step 32
iPCR1b	5'-GACAATCGATAAATATTTACGTTTGGAGAC-3'	Step 32
iPCR2b	5'-CATCTATATGTTGCAACCGACATTCCC-3'	Step 34



## PROTOCOL

**▲ CRITICAL STEP** It is important to track the number of generations where the transposon array and the transposase arrays are combined as transposition efficiency tends to decrease over time. In addition, it is possible that leaky expression of the transposase causes random insertions in the background of doubly transgenic worms. However rare, this could make it more difficult to identify mutagenic insertions in later mapping stages. Therefore, generate new doubly transgenic strains after 15 generations or sooner if transposition frequency decreases.

4| Maintain and amplify the population of doubly transgenic worms by picking pools of three doubly transgenic hermaphrodites to fresh plates and growing them at 25 °C. Repeat until a sufficiently large population is obtained.

**▲ CRITICAL STEP** The plates of each successive generation should be labeled TT2, TT3 and so on.

**▲ CRITICAL STEP** Set apart ten young TT2 adults to test the transposition efficiency (Steps 5–22).

### Measuring transposition rate: heat-shock-induced *Mos1* transposition ● TIMING ~ 4 h

5| While the population of doubly transgenic worms grows, perform a test heat shock on ten doubly transgenic young adults from the TT2 generation to verify the transposition rate of the strain. Equilibrate a water bath to 33 °C ahead of time.

**▲ CRITICAL STEP** Heat shocking is deleterious for worms that contain both the transposase and the transposon arrays, most probably because the heat-shock promoter is driving high levels of transposase expression in somatic tissues and causes extremely high rates of transposition in somatic cells. We estimate that, on average, ten copies of *Mos1* insert into each haploid genome in somatic tissues. Thus, heat-shock conditions have been optimized to achieve efficient transposition without overly affecting brood size. On average, heat-shocked worms will produce around 50 F<sub>1</sub> progeny, which is significantly lower than wild type.

6| Use two layers of parafilm to seal the plate containing the ten doubly transgenic young adult TT2 worms set apart in Step 4.

**▲ CRITICAL STEP** Heat shock only young adult stage doubly transgenic worms. Do not heat shock L4 larvae because they are more likely to die or be sterile after a heat shock.

7| Submerge the plate in the water bath at 33 °C for 1 h.

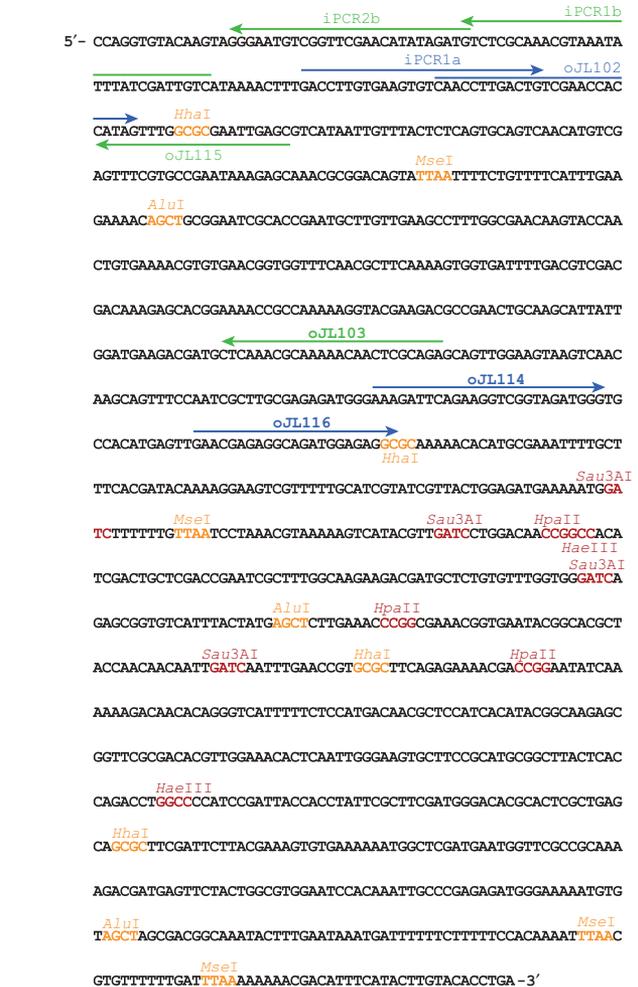
8| Remove the plate from the water bath and place it at 20 °C for 1 h.

9| Put the plate back at 33 °C for 1 h.

10| Take the plate out of the water bath, remove the parafilm and let the worms recover and lay their first eggs for 12 h at 20 °C.

**TABLE 3** | Summary of heat-shock treatment.

Heat shock
1 h at 33 °C
1 h at 20 °C
1 h at 33 °C
12 h at 20 °C
Transfer worms to fresh plates at 20 °C



**Figure 4** | *Mos1* sequence features. Sequence of the *Mos1* transposon (accession number X78906). When *Mos1* is inserted in the genome, TA dinucleotides flank this sequence. Primers and restriction sites used in this protocol are indicated. Cleavage sites for class A enzymes are shown in red. Cleavage sites for class B enzymes are shown in orange. This figure has been adapted with permission from Bessereau<sup>29</sup>.

11| After 12 h, transfer pairs of worms onto five fresh plates and let them grow at 20 °C. The heat-shock regime outlined in Steps 7–11 is summarized in **Table 3**.

12| At 24–30 h after heat shock, randomly pick, under visible light, six individual F<sub>1</sub> progeny from each of the five plates of heat-shocked worms from Step 11. Move each worm to individual plates. Grow these F<sub>1</sub> worms for 3 days at 25 °C.

13| After 3 days, pick ten F<sub>2</sub> worms that do not carry the “transposon array” *oxEx229* (i.e., have no GFP in the pharynx) from each clonal F<sub>1</sub> plate from Step 12 together onto a new plate. Grow these worms at 25 °C for 3 days.

▲ **CRITICAL STEP** As the PCR assay to detect *Mos1* insertions in the genome also detects *Mos1* in the “transposon array”, it is essential to pick only those worms that do not contain the array (i.e., show no *gfp* expression in the pharynx; see Fig. 5). Note that presence of the “transposase array” *oxEx166* (labeled by *gfp* expression in coelomocytes; see Fig. 5) is inconsequential, as the PCR assay used to detect *Mos1* transposons does not amplify the transposase array.

**Measuring transposition rate: preparation of worm lysates for PCR** ● **TIMING** ~ 2 h

14| In the F<sub>3</sub> generation, prepare worm lysates from each clone by lysing 20 worms that do not carry the “transposon array”.

▲ **CRITICAL STEP** Contamination of these worm lysates with worms that contain the “transposon array” will lead to false-positive PCR and erroneous values of transposition rates. If some of the F<sub>3</sub> worms still carry the *oxEx229* “transposon array”, either (a) pick ten worms that do not carry the “transposon array” to a fresh plate, wait for 4 days and proceed to Step 15 if none of the F<sub>4</sub> worms carries the *oxEx229* array; or (b) prepare worm lysates as described in Step 15 and verify that none of the lysed F<sub>3</sub> worms is mosaic for the “transposon array” by performing an oJL103/oJL104 PCR, which detects the presence of the “transposon array” (see Step 20). Use worms carrying the *oxEx229* “transposon array” as a positive control for this experiment.

15| For each clone, place 20 worms in 10 µl of worm lysis solution and freeze them for 15 min at –80 °C. Freezing is a crucial step that disrupts the envelope of the worm and promotes efficient lysis.

▲ **CRITICAL STEP** Remember to include positive controls (worms carrying the “transposon array”) and negative controls (N2 worms).

16| Incubate for 1 h at 65 °C to lyse the worms.

17| Incubate for 15 min at 95 °C to inactivate proteinase K in the lysis solution.

■ **PAUSE POINT** Keep the lysates on ice or freeze them at –20 °C.

**Measuring transposition rate: PCR for *Mos1* transposon detection** ● **TIMING** ~ 3 h

18| Set up a PCR for each worm lysate as detailed below, using oligonucleotides oJL102 and oJL103 to detect *Mos1* insertions (see REAGENTS and Fig. 4).

Reagent	Volume (25 µl total)	Final
oJL102 (25 µM)	0.5 µl	500 nM
oJL103 (25 µM)	0.5 µl	500 nM
dNTP (10 mM)	0.5 µl	200 µM
10× PCR buffer (–MgCl <sub>2</sub> )	2.5 µl	1×
MgCl <sub>2</sub> (50 mM)	0.75 µl	1.5 mM
<i>Taq</i> (5 U µl <sup>-1</sup> )	0.5 µl	2.5 U
H <sub>2</sub> O	18.75 µl	
Worm lysate	1 µl	

19| Run the PCR program detailed below.

Cycle number	Denaturation	Annealing	Extension
1	94 °C, 3 min	None	None
2–31	94 °C, 45 s	56 °C, 1 min	72 °C, 45 s
32			72 °C, 4 min

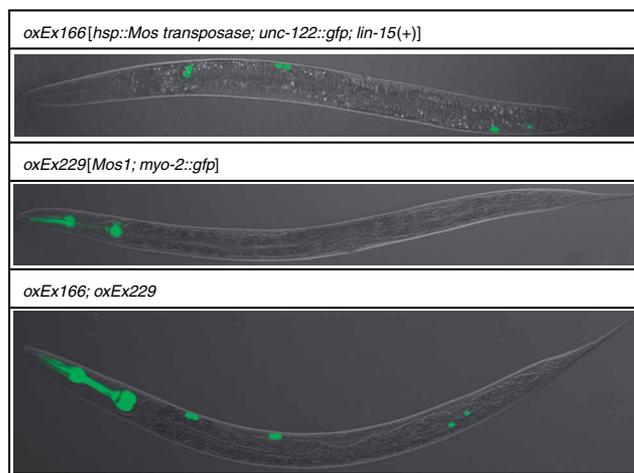


Figure 5 | Representative pictures of transgenic strains used in this protocol. See Table 1 for detailed information about these strains.

## PROTOCOL

**20** | If necessary (see Step 14), check for the absence of contaminant *oxEx229* DNA by performing a PCR using  $\alpha$ JL103 and  $\alpha$ JL104 as outlined below. Presence of *oxEx229* contaminant DNA will result in a PCR product of 726 bp. Use the PCR program detailed in Step 19.

Reagent	Volume (25 $\mu$ l total)	Final
$\alpha$ JL103 (25 $\mu$ M)	0.5 $\mu$ l	500 nM
$\alpha$ JL104 (25 $\mu$ M)	0.5 $\mu$ l	500 nM
dNTP (10 mM)	0.5 $\mu$ l	200 $\mu$ M
10 $\times$ PCR buffer (–MgCl <sub>2</sub> )	2.5 $\mu$ l	1 $\times$
MgCl <sub>2</sub> (50 mM)	0.5 $\mu$ l	1 mM
<i>Taq</i> (5 U $\mu$ l <sup>-1</sup> )	0.5 $\mu$ l	2.5 U
H <sub>2</sub> O	19 $\mu$ l	
Worm lysate	1 $\mu$ l	

**21** | Analyze PCR products on a 1% agarose gel. Presence of a *Mos1* transposon will result in a PCR product of 355 bp.

**▲ CRITICAL STEP** Use a dedicated pipetman to load these PCR products to avoid contamination of future PCR experiments by *Mos1* PCR products.

**22** | Calculate the transposition rate by dividing the number of *Mos1*-positive clones by the total number of clones that were analyzed. It should be 50  $\pm$  15%. If the transposition rate is lower, refer to the TROUBLESHOOTING section.

**? TROUBLESHOOTING**

### Screening with *Mos1* ● TIMING variable

**23** | To start a screen, select doubly transgenic early adult stage worms using an epifluorescence dissecting microscope from the population that has been amplified in Step 4. Pool 50 doubly transgenic worms onto one plate. Make as many pools as necessary. These animals are the P<sub>0</sub> population.

**▲ CRITICAL STEP** Remember that each heat-shocked P<sub>0</sub> worm will produce only ~50 F<sub>1</sub> progeny. As *Mos1* mutagenesis is about ten times less efficient than chemical mutagenesis with EMS, scale your experiment to screen the appropriate number of haploid genomes. Results from an EMS pilot screen are valuable to estimate this number. In addition, note that some of the P<sub>0</sub> worms might die because of the heat shock. Therefore, more worms should be heat-shocked than theoretically necessary.

**24** | Heat shock the worms immediately, as outlined in Steps 7–11 and **Table 3**.

**25** | Once the worms have recovered for 12 h and depending on the screening strategy, move individual or groups of P<sub>0</sub> worms onto small or large plates. Alternatively, let multiple heat-shocked worms lay eggs for a given period of time on a fresh plate (e.g., 12 h) and retransfer them to fresh plates at regular intervals. This will synchronize the F<sub>1</sub> population and make it easier to manage large numbers of worms. Stop harvesting F<sub>1</sub> progeny 36 h after heat shock as these worms will not carry *Mos1* insertions<sup>17</sup>.

**26** | When the population of F<sub>1</sub> animals is grown, set apart 30 F<sub>1</sub>s on individual plates to measure the transposition rate that was obtained in the heat-shocked P<sub>0</sub>s from Step 24. Proceed with these F<sub>1</sub>s as described in Steps 13–22.

**▲ CRITICAL STEP** A test of transposition rate must be performed during the screen to control the efficacy of the mutagenesis step.

**? TROUBLESHOOTING**

**27** | Screen the F<sub>1</sub> or the F<sub>2</sub> population for the phenotype you are interested in. Clone individual mutants to establish mutant lines. Unless two worms with clearly different phenotypes are found on a given plate, assume that mutants on a plate are all siblings and represent a single mutant line.

**? TROUBLESHOOTING**

### Identifying *Mos1* mutants: testing for mendelian segregation of the mutation

**28** | Once a candidate mutant has been isolated from a screen, confirm that its phenotype breeds true to the next generation and test whether worms that have lost the “transposon array” *oxEx229* are positive for *Mos1* by PCR (Steps 15–19). After the presence of *Mos1* in the mutant strain is confirmed, use either option A or B. Option A involves outcrossing the mutant against N2 or another relevant genetic background. This is often the simplest approach. Option B involves rough genetic mapping using SNPs or genetic markers. Mapping is more informative but often requires more work and reagents.

**▲ CRITICAL STEP** Once a *Mos1*-carrying mutant line is established, you could theoretically perform inverse PCR (Steps 29–43) immediately. However, we recommend applying inverse PCR only after outcrossing the mutant line once to increase the chances of identifying relevant *Mos1* insertions. This additional step also serves to obtain preliminary mapping or genetic linkage information, which will narrow down the list of candidate insertions when multiple insertions are localized in a strain.

#### (A) Outcrossing against N2 or another relevant genetic background ● TIMING variable

- (i) Outcross the mutant strain against N2 (or another appropriate genetic background) and generate 24 independent outcrossed clones that have been reselected for the phenotype of interest.

- (ii) Test whether all of these independent clones carry at least one *Mos1* insertion by PCR, as detailed in Steps 15–19.
- (iii) If all independent clones are positive for *Mos1*, select one clone on which to perform inverse PCR (Steps 29–43).
  - **PAUSE POINT** The remaining clones can be kept at 15 °C and used later to validate a mutagenic insertion (see Step 44A).
  - ▲ **CRITICAL STEP** If one or multiple mutant clones are found to contain no *Mos1* transposon, then the mutation is unlikely to be tagged with a *Mos1* insertion and will have to be identified by classical genetic mapping.

**(B) Mapping using SNPs or genetic markers ● TIMING variable**

- (i) Map the mutation to a chromosome by using SNPs or visible genetic markers as outlined in the WormBook ([http://www.wormbook.org/chapters/www\\_introandbasics/introandbasics.html](http://www.wormbook.org/chapters/www_introandbasics/introandbasics.html)). This will provide genetic mapping information, which can be used to confirm or exclude *Mos1* insertions identified by inverse PCR.
- (ii) Analyze independent mutant lines generated during the mapping process for the presence of *Mos1* insertions by PCR, as detailed in Steps 15–19.
- (iii) If all independent clones are positive for *Mos1*, select one clone on which to perform inverse PCR (Steps 29–43).

**Identifying *Mos1* mutants: localizing *Mos1* insertions by inverse PCR ● TIMING variable**

**29|** Prepare genomic DNA.

▲ **CRITICAL STEP** Inverse PCR can be performed on purified genomic DNA (DNEasy DNA extraction kit; Qiagen) or on a worm lysate. Worm lysates work most of the time; a 20 µl lysate can be prepared from 20 worms as detailed in Steps 15–17, half of which should be used for digestion. However, we prefer to use purified genomic DNA because it gives more reliable results.

**30|** Digest approximately 150 ng of genomic DNA in a final volume of 30 µl, as detailed in the table below. Incubate the digestion mix for 3 h to overnight at 37 °C. Inactivate the restriction enzyme by incubating at 75 °C for 15 min. We recommend that each DNA sample be analyzed using at least two different enzymes.

Component	Amount	Final
DNA sample	10 µl (or 150 ng)	150 ng
Restriction buffer (10×)	3 µl	1×
Restriction enzyme (10 U µl <sup>-1</sup> )	1 µl	10 U
H <sub>2</sub> O	16 µl	

▲ **CRITICAL STEP** Six different restriction enzymes can be used to digest the genomic DNA (see Fig. 4, REAGENTS and Step 37). Each enzyme will cut in a different position in *Mos1* and the flanking genomic DNA sequence (see Fig. 4). Enzymes are grouped into two classes depending on where they cut in *Mos1* and therefore which set of primers is used to perform the inverse PCRs: *class A*: *Hae*III, *Hpa*II and *Sau*3AI; *class B*: *Alu*I, *Hha*I and *Mse*I. When there are multiple insertions in a strain, often only a subset of insertions will be identified using one restriction enzyme. Indeed some insertions can be missed if the closest restriction site in the flanking genomic sequence is too far (resulting in no PCR product) or too close (resulting in too little sequence to be informative).

**31|** Set up ligations of digested genomic DNA as detailed below. Setting up the ligation in a large volume will ensure that intramolecular reactions are favored. Incubate overnight at 4 or 15 °C.

Component	Amount	Final
Digestion mix from Step 30	10 µl	
10× ligation buffer	10 µl	1×
T4 ligase (10 U µl <sup>-1</sup> )	1 µl	10 U
H <sub>2</sub> O	79 µl	

■ **PAUSE POINT** The ligation reactions can be kept frozen at –20 °C indefinitely.

**32|** Set up inverse PCRs, as detailed below, using 3 µl of ligation mix (from Step 31) using the appropriate primer combination depending on whether a class A (primers oJL103 and oJL114) or class B (primers iPCR1a and iPCR1b) enzyme was used to digest the genomic DNA.

Component	Amount	Final
Ligation mix from Step 31	3 µl	
Primer 1 (25 µM)	0.5 µl	500 nM
Primer 2 (25 µM)	0.5 µl	500 nM
dNTP (10 mM)	0.5 µl	200 µM
MgCl <sub>2</sub> (50 mM)	0.75 µl	1.5 mM
PCR buffer (10×) (–MgCl <sub>2</sub> )	2.5 µl	1×
<i>Taq</i> polymerase (10 U µl <sup>-1</sup> )	0.5 µl	5 U
H <sub>2</sub> O	16.75 µl	



## PROTOCOL

**33|** Perform PCR using the program outlined below. Choose an annealing temperature appropriate for the primer pair used in Step 32.

Cycle number	Denaturation	Annealing	Extension
1	94 °C, 3 min	None	None
2–31	94 °C, 45 s	60 °C (class A) or 59 °C (class B), 1 min	72 °C, 1 min
32			72 °C, 5 min

**34|** Set up a nested PCR using a 1:200 dilution of the PCR products from Step 33 and the appropriate primer pair: primers oJL115 and oJL116 for class A; primers oJL102 and IPCR2b for class B.

Component	Amount	Final
1:200 dilution of inverse PCR from Step 33	1 µl	
Primer 3 (25 µM)	0.5 µl	500 nM
Primer 4 (25 µM)	0.5 µl	500 nM
dNTP (10 mM)	0.5 µl	200 µM
MgCl <sub>2</sub> (50 mM)	0.75 µl	1.5 mM
PCR buffer (10×) (–MgCl <sub>2</sub> )	2.5 µl	1×
<i>Taq</i> polymerase (10 U µl <sup>–1</sup> )	0.5 µl	5 U
H <sub>2</sub> O	18.75 µl	

**35|** Perform PCR using the appropriate program (for class A or for class B) as outlined below.

Cycle number	Denaturation	Annealing	Extension
1	94 °C, 3 min	None	None
2–26	94 °C, 45 s	62 °C (class A) or 59 °C (class B), 1 min	72 °C, 1 min
27			72 °C, 5 min

**36|** Run PCR products from Step 35 on a 1.8% agarose gel. Each PCR can generate multiple products of different sizes, which can correspond to independent *Mos1* insertions or to a single *Mos1* insertion (owing to partial digests, religation of degraded DNA fragments, illegitimate PCR priming, etc.). Three examples of inverse PCR products are shown in **Figure 6a**.

▲ **CRITICAL STEP** Use a dedicated pipetman to load these PCR products.

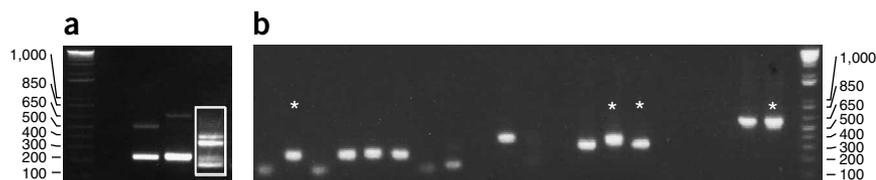
**37|** Gel-purify candidate PCR products using the QIAquick gel purification kit. Purify only those fragments that are longer than the minimal sizes listed in the table below. Shorter fragment would not be informative.

Enzyme class	Restriction enzyme	Minimal size (bp)
A	<i>Sau3AI</i>	~250
	<i>HpaII</i>	~300
	<i>HaeIII</i>	~300
B	<i>HhaI</i>	~80
	<i>MseI</i>	~160
	<i>AluI</i>	~190

**38|** Gel-purified fragments could be sent for sequencing directly if individual bands can be clearly separated. However, we recommend TA-cloning the gel-purified PCR products in order to obtain individual clones that will guarantee successful sequencing. TA-clone gel-purified PCR products according to the manufacturer's protocol. Transform the TA-cloning reactions and select transformants using the appropriate antibiotic selection.

■ **PAUSE POINT** TA-cloning ligations can be stored at –20 °C, or remain at 4 °C for 2 days before being transformed. Transformant colonies can be stored for some time at 4 °C.

**39|** Perform colony PCRs on individual transformant colonies from the TA cloning using the PCR protocol described in Steps 34 and 35, that is, pick a single colony using a sterile pipet tip and swirl the tip in the PCR tube containing the PCR mix. It is usually possible to recover clones representing all the principal PCR products obtained in Step 36.



**Figure 6 |** Inverse PCR examples. **(a)** Examples of inverse PCR on three different independent strains using the *MseI* restriction enzyme. The products in lane 3 (white box) were TA-cloned by cutting out the four most prominent bands. All visible bands were of sufficient size to be informative. **(b)** Colony PCRs derived from the TA cloning of the inverse PCR (white box in **a**). Stars indicate four different samples that were sequenced.

40| Analyze PCR products on a 1.8% agarose gel (see **Fig. 6b**).  
**▲ CRITICAL STEP** Use a dedicated pipetman to load these PCR products.

41| If PCR products of different sizes are obtained, select one representative sample for each size class and purify it using the QIAquick PCR purification kit (see **Fig. 6b**). Do not use samples containing multiple bands because sequencing will fail.

42| Sequence samples using either oJL115 (for class A enzymes) or oJL102 (for class B enzymes) as a primer.

43| Analyze sequencing results. Legitimate PCR products must contain the 5' end of the transposon sequence directly followed by the *C. elegans* genomic sequence starting at a TA dinucleotide (**Fig. 2**). *Mos1* insertions can be mapped to the genome by using BLAST or by using the Genome Browser function of wormbase (<http://www.wormbase.org/db/seq/gbrowse/wormbase/>).

### Identifying *Mos1* mutants: validating *Mos1* alleles

44| Once a candidate insertion has been identified by inverse PCR, use one or more of the five basic strategies (options A–E) described below to validate the mutant.

#### (A) Test for genetic linkage

- (i) This approach tests whether a mutant phenotype cosegregates with a given *Mos1* insertion. First, design PCR primers located on each side of the *Mos1* insertion. In combination with a primer inside *Mos1*, this allows one to differentiate between loci that do or do not contain a *Mos1* insertion.
- (ii) Next, in the case of a recessive mutation, outcross the mutant against N2 (or another appropriate genetic background) and generate 24 independent mutant lines. If a candidate *Mos1* insertion is homozygous in all 24 independent lines, the mutation is less than two genetic map units away from the *Mos1* insertion. As *Mos1* transposons are usually scattered throughout the genome, the likelihood that there will be no other *Mos1* insertion in the vicinity is high. It is possible to further narrow down the genetic interval by increasing the number of independent clones, for example, 49 independent clones will place the mutation within less than one genetic map unit of the *Mos1* insertion. Conversely, if a mutant clone that lacks the *Mos1* insertion is found, this strain should be used to start a new mapping cycle.

#### (B) Map the mutant phenotype to a chromosomal region

- (i) Use genetic mapping data to confirm or reject a candidate *Mos1* insertion, as discussed in Step 28B.

#### (C) Independent alleles

- (i) If a screen produces independent *Mos1* mutants in the same gene, they validate each other. In addition, alleles previously generated in other screens (e.g., using chemical mutagens) or by reverse genetic approaches ([http://www.wormbook.org/chapters/www\\_introreversegenetics/introreversegenetics.html#d0e928](http://www.wormbook.org/chapters/www_introreversegenetics/introreversegenetics.html#d0e928)) can be used to validate a *Mos1* mutant candidate using genetic complementation tests ([http://www.wormbook.org/chapters/www\\_complementation/complementation.html](http://www.wormbook.org/chapters/www_complementation/complementation.html)).

#### (D) Phenocopy by RNAi

- (i) If no other alleles are available, RNAi can be used to phenocopy the candidate mutant ([http://www.wormbook.org/chapters/www\\_introreversegenetics/introreversegenetics.html](http://www.wormbook.org/chapters/www_introreversegenetics/introreversegenetics.html)).

#### (E) Rescue experiments

- (i) A definitive way to validate a candidate mutant consists of complementing the defect by providing a wild-type copy of the gene via a transgene ([http://www.wormbook.org/chapters/www\\_transformationmicroinjection/transformationmicroinjection.html](http://www.wormbook.org/chapters/www_transformationmicroinjection/transformationmicroinjection.html)).

### ● TIMING

Steps 1–4: generating doubly transgenic worms, 12 days

Steps 5–13: measuring transposition rate, heat-shock-induced *Mos1* transposition, ~4 h

Steps 14–17: measuring transposition rate, preparation of worm lysates for PCR, ~2 h

Steps 18–22: measuring transposition rate, PCR for *Mos1* transposon detection, ~3 h

Steps 23–27: screening with *Mos1*, variable

Step 28: identifying *Mos1* mutants, testing for mendelian segregation of the mutation, variable

Steps 29–43: identifying *Mos1* mutants, localizing *Mos1* insertions by inverse PCR, variable

Step 44: identifying *Mos1* mutants, validating *Mos1* alleles, variable

### ? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 4**.

**TABLE 4** | Troubleshooting table.

Step	Problem	Solution
22, 26	Low transposition rate	Thaw fresh stocks of EG2762 and EG1470 strains
	Exceptionally high transposition rate	(A) The doubly transgenic strain used for heat shock may contain a background insertion. (B) Possible contaminations of reagents and equipment with <i>Mos1</i> PCR fragments. Change all reagents. Clean bench space and pipettes thoroughly
	Background insertions	Generate new doubly transgenic strains by crossing <i>oxEx166</i> and <i>oxEx229</i>
27	No mutant of interest after mutagenesis	(A) Might be explained by low transposition. Check transposition rates (B) Too few genomes were mutagenized. Scale up the screen based on the results of a pilot EMS screen

**ANTICIPATED RESULTS**

**Transposition rate**

The average transposition rate should be  $50 \pm 15\%$ . If the transposition rate is consistently lower than average, thaw the “transposon array” and “transposase array” strains again and generate new doubly transgenic strains.

**Number of *Mos1* insertions per strain**

The number of *Mos1* insertion per strain can range from 1 to 10 and averages 2.5 (see ref. 17).

**Mutant yield**

We estimate that *Mos1* mutagenesis is roughly ten times less efficient than chemical mutagenesis with EMS<sup>17</sup>. This number was calculated by measuring the relative mutagenicity of EMS and *Mos1* in a screen for mutants resistant to the antihelminthic drug levamisole. The mutagenic frequency of EMS was found to be  $2.34 \times 10^{-3}$  versus  $4.3 \times 10^{-4}$  for *Mos1*. Taking into account that *Mos1* only affects gametes of the maternal germline, this means that ten times more genomes must be analyzed to obtain the same number of mutants. This is a critical parameter to take into account when deciding to undertake a *Mos1* genetic screen and for future scaling of the experiment.

**Nature of mutations**

Most *Mos1* insertions will lead to strong loss-of-function or null alleles. However, some insertions can generate hypomorphic alleles too. Insertions in promoters, introns or in late exons of genes have been found to partially decrease gene activity. In rarer cases, semi-dominant alleles have also been isolated<sup>17</sup>.

**ACKNOWLEDGMENTS** We thank Iskra Katic for critical reading of the manuscript and members of the Bessereau lab and the Jorgensen lab for optimizing technical aspects of the protocol. T.B. is an EMBO long-term fellow. This work was funded by a European Union Grant (6th Framework program, PL-503334, code NemageneTAG) and an AVENIR grant from the Institut National de la Santé et de la Recherche Médicale.

**COMPETING INTERESTS STATEMENT** The authors declare no competing financial interests.

Published online at <http://www.natureprotocols.com>  
Rights and permissions information is available online at <http://npg.nature.com/reprintsandpermissions>

- Ellis, H.M. & Horvitz, H.R. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* **44**, 817–829 (1986).
- Hedgecock, E.M., Sulston, J.E. & Thomson, J.N. Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science* **220**, 1277–1279 (1983).
- Ketting, R.F., Haverkamp, T.H., van Luenen, H.G. & Plasterk, R.H. Mut-7 of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNaseD. *Cell* **99**, 133–141 (1999).
- Tabara, H. *et al.* The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*. *Cell* **99**, 123–132 (1999).
- Jorgensen, E.M. & Mango, S.E. The art and design of genetic screens: *Caenorhabditis elegans*. *Nat. Rev. Genet.* **3**, 356–369 (2002).
- Wicks, S.R., Yeh, R.T., Gish, W.R., Waterston, R.H. & Plasterk, R.H. Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. *Nat. Genet.* **28**, 160–164 (2001).
- Hodgkin, J. & Doniach, T. Natural variation and copulatory plug formation in *Caenorhabditis elegans*. *Genetics* **146**, 149–164 (1997).
- Kamath, R.S. *et al.* Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421**, 231–237 (2003).
- Sonnichsen, B. *et al.* Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. *Nature* **434**, 462–469 (2005).
- Rual, J.F. *et al.* Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. *Genome Res.* **14**, 2162–2168 (2004).
- Timmons, L., Court, D.L. & Fire, A. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263**, 103–112 (2001).
- Kennedy, S., Wang, D. & Ruvkun, G. A conserved siRNA-degrading RNase negatively regulates RNA interference in *C. elegans*. *Nature* **427**, 645–649 (2004).
- Simmer, F. *et al.* Genome-wide RNAi of *C. elegans* using the hypersensitive rrf-3 strain reveals novel gene functions. *PLoS Biol.* **1**, E12 (2003).
- Wang, D. *et al.* Somatic misexpression of germline P granules and enhanced RNA interference in retinoblastoma pathway mutants. *Nature* **436**, 593–597 (2005).
- Jacobson, J.W., Medhora, M.M. & Hartl, D.L. Molecular structure of a somatically unstable transposable element in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **83**, 8684–8688 (1986).
- Bessereau, J.L. *et al.* Mobilization of a *Drosophila* transposon in the *Caenorhabditis elegans* germ line. *Nature* **413**, 70–74 (2001).
- Williams, D.C., Boulin, T., Ruaud, A.F., Jorgensen, E.M. & Bessereau, J.L. Characterization of *Mos1*-mediated mutagenesis in *Caenorhabditis elegans*: a method for the rapid identification of mutated genes. *Genetics* **169**, 1779–1785 (2005).



18. Ruaud, A.F. & Bessereau, J.L. Activation of nicotinic receptors uncouples a developmental timer from the molting timer in *C. elegans*. *Development* **133**, 2211–2222 (2006).
19. Ruaud, A.F. & Bessereau, J.L. The P-type ATPase CATP-1 is a novel regulator of *C. elegans* developmental timing that acts independently of its predicted pump function. *Development* **134**, 867–879 (2007).
20. Gally, C., Eimer, S., Richmond, J.E. & Bessereau, J.L. A transmembrane protein required for acetylcholine receptor clustering in *Caenorhabditis elegans*. *Nature* **431**, 578–582 (2004).
21. Yook, K. & Hodgkin, J. Mos1 mutagenesis reveals a diversity of mechanisms affecting response of *Caenorhabditis elegans* to the bacterial pathogen *Microbacterium nematophilum*. *Genetics* **175**, 681–697 (2007).
22. Robert, V. & Bessereau, J.L. Targeted engineering of the *Caenorhabditis elegans* genome following Mos1-triggered chromosomal breaks. *EMBO J.* (2006).
23. van Luenen, H.G., Colloms, S.D. & Plasterk, R.H. The mechanism of transposition of Tc3 in *C. elegans*. *Cell* **79**, 293–301 (1994).
24. Dawson, A. & Finnegan, D.J. Excision of the *Drosophila mariner* transposon Mos1. Comparison with bacterial transposition and V(D)J recombination. *Mol. Cell* **11**, 225–235 (2003).
25. Ochman, H., Gerber, A.S. & Hartl, D.L. Genetic applications of an inverse polymerase chain reaction. *Genetics* **120**, 621–623 (1988).
26. Granger, L., Martin, E. & Segalat, L. Mos as a tool for genome-wide insertional mutagenesis in *Caenorhabditis elegans*: results of a pilot study. *Nucleic Acids Res.* **32**, e117 (2004).
27. Salzberg, A. *et al.* P-element insertion alleles of essential genes on the third chromosome of *Drosophila melanogaster*: mutations affecting embryonic PNS development. *Genetics* **147**, 1723–1741 (1997).
28. Hiesinger, P.R. & Bellen, H.J. Flying in the face of total disruption. *Nat. Genet.* **36**, 211–212 (2004).
29. Bessereau, J.L. Insertional mutagenesis in *C. elegans* using the *Drosophila* transposon Mos1: a method for the rapid identification of mutated genes. *Methods Mol. Biol.* **351**, 59–73 (2006).