Mos1-MEDIATED MUTAGENESIS

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This protocole has been assembled based on the work of multiple people in the Bessereau lab (Paris) and Jorgensen lab (Utah).

GENERALITIES:

Mos1-mediated mutagenesis relies on the mobilization of the *Drosophila* transposon *Mos1* in the *C. elegans* germline (*Nature*, 2001, 413, 70-74). *Mos1* insertions introduce unique sequences in the *C. elegans* genome which can be easily localized using inverse PCR. Thus the *Mos1* transposon can be used as a molecular tag to rapidly identify a gene that has been mutated by insertion.

The mobilization of *Mos1* requires two extrachromosomal arrays. *oxEx166[hsp::MosTransposase; unc-122::GFP]* is the "enzyme array" which expresses the transposase under the control of a heat shock promoter. *oxEx229[Mos1; myo-2::GFP]* is the "substrate array" which contains multiple copies of the transposon.

The two transgenes are maintained in independent strains because transposition efficiency decreases when double transgenic animals are propagated for many generations. Moreover, we observed that the substrate array can be "silenced" over time regardless of maintenance protocol. Therfore there are two steps which should always be followed:

1- CULTIVATE *oxEx166* AND *oxEx229* INDEPENDENTLY AND SET UP NEW CROSSES FOR EACH NEW SCREEN.

2- IT IS CRITICAL TO MEASURE THE RATE OF TRANSPOSITION IN EACH SCREEN TO MAKE SURE THAT THE MUTAGENIC RATE IS SUFFICIENT.

Under these conditions, *Mos1* mediated mutagenesis is about 10 times less efficient than chemical mutagenesis. *Mos1* is clearly a less efficient mutagen than EMS but the identification of a mutated gene is extremely fast. Before using *Mos1* for mutagenesis, one must evaluate the trade-off between time spent screening for mutants versus the time spent mapping and rescuing a mutation.

STRAINS:

"Catalytic array":

EN547 or EG2762 *oxEx166[hsp::MosTransposase; unc-122::gfp; lin-15(+)].*
EG1642 *lin-15(e765); oxEx166[hsp::MosTransposase; unc-122::gfp; lin-15(+)].*
Drives expression of GFP in cœlomocytes.

Propagate the strain at 25°C (this is perhaps voodoo, but we think that it tends to prevent or decrease the silencing of the array). This strain segregates sick animals (Ste, pVul, Unc,...) and Dpys. These phenotypes never breed through and are most probably caused by leaky expression of the Mos Transposase in somatic tissues.

Freeze multiple vials of the strain when you receive it.

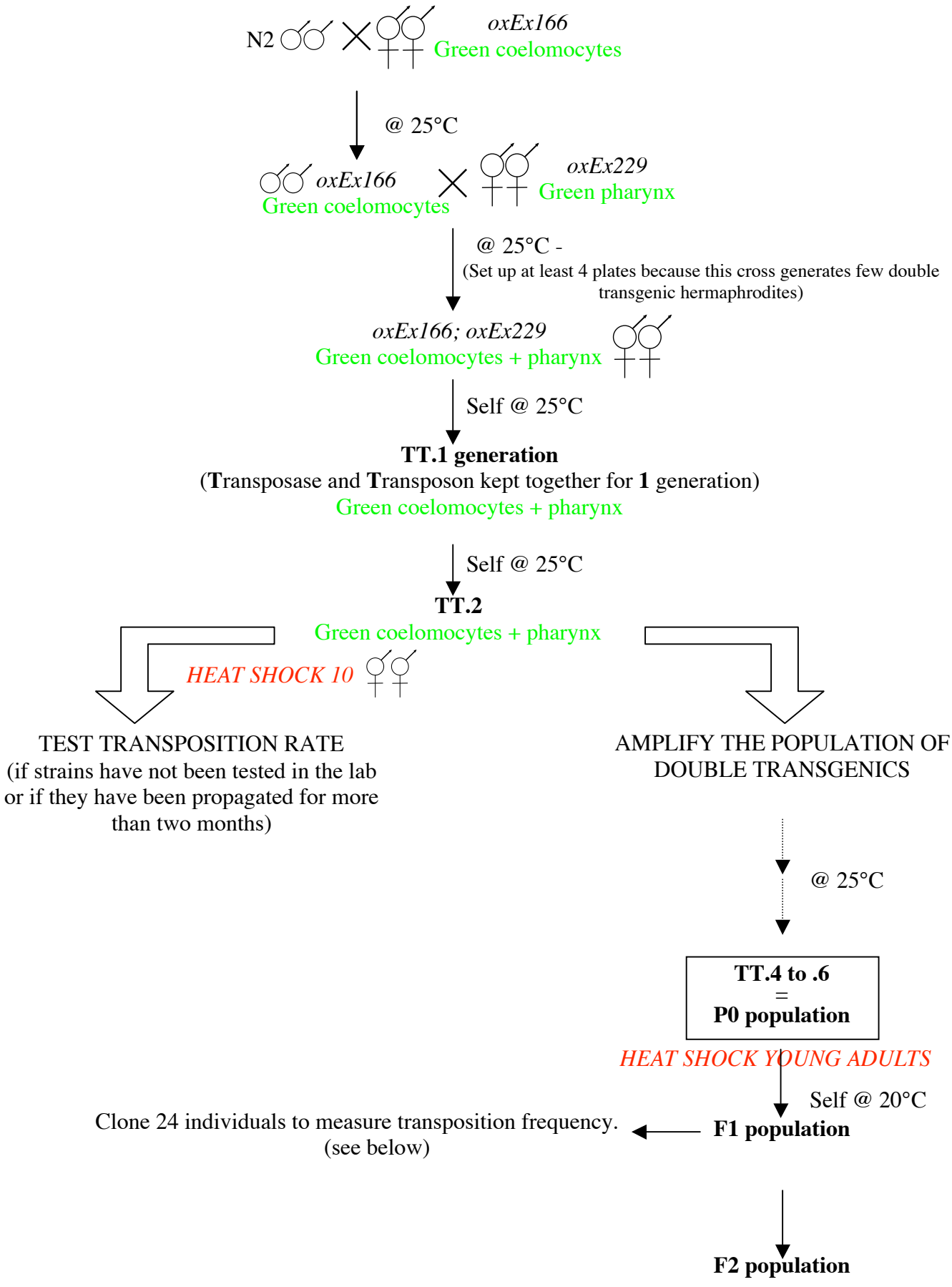
"Substrate array":

EG1470 *oxEx229[Mos1; myo-2::GFP]*
GFP expressed in pharynx

Propagate the strain at 25°C (again, we think that it tends to prevent or decrease silencing).

Freeze multiple vials of the strain when you receive it.

CONDUCTING A *Mos1* SCREEN:



Heat-shock conditions:

Heat-shock is very toxic for worms that contain both the catalytic and the substrate arrays. 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, 10 copies of *Mos1* insert into each haploid genome in somatic tissues. Heat-shock conditions are a compromise between efficiency and toxicity. We are using the following protocol:

Heat-shock **YOUNG ADULTS**. DO NOT HEAT-SHOCK L4 LARVAE (most L4s will die or be sterile)

Heat-shock : 1 hour @33°C in a water-bath
1 hour @20°C;
1 hour @33°C
Let worms recover overnight @ 15°C. It usually takes 12 to 16 hours for heat-shocked worms to start laying eggs again.

The brood size from heat-shocked doubles transgenics is small (10 to 50 F1s). In a screen, you have to heat-shock MANY P0s in order to get a decent number of mutagenized genomes.

MEASURING TRANSPOSITION RATE:

After heat-shocking double transgenic hermaphrodites, the transposition frequency is defined as the FRACTION OF THE PROGENY THAT CONTAINS AT LEAST ONE GENOMIC *Mos1* INSERTION *i. e.* animals that lost the substrate array *oxEx229[Mos1; myo-2::GFP]* and are positive for *Mos1* by PCR .

oxEx166; oxEx229 ♀♀
Green coelomocytes + pharynx

HEAT SHOCK:
1 hour @33°C; 1 hour @20°C; 1hour @33°C
Overnight @ 15°C.
Transfer to fresh plates at 20°C and collect F1s from those plates.

↓

Clone at least 24 individual F1s
(under visible light - do not select for animals that already lost the *oxEx229* array at this step because the transposition frequency is lower in this population.
You want to sample the F1 generation "blind")

Self
(@25°C if you want faster results)

From each plate, pick 5-10 F2s that lost *oxEx229 i. e.* have no GFP in the pharynx (fluorescent cœlomocytes do not matter)

*Mos1*insertions can be checked by PCR directly on these worms using oJL102/103. However, some of these animals will be mosaic for the *Mos1* array if the parent did contain *oxEx229*. The absence of the substrate array can be tested using oJL103/104 which detects the *Drosophila* genomic sequence that flanks *Mos1* in *oxEx229*.
We usually transfer the GFP(-) worms on one new plate.

Pick 20 GFP(-) F3 larvae per plate and PCR for the presence of *Mos1* using oJL102/103 primers.

The average transposition rate should be 50 ± 15 %.
If you get less than 30 %, something is wrong...

PCR for *Mos1* detection:

Worms are placed in 10 µl of Worm Lysis Buffer
(50mM KCl, 10mM Tris-Cl (pH 8.3), 2.5mM $MgCl_2$, 0.45% Tween-20, 0.45% NP-40, 0.01% Gelatin and 1 mg/ml Proteinase K (added fresh))
and incubated at 65°C for one hour, then at 95°C for 15 minutes

1 µl of the lysate is used in the PCR reaction.

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Mos1 + : oJL102/oJL103
MgCl2 1.5 mM
94 C 45"/ 56 C 1' / 72 C 45" x30
product = 355 bp

Flanking : oJL103/oJL104
MgCl2 1 mM
94 C 45"/ 56 C 1' / 72 C 45" x30
product = 726 bp

Do not forget to include *oxEx229* worms as a positive control and N2s as a negative control.

IDENTIFYING THE MUTAGENIC INSERTION

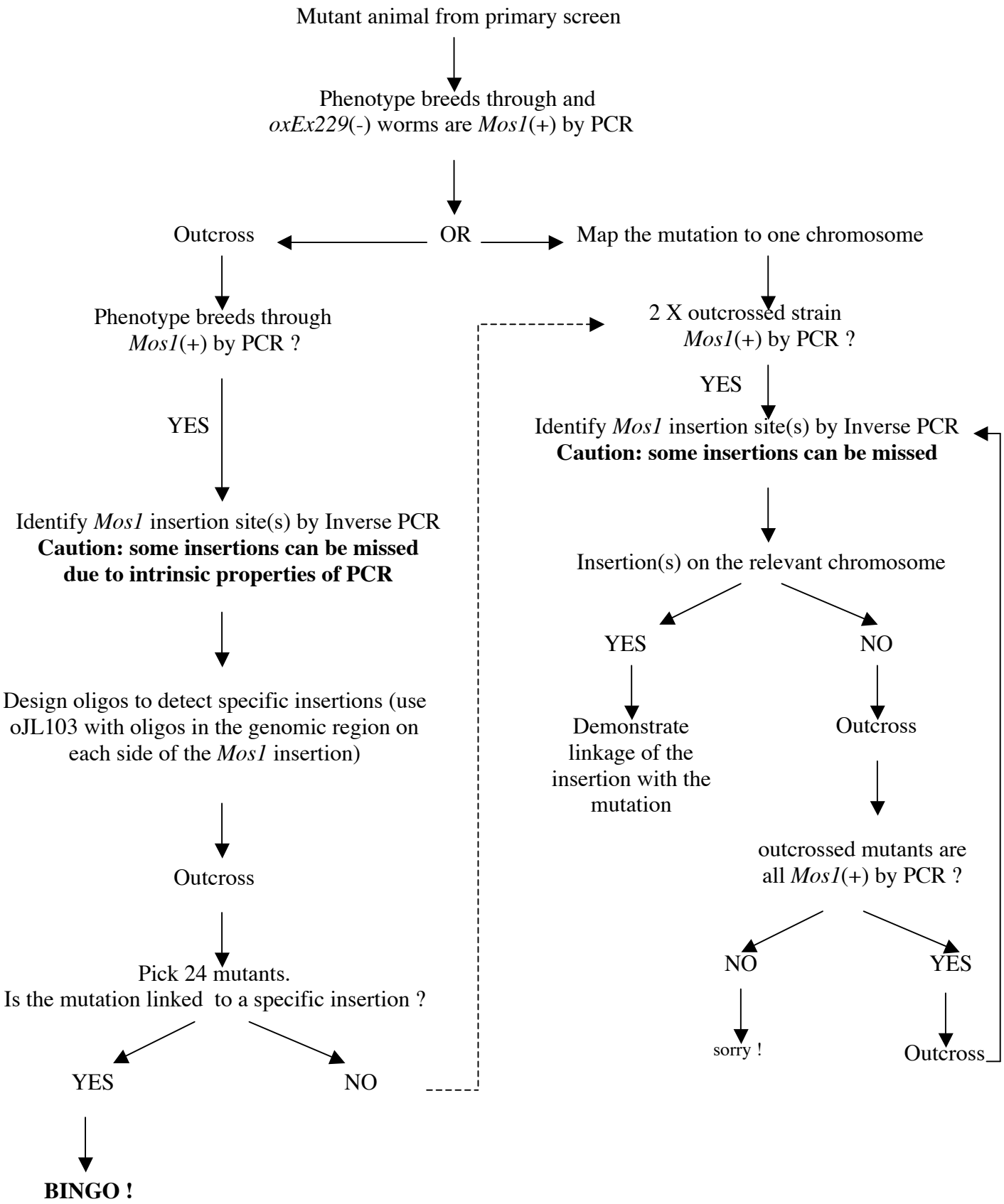
Mos1 insertion is a molecular tag that enables the rapid identification of the mutated gene. Identification of *Mos1* insertion site is very easy. However, there are two concerns to keep in mind:

1/ A mutant strain usually contains multiple insertions. The number of insertions is variable, from 1 to 10. In our experience, multiple insertions are scattered in the genome. Therefore it is easy to identify the relevant insertion by outcrossing the strain or getting rough mapping data.

2/ Mobilization of transposons can generate mutants that do not contain a copy of a transposon ('hit and run' events). However, these events are rare with *Mos1*: among the mutants that were identified in different *Mos1*screens conducted in our laboratory, a *Mos1* insertion was present in 14 of 15 mutated genes.

It is therefore essential to confirm the identity of the mutated gene with other criteria *i. e.* genetic position and rescue experiments.

SCHEMATIC STRATEGY TO IDENTIFY A MUTAGENIC INSERTION



INVERSE PCR PROTOCOL

I-PCR can be performed on a worm lysate or on purified genomic DNA. Worm lysis works fine most of the time. However, for reasons that we did not try to identify, we had a few experiments fail with worm lysates while purified DNA gave a positive result. Because it is faster, we usually try worm lysates first.

Different enzymes can be used to digest DNA:

- MboI, HpaII and HaeIII, then PCR with oJL103/oJL114 then oJL115/oJL116
- MseI, AluI and HhaI, then PCR with IPCR1a/IPCR1b then oJL102/IPCR2b.

When there are multiple insertions, only a fraction of these insertions are identified using one restriction enzyme. We recommend to use at least two (we routinely use MboI and MseI).

1. Using genomic DNA: Digest ≈ 150 ng of DNA in a 30µl final volume.

1'. Worm lysis: put 5 worms in 20 ul single worm lysis buffer, freeze -80°C ~25 minutes, 65° 1 hour, 95° 15 minutes. Digest 10µl

2.DNA digestion:

Typical mix is:		
	DNA	10 µl
	Boehringer buffer A	3 µl
	Mbo I (10u/µl)	1 µl
	Water	16 µl

Incubate 3 hours @ 37 C.

Run 10 µl on a gel (you expect to see the disappearance of the slow migrating band that is seen when runing intact genomic)

Inactivate the enzyme: 15 ' @ 75 C

3. Ligation:

Digestion mix	10 µl
5 X ligation buffer	10 µl
T4 ligase (~10 U)	1 µl
Water	29 µl

Incubate overnight @15 C. The ligation mix can be kept frozen @-20 C after ligation.

PCR:

1st reaction

Ligation mix	3 µl
oJL103 25 µM	0.5 µl
oJL114 25 µM	0.5 µl
dNTPs 10 mM	0.5 µl
MgCl2 50mM	0.75 µl
10 X Buffer	2.5 µl
Taq	0.5 µl
Water	16.75 µl

oJL103/oJL114
30 cycles 45"@94C / 1'@60 C / 1'@72C

IPCR1a/IPCR1b
30 cycles 45"@94C / 1'@59 C / 1'@72C

2nd reaction

Dilute 1 µl of the PCR in 200 µl of water

Dilution	1 µl
oJL115 25 µM	0.5 µl
oJL116 25 µM	0.5 µl
dNTPs 10 mM	0.5 µl
MgCl2 50mM	0.75 µl
10 X Buffer	2.5 µl
Taq	0.5 µl
Water	18.75 µl

oJL115/oJL116
25 cycles 45"@94C / 1'@62 C / 1'@72C

oJL102/IPCR2b
25 cycles 45"@94C / 1'@59 C / 1'@72C

Run a 1.8 % agarose gel.

Using Mbo1 or MseI, the smallest specific bands are respectively 250 bp and160 bp long.

Be aware that this protocol often generates mutliple bands of different sizes from a single insertion (partial digest, religation of degraded DNA fragments, illegitimate PCR priming,...).

Gel-purify candidate bands and sequence using oJL115 or oJL102 as a primer. We tend to systematically TA-clone the bands. Then, directly PCR the bacteria colonies using oJL115/oJL116 or oJL102/IPCR2b and sequence these PCR products (it takes one more day but gives much better sequence results).

Be aware that some PCR products are fake. To be sure that you are looking at a true insertion, you must read the sequence of the left end of the transposon directly followed by the *C. elegans* genomic sequence starting at a TA dinucleotide.

***Mos1* SEQUENCE.**

OLIGOS

oJL102: CAACCTTGACTGTCGAACCACCATAG
oJL103: TCTGCGAGTTGTTTTTGCGTTTGAG
oJL104: ACAAAGAGCGAACGCAGACAGT
oJL114: AAAGATTCAGAAGGTCGGTAGATGGG
oJL115: GCTCAATTCGCGCCAAACTATG
oJL116: GAACGAGAGGCAGATGGAGAGG
IPCR1a: GACCTTGTGAAGTGTCAACCTTGACTG
IPCR1b: GACAATCGATAAATATTTACGTTTGCGAGAC
IPCR2b: CATCTATATGTTCGAACCGACATTCCC