*Mos*TIC

(*Mos1* excision induced Transgene Instructed gene Conversion)

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Introduction: *Mos*TIC (Robert and Bessereau, 2006) is a technique which enables the engineering of the *C. elegans* genome by homologous recombination (Figure 1). In *Mos*TIC experiments, a double strand break (DSB) is introduced in the locus to engineer by remobilizing a pre-existing insertion of the *Mos1* transposable element. A transgene containing engineered sequences homologous to the DSB flanking regions is provided as a repair template. During DSB repair by gene conversion, the modifications present in the transgene are copied into the genome. *Mos*TIC events are obtained with frequencies ranging from 10^{-4} to 10^{-5} events per generation. *Mos*TIC is efficient in a 1 kb region of the genome centered on the remobilized *Mos1* insertion.

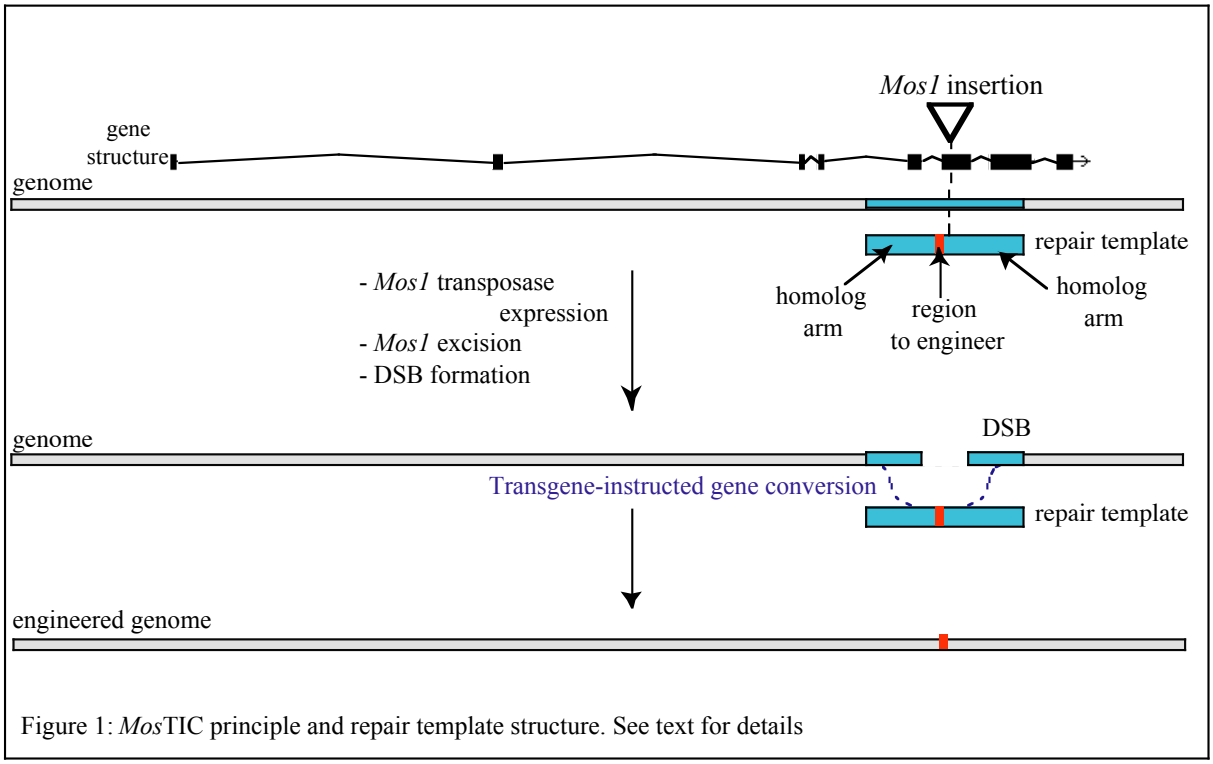

Figure 1: *Mos*TIC principle and repair template structure. See text for details

General outline:

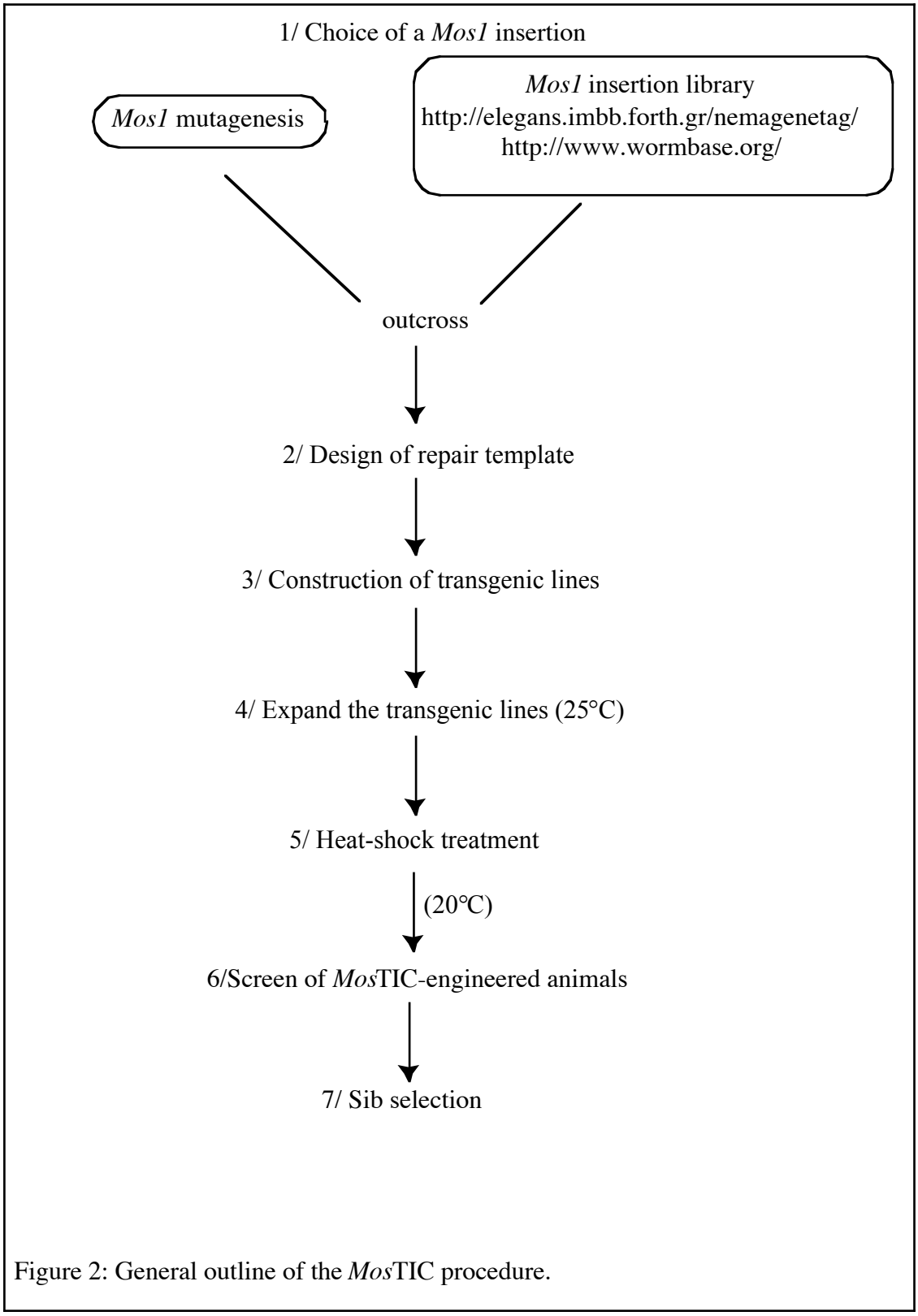

Figure 2: General outline of the *Mos*TIC procedure.

1- How to find *Mos1* insertions ?:

The area that can be engineered by *Mos*TIC is constrained by the position where *Mos1* is inserted into the genome. We characterized *Mos*TIC conversion tract at the *unc-5* locus (see Robert and Bessereau, 2006, figure 2) and demonstrated that a point mutation localized in a 1 kb long region centered on the *Mos1* insertion point will be copied in at least 50% of the animals modified by *Mos*TIC. Point mutations can be introduced 3 kb further away from the *Mos1* insertion point with an efficiency which is 20 times lower that the one obtained when introducing a point mutation at the *Mos1* insertion site.

Finding a strain with the insertion of your dreams can be obtained in two ways:

a- A *Mos1* mutagenesis (Bessereau et al., 2001; Williams et al., 2005) can be performed to find *Mos1* insertions in your favorite genes (see Bessereau, 2006; protocol available at http://www.biologie.ens.fr/bcsgnce/article.php3?id_article=27).

b- A comprehensive *Mos1* insertion library is being generated by the NemaGENETAG consortium (http://elegans.imbb.forth.gr/nemagenetag/). Identified insertions are annotated in Wormbase (http://www.wormbase.org/). *C. elegans* stocks carrying these insertions are distributed upon request.

Mos1-mobilization in the *C. elegans* germline generates more than one *Mos1* insertion per haploid genome (mean=2.6/genome, Williams et al., 2005) on average. These insertions are usually not linked. Since the remobilization of *Mos1* will not be limited to a specific insertion, we recommend to outcross the line carrying the insertion of interest in order to reduce the number of background insertions as much as possible.

2-Design of the repair template:

Repair templates used for *Mos*TIC experiments contain:

- the modifications (point mutations, deletions, tags, ...) to be introduced into the locus (in red in Figure 1);
- 2 "arms" (blue regions on Figure 1) homologous to the genomic region broken by *Mos1* excision. We performed successful *Mos*TIC experiments with repair templates carrying 1.5 kb long homology regions on each side of the modification to introduce into the genome. Increasing the length of the arms did not improve *Mos*TIC efficiency. Reducing the length of 1 of the arms to 700 nt significantly reduced *Mos*TIC efficiency (Robert and Bessereau, 2006).

The repair template is built in a standard plasmid.

3- Construction of transgenic lines:

The repair template is introduced into *C. elegans* by micro-injection (Stinchcomb et al., 1985). To induce *Mos1* excision, we use pJL44 (*hsp::Transposase*) to drive Mos transposase expression under the control of a heat-shock promoter (plasmid available upon request). During the establishment of *Mos*TIC, we noticed that it is essential to have the transposase source and the repair template on the same array. Usual injection mix contains:

- the repair template (50 ng/µl)
- pJL44 (*hsp::Transposase*) (50 ng/µl)
- pPD118.33 (*myo-2::GFP*) (5 ng/µl) (used as a transformation marker)

We usually perform injections directly into the strain homozygous for the selected *Mos1* insertion. Alternatively injections can be performed in N2 and transgenes are introduced into the *Mos1* carrying strain by crosses.

4- Expanding the transgenic population:

Transgenic lines are usually amplified and maintained at 25°C to minimize potential transgene silencing. However, if the *Mos1* containing strain is temperature sensitive, transgenic lines can be grown at 20°C without significant loss of *Mos*TIC efficiency.

5- Triggering *Mos1* excision by heat-shock:

Heat-shock young adults by immersing parafilm-sealed plates in a water bath:
- 1 hour @ 33°C
- 1 hour @ 15°C
- 1 hour @ 33°C

Let animals recover overnight @ 20°C.
Transfer transgenic animals (P0) on fresh plates and let them lay eggs to get around 100 F1 animals per plate. Depending on the fertility of the heat-shocked transgenic animals, 1 to 5 animals can be put on the same plate.

6- Screen for *Mos*TIC-engineered animals:

In some cases, locus engineering will cause phenotypic changes that could be used to identify *Mos*TIC events. Specifically, *Mos*TIC might cause a loss of function of the gene to engineer. Alternatively, it could revert the mutant phenotype caused by the *Mos1* insertion to wild type. Such screening strategies proved to be very efficient in some of our experiments. However, be aware that DSB caused by *Mos1* excision are not repaired only by *Mos*TIC. Several mechanisms, including non-homologous end joining, are at work and can sometimes regenerate functional alleles after *Mos1* excision or introduce deleterious footprints at the excision site (Robert and Bessereau, 2006). Therefore, animals selected based on phenotypic changes of the starting strain must be analyzed at the molecular level to identify real *Mos*TIC engineered strains.

In most cases, screening relies on the PCR identification of the molecular changes introduced in the engineered locus (Figure 3).
PCR design.
The way we designed primers in our previous experiments is featured in Figure 3A. One primer (P1) is present in the repair template whereas the second primer (P2) is present in the genome but absent from the repair template. According to this strategy a specific PCR product should be amplified only if the modifications present in the template were introduced into the genome. However, we noticed that a PCR product having the same size as the specific one could also be amplified from transgenic animals containing a non-engineered locus. Amplification arises from annealing between single-strand DNA generated from the transgenic array on the one hand and the genome on the other hand (Figure 3B). This process is known as 'PCR jumping' or 'PCR bridging' and will give false-positive *Mos*TIC signals. To minimize PCR bridging, it is recommended to optimize PCR conditions. In our experience, PCR jumping could be minimized by:
- reducing the annealing time,
- increasing the annealing temperature,

- diluting the worm lysates before starting PCR 100 times.

To increase the sensitivity of the PCR screening, a second step of nested PCR can be performed.

Screening steps.

Initial pools contain about 200 genomes (Figure 4). Once the F1 plates are starved, wash half of the plate with M9 buffer and transfer the animals to a 1.5 ml tube. Let the worms sediment on ice and eliminate M9. Then transfer the worms to PCR tubes or plates and perform lysis at 65°C for 2-3 hours in 50 µl of lysis buffer containing proteinase K (final concentration: 1 mg/ml). Perform PCR on 1 µl of 10 times diluted lysate. If a nested PCR is required, dilute PCR#1 100 times to set up PCR#2.

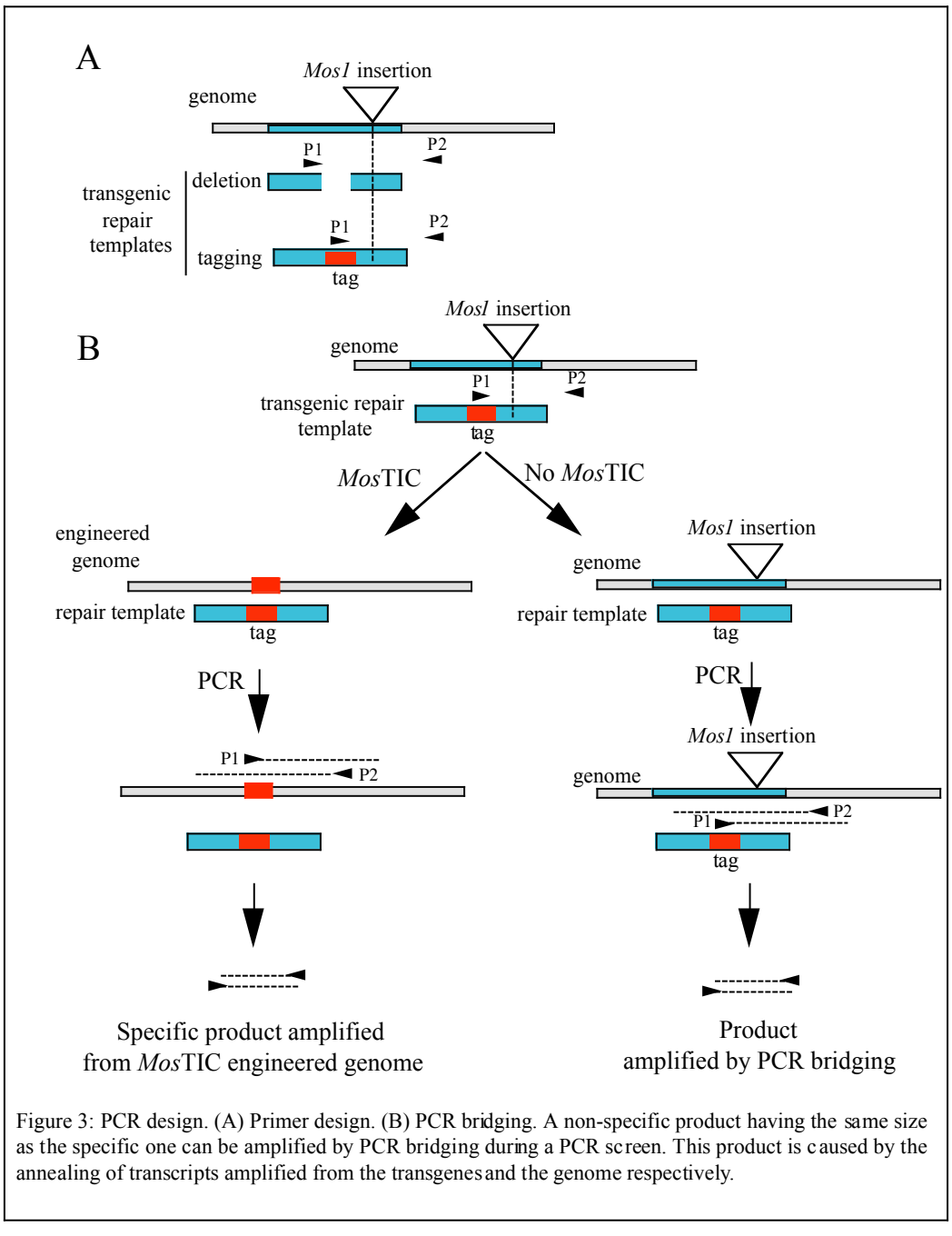

Figure 3: PCR design. (A) Primer design. (B) PCR bridging. A non-specific product having the same size as the specific one can be amplified by PCR bridging during a PCR screen. This product is caused by the annealing of transcripts amplified from the transgenes and the genome respectively.

7- Sib selection:

Once you have a positive PCR signal, chunk the corresponding plate to a fresh plate.
From the developing population, make 15 pools of 20 non-transgenic animals (only use non-transgenics at this step to get rid of putative PCR bridging problems). Analyze the pools as described above.
From one positive sub-pool, clone 40 individuals to separate plates to identifiy the *Mos*TIC engineered strain.

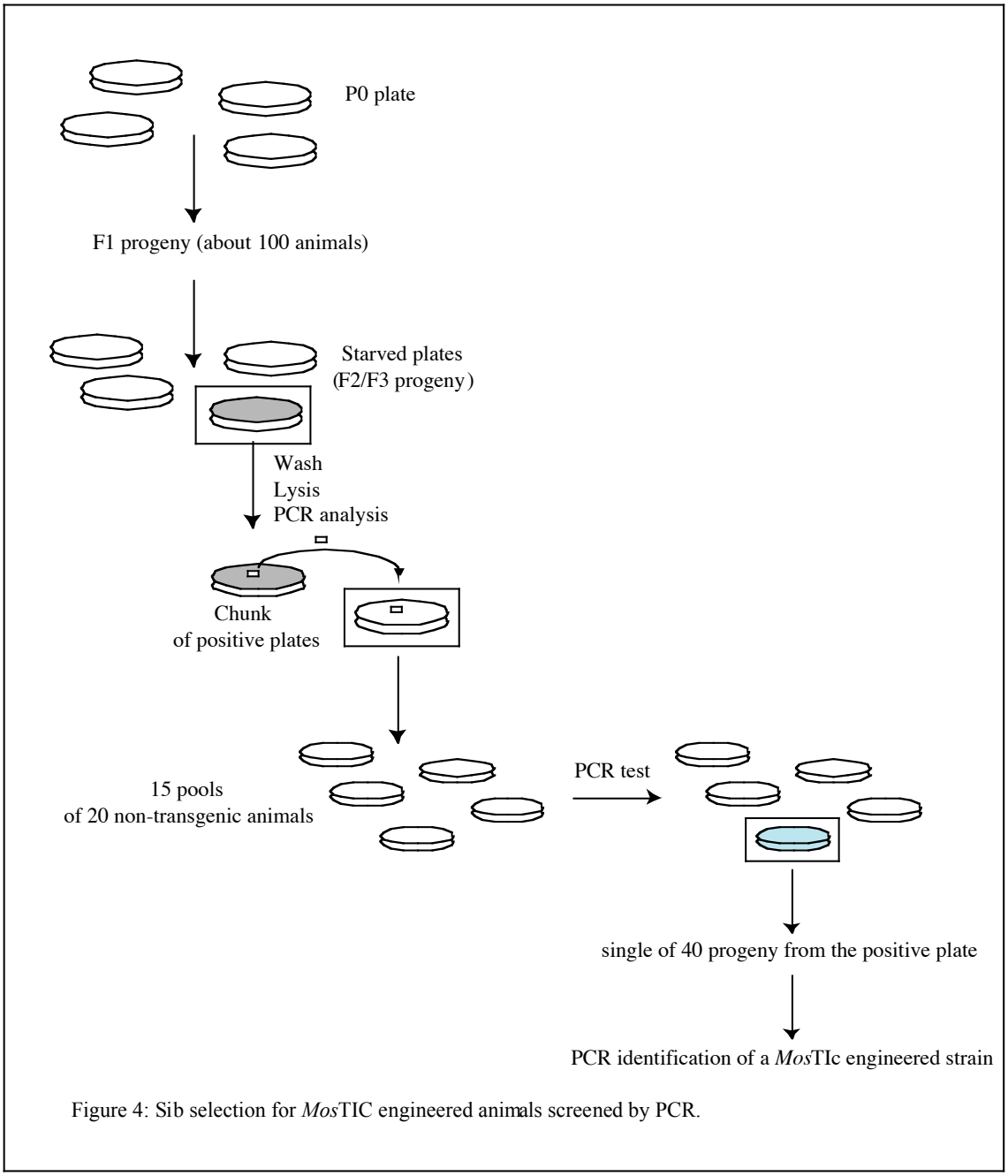

Figure 4: Sib selection for *Mos*TIC engineered animals screened by PCR.

The *Mos*TIC technique is young. We think that it would be extremely valuable for the community to identify the strengths and the limits of this strategy. We would greatly appreciate feed back (efficiency, type of modification, distance from the *Mos1* insertion, technical improvements, failures, ...) in order to warn people about potential limitations of the technique and provide relevant suggestions. Do not hesitate to contact us (vrobert@biologie.ens.fr, jlbesse@biologie.ens.fr).

- **Solutions**

Lysis buffer: 50mM KCl, 10mM Tris pH 8.2, 25 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween-20, 0.01% Gelatin.
M9 buffer: 1 liter 10X: Na_2HPO_4.12 H20 146 g, KH_2PO4 30 g, NaCl 5 g, NH_4Cl 10 g

- **References**

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