### Supplementary protocol 1 - miniMos insertion

This protocol describes how to generate miniMos inserts directly by injection. The protocol is very similar to the protocol used to generate MosSCI insertions and many of the necessary reagents are identical.

Please see the webpage www.wormbuilder.org we maintain for updates to the protocol and a FAQ about common problems.

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#### Reagents

Co-injection plasmids

pGH8 P*rab-3:mCherry:unc-54*UTR

pCFJ90 P*myo-2:mCherry:unc-54*UTR

pCFJ104 P*myo-3:mCherry:unc-54*UTR

pCFJ601 P*eft-3:mos1 transposase:tbb-2*UTR

pMA122 P*hsp16.41:peel-1:tbb-2*UTR

Cloning plasmids (miniMos vectors)

Various There are different vectors based on *unc-119*, neoR and puroR selection. All vectors are available as three-fragment [4-3] Gateway vectors or as multiple cloning site vectors. We recommend using the vectors with *peel-1* in the backbone for direct insertions and vectors without *peel-1* for heat-shock based insertion.

Plasmids can be requested from Addgene.

Strains

EG6207 *unc-119(ed3)*. 11x outcross. Outcrossed by Amir Sapir in Sternberg lab.

Wildtype For NeoR and PuroR selection

Antibiotics

G418 for NeoR selection. We purchase powder from Gold Biotechnology and make up our own solution. Make 25 mg/ml solution in water.

Important: Filter sterilize to avoid contamination. Store working stock in refrigerator, keep stocks in -20C freezer.

Puromycin for PuroR selection. We purchase 10 mg/ml solution from Invivogen. Store working stock in refrigerator and stock in -20C freezer.

*Note: In our hands, G418 selection is more effective and considerably cheaper than puromycin*.

### Protocol

**Before injection**

#### 1. Insert transgene into miniMos vector.

Insert the transgene of interest into the appropriate miniMos vector (*unc-119*, NeoR, PuroR) by your prefered cloning method (for example, Gateway cloning, restriction enzyme cloning or multiple fragment assembly). Or generate a fosmid-based vector by inserting the miniMos-*unc-119* cassette into the backbone of the fosmid by recombineering.

#### 2. Make injection mix.

MiniMos-based vector 10 ng/ul

pGH8 10 ng/ul

pCFJ90 2.5 ng/ul

pCFJ104 10 ng/ul

pCFJ601 50 ng/ul

pMA122 10 ng/ul

*Making the injection mix is much easier if you make a 2x stock solution of all the co-injection plasmids. Lower the concentration of the miniMos vector if your transgene is toxic. Omit pMA122 if you are using a miniMos vector with peel-1 selection in backbone of vector. We think the purity of the DNA is important for good success so we suggest using a kit that gives better quality DNA than a miniprep kit or that you do an ethanol precipitation after isolating DNA with the miniprep kit (see Morris Maduro's description in Worm Breeders Gazette).*

#### 3. Grow injection strain at 15°C to 20°C on HB101 bacteria.

*unc-119* animals are much healthier (and easier to inject) if they are grown at lower temperatures on HB101 bacteria. We generally grow N2 on OP50 at room temperature.

**Injection**

#### 4. Inject worms.

Inject into the appropriate injection strain. Put 1-3 animals on each NGM plate seeded with HB101 or OP50.

*It is difficult to give guidelines for how many injections to perform to generate an insertion. In our hands, the technique is as efficient as generating extra-chromosomal arrays for plasmids and less efficient for fosmids.*

**After Injection**

#### 4. Place injected worms at 25°C. (Day 1)

Place the plates with injected worms at 25°C.

*The insertion frequency is strongly temperature dependent, with more insertions happening at higher temperatures. Although the insertion appears to happen in the F1 generation, we place the injected animals at 25°C within a few hours of injection.*

#### 4b. Add antibiotic to the injection plates. (Day 2)

If you are injecting into unc-119 animals then skip this step. For NeoR selection, add 500 ul of the stock solution (25 mg/ml) directly to the plate the day after injection. For PuroR selection, add 500 ul of the stock solution (10 mg/ml) directly to the plate the day after injection. Let plates dry with the lid off. Keep plates at 25°C.

*This is a modified protocol from the protocols described in Giordano-Santini et al. (2010) and Semple et al. (2010). We prefer to add the antiobiotic directly to the seeded plates because it requires less planning ahead. In our hands the protocol is efficient but it is quite possible that making NGM plates with antiobiotic already added is more efficient. Please see the two references for the standard protocol for antibiotic selection.*

*The amount of antibiotic added is based on our NGM plates weighing approx. 8 g each. Adjust the volume added based on the weight of plates in your lab.*

#### 5. Let worms starve out at 25°C. (Days 2-7)

This takes approximately 1 week. The protocol works best if the worms are fully starved before you proceed to the next step.

*We do not pick off individual F1 progeny from each plate but let them starve out as a population. As we show, you can generate several independent insertions if you pick off individual F1 progeny. However, we find that picking F1 progeny takes a lot of time and uses a fair amount of ressources so generally we prefer to inject more animals instead.*

*Can you find insertions before the plate starves out? Yes. But again, it's much harder and usually more work to find these rare early inserts relative to waiting a few days and letting the plate starve fully.*

#### 6. Heat-shock animals for two hours at 34°C in air incubator. (Day 7)

This step kills animals that are carrying the extra-chromosomal array by activating the *peel-1* toxin. Wait until the plates are fully starved. Insertions happen relatively long after injection and if you heat-shock too early you will kill the animals with insertions before they can get rid of the extra-chromomal arrays.

*This works very efficiently if the plates actually heat up relatively fast to 34°C for the duration of the heat-shock. For example, it works well in our incubator that has a fan but is much less effective in a similar incubator without a fan, probably because it takes longer to heat the plates up. Don't heat-shock a full box of plates in a closed box in an air incubator. Separate out plates so they are only stacked one or two high. Can you use a water incubator? Yes. In fact, it is more efficient that way but it is also a lot of work to wrap and un-wrap a lot of plates. So, depending on how many plates you have you should choose the most convenient method.*

#### 7. Screen plates for insertions. (Day 8)

Screen at least four hours after heat-shock and preferably the next day. Look for animals that are alive and move well but lack the flourescent co-injection markers.

*We screen the plates on a normal dissection microscope and then secondarily verify on a flourescence dissection microscope. We typically do not see hardly any false positives. Adjust the heat-shock if you are not killing all the extra-chromosomal array animals.*

#### 8. Chunk or pick rescued animals. (Day 8 - 10)

Chunk plates with insertion animals to a seeded NGM plate. Pick off a single, healthy adult animal two days later.

*We prefer to chunk animals and then pick a healthy adult animal two days later instead of picking off individual starved animals. The starved L1 animals have a relatively high incidence of sterility so you often have to go back and re-pick. Chunking also often lets you screen visually for the transgene (germline expression, for example) before picking a clonal worm. Since multiple independent insertions are often generated, this can save some work in finding the animal that will work for your experiment.*

*Can you pick several independent insertions from a single plate? Yes. But you have to be careful to verify that the insertions are independent - most insertions on a plate will not be independent.*

#### 8. Determine insertion site. (~ 2 days of molecular biology)

If necessary, use the inverse PCR protocol to determine the insertion site (see Supplementary Protocols 2 and 3). For some experiments this may not be necessary; for other experiments this may be crucial.

*Treat the insertions as you would treat different alleles of a gene. It's always nice to have more than one allele. Some insertions will be affected by genomic environment (for example, X chromosome inactivation in the germline). Other insertions will disrupt a genomic locus that is important.*