**Integration of worm transgenes**

*This protocol illustrates how to transform worms and select for stable integrants in the MOSSCI sites.*

**Plasmids:**

Plasmids to inject are:

(1) a pCFJ150 backbone plasmid carrying the transgenes of interest

(2) pDD122 (*cas-9 + guide RNA for pCFJ150*) -- saved as pEO93

(3) pMA122 (*peel-1*) -- saved as pEO94

(4) pGH8 (*prab-3::mCherry*) Addgene #19359-- saved as pEO95

(5) pCFJ104 (*pmyo-3::mCherry*) Addgene #19328 -- saved as pEO96

(6) pCFJ90 (*pmyo-2::mCherry*) Addgene #19327 -- saved as pEO97

**Reagents:**

* M9
* NGM plates
* Hygromycin (50 mg/ml) stock solution
* 2 – 10 x co-injection markers

**Preparation:**

* Purify all the plasmids using PurLink Quick Plasmid Miniprep kit (Invitrogen).
* Make the 2x or 10x co-injection plasmid mix:
* 10 x CoInjection Marker solution is:
  + 500 ng/ul pDD122
  + pMA122
  + 100 ng/ul pGH8
  + 50 ng/ul pCFJ104
  + 25 ng/ul pCFJ90
* Prepare several glass capillary needles using the needle puller (setting #21)
* Grow N2 or desired worm strain to gravid stages on OP50-seeded NGM plates.

**Injection Day:**

* ***Go to the injecting scope.*** Bring the following items to the injection scope:
  + P2 or P10 pipet
  + Pulled glass capillary needles
  + M9 buffer
  + Early adult N2 worms
  + NGM plates – small, unseeded.
  + NGM plates – small, seeded with OP50.
* ***Load the needle.*** Add 0.3 ul of plasmid + co-injection plasmids into the back end of a glass capillary need. Let it sit to absorb the fluid.
* ***Wash worms*** by picking several young adult worms and dropping them into 100 ul of M9 on a fresh NGM (unseeded) plate.
* ***Break the needle*** by “ruffling” up the agar edge of on an agar coverslip. Add a drop of oil to the ruffled agar edge. Place the coverslip with ruffled edge in the dissecting scope. Place the needle in the micromanipulator arm. Zoom in using the 20 x or 40 x objective. Break the needle by touching the needle to the ruffled edge (continuously press on the foot pedal). Once liquid appears out of the needle, lift it up from the agar edge.
* ***Calibrate droplet.*** Check to see that the broken needle has a pore of the right size and calibrate the droplet. At 20 x a one-pedal-droplet should measure the size of the area within the crosshairs. If it is not this size, turn up the volume or pressure until it is as close as possible to that size.
* ***Load worms onto the microscope.*** Add a drop of oil onto the agar pad. Pick 5 – 20 worms using a worm picker and drop into the oil. As worms start to settle down, try to line them up using the worm picker or an eyebrow hair.
* ***Inject worms.*** Mount the worms on the scope and place the needle under the oil close to a worm. With highest magnification objective, inject into the worm gonad and use the pedal several times to inject fluid. Repeat for all the worms in the oil droplet.
* ***Recover worms.*** Add 100 ul of M9 to the top of the oil droplet containing the injected worms. Some worm will float out of the oil. Use a worm picker to lift worms off of the agar pad and move onto an OP50-seeded NGM plate.
* ***Distribute worms.*** At the end of the day, distribute the worms so that there are 3 worms per small OP50-seeded plate. Also grow a plate of 3 uninjected worms to serve as a positive control.
* Grow worms at 25 C (benchtop).

**2-days later:**

* Two days later, dilute hygromycin to 10 mg/ml.
* Pipet 500 ul of hygromycin onto each 6cm plate (1ml if using 10 cm plates).
* Grow worms at 25 C.

*Note from Dan: “If you do not see any candidate knock-ins at this stage, or if you have fewer lines than you’d like, wait 3 days and then examine the plates again. We sometimes find that knock-ins “appear” 10-11 days after injection, when the F3 are young adults. Most likely, these animals are the progeny of knock-in worms that were present earlier, but in low numbers, and thus were missed during the initial screening.”*

**3 – 7 days later**

* Repeatedly check to determine whether worms were successfully injected by looking for mCherry fluorescence.
* Grow worms at 25 C.

**7-days after injection**

* Select against worms with chromosomal arrays using the peel-1 heat shock system.
* Move worms to 34 C for 4 hours (the 34C incubator is on the 6th floor of Fordham right opposite the glass capillary puller).
* Return to 25 C.

**8-days after injection**

* check for survivors
* If worms are low on food, add some OP50 or HB101 (depending on how many worms there are.
* Check for counter selection… look to see that mCherry fluorescence is gone in survivors.
* Look to see that worms are growing.
* Pick individual worms that are surviving peel-1 and do not have mCherry fluorescence onto their own plate. Make sure to save at least three individual worms from at least three plates.
* Also, chunk plate for survivors if there are a lot of surviving worms.

**Next few days**

* Propagate worms from individual selected and chunked plates.
* Isolate gDNA of worms that were selected and chunked plates. Also freeze worms that were selected and also the chunked plates.
* Test isolated gDNA for an insertion.