Protocol for *C. elegans* transgenesis

A) PREPARING INJECTION PADS

1) Boil 2% agarose in water.

2) Place 100μl onto the center of 48X60 mm glass cover slip.

3) Immediately flatten drop with a second cover slip.

4) Let dry for few minutes then slide off top cover slip.

5) Label the side with the agarose pad up by drawing 'R' for convenience since the agarose dries clear. Prepare at least 10 slides every time.

6) Keep for several days at room temp before use[1] (the more the better). Store slides in boxes to prevent dust accumulation. Once slides are dry they can be used indefinitely.

B) Preparing DNA for injection

1) Prepare 20 μl injection mix of the different DNAs in H2O to final concentration of 100~150 ng/μl. Add Bluescript or Genomic DNA if the concentration is less then 100 ng/μl [2].

2) Spin 12,000 g for 30 minuets @ 4C.

3) Transfer 10-15 μl of the upper phase to new eppendorf tube. This mix will be load on the needles. You can store this injection mix in -20/-80 for future injections.

C) Needles preparations, loading and opening

I) Ramp test

1) Every few weeks equilibrate the needle puller (ramp test).

2) Enter any program number and press the clr button.

3) Do you wish to clear all values? Press 0 (no).

4) Do you wish to run a test? Press 1 (ramp test).

5) Load capillary on the needle puller and press pull. At the end of the test you will get a number- write it down on the paper attached to the wall near the needle puller.

6) Go back to the main menu and put this number as the head value. The other values should be equal to150.

II) Pulling and loading needles

1) Load the capillary by sliding it inside the notch until it is reaches almost the end of the notch. This should give 2 needles (One a little shorter then the other) from every capilara[3].

2) Tighten the capillary by screwing the two screws.

3) Close the cover and hit the pull button. Make 4 needles from 2 capillaries for every injection[4].

4) Store needles in needle holder. It is not recommended to store needles more then 2 weeks due to the liquid properties of the glass.

5) Place 4 needles vertically by attaching them on the table modeling clay patch so that the pulled side is facing to the floor.

6) Using 1-20 ul Gilson take 1ul of the injection mix place ~ 0.5 ul drop of the solution on the back (unpulled) end of the needle, wait for 5-10 minutes and look under the dissecting scope to see if the solution went to the needle's pulled end and whether air bubbles are trapped in the liquid. Tiny bubbles near the needle tip- proceed to break the needle and blow the bubble out the tip; big bubbles- throw the needle.

D) Opening the needle: (the most difficult part)

1) Switch on the air controller and the light source. Open the air tank and the pressure screw and be sure that the pressure is 80 psi- if less adjusts pressure by twisting the black screw.

2) Remove the old needle by unscrewing the assembly that holds the needle.

3) Enter the needle and screw the assembly ~ half of the needle length should be inside the holder. Make sure that you tightly screw the needle so it will not release by the air pressure during injections.

4) Over a Bunsen burner draw out a standard (not microinjection) 10 ul micropipette to about 1/5 its starting thickness. Place a stretch of the drawn out part on a 48X60 mm cover slip, and put a drop of microinjection oil on top[5].

5) Mount this on the microscope with an angle of ~30 Deg and focus on the micropipette- see a sharp black line on the edge when you are focused on the middle.

6) Using the fine controls, carefully lower the injection needle towards the stage until it is in the same focal plane as the micropipette.

7) Switch to X20, focused again the glass rod and the needle. With your left hand move gently the table until the injection needle touches the micropipette, and then pull the table back.

8) Check the needle by pressing the orange button, look for flow out of the needle. A bubble should appear in the oil. Make sure that the bubble is big enough (if you are not sure- ask someone).

E) Mounting worms on an injection pad.

1) Picks L4 animals and age them 12-24 hours @ 20C before injection.

2) Prepare injecting picks by attaching one of your leases to a toothpick using a sellotape.

3) Take out an agarose pad and breathe on it (about 1 long breath) and drew a target or a small circle on the agarose free side[6].

4) Using a long stick rod place a drop of microinjection oil on the pad and spread the oil drop around with a worm pick so that the oil isn't too deep.

5) Using a worm pick with oil on it as glue (want to minimize the amount of bacteria you transfer) transfer adult hermaphrodites to the oil drop on the pad. If there is still adhering bacteria, push the worms around in the oil with a pick until the bacteria come off.

6) Stick 1-2 animals on a pad (experts do up to 9 animals at once).

7) To stick the animal right, wait until it is floating in the oil so that its body flexures go sideways, not up and down, and stroke the animal down on the agarose pad with your injection eye leash pick. If during this process the oil was spread- cover the animal with oil again[7]. Once the animals are in the oil, work reasonably fast to get the procedure over with before the animals dehydrate.

F) Injection

1) Before injection look in the internet for movies on C. elegans transgenesis so you will have idea on how things should be look like:
http://www.youtube.com/watch?v=CcvstfwArrs
http://www.jove.com/index/Details.stp?ID=833

2) Place the cover slip on the stage 10X objective find the worm; make sure it is in the correct orientation (vulva away from the needle). Move or rotate the entire stage to move the worm or move the cover slip itself. It is best to have the worm at a 45deg. angle to the needle; this maximizes the path length for the needle inside the gonad, helping to make sure you get the tip in the gonad instead of going all the way through and out the other side.

3) Carefully lower the needle into the focal plane with the fine adjuster. At this point, you only need to move the needle up and down with the micromanipulator and move the worm left/right by moving the whole stage.

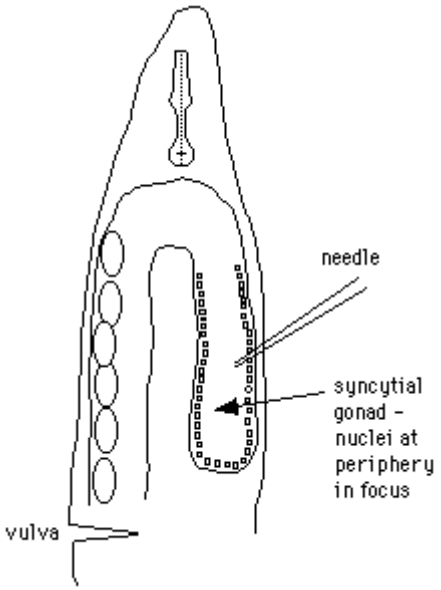
4) Move to the 20X objective. Focus on a syncytial gonad arm; this is recognized as a sausage shaped clear area surrounded by nice round nuclei. Kimble and Sharrock (Dev. Biol. 96:189-196 (1983)) show an excellent photograph of a dissected gonad that should give you a good idea of what to look for if you're new to worm anatomy. Focus on the center of the sausage so that you see a nice row of nuclei on either side of the sausage.

5) Using the fine adjuster, move the needle up/down until its very tip is in focus. Gently move the stage so the worm is pressing gently against the needle at a point where the syncytial gonad is pressed up against the body wall, and so that the needle tip will end up inside the gonad after it penetrates the body wall. Gently move the stage so the needle penetrates the gonad.

6) Press the orange button to start the flow of DNA. If you're in the gonad it should be obvious; as the gonad is flooded it bloats like you're filling a sausage, and you can sometimes see the nuclei in the syncytium reacting to the flow.

7) Usually one gonad arm is much easier to see well than the other, so some people only inject the easy gonad arm. Others try to inject both. If you miss the gonad, you will see liquid filling the pseudocoelom. Usually, the animal is ok, and you can just try again. It is surprisingly hard to kill the worm by jabbing and injecting it incorrectly.

8) Move the stage away and move to the other animal (in case you are injecting more then one.



G) Recovery

Put the pad under the dissecting scope (on the inverted plate lid) and using the eye leash detaches the worms from the pad and transferred them to a plate with a drop (20 ul) of M9. Make sure that the worm gets out of the oil.
Keep every injected worm in a different plate.

H) Results

1) Three days after injection, score the F1 for the marker gene phenotype. Isolate positive F1s into new plates- mark on the F1s plates from which injected hermaphrodite the worms came (to choose independent lines you should work with progeny of different injected animals).

2) Grow and look for positive F2s. Positive F2s will indicate that germ line transmission occurred and will be the source for the different lines.

Appendix I: choosing the selection

There are two ways of marking your transgene.

Dominant: adding to the injection mix dominant marker that will pass between generations and between different strains. **Pros:** enable array identification regardless of the genetic background. **Cons:** Affect worm's morphology and

health (non fluorescent markers), requires fluorescent selection every generation (Fluorescents markers).

Recessive: Adding to the mix a rescue construct that will rescue a mutant background. **Pros:** providing an advantage to the rescued worm vs. their non-transgenic siblings- easy to maintain and select. Easy to integrate. **Cons**: The selection is lost if the worms are crossed to WT worms. Might have some effect on worm's morphology and behavior.

Different markers:

Selection	**Marker**	**Selection**	**Conc (ug/ul)**	**Comments**
Dominant	Rol-6	Roller (stronger @ 25)	20-100	No good for hypodermis studies: the hypodermis is rolled
Dominant	Twk-18	Paralyzed @ 25C	20	
Dominant	Myo-2:: GFP/dsRED	Fluorescent	5-15	Not that easy to maintain: every generation- fluorescent selection
Recessive	unc-119 (+)	WT crawling worms	20-100	Pre-injection grow worms on DA7166 bacteria@ 15C. Problematic background for neurobiology studies
Recessive	pha-1 (+)	Live @ 25C	20	
Recessive	lin-15 (+)	WT vulva	20-50	Problematic background for Vulva development studies

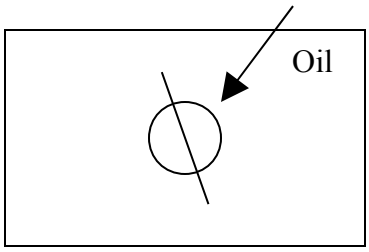
Comments:

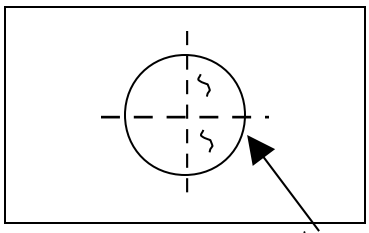
1) If you are in a hurry you can bake the slides for 1 hr in 65C or 15 minuets at 80C.

2) Less then 100ng/ ul: instable extra-chromosomal array; more then 200ng/ ul: needle blockage.

3) If needle is too long (the capillary was not slide to the end) a bubbles will form while loading resulting in needle blocking!

4) You can try making only 2 needles but according to injectors myth: if you make 2, you will need to make 2 more but if you make 4 you will inject all worms with the first needle!





5) Sophisticates can stick down a whole set of animals in a line in the same orientation for assembly line injecting.