Drosophila germline transformation

Nicolas Gompel, September 2003

This protocol is designed for P-element based transformation and also works for other retrotransposons such as Hermes (Horn and Wimmer, 2000).

Preparing the DNA (for P-element based constructs)

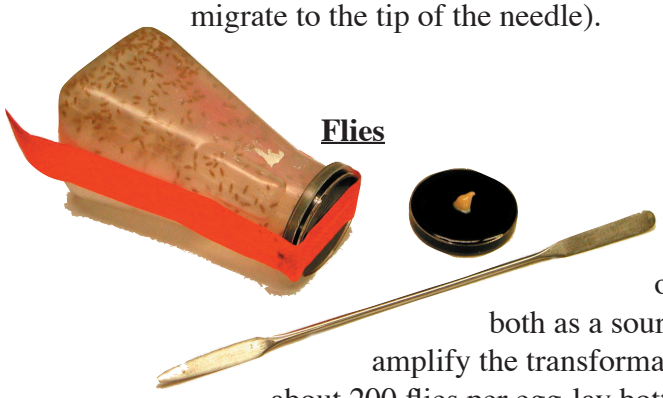
• Make a midiprep of the construct of interest and of the helper plasmid pπ25.7 Δ2-3 wc (Spradling and Rubin, 1982) (e. g. using a Qiagen® Plasmid Purification Kit). Avoid phenol purification. Resuspend DNA at 1 μg/μl.

• Mix

Construct plasmid (1 μg/μl)	10 μl
Helper plasmid (1 μg/μl)	9 μl
Filtered (0.2 μm) food dye	1 μl

• Vortex, spin 15 min at max speed. Carefully harvest 17 μl from the top and transfer to a new tube. Use 1 μl or less to fill the needles (capillaries have an internal filament and a droplet of mix added at the open end will migrate to the tip of the needle).

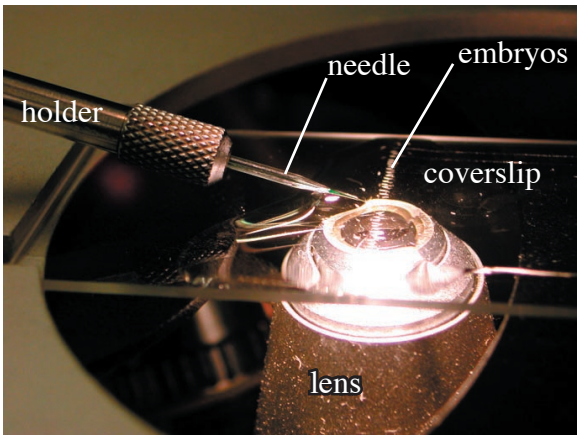
Flies



A *yw* mutant strain is best suited for this protocol, since it allows detection of *white* rescue phenotypes or expression of a fluorescent protein in the adult eye. These flies are used both as a source of embryos for the injection and as a backcross stock to amplify the transformants. Flies are kept in bottles on standard medium. Transfer about 200 flies per egg-lay bottles (figure on the left) 2-3 days before the first injection, to allow them acclimate to these egg-lay conditions. Egg-lay caps should be changed at least twice a day when not injecting. The population should be kept large enough to permit easy female virgining for backcrosses.

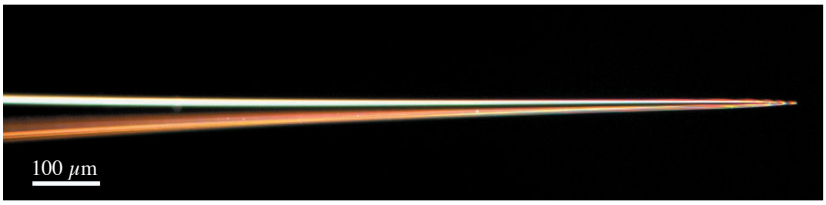
Set-up

The injection set-up consists of two parts: an inverted microscope equipped with a 20x lens and a micromanipulator, and an air-pressure injecting device (e.g., Narishige IM-300 Microinjector) connected to the needle holder. Bright field diascopy is most appropriate to monitor injections. The micromanipulator can be attached to the stage or not, but if it is not, it should be firmly attached to the bench.
A set-up installed in a cool room (18˚C) gives more flexibility as the embyos develop more slowly and the appropriate stage for injection lasts longer.



Needles

The quality of the needles is critical for high through-put. Needles should be pulled on any horizontal puller of the Sutter brand series using 1.0 mm OD borosilicate capillaries with omega dot fiber (e.g. Frederick Haer & Co, # 30-30-0). The settings will be different for each machine and will need to be updated each time the heating filament is replaced or a new type of capillaries is used. Several parameters influence the shape and properties of the needle and the effect produced by changing any of them (heat, velocity of pull, pressure of gas flow, number of steps) is difficult to predict. However, a paper by Miller et al. (2002) is a very useful guideline for designing suitable needles.



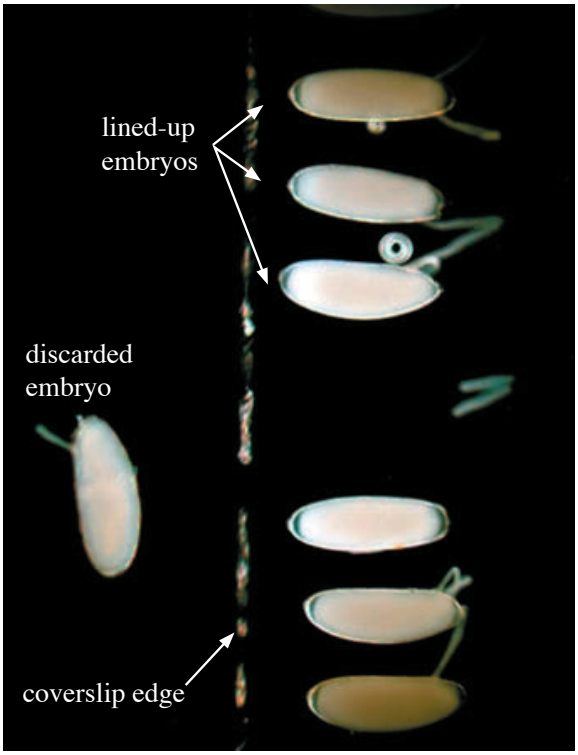
Optimal needles should deliver a droplet as in the figure on next page (dashed circle) when the injection time is between 10 and 40 ms.

Preparing the embryos

• Harvest embryos from a 20-40 min laying period. Egg-lay caps (with a bit of yeast paste) should be changed at least twice in the 2 hours preceding this harvest, to empty females of older embryos. A spatula or a large brush is best suited to collect the embryos.

• Transfer the embryos to a mesh basket and wash thoroughly with a squeeze bottle of distilled water.

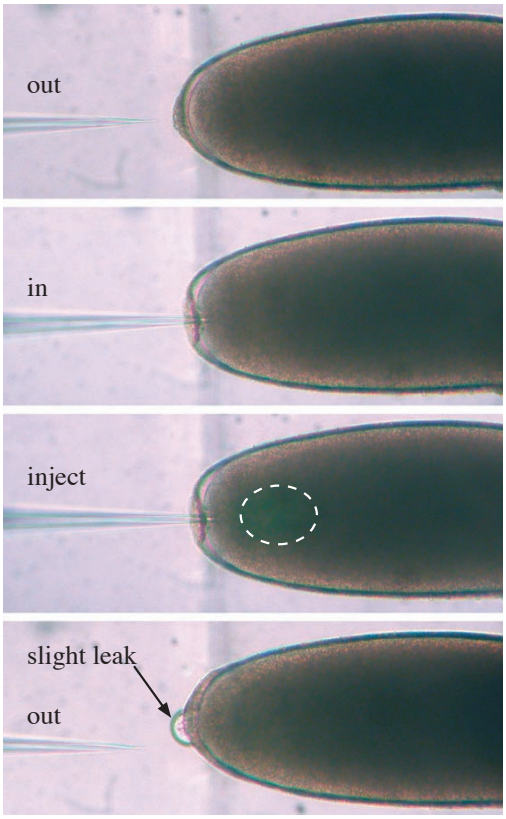
• Set a 18 x 18 mm coverslip on a microscope slide. Put a droplet of water between the coverslip and he slide to immoblize the coverslip.



• Transfer the clean embryos in a small quantity of water to the center of the coverslip with a clean thin pointed brush. Remove most water from the brush by touching a clean Kimwipe. Line up the moist embryos with the brush, one at a time, near one edge of the coverslip (but not crossing or touching the edge), with the posterior pole pointing to the edge. Dorso-ventral orientation doesn't matter. The shape and the moisture of the brush are key to easy arrangement of the embryos. Pack up to 60 embryos per coverslip, but make sure they don't touch each other.

• Remove leftover embryos (and save for another slide if they are not too old), and let the arranged embryos dry for a few seconds. Cover them with as little halocarbon oil mix as possible. Wait 5-10 min until the oil has penetrated between the chorion and the vitelline membrane, clearing the embryo and allowing a rough staging under the dissecting scope.

• Discard embryos older than stage 2 (figure on the right, Campos-Ortega and Hartenstein, 1997). They won't integrate the DNA and will add unnecessary work later. Transfer the slide to the microscope stage.



Injecting

• Move the slide on the microscope stage until the first embryo to be injected is positioned in the middle of the field in the eyepiece.

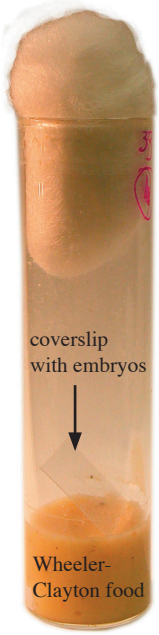
• Put a filled needle in the holder and tighten the screw with the rubber gasket gently but firmly. Bring the tip of the needle as close as possible to the first embryo, first manually, then using the micromanipulator. From this point, the stage rather than the needle should be moved for *xy* movements (including impaling the embryo on the needle). Use the micromanipulator for *z* adjustments to bring the tip of the needle into the focal plane of the embryo.

• Before injecting any embryo, set the injection time such that a droplet of the optimal size (see figure on the left) is released. If no liquid comes out, try breaking the tip of the needle very gently by barely touching the edge of the coverslip (i.e. move the edge of the coverslip away from the needle, focus on any irregularity of this edge, bring the tip of the needle to this focal plane, slowly bring the edge of the coverslip in contact with the tip of the needle). Adjust injection time again.

• Gently impale the first embryo onto the needle tip. Don't go further in than one-fourth the lengh of the embryo. Inject once, making sure you see a droplet diffusing into the embryo. Otherwise inject again. Overloaded embryos won't explode in many cases, but too much injected volume seems to be lethal.

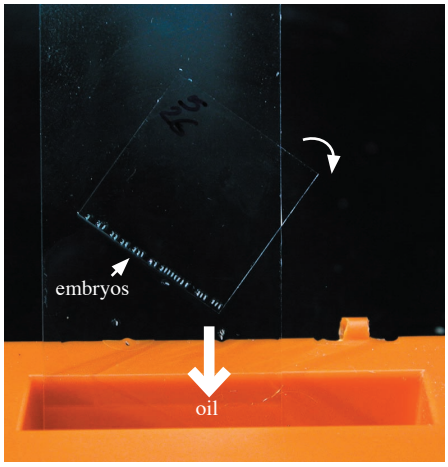
• Move the embryo off the needle in a quick motion. The speed limits cells leaking out of the embryo. Don't worry about a few cells coming out, most embryos will survive.

• Move the stage down to the next embryo and repeat.



After the injection

Drain most oil off the coverslip as shown below. Transfer coverslip to a Wheeler-Clayton food vial, placing the edge with the embryos against the food. Keep vials at room temperature or at 25˚C until adults hatch.



Back-crossing the injected flies

Collect hatching adults (Po) and separate the sexes. Cross each male to 3 virgin *yw* females and each female, even if obviously not virgin to 2 *yw* males. Perform crosses in separate vials of standard sugar food (e.g. http://flystocks.bio.indiana.edu/media-recipes.htm). Expect a good proportion (10-20%) of candidates to be sterile.
Wait until at least 20-50 adult F1 flies hatch in each vial before screening for transformants.

Recipies and references

Halocarbon oil mix
series HC-700 35 ml
series 27 5 ml

Egg-lay caps
for 120 caps (3 cm diameter petri dishes lids)
Melt 37.5 g of agar (bacterial is better) in 1 l dH_2O (microwave).
Add 12 ml Tegosept (10 g in 100 ml 95% ethanol).
Add 400 ml molasses
Stir well
Pour.
Store caps in large Petri dishes at 4˚C after caps have solidified.
Wipe off condensation before use.

Tucson Stock Center Wheeler-Clayton mix
(makes approx. 80 glass vials)

Top-layer ingredients	Amount
Cornmeal	20.1 g
Special K	6.75 g
Wheat Germ	6.75 g
Product 19	3.78 g
Brewer's yeast	13.5 g
Hi-protein baby cereal	10.3 g
Green banana	30 g
Tap water	675 ml
95% EtOH	4.3 ml
Propionic acid	4.3 ml
Agar (powder)	3 g

Bottom-layer ingredients	Amount
Instant Drosophila medium (Carolina biological supply)	27 g
Boiled tap water	120 ml

Directions for cooking on stove top

1. Mix the instant Drosophila medium with the boiled tap water in a small Ziploc baggie. Allow closed Ziploc to cool to a touchable temperature. Cut a corner out of the baggie and squeeze a small dab (about 1 ml) into the bottom of each vial.

2. Bring 300 ml of water to a rapid boil. Add the agar and *stir constantly*. Cook at a boil until agar is melted, about 5-10 minutes. Be careful not to burn the agar.

3. Take out the dry cereal mix (containing all of the cereals, blended to a powder – dry mixes are prepared ahead of time and stored a -20˚C in a Ziploc bag for up to 6 months in advance).

4. Blend the bananas, the dry mix, and 175 ml tap water in a blender.

5. Once the agar is melted, add in the banana mixture and 200 ml of water. (Use this water to wash all of the cereal mix from the blender into the pot of agar).

6. Lower the temperature slightly to prevent scalding.

7. Stir constantly; simmer for 10 minutes.

8. Carefully add 95% EtOH, then propionic acid; mix thoroughly.
9. Simmer 5 more minutes.
10. Remove from heat and pour into vials.

References

Campos-Ortega, J. A. and Hartenstein, V. (1997). The embryonic development of Drosophila melanogaster. Berlin ; New York: Springer.
Horn, C. and Wimmer, E. A. (2000). A versatile vector set for animal transgenesis. *Dev Genes Evol* **210**, 630-7.
Miller, D. F., Holtzman, S. L. and Kaufman, T. C. (2002). Customized microinjection glass capillary needles for P-element transformations in *Drosophila melanogaster*. *Biotechniques* **33**, 366-7, 369-70, 372 passim.
Spradling, A. C. and Rubin, G. M. (1982). Transposition of cloned P elements into Drosophila germ line chromosomes. *Science* **218**, 341-7.

***

(Please send feed-back comments to improve this protocol to ngompel@wisc.edu)