Drosophila germline transformation

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This protocol is designed for P-element based transformation (Spradling and Rubin, 1982) 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.

• Injection mix

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

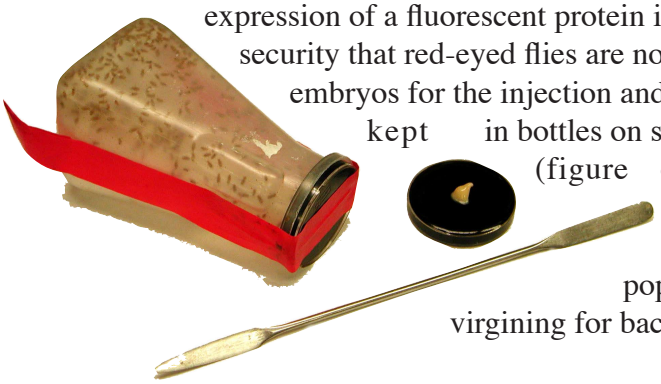
Alterantively (but I haven't tested it myself), the construct can be injected in flies that have a stable source of transposase (progeny of w; Δ2-3 98B; + x w; +; +. The w; Δ2-3 98B is available from the Bloomington center, #...). In this case the injection mix would be:

Construct plasmid (1 μg/μl)	19 μl
Filtered (0.2 μm) food dye	1 μl

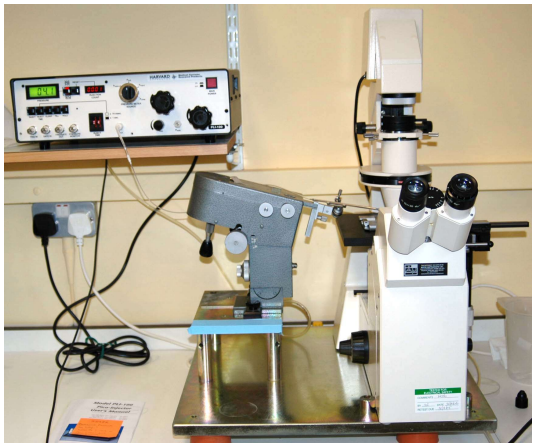
• Vortex, spin 15 min at max speed. Carefully harvest 17 μl from the top and transfer to a new tube. Use 1 μl or less to fill the needles (capillaries have an internal filament and a droplet of mix added at the open end will migrate to the tip of the needle). Sometimes, a tiny air bubble forms at the tip of the needle and will make injection difficult. It will go off 30 min or so after filling the needle. Once filled, the needles can be stored in a humid chamber (on a bar of plasticin in a Petri dish with a wet Kimwipes in the lid). They can be kept at 4˚C for a few days.

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. Also, the *y* phenotype provides an extra security that red-eyed flies are not contaminants . 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 300 flies per egg-lay cage (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.

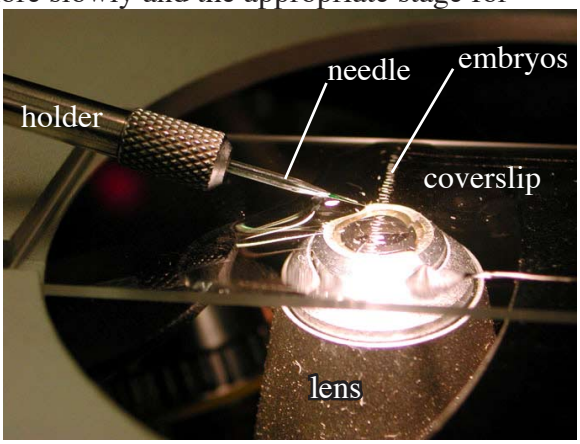


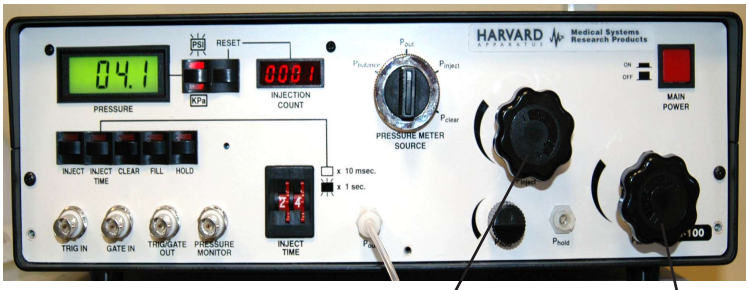
Set-up



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 or Nomarski diascopy are 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 time flexibility as the embyos develop more slowly and the appropriate stage for injection lasts longer.

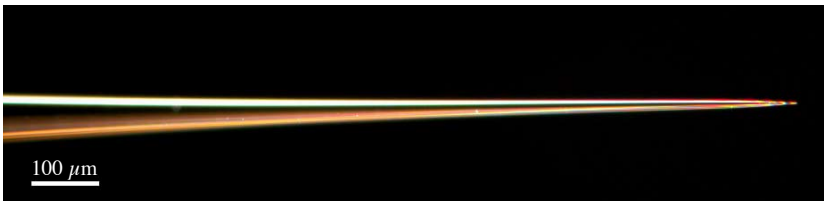




pressure out knob

pressure balance knob

Needles



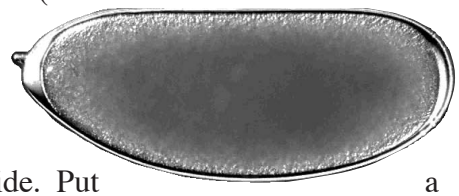
The quality of the needles is critical for high through-put. Needles should be pulled on any horizontal puller of the Sutter brand series using 1.0 mm OD borosilicate capillaries with omega dot fiber (e.g. Frederick Haer & Co, # 30-30-0). The settings will be different for each machine and will need to be updated each time the heating filament is replaced or re-shaped, 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. The needle should be progressively but shortly tapered and have no discontinuity or step. Needles that are too elongated will bend and brake when impaling the chorion. Needles that are too blunt, on the other hand, won't bend, but will damage the embryos more severely and lower the overall survival. Once the needles are suitable to penetrate the embryos smoothly, the amount of injection mix coming out can be adjusted by playing with the injection time (should be between 10 and 40 ms) and the pressure knobs (Pout and Pbalance). The droplet should be as in the figure on next page.

Preparing the embryos

• Harvest embryos from a 20-40 min laying period. Egg-lay caps (with a bit of fresh 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 (Costar Netwell™, Corning 3477, 74 μm mesh, picture above) and

wash thoroughly with a squeeze bottle of distilled water, then with 95° alcohol (that gets between the chorion and the vitelline membrane, making the chorion transparent which ease the staging) , and with water again.



• 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. The edges of the coverslip should be parallel to those of the slide.

• 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 Kimwipes. 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, but it is easier to inject if all embryos have the same orientation as there is no need to refocus. The shape and the moisture of the brush are key to easy arrangement of the embryos. Pack up to 100 embryos per coverslip, side by side.

• Remove leftover embryos (and save for another slide if they are not too old), and let the arranged embryos dry for a few minutes to attach them firmly to the coverslip. 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.

• Poke 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.

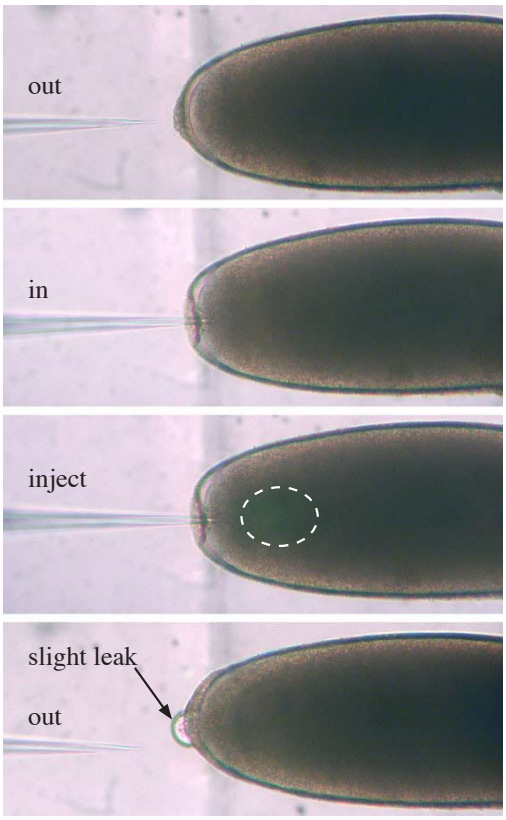
Injecting

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

• Put a filled needle in the holder and tighten the screw containing 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 and pressure such that a droplet of the optimal size (see figure) is released. If no liquid comes out, try widening the tip of the needle very gently by tickling an embryo (put the tip in conatct with an embryo and move the slide back and forth along the y axis).

• Gently impale the first embryo onto the needle tip. Don't go further in than one-fith 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 mix seems to be lethal.

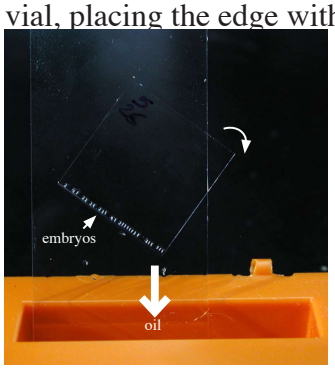


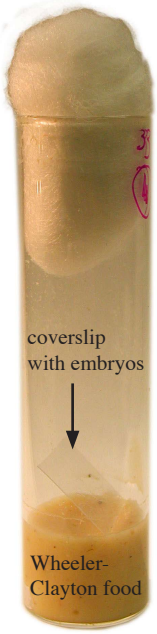
• Move the embryo off the needle in a quick motion. The speed limits cytoplasm/nuclei leaking out of the embryo. Don't worry about a few cells coming out, most embryos will survive this.

• 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 the vials at room temperature or at 25˚C until adults hatch. REMOVE COVERSLIPS FROM THE VIALS IF YOU RECYCLE THEM. Keeping the vials in a humid chamber (a beaker with wet tissue covered with Saran) for the first 48 hours seems to increase the viability.





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.
It is worthwile to mark on each cross if the candidate transformant was a male or a virgin female, as each will represent a clear independant recombination event.

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.

*these dark molasses caps are nice because they make the embryos very conspicuous, contrary to apple juice-based caps, and the flies like them as much

Tucson Stock Center Wheeler-Clayton mix (Wheeler and Clayton, 1965)
(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.

Wheeler, M. R. and Clayton, F. E. (1965). A new Drosophila culture technique. *Drosophila Information Service* **40**, 98.

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(Please send feed-back to improve this protocol to ng293@cam.ac.uk)