**Embryo injections**

**Make heptane/adhesive solution**

Note: the toxicity of adhesives vary from batch to batch. You may have to try several rolls of tape.

- Roll a strip (3-4 inches) of double stick Scotch tape into vial (scintillation vial).
- Add enough heptane to completely immerse tape.
- Cap vial and let tape soak for at least a few hours (overnight is fine).
- Pour heptane/adhesive solution into a coverslip jar.

**Coat coverslips**

- Dip 10-15 coverslips in heptane/adhesive solution (3-5 mm deep). Let dry overnight.

**Co-precipitate P-element vector and Transposase vector** (e.g. pTurbo):

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-element vector</td>
<td>6 ug</td>
</tr>
<tr>
<td>pTurbo (transposase)</td>
<td>2 ug</td>
</tr>
<tr>
<td>3 M Sodium Acetate</td>
<td>1/10 volume</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>2 volumes</td>
</tr>
</tbody>
</table>

- Place on ice or freezer at least 30 minutes.
- Spin. Wash 3x with 70% Ethanol. Dry.
- Resuspend in 20 ul loading buffer (5 mM KCl, 0.1 mM Sodium Phosphate pH 7.8)

**Prepare solutions** on the day of injections:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypochlorite solution</td>
<td>100 ml (50% Chlorox): 50 ml Chlorox, 50 ml water</td>
</tr>
<tr>
<td>Wash solution</td>
<td>500 ml (0.7% NaCl, 0.03% TritonX-100): 17.5 ml 20% NaCl, 1.5 ml 10% TritonX-100, 481 ml water</td>
</tr>
<tr>
<td>Water</td>
<td>500 ml water</td>
</tr>
</tbody>
</table>

**Dessicant boxes**: add enough Drier-rite dessicant to cover (one-layer thick) empty mounting slide boxes.

**Bubble oxygen through Halocarbon oil** (400 series; Halocarbon Products catalog # 9002-83-9) for at least 10 minutes

**Loading/mounting the injection needle**:

- Pipette 1 ul DNA solution into a P-2 tip.
- Carefully detach tip from pipette. If necessary, tap wide end of tip to move DNA solution close to narrow end of tip.
- Set P-20 to 10 ul, and use a microloader tip (Eppendorf catalog # 5242-009-012) to pipette/transfer DNA solution from P-2 tip to load into injection needle (Eppendorf catalog # 5242-952-008). If necessary, use a dark background and/or dissecting scope.
- Unscrew needle cap and place needle in injection apparatus (simple micromanipulator, Prior model # 97655?).
- Carefully break the tip of the needle against the edge of a coverslip mounted on a slide. The size of the
aperture and the shape at the needle tip are critical to good injections. The aperture should be just large enough allow the smallest volume to pass through. The ideal shape of the needle tip should be bevelled, as opposed to flat.
-Place the needle tip in halocarbon oil on coverslip.
-Check volume of injection pulse. Adjust if necessary.
Embryo collection and dechorionation

- Collect embryos on egg-laying plates from 6-12 day old flies. Use 0-1 hr old embryos (60 minute interval collections at 18-20 deg C).
- Dislodge embryos from plate by squirting 15-30 ml hypochlorite solution, collecting the embryos and solution in a 50 ml beaker.
- Let embryos soak in the hypochlorite solution 2-3 minutes.
- Prepare 3-4 slabs of agar (from egg-laying plate) to line up embryos.
- Add equal volume of wash solution (0.7% NaCl, 0.03% TritonX-100) to embryos in hypochlorite solution.
- Filter embryos from solution through Nitex mesh.
- Wash 3x 50 ml with wash solution.
- Rinse 3x 50 ml with water (milliQ or RO).
- Drain excess water from Nitex mesh with paper towel.

Mounting and dessication of embryos

- Transfer embryos to agar slabs. Distribute at least 40 embryos per slab.
- Line up embryos, parallel to each other, posterior ends on the edge of the agar slab. 20-30 embryos should easily fit on one slab. Spacing between embryos should be at least 1 embryo width apart.
- Transfer embryos to coverslip (adhesive-coated) by placing coverslip over embryos, with posterior ends pointing toward center of cover slip. Leave space (at least on embryo length) between edge of coverslip and anterior ends of embryos.
- Place coverslip with embryos in dessicant box. Dessication time will vary depending on factors such as temperature, relative humidity, and length of time to mount embryos (exposure to air). At 20 deg C, 55%-65% relative humidity, start with 9-10 minute dessication time and, if necessary, adjust subsequently.

Dessication time is critical to post-injection embryo survival and transformation efficiency.

- While the first set of embryos is dessicating, line up a second (and third) set for dessication.
- After appropriate dessication, attach the coverslip (with dessicated embryos) onto a mounting slide with a small drop of water. Immediately place halocarbon oil over embryos to prevent further dessication.

Injecting the embryos

- Place needle tip in center of field of view. Start at low magnification and optimize at higher magnifications. (Simple inverted scope, Nikon TM3, 4X, 10X, 20X lens)
- Observe injections at highest magnification (20 X).
- Set injection pulse at 15-20 milliseconds (General Valve Co., Picospritzer II).
- Pulse inject in oil to check the volume. Adjust time (milliseconds) accordingly. If necessary, adjust pressure.
- Adjust height of needle for proper contact with the posterior end of the embryo.
- Use a quick yet smooth motion to puncture the posterior end of the embryo. Try to minimize the depth of the puncture. (Needle is stationary, embryo is moved into the needle using the stage controls).
- Withdraw from the needle until just the tip is within the embryo's vitelline membrane.
- Pulse inject.
- Completely withdraw from needle.
- Move to next embryo, repeat.
- If embryos consistently leak, they are under-dessicated. Increase dessication time.
- If embryos are shriveled, they are over-dessicated. Decrease dessication time.
- Frequently, the needle tip gets clogged. This usually happens when the tip touches the embryo membrane without puncturing through, which occurs when the embryo is not anchored strongly enough to the coverslip (reasons: weak adhesive, wet embryo, or improper dechorionation). Continue to inject the next embryo. Repeatedly puncturing the embryo should unplug the tip. A temporary adjustment to the pulse time (up to hundreds of milliseconds) may also be necessary. If the clog persists, try to rub and/or break the tip on the edge of the coverslip.
Post-injection care

- Keep embryos under enough oil at all times. Low levels of oil on embryos will result in the exposure of embryos to the oil surface tension, leading to deformation/death of the embryos.
- Cut edge (4-6 mm width) of cover slip with embryos.
- Place on platform (with platform perimeter well within the perimeter of the cut coverslip). This will prevent oil from leaking/dispersing from the cover slip. Platforms (2 x 2 x 10 mm) can be made from rubber erasers.
- Remove/destroy un-injected and leaking embryos.
- Allow embryos to develop in moist oxygen chamber (optional?).
- Most of the larvae will hatch approximately 30-35 hours (20 deg C) after injection.
- Transfer larvae to food.