NYU/Desplan lab

MICRO INJECTIONS FOR DROSOPHILA EMBRYOS

Set-up egg laying cages to collect early embryos on grape plates with a smear of yeast paste.

Allow 30 minute laying period in 18 degree room. (We collect and inject at 18° C to give more time flexibility as the embryos develop more slowly. This gives you a longer time frame for injections.)

Start collecting embryos immediately from the grape plate using a small brush (try not to collect from the yeast paste as it can be messy). Transfer the embryos onto the double sided tape on the slide. Using a probe gently push the around to dechorionate them. (You can also use another protocol to dechorionate many embryos at the same time using bleach but we find that manually dechorionating is better for the efficiency of the injections).

The prepared slide consists of a thin piece of double sided tape approximately 4mm wide (3M/scotch brand) with a thin semi circle, drawn with colored nail polish, creating a barrier for the mineral oil.

Use the probe to line up the embryos with the very tip of the posterior end on the edge of the tape and the anterior side fully on the tape. I usually line up about 50 embryos per slide. You don’t want to take any longer than a half hour to line up (as the embryos will get too old to inject).

Place the slide with the embryos in a drying chamber with Drierite (Desiccant) for 5-15 minutes. The time in the drying chamber will depend on the humidity on the day you are injecting. This is something you will have to play with each day. You do not want them too dry because then the embryos will not survive and you do not want them too moist because the injections will cause all the matter inside the embryo to come flooding out, also killing them.

After drying take the slide and put a few drops of mineral oil over the embryos, enough to cover them fully plus a little more (so they don’t dry out during and after injections).

INJECTIONS

Place the slide on the microscope stage until the first embryo to be injected is positioned in the eyepiece. We inject a small amount of injection mix into the posterior region of the embryo.

We use the eppendorf Femtojet to keep a constant pressure for injections.

After injections we put the slides on a flat, covered surface for about 48 hours (or until the embryos hatch).

Use a probe to collect larvae and put them onto fresh food (mash surface of food so larvae can move freely).