Mass Dechorionation for Embryo Microinjection

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1. Establish healthy, low density cultures of yw flies by allowing several hundred adults to lay for one day in fly bottles. Transfer adults each day for 3-4 days to new bottles. After this, begin the process with new flies.

2. Two to three days before you inject, set up a population cage with 0-3 day old yw adults raised at low density. Feed with grape agar plates on which a small amount of yeast paste has been spread. Feed cage twice daily.

3. On the day you wish to inject, feed the cage every hour to encourage females to lay eggs.

4. Using tap water, loosen the embryos on the plate and rinse into a nylon mesh basket. Rinse thoroughly to remove excess yeast paste.

5. Chemically dechorionate by incubating embryos in a solution of 50% bleach for 2-4 minutes. Gently agitate to facilitate the mixing of embryos in bleach solution.
Hyung-Don Ryoo:
100% bleach=2 minutes
66% bleach=3 minutes

6. Using tap water, wash embryos extensively in basket to remove all traces of bleach. Test by placing basket on paper towels. Any trace of bleach will turn the paper pink. If so, continue rinsing.

7. Using a brush, transfer dechorionated embryos immediately to cube of 3% agarose/ultrapure water containing enough charcoal to make it quite black. Cut a chunk of agarose approximately the width of the non-frosted area of the slide in length, and approximately half the width of the slide. Line up embryos on agarose, making sure to keep them all oriented in the same direction. Use a metal needle to transfer and line up embryos.

8. Prepare a glass slide with a piece of double stick tape. Use acid-free archival double stick tape! Take the cube of agarose containing the lined up embryos and gently invert double stick taped slide onto embryos to transfer embryos to slide.

9. Place embryos in desiccation chamber for the necessary period of time to obtain well desiccated, but not overdesiccated embryos. Start with 10-15 minutes.

10. Cover embryos with halocarbon oil and inject with DNA mixed with helper DNA.

11. Allow embryos to develop at 18^{o}C for 2 days, then transfer to vials as 1st instar larvae.