

# Microinjection

## The Belote Lab Protocol

### A. DNA & needle preparation

#### DNA

1. Prepare DNA for injection using your favorite Plasmid Purification Kit<sup>a</sup>.
2. Mix your plasmid of interest plus helper plasmid<sup>b</sup> (15 $\mu$ g + 5 $\mu$ g), then ethanol precipitate the mixture using 1/10 volume 3M NaOAc and two times volume 100% ethanol. Spin for 30 min @ max speed, wash with 70% ethanol and bring the pellet up in 50 $\mu$ l injection buffer<sup>c</sup>.

#### Needle<sup>d</sup>

1. Adjust the appropriate setting for the needle puller (see suggested settings - Tip # 1) and placed the pulled needles in a clean box with a lid.

### B. Maintaining fly stocks<sup>e</sup>

1. Maintain flies for 2 weeks cycle (25°C, 12L:12D).
2. Clear the bottles and transfer newly enclosed adults to fresh food bottles (medium density). Add some dry yeast (see Tip # 2).

### C. Setting up collection bottles

1. For embryo collection use 4-6 day old adults.
2. Transfer the flies to empty glass bottle and place a small Petri dish<sup>f</sup> containing collection medium<sup>g,h</sup> on top of the bottle.
3. Invert the bottle and place in a dark site.
4. After 60 min replace the collection plate with a fresh one and from that point you can replace the plate every 30-min and use the embryos for injections.

### D. Preparing for injection:

#### Chorion removal

##### a: preparation

1. Place a strip of Double Stick Tape<sup>1</sup> (scotch tape) on a microscope slide.
2. Cut the tape into narrow strips with a razor blade and transfer one strip to the middle of a new microscope slide (see Fig. 1).
3. Collect a small ball of scotch tape in the tips of your forceps<sup>2</sup>. This will allow for dechoriation and transfer.

##### b:

1. Transfer embryos from the collection plate to the tape on the slide with a micro-hook<sup>3</sup> (or fine brush) (Fig. 1a)

<sup>a</sup>Superscripts: letters and numbers see materials-section.

2. Put the slide in a humid environment (Petri dish with moisture Kimwipe) for 2-5 min. Meanwhile you can transfer another set of embryos to be injected.
3. Rupture the chorion by gently rolling the ball of scotch tape on the tip of your forceps, upon the middle of the embryo.
4. Transfer the embryo to the edge of the strip and orient it on its side with the posterior end just hanging off the tape (see Fig. 2).
5. Space the embryos along the length of the tape.
6. Pay attention to the embryo quality and developmental stage. Discard low quality, old embryos and the remaining unaligned embryos (see Fig. 3 & Tip # 3).
7. Place the slide in a Petri dish containing desiccant for about 5 min (adjust desiccation conditions as needed, see Tip # 4).
8. While the embryos are desiccating, dechorionate the embryos on the next slide.
9. When desiccation is complete, remove the slide from the desiccant and cover the embryos with a thin layer of halocarbon oil<sup>4</sup> using a transfer pipette.

### DNA & Needle preparation

1. Place 15 $\mu$ l of the DNA ready for injection (section A) in a 0.65ml Eppendorf tube. Add 4 $\mu$ l of green dye<sup>5</sup> and mix.
2. Load the pulled needle (from section A) with DNA by back filling (place the back part of the needle in the tube with the DNA mix).
3. Connect the loaded needle to air-filled plastic tubing<sup>6</sup> that, in turn, is connected to a 10ml syringe. Seal the needle to the tubing by wrapping parafilm around the junction.
4. Place a microscope slide loaded with a drop of halocarbon oil or the slide that is loaded with the embryos for injection, on the microscope stage, and place a coverslip at the right side of the slide. Break the needle tip against the coverslip (see Fig. 4). Test the tip quality under the oil by dispensing DNA (see also Tip # 5).

### E. Injection

1. Position the needle slightly away from the middle of the posterior tip of the embryo. Once aligned penetrate the embryo. After penetration, draw back the needle as far as possible, without leaving the embryo, then deposit the DNA in the posterior most region of the embryonic cytoplasm (Fig. 3.1).
2. If it is possible (depends on the tip of your needle) dispense the DNA before penetrating the embryo (when the needle is aligned at the right position). This will allow DNA deposition without increasing pressure inside the embryo.
3. A "good embryo" for injection is an embryo at stage 2 (early cleavage) when a cap of clear cytoplasm becomes visible at the posterior pole. In this case, dispense the DNA before penetration and deposit the DNA in the space (see Fig. 3.2).

### F. Post injection

1. Remove the uninjected embryos.
2. Place the slide in a humid environment (Petri dish<sup>7</sup> with humid paper - see Fig. 5).  
Optional: add yeast and a drop of water on top of them near the injected embryos.
3. Transfer hatched larvae using a micro-hook or a fine hair-hook to food vial. - *NOTE: usually takes 2 days to hatch*
4. Cross each enclosed adult ( $G_0$ ) to 2 W strain partners.

5. Screen for **RED EYE** flies ( $G_1$ )- **TRANSFORMANTS!!!**

## G. Materials

### Sections: A-C

- a **Plasmid Purification Kit:** Promega, QIAGEN: EndoFree Plasmid Maxi Kit.
- b **Helper plasmid:**  $\Delta 2-3$  wc .
- c **Injection buffer:** 5 mM KCL; 0.1 mM NaPO<sub>4</sub> buffer pH 7.5).
- d **Injection needle:** World Precision Inc.; glass 1BBL w/FIL 1.0 mm, 6 IN, 1B100F-6; 500, 011-1.
- e **Fly strain:** W
- f **Petri dish:** FALCON; 351008 Easy grip Petri dish, 35 x 10 mm style.
- g **Apple agar plate: (500mL flask)**
  - 100mL H<sub>2</sub>O
  - 100mL apple juice
  - 6g agar

Microwave to dissolve the agar. Add 2mL-mold inhibition (10% methyl P hydroxy benzoate in ethanol) and 2mL glacial acetic acid (optional: 5mL ethyl acetate). Pour into small Petri dishes.

### h **Grape agar plate:**

- Mix: 271.5mL distilled water  
227.5mL Grape Juice

Bring to a boil. Then add:

- 11g Agar
- 29g Dextrose
- 14.5g Sucrose
- 9g Yeast

Let come to a rolling boil. Remove from heat. Add 11mL of 1.25 M NaOH and mix in. Cover with foil. Autoclave, 10 min.--slow exhaust. Cool to 45°C (using thermometer to measure). Add 0.56mL ACID-MIX and mix in. Continue to stir periodically while dispensing. Dispense slowly as not to create foam atop media. Fill dish until half full.

1.25 M NaOH: For 1 Liter: Add 50g NaOH to 900mL distilled water, dissolve. Bring to volume with distilled water.

### Sections: D-F

- 1. **Double Stick Tape:** Scotch™ ; (472 in x 250 in 3M). *outer surface up!*
- 2. **Dumont forceps:** FST; Biologie tip, 0.05 mm x 0.02 mm, Inox 11 cm, No. 11255-20.
- 3. **Micro- hook:** FST; 15.5 cm, No. 10140-01.
- 4. **Halocarbon oil:** Halocarbon™; Halocarbon 700 oil, 1 lb.
- 5. **Green Food Color:** McCormick, 29 ml. Autoclave before using for injection.
- 6. **Tygon Plastic Tubing:** Tygon™.
- 7. **Petri dish:** VWR; Polystyrene disposable, 150x15mm (cat No. 25384-139).

## H. TIPS

1. Parameters for pulling needles: Using needle puller model:

H=505

P=125

V=100

T=170

*This can vary!! Adjust H settings to modify needle tips.*

2. Newly enclosed flies that will be used for embryo collection can be maintained on reversed photoperiod - 12D:12L. Having females at the dark period of the day in addition to placing them at a dark place will increase their egg-laying rate.
3. Avoid selecting yellowish embryos (too old or low quality) or dehydrated ("sad looking") embryos (low quality).
4. The appropriate time of desiccation depends on the humidity at the specific injection day and the time it takes to dechorionate and aligns the embryos on the slide. Find the right time between the appropriate desiccation (no cytoplasm leakage when the needle penetrates or is withdrawn from the embryo) and lethal overdesiccation (embryos take up large amounts of DNA or looks saggy).
5. Slide the needle tip against and along the slide edge as indicated in Fig. 4. A **Very Good** needle has a sharp bevelled opening and dispenses small drops of DNA.

Figure: 1

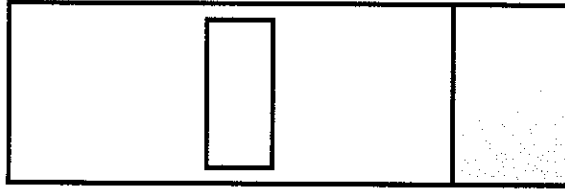


Figure: 1a

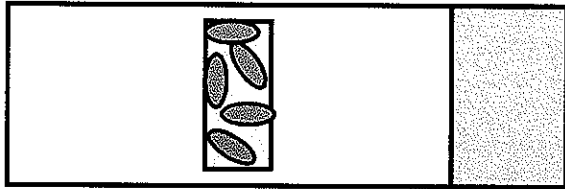
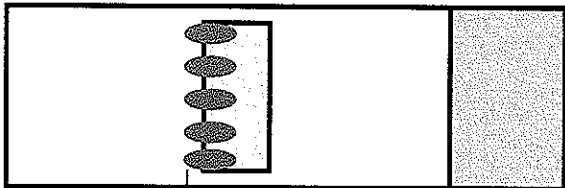
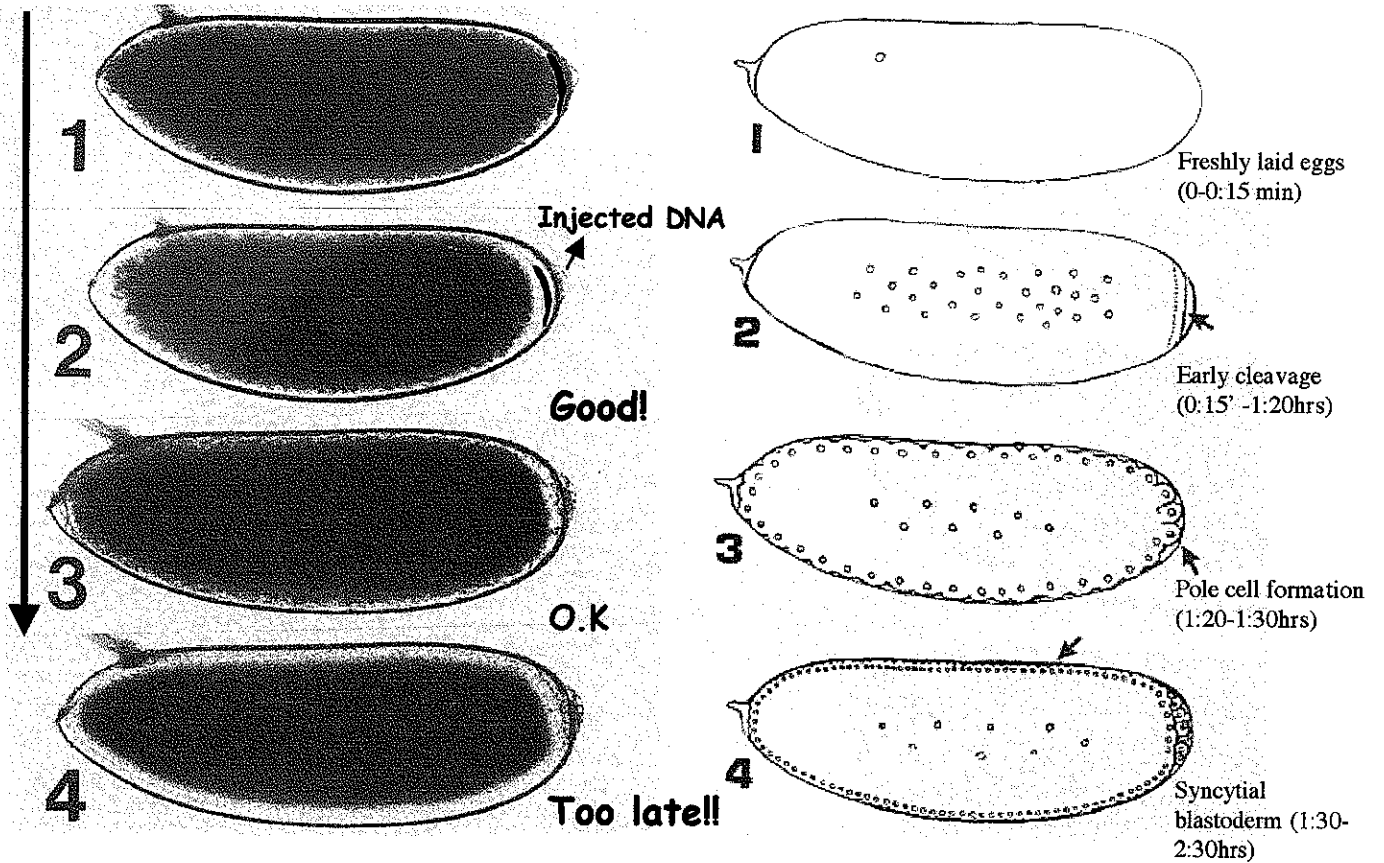


Figure: 2



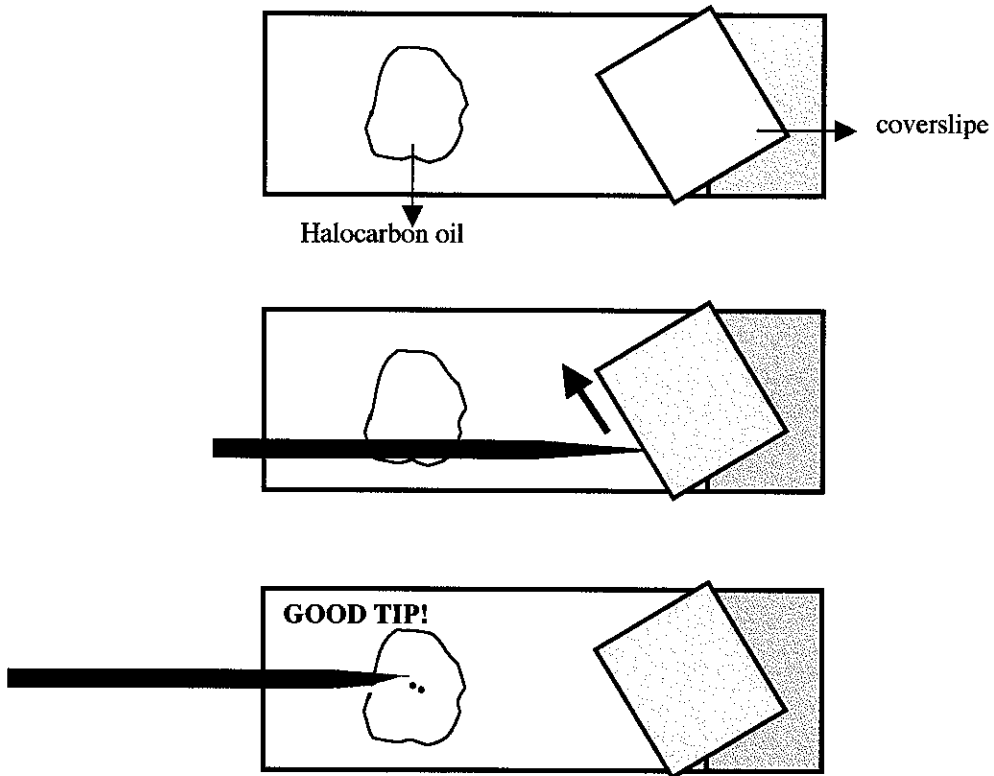
dechorionate embryos

Figure: 3



(Figure adapted from Wieschaus & Nusslein-Volhard, 1986)

**Figure: 4**



**Figure: 5**

