Protocol 2: Growing *Archegozetes longisetosus* Embryos *De Mater*

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Materials

Bleach diluted 1:1 in double-distilled H_2O
Sigma halocarbon oil 27
Sigma halocarbon oil 700
An oil solution of 70% halocarbon 27 and 30% halocarbon 700
Heptane
Methanol
1X phosphate buffered saline (PBS)
Formaldehyde
Fine paintbrush
Petri dish, cleaned and autoclaved
Small glass dish
Standard microscope slide, cleaned
Cotton
0.25 mm glass capillary

**All of the proceeding steps should take place in a small weighing dish.

1. Collect and prepare early-stage *A. longisetosus* embryos as specified in Protocol 1.

2. Remove as much of the 1X PBS as possible, and rinse 5 times with ddH_2O. Do this quickly as to not provide too much of an osmotic shock to the embryos.

3. Quickly add roughly 500 µl of the diluted bleach to the embryos, and gently swirl the solution with a pipette for 5 minutes to dechorionated them.

> **This selects for early germ-band stage embryos, as these tend to have stronger vitelline membranes that prevents them from being destroyed during the dechorionization step.

4. Remove as much of the bleach as possible, and rinse the embryos 5 times with ddH_2O.

5. Place the 0.25 mm glass capillary onto a glass microscope slide, with the capillary being parallel to the long side of the slide (Figure 1).

6. With the fine paintbrush, collect the dechorionated embryos and place them along the capillary. It is difficult to assess which portions of the embryos are dorsal/ventral during this step. However, one should place the embryos so that their long axis is parallel to the slide. Also, space the embryos so that they are not in contact with one another.

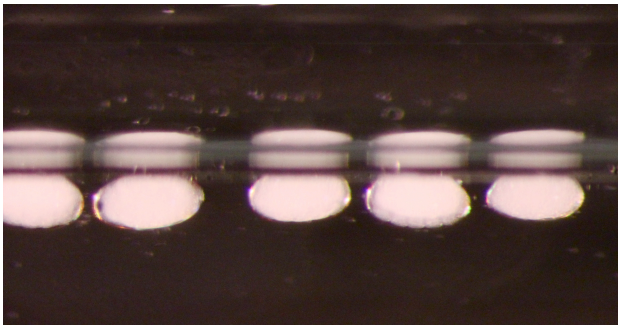

Figure 1: Mid-stage *A. longisetosus* embryos aligned on a capillary in the oil mixture.

> ** It is also important to ensure that the embryos do not dry out at this step. When transferring embryos, make sure to add water to the capillary.

7. After all embryos are positioned on the slide, dry the embryos for 20 seconds. Quickly spread roughly 200 µl of the 27/700 halocarbon mixture across the capillary. Make sure that each embryos is covered in the solution.

8. The embryos are now ready for microinjection (Protocol 3).

9. Following step 6, or following microinjection, place the slide with the embryos into a clean, autoclaved Petri dish. Line the Petri dish with water-soaked cotton to provide humidity. Place the cover on the Petri dish, and place it in an incubator at 27°C.

10. Allow the embryos to grow to the desired stage (Barnett and Thomas 2012). The embryos will become prelarvae roughly seven days following the germ band.

Embryo Fixation

1. After embryos have grown to the desired stage, carefully remove the embryos from the oil using the fine paintbrush under a dissecting microscope. The embryos will stick to the paintbrush.

2. Slowly manipulate the paintbrush in a small glass dish filled with heptane. The heptane will dissolve the oil. Remove the heptane from the glass dish, avoiding the embryos. Wash the embryos with 1X PBS once. Place the embryos into a 1.5 ml microcentrifuge tube with 1X PBS supplemented with 4% formaldehyde. Fix the embryos at room temperature for 1 hour. In the meantime, place 500 µl of heptane into an -80°C freezer.

3. Remove the formaldehyde, and place the -80°C heptane into the tube containing the embryos, and wait 30 sec. Quickly add 500 µl of room temperature methanol, close the tube and shake violently for 1 min.

4. Collect the de-vitellinized embryos (most should sink to the bottom of the tube). Rinse the embryos twice in methanol, and store in methanol at -20°C.

Reference

Barnett, A.A., Thomas, R.H. (2012). The expression of limb gap genes in the mite *Archegozetes longisetosus* reveals differential patterning mechanisms in chelicerates. MS *in prep*.