Whole Embryo Z.1 Mounting, E5.5-E8.5
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December 2019

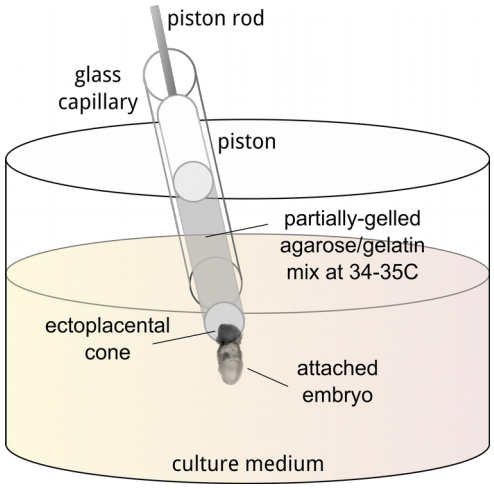

Figure 1

Embryo Mounting:

A. Prepare Mounting Medium

1. Place 300mg agarose (Fisher BP165-25) and 600mg gelatin (Sigma G1890) in a 20mL conical vial, add 20mL sterile PBS, and vortex.
2. Close conical vial tightly and microwave repeatedly on high for 5-7 seconds each time until melted, stopping to remove and replace lid for venting hot vapor between each cycle.
3. Optional for multiview registration: Add 1uL red and 1uL green latex beads (Sigma L1030 and L2778) and vortex.
4. Aliquot melted gel mix into sterile microcentrifuge tubes and cool to room temperature.

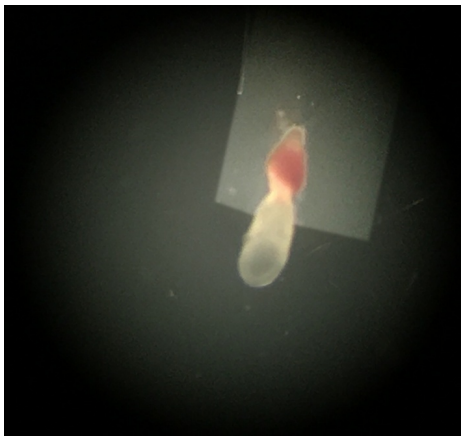
Figure 2

B. Mounting Protocol

1. Melt two tubes of embedding medium at 75C, then cool to and maintain 34.5C.
2. Once at 34.5C, use P200 pipette to fill a glass capillary (orange for E6.5, black for E7.0, green for E7.5, blue for E9.0+) with liquid embedding medium. Once filled, orientate capillary vertically, remove pipette, and allow medium to start to drip out of bottom. As it drips down, insert plunger into that end to make a good seal with medium (with no air bubbles at the piston/gel interface). Place capillary/piston setup horizontally in oven at 34.5C to maintain liquid state.
3. Repeat step 2 until 3-5 capillaries are prepared and ready.
4. Sacrifice dam, dissect embryos, and transfer 3-5 embryos to a dedicated dish containing culture medium. Maintain on warmer to maintain 37C as closely as possible.
5. Remove one capillary/piston setup at a time, and keeping as sterile as possible, cool at RT until the mix is starting to gel (when plunger/piston is pushed, the extruded gel is able to maintain the cylindrical shape of the capillary—but barely). Immediately proceed to mounting embryo.
6. Push plunger to extrude at least 25-33% of the overall capillary length of gel, then cut this off sharply with dissecting forceps; remove this portion.
7. Place free end of capillary/piston set-up into the dish containing embryos, holding it with non-dominant hand. Use dominant hand to push piston to extrude a small amount of gel ~1-2mm, then pick up dissecting forceps with dominant hand.
8. Holding capillary with non-dominant hand, use dominant hand with dissecting forceps to lightly grab the ectoplacental cone from one embryo, pushing/shoving it into the extruded agarose from the capillary (Figures 1-2). Once the embryo is immobilized by adquate embedding of the ectoplacental cone into the gel, use dominant hand (first return forceps to tabletop) to pull on piston/plunger to suck gel with mounted embryo and excess culture medium back into the capillary. Park embryo about 4-5mm from the end of capillary.
9. Allow capillary to cool to room temperature for a few minutes, while steps 5-8 are repeated for each embryo. After a few minutes at room temperature, transfer each embryo/capillary/piston setup to an empty 50mL conical vial taped down on its side, for transfer to LSFM. Preferably, maintain 37C as best as possible by taping 50mL conical vial to a heat block to stay warm.