

Materials List for:

## Ablation of a Single Cell From Eight-cell Embryos of the Amphipod Crustacean *Parhyale hawaiensis*

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## **Materials**

Name	Company	Catalog Number	Comments
15 cm Pasteur pipette	Wheaton	53499-630 VWR	For transferring intact and blastomere-ablated embryos from dish to dish.
22 cm Pasteur pipette	Wheaton	53499-632 VWR	For making mouth pipette tips.
3 cm Petri dishes	Thermo Scientific	25382-334 VWR	Use some unaltered to collect and store embryos; coat some with a layer of Sylgard (see below) to create a working surface for blastomere ablations.
48-well Plates	Greiner Bio-One	82051-004 VWR	For culturing embryos following ablation.
Bottle top filters 0.2 µm	Nalge Nunc International	28199-296 VWR	For creating FASW (See below).
Bunsen burner	VWR	89038-530 VWR	For creating tungsten wire tool and fine tip of mouth pipette.
Diamond scribe	Musco Sports Lighting	52865-005 VWR	For creating wide-mouthed Pasteur pipettes for collecting couples.
Forceps	Fine Science Tools	11050-10 Fine Science Tools	For holding anaesthetized females during removal of embryos from brood pouch. These do not have to be fine-tipped Dumont forceps - the tips can be at least as blunt as those of the forceps for which the catalogue number is listed here.
Fungizone-Amphotericin B	Sigma	A2942 Sigma Aldrich	Add to FASW to a final concentration of 1 mg/ml, and use this solution to culture blastomereablated embryos.
Glass capillaries 4 in long, 1/0.58 OD/ID (mm)	World Precision Instruments	1B100-4 World Precision Instruments	For making needles for puncturing the blastomere to be ablated.
Glass watchglass 300 mm diameter	Electron Microscopy Sciences	70543-30 Electron Microscopy Sciences	For use in removing embryos from the brood pouch of anesthetized females.
Instant Ocean Artificial Sea Water (ASW)	Instant Ocean	IS160 Aquatic EcoSystems	Make ASW by dissolving salt in deionized water to a salinity of 1.022-1.024.
Instant Ocean Filtered Artificial Sea Water (FASW)	Instant Ocean	IS160 Aquatic EcoSystems	Use 0.2 µm filters to sterilize ASW. Make up in small amounts (< 250 ml) as needed.
Kimwipes	Kimberly-Clark	21905-026 VWR	For safely breaking off the fine end of Pasteur pipettes modified for collecting couples.
Mouth pipette adaptor	Drummond Labware	A5177-5EA Sigma Aldrich	Insert the fine pulled Pasteur pipette tip into this end, to be used

			for removing extruded cell contents during the ablation procedure.
Mouth pipette tubing	Aldrich	Z280356 Sigma Aldrich	Cut this to be long enough to comfortably hang around your neck during the ablation procedure.
Needle Puller	Sutter Instrument Company	P97 Sutter Instrument Company	For making needles from glass capillaries for puncturing the blastomere to be ablated.
Penicillin-Streptomycin Solution	Mediatech	45000-650 VWR	Add to FASW to a final concentration of 100 units/ ml penicillin and 100 mg/ml streptomycin, and use this solution to culture blastomere-ablated embryos.
Rubber bulb	Electron Microscopy Sciences	100488-418 VWR	For using Pasteur pipettes to transfer embryos from dish to dish.
Sylgard 184	K. R. Anderson, Inc.	NC9659604 Fisher Scientific	Make up according to manufacturer's instructions. Pour a layer 2-5 mm thick into a 3cm petri dish to create a working surface for blastomere ablations.
Tungsten wire 0.004 inches diameter	A-M Systems	719000 A-M Systems	Use this to make a tool for removing embryos from the brood pouch of anaesthetized females.