2.6. *Parhyale* single cell ablation at 8-cell stages

In General there are several alternative methods to ablate cells. In these experiments two methods were performed, photoablation (2.6.1) and manual ablations (2.6.3), both of which are well established for *Parhyale* embryos.

2.6.1. Photoablation

This method of ablation via dye sentisized photo-oxidation requires the injection of a green fluochrome (e.g. Dextran green) followed by an exposure to blue light (λ=490nm) for 10-20 min.
The great advantage of this method is that the time point of ablation can be performed directly after injection, or at much later time points, targeting the cell progeny of the injected cell, given the signal of the fluochrome is strong enough to ensure the compete ablation of all cells.
Dextran-green is injected at a concentration of 0.5 mg/ml. The embryos get exposed at max fluorescence and 5x magnification for max. 20min.

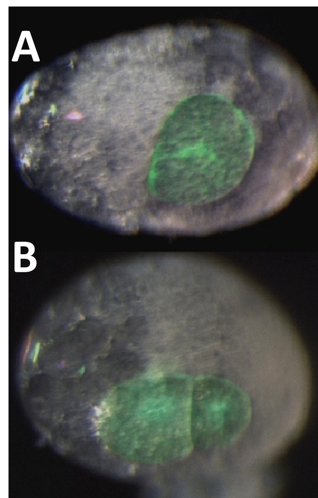
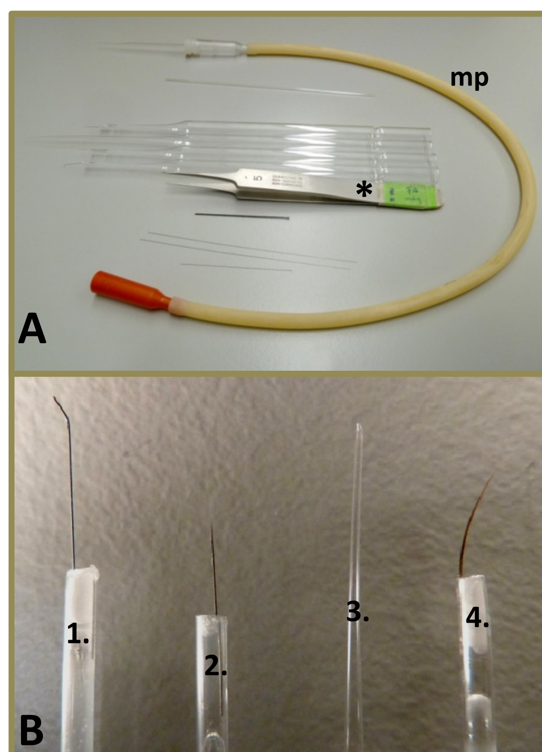


Fig. 6 Superimposed images of embryos at the germ disc stage. The green fuorescent areas are dead cells rests photoablated at the 8 cell stage. In **A** the cell did not undergo any further division, whereas in **B** the cell underwent one more division and then both daughter cells died.

The cells may undergo one more division and the will remain in the embryo as dead component. The rest of the embryo develops normally.

2.6.2. Helpful tools especially for manual ablations

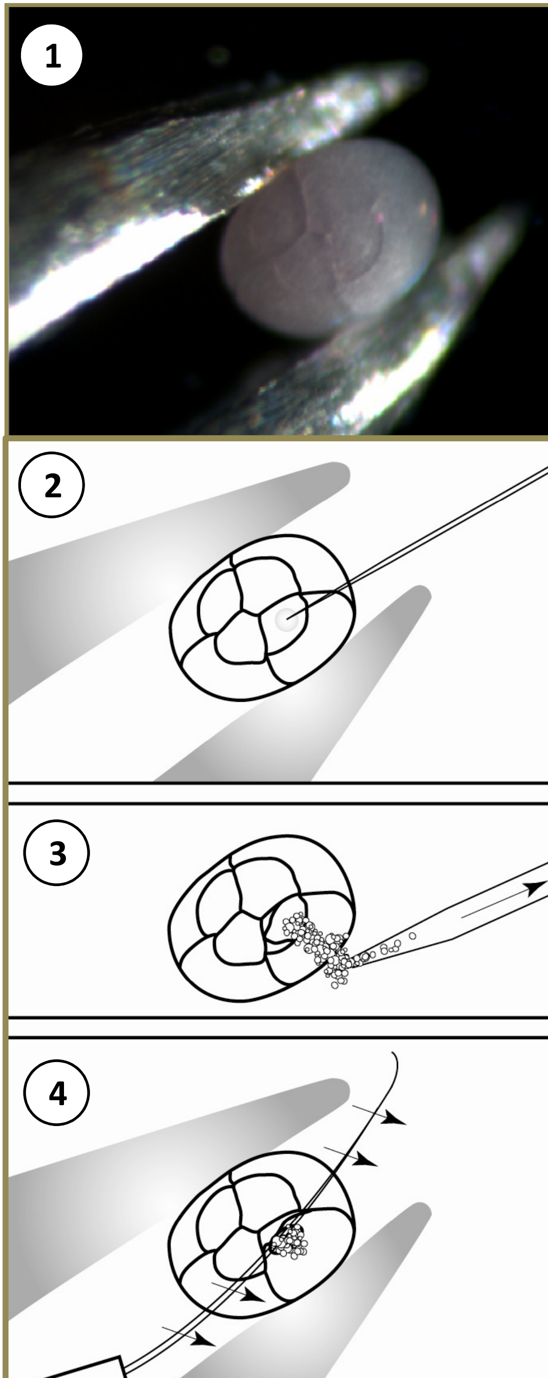


Mouth pipettes (Fig. 6A,mp): can be purchased and are very helpful for removal of fine cell contents. In one end of a rubber tube is the mouthpart, in the other end is a holder of a fine glass capillary, which addidionally can be drawn out over a flame to make it a very fine tool.
Forceps (Fig. 6A,*): should be as fine as possible, usually Dumont forceps are most useful.
Old injection needles: When pulling needles for injections it is recommended to not use the first few needles, because the needle puller might not have reached the optimal conditions. These needles are perfectly suitable for poking the initial hole during manual ablations.
Tungsten needles: glued into pasteur pipette tip can be either useful for opening the ventral brood pouch of females (bent as in Fig.6B,1.), or as sharp dissection tools or ablations (Fig.6B,2.). Sharpening by holding over a flame, until the tip is burned off and falls down (for other methods see also Rehm et al 2009).
Pasteur pipettes: the opening of the regular pipettes can be drawn out over a flame to that the opening gets finer.
Eye lash tool: eye lashes are glued into a pasteur pipette tip.

Fig. 7 Various tools that are useful for any micro dissections on small embryos.

2.6.3. Manual ablations of blastomeres

Ablating manually ensures that the respective cells with cell content and products are completely removed. This ablation method allows the removal of a micromere at a survival rate of around 60% until hatching. The removal of macromeres at the 8-cell stage, however, is less successful and photoablation might be more suitable for macromere removal.



In order to ablate a micromere at the 8-cell stage, transfere one or few embryos onto a Silgard dish in a droplet of FASW. Have a petidish with ddH2O and one with

1 Hold the embryo with forceps to that the micromeres are facing up and are accessible.

2 Poke the aimed blastomere with an injection needle (use those first pulled needles that usually are to be not used for injections). Adapt the osmotic pressure of the egg by changing the salinity (and therefore the osmotic pressure of the cells in the egg) by adding small amounts of ddH20.

3 After poking a hole, some cell content will flow out. Leave the eggs in the seawater for few minutes, adapt salinity if necessary. Remove the yolky cell content with a mouth pipette.

4 To completely remove the rest of the cell with an eyelash by carfully striping over the egg surface while holding the egg with the forceps and pressing the remaining cell content out through the hole.

Check carefully under high magnification whether the cell content is completely removed (view from different angles with light coming from various angles).

When cell is completely removed, transfere embryo into sterile petridish (small) with FASW + Antibiotica and Fundizide. Change FASW daily thereafter for ca. 5 days.

2.9. References and helpful literature/links

Parhyale embryology:

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• Zeng, V., Ewen-Campen, B., Alwes, F., Browne, W.E., Extavour, C.G., 2011. *De novo* assembly and characterization of a maternal and developmental transcriptome for the emerging model crustacean *Parhyale hawaiensis*. BMC Genomics 12(1): 581.

Parhyale transgenesis:

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More protocols, images and other information:

• http://patelweb.berkeley.edu/
• http://extavourlab.com/protocols/index.html

Amphipod development:

• Scholtz, G., Wolff, C., 2002. Cleavage, gastrulation, and germ disc formation of the amphipod *Orchestia cavimana* (Crustacea, Malacostraca, Peracarida). Contributions to Zoology 71, 9-28.
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