Targeted Laser deletion in embryos and larvae of *Capitella teleta*

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***Note**: This protocol was developed using a 20X XYClone infrared laser from Hamilton Thorne.*

- Turn on compound microscope with the XYClone laser (20X objective) and the associated PC laptop.
- Double click to open Hamilton-Thorne XYClone software.
- There should be a small red laser pointer (RED-i) that appears on the screen.
- In the settings panel on the right side of the screen:
    - set laser to "single"
    - set power to 100%
    - pulse is variable depending on what cell is being deleted: e.g., 270 us for a 1q micromere, between 500 to 1000 us for a macromere, 150 us for a 1q1 micromere, 300 us for a larval eye (direct deletion).

- Mount the animals onto a Rain-X coated slide
    - make sure the polar lobe has been reabsorbed completely (generally it's a good idea to wait 10-15 minutes after resorption before mounting)
    - mouth pipet embryos in a small drop of 0.2 um filtered sea water
    - use an eyelash brush to roll embryos to the correct orientation (e.g., animal side up)
    - it is o.k. to mount several embryos in one drop, usually no more than 5 or 6
    - gently lower the modified cover slip (see ModifiedCoverSlip.docx) onto the embryos
    - if doing direct eye deletion, mount st5 or early st6 larva in lateral view in MgCl2:FSW

- Laser deletion
    - identify the cell being targeted
    - focus up and down to get a sense for the depth of the cell and where its membrane is
    - if deleting a micromere, adjust stage so that the RED-i dot fits completely within the cell being targeted and focus to the most animal edge (the rest of the embryo will look slightly blurry)
    - if deleting a macromere, adjust so the middle of the RED-i dot is along the outermost edge of the cell membrane and the cell membrane is in focus
    - hit the foot pedal 1-3 times and a small "crater" should be visible (micromeres) or there will be a gap in the egg envelope/cell membrane (macromeres)
    - wait about 30 seconds – 1 minute to see if there is any yolk moving around in the targeted cell
    - the cell will generally start to leak cytoplasm in a little "bubble" that grows as more cytoplasm leaks. The bubble can be "popped" by focusing up to its surface and hitting the foot pedal a couple of times, but be careful not to accidentally hit another cell in the embryo.
    - if nothing happens, then try hitting the cell again, but be judicious about how many times!
    - (I usually hit a micromere three times quickly, check for cytoplasm movement, go to the next embryo in the FSW drop, hit the micromere three times quickly, etc. By the time I reach the last embryo in the drop, the first one is usually starting to form the "bubble")

- Following deletion
    - use NEW 35mm gelatin-coated dishes with 1X penicillin/streptomycin in 0.2 um FSW
    - carefully remove modified cover slip and pipet embryos into antibiotic seawater
    - check embryos a few minutes after and 30-45 minutes after the deletion to confirm that nearby cells are still developing normally
    - change out antibiotic FSW daily, twice daily if necessary

References

Pernet, B., Amiel, A. and Seaver, E. C. (2012). Effects of maternal investment on larvae and juveniles of the annelid *Capitella teleta* determined by experimental reduction of embryo energy content.**Invertebrate Biology** 131(2): 82-95

Amiel, A., Henry, J. Q. and Seaver, E. C. (2013). An organizing activity is required for head patterning and cell fate specification in the polychaete annelid *Capitella teleta*: new insights into cell-cell signaling in Lophotrochozoa. **Developmental Biology** 379: 107-122

Yamaguchi, E., and Seaver, E. C. (2013). The importance of larval eyes in the polychaete *Capitella teleta*: effects of eye deletion on formation of the adult eye. **Invertebrate Biology** 132 (4): 352-367