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Title of protocol: Microscopy techniques for *Pelagia noctiluca* embryos
Rationale and background: to develop a time series for *P. noctiluca* development, and image muscle formation

I visited the Observatoire Oceanographique de Villefranche Sur Mer from October to the end of the November, 2012. My intention was the study the development of the direct developing scyphozoan jellyfish *Pelagia noctiluca*, with a particular focus on establishing a time series and imaging muscle formation. I was motivated to visit France because there is a stable population of *P. noctiluca* that occur off the coast. Although I had difficulty collecting animals, I was able to collect enough embryos to generate a full developmental time course and collect data on muscle formation over time. I was able to characterize how muscle forms through development in *P. noctiluca*, and will be applying these techniques to sister species.

Handling of *P. noctiluca* embryos

This is not a technique so much as a series of guidelines that should be used when working with *P. noctiluca* embryos:

-- only non-stick low retention tips should be used when transferring embryos, multiple brands were used with similar rates of success. If regular tips are used, embryos will stick to the tip walls

-- embryos will stick to plastic petri dishes, this can be desirable for microinjection or to follow an embryo through early development. If this is not desired, embryos should only be placed in petri dishes that are coated with a 1% agarose and seawater solution, and allowed to solidify.

--embryos should be stored in non-stick tubes.

Time Lapse imaging of *P. noctiluca* embryos on an inverted scope

Material needed:
-- a small petri dish with a hole bore through the center, and a coverslip fastened over the hole
-- low melting temperature agarose
-- pipettes (mouth pipettes may be necessary)

1) prepare 0.2% solution of low temperature agarose, and sea water. Place in the dish

over the coverslip, let set until around 25 degrees celsius (not quite firm).

2) Under a scope, gently pipette embryos into the agarose solution, as close to the coverslip as possible. The embryos will be in their own liquid (in our case, seawater). Transfer as little liquid as possible, and remove as much of this liquid as quickly as possible by pipetting it back up.

3) allow to set on the benchtop, or in a cool room

4) gently add seawater to the dish, so that the embryos are embedded in agarose, with seawater around the agarose. Cover the dish and wrap it in parafilm. The embryos are now ready for time lapse imaging on an inverted scope.

Muscle imaging in *P. noctiluca* with Phalloidin staining

1) Fix embryos in a 0.2% glutaraldehyde, 4% paraformaldehyde, seawater solution for 2 hours.
2) Wash embryos in seawater once for 10 minutes, then begin a dilution series from seawater to PBT:

-- 25% PBT	75% Seawater	15 minutes rocking at room temperature
-- 50% PBT	50% Seawater	15 minutes rocking at room temperature
-- 75% PBT	25% Seawater	15 minutes rocking at room temperature

3) 2x 10 minutes with PBT
4) 2x 10 minutes with PBS
5) add 1 microliter Phalloidin stock solution to 100 microliters PBS and sample
6) Rock for 2 hours at room temperature in the dark (cover the tube with foil)
7) was 3x 15 minutes with PBS
(can stain with Dapi or Hoechst at this point)
8) mount and image with a confocal or fluorescent scope

<u>Phalloidin stock solution</u>
10 micromolar phalloidin in DMSO
<u>PBT</u>
1x phosphate buffered saline (PBS) with 0.1% TritonX