Embedding living larvae in low-melting agarose for imaging and recovery thereafter

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Abstract

This protocol describes how to use agarose with a low gelling point (27-30°C; often called low-melting agarose) to immobilize Aiptasia larvae for microscopy.
Larvae stay alive and in one place for prolonged periods of several hours but continue to mainly spin around the longitudal axis. After imaging, larvae can easily be retrieved from the agarose and transferred back to artificial sea water.

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Guidelines

A protocol for making the Valap for sealing the sample can be found under the following link:
http://cshprotocols.cshlp.org/content/2010/12/pdb.rec12380
Valap is basically a wax-like substance which has the advantages over other ways of sealing (for example nail-polish) that it is hydrophobic and doesn't contain any solvents which might mix with the aqueous sample and interfere with viability and fluorescence.

We are actually using a device with a small resistance wire loop to take a small amount of cold, solid Valap and then to melt it directly at the slide-mounted cover slip.

Before start

Preparation of the agarose:

1. **Weight 1 g low melting agarose into 50 ml ASW (2% w/v) in a small glass flask.**
Using higher concentration (up to 8%) makes the agarose harder to handle but doesn't improve the embedding notably (at least in my experience).
2. **Heat the mix without lid in a microwave to completely dissolve the agarose.**
3. **Transfer 1 ml of the agarose into a sterile microcentrifuge tube, pour the rest into a sterile 50 ml tube.**
4. **You can let the agarose cool down and solidify.**

Materials

✓ Microcentrifuge Tubes by Contributed by users

- Microscope slides by Contributed by users
- Standard square cover slips 18×18 mm by Contributed by users
- UltraPure™ Low Melting Point Agarose 16520100 by Invitrogen - Thermo Fisher
- modeling clay by Contributed by users
- Water bath or heat block at 37°C by Contributed by users
- Water bath or heatblock at 65°C by Contributed by users
- Micropipettes and tips by Contributed by users
- Stereomicroscope by Contributed by users
- Artificial seawater by Contributed by users

Protocol

Preparation

Step 1.

Before usage, melt the 1 ml agarose aliquot at 65°C in either a water bath or a heat block. This takes only a few minutes for the small aliquot.

Melting the agarose in the 50 ml tube takes a little longer. 2% agarose stock (so 1% end concentration) works best in regards to keeping the larvae in place and being easy to pipette and mix.
Higher concentrations (up to 8% for the stock) have no obvious benefit but make pipetting and mixing difficult.

Step 2.

Place the melted agarose at 37°C to keep it liquid and ready for use.

Step 3.
Label the microscope slide, prepare a coverslip with clay feet and place both next to a stereomicroscope.

Embedding the larvae

Step 4.
Under the stereomicroscope transfer your larvae in 5 to 10 µl ASW into a 1.5 ml microcentrifuge tube and close the lid.
10 µl should allow to take up to 10 larvae. Bigger volumes don't fit under standard 18×18 mm coverslips.

Step 5.
Adjust a second micropipette to double the volume you have your larvae in.

Step 6.
Place the microscope slide under the stereomicroscope and adjust focus and magnification to be able to later see the larvae on top.

Step 7.
Take the 37°C warm agarose and place it next to the stereomicroscope and your larvae.
The agarose will cool down to room temperature quickly, so you can directly proceed to the next step. However, if you are worried about heat stress you can use a 27°C heat block and wait a few minutes. ATTENTION: If you wait too long, the agarose will solidify at this temperature.

Step 8.
Add one volume of agarose to your larvae and immediately mix by gently pipetting up and down several times.

Step 9.
Take the second pipette and transfer the complete mix to the microscope slide and, while watching through the microscope, put the cover slip on top and press it a little bit down to obtain a roughly larval-thick layer of agarose.
CAUTION: Be careful to keep all larvae under the cover slip and not to squeeze them too much. Make also sure to apply the cover slip with the clay feet below, otherwise it will squash the larvae immediately!

Step 10.
Add some of the 2% agarose to the edges of the coverslip to fill the complete area under it.

Step 11.
Seal the edges of the coverslip airtight with hot Valap.

Step 12.
After sealing check the larvae a last time under the stereo microscope.

It helps if you mark their positions with dots next to them on the cover slip to find them easier with a compound microscope.

Step 13.
Take your images.
The larvae should be fine for at least 2-4 h.

Retrieving the larvae after imaging

Step 14.
Use another coverslip, razor blade or scalpel to remove the wax around the sample under the stereo microscope.

Step 15.
Use a scalpel blade to carefully and slowly lift the cover slip.
Doing this under a stereo microscope helps to follow the positions of the larvae.
CAUTION: As soon as you remove the cover slip the sample will dry out very fast.

Step 16.
Place the cover slip upside down on a clean surface.

Step 17.
Cover the cover slip as well as the sample area on the slide with ASW.

Step 18.
Locate and collect the larvae with a micropipette and transfer them back into their dish.
If some larvae are still embedded in the agarose, you can just wait until they free themselves or transfer the larvae together with the small piece of agarose.

Warnings

Don't eat it!