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Drosophila Egg Dechorionation

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Katy M Monteith[1]

[1]University of Edinburgh

Vale Lab

Katy M Monteith
University of Edinburgh

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We use this protocol and it's working

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Abstract

This protocol describes how to make an egg dechorionator using available lab equipment before describing how to successfully use the egg dechorionator to remove the outer chorion layer from a *Drosophila* egg.

Egg dechorionation allows cellular analysis, quantitative microinjection and the rearing of developing offspring in a gnotobiotic or axenic environment.

This protocol is written with the use of dechorionated eggs for rearing fruit flies in a gnotobiotic or axenic environment in mind. Therefore sterile techniques are described. For cellular analysis and/or quantitative microinjection the protocol can be followed but with a less stringent sterile procedure where unneccesary.

Guidelines

Gloves should be worn during all steps of the protocol.
Step 1 should be performed in a fume hood.
Step 7-onwards should be performed in a laminar air flow hood with sterile conditions. The flow hood and all equipment added to the hood must be cleaned thoroughly with 70% ethanol before commencing.

Following these guidelines will reduce the possible contamination of your eggs with any microorganisims.

Safety warnings

1. Extreme caution should be taken when using a soldering iron and supervision should be provided if neccesary. The iron can become extremely hot and should not make contact with any surface other than the item requiring melting.
2. Do not touch the melted plastic edges of the egg dechorionator until cooled and reset.

Making the egg dechorionator

1 In a fume hood set up a soldering iron and leave for a few mins to heat up. When ready, carefully melt the plastic around the circumference of a 50 mL falcon tube, no more than 2 inches from bottom (fig 1). Discard the bottom of tube. Remove the lid from tube and carefully melt the plastic around the circumference of the lid top until the centre of the lid pops out (fig 2). Discard the centre of the lid. Once cooled and the plastic edges reset, place a small square of gauze fabric around the top of the falcon tube and screw on the lid (fig 3). Your egg dechorionator is now ready to use.

Fig 1: 50 mL falcon tube with the bottom ~2 inches removed.

Fig 2: 50 mL falcon tube lid with the centre of the lid top removed.

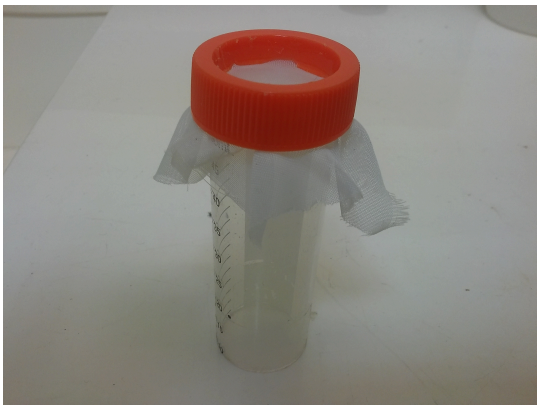

Fig 3: Ready to use egg dechorionator. Gauze fabric is screwed into the lid providing a mesh barrier to collect the eggs.

Preparing apple agar

2 Prepare apple agar petri dishes

Protocol

NAME

Recipe for apple-agar embryo collection plates

CREATED BY

Katy M Monteith

PREVIEW

2.1 Add 9 g of agar and 10 g sucrose to 300 mL distilled H_2O in a pan.

2.2 Mix well, place pan on heat and bring mixture to boil while stirring continuously.

2.3 Once boiling, turn off heat and add 100 mL apple juice to mixture.

2.4 Allow mixture to cool to ~70 °C and then stir in 2.1 mL of tegosept anti-fungal solution (dissolve Methyl-4- hydroxybenzoate in 95% Ethanol at 150g/L).

2.5 Dispense apple-agar into plates before it sets.

Setting up the egg cage

3 Set up egg cage with appropriate flies as described in the Drosophila embryo collection protocol. Carry out steps 1-4, so that your final step is removing any debris/dead flies from the petri dish using the soft tweezers.

Protocol

NAME

Drosophila embryo collection

CREATED BY

Katy M Monteith

PREVIEW

3.1 Place an apple-agar plate in the embryo collection cage to provide a substrate for egg laying. Prior to adding the plate, smear a small quantity of yeast paste (dry yeast mixed with water to a peanut-butter like consistency) onto the centre of the apple-agar. The addition of yeast will encourage egg laying. Water soaked absorbent cotton wool is also added to the cage to provide moisture.

3.2 Add fly population to cage and place cage at 25±1 °C in a 12 h:12h light:dark cycle for 24 hours.

3.3 Check the surface of the apple-agar plate for eggs. If If there are too few eggs, flies may require a longer habituation period. Replace with a fresh apple-agar plate, re-soak the cotton wool and leave the fly population in cage for a further 24 hours.

3.4 Take egg laden apple-agar plate from the cage and remove remaining yeast paste and any dead flies from the agar surface.

3.5 Submerge the apple-agar plate in 1x PBS and gently dislodge eggs from the agar surface with a fine paintbrush– most eggs will be found on the outer edge of the agar. While suspended in PBS, transfer the eggs to a falcon tube and leave for 5 min, allowing the eggs to sink to the bottom of solution.

3.6 Cut the bottom 4mm off a p1000 filtered pipette tip, and use this to draw 1 mL of 'eggy solution', taken from the bottom of the falcon tube. Transfer this to a 1.5 mL microcentrifuge tube and allow to settle. When drawing up solution a more consistent number of eggs is achieved by snap-releasing the pipette plunger rather than by gentle-release.

3.7 Cut the bottom 4 mm off a p20 filtered pipette tip. Set the pipette to a desired volume and draw this from the bottom of the microcentrifuge tube, again using the snap-release pipetting method. With practice a consistent number of eggs can be obtained, a volume of 5µL will produce 102±4 eggs.

Note

See 'egg squirt graph' in abstract

3.8 Dispense collected eggs onto food source and leave to develop until required for experiments.

Egg dechorionation

4 Pour triple distilled water into the petri dish until the agar is fully submerged. Carefully use the paintbrush to dislodge the eggs from the agar surface

5 Once all the eggs are dislodged, pour the water/egg solution from the petri dish into the egg dechorionator placed in an empty beaker- allowing the eggs to collect on the gauze and the water to flow through into the beaker

6 Repeat steps 4 & 5 if eggs still remain on the agar surface

7 Place 2× 250ml beakers (one containing ~90 mL of 7-10% bleach and one containing ~90 mL of triple distilled water), hard tweezers, paintbrush, container of 7-10% bleach, container of triple distilled water, timer and premade autoclaved food tubes into a laminar flow hood- see guidelines.

8 Using the hard tweezers, pick up the egg dechorionator and place immediately into the beaker containing bleach within the flow hood.

9 Gently and periodically move the egg dechorionator up and down in the bleach for 2min 30sec using the hard tweezers, ensuring the eggs are submerged in the bleach.

10 Discard the bleach, add another 90 mL of 7-10% bleach to beaker and repeat step 9.

11 Move the egg dechorionator from the bleach directly into the beaker containing triple distilled water, rinse in the distilled water for ~30 seconds by gently and periodically moving up and down. Discard the water and repeat a further two times until the eggs have been completely rinsed of bleach solution.

12 The eggs should now be dechorionated and ready to use.
If rearing the resulting offspring, immediately move the eggs from dechorionator to autoclaved food vials/bottles using a paintbrush. Alternatively, if density controlling is required, re-suspend the eggs in triple distilled water in a falcon tube and squirt into food vials/bottles following steps 6-8 of the Drosophila embryo collection protocol (see step 3.6-3.8 above). For both options, perform within a laminar flow hood with **sterile** equipment.

Note

It is possible to check if the eggs have been successfully dechorionated by inspection under a microscope prior to adding to food media. Eggs will look like smooth prolonged ovoids lacking the characteristic dorsal appendages.