Schistocephalus solidus culturing

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Abstract

This protocol describes how the parasitic flatworms (S. solidus) were cultured in the lab for:

Hebert, F, O; Grambauer, S; Barber, I; Landry, C, R; Aubin-Horth, N (2016): Reference transcriptome sequence resource for the study of the Cestode Schistocephalus solidus, a threespine stickleback parasite. GigaScience Database. http://dx.doi.org/10.5524/100197

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https://www.protocols.io/view/Schistocephalus-solidus-culturing-ew8bfhw
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Guidelines

Equipment required:

- Culture Tube - Tube culture screw cap borosilicate glass phenolic cap with PTFE liner use up to 121°C 26mm x 200mm Pyrex from Fisher product code 12349339
- Dialysis Membrane - 30m SIZE 1 DIALYSIS TUBING 12-14000 DALT from Medicell product code DVT.12000.01
- RPMI Media – RPMI-1640 Medium , With L-glutamine, without sodium bicarbonate, powder from Sigma product code R6504-10X1L
- Horse Serum -Horse Serum Heat inactivated sterile-filtered from Sigma product code H1138-6X500ML
- Penicillium - HyClone; Pen/Strep/Glutamine from Fisher product code 12340243

Before culturing worms:

- Culture tubes need to be set up with membrane and autoclaved and RPMI media made up and autoclaved.
- Cut a piece of dialysis membrane twice the length of the tube. Make into a U shape to fit inside the tube (do not fold the membrane as the crease can affect the set up) leaving 1-2 cm folded over the top on each side and screw the lid on. Wrap in foil and autoclave.
- Fresh RPMI media should be prepared each time you culture worms. Weigh out the appropriate quantity of powder (10.4g/L) and make up to the required volume in a 400ml Duran bottle with ddH20 and autoclave

Before start

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Protocol

Parasite collection

Step 1.
For each culture tube fill half way up with horse serum and then fill up with RPMI – leaving about 2 cm gap from the top. Add 500µl of penicillium

NOTES

GigaScience Database 28 Apr 2016
• Horse serum and Penicillium are kept in -20 and need to be defrosted. Use the 40°C water bath to defrost/warm the horse serum and autoclaved RPMI, the autoclaved culture tubes can warm in the 37°C incubator in the lab. Leave the aliquots of penicillium at RT°C
• If dissecting several fish at once keep the worms on a petri dish with a few drops of RPMI on them so they don't dry out before setting up the culture tube. Worms over 50mg should be used for the culture – any smaller and it is unlikely to produce any eggs.

GigaScience Database 28 Apr 2016
On the day of fish dissection/worm culturing switch on the shaking water bath, this should be set at 40°C and 120rpm.

Parasite collection

Step 2.
With a pair of blunt forceps gently open the top of dialysis membrane and place the worm into the membrane.

NOTES

GigaScience Database 28 Apr 2016
This can be tricky as you do not want to open up the membrane too much as the pressure is required for the worm to develop. If you hold the worm between your finger and them at both ends and gently squeeze you can feel the way the worm is moving so which end to put into the membrane first. Try to squeeze the worm down toward the bottom of the membrane – a bit like squeezing a toothpaste tube!

Parasite collection

Step 3.
Screw the top on to the tube – and loosen slightly and place into the shaking 40°C water bath

Parasite collection

Step 4.
Check the worms each day – the worms may start to shed eggs after day 5 so can be collected after day 6 – if not collect eggs on day 7 – if the worms are left longer they may dissolve and the eggs will be difficult to collect cleanly

Egg collection

Step 5.
Remove the dialysis membrane from the culture tube and cut it open above any visible eggs, flush

out onto a petri dish using ddH20. Fill the petri dish to about ¼ full of ddH20

Egg collection

Step 6.
Under the stereo microscope with cold light source tilt the plate and the eggs should line up on the surface.

Egg collection

Step 7.
Collect the egg layer with a glass pipette and transfer to fresh petri dishes – up to 4 depending on the number of eggs.

Egg collection

Step 8.
Fill all the plates up to about half full with ddH20 including the original petri dish. Label each plate with an identification number/name

Egg collection

Step 9.
Wrap each plate individually in foil and label the outside with the same identification plus your initials.

Egg collection

Step 10.
Place the wrapped plates in the 20°C incubator and leave in there for at least 21 days before checking for hatching