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We use this protocol and it's working

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Schistosoma mansoni cercariae transformation (without needle) V.2

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Schistosoma mansoni

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ABSTRACT

Free-living aquatic *S. mansoni* cercariae transform into the first intramammalian stage, called schistosomula or somules, by burrowing in the host skin. Upon contact, cercariae begin to enter the skin and lose their tails, becoming schistosomula. Somules migrate through the epidermis to the dermis to find a small venule or lymphatic vessel to enter the vasculature.

Transformation of cercariae to schistosomula can be mimicked in the laboratory by centrifuging cercariae to remove tails and then culturing the somules in somule media. This method is particularly useful when the number of cercariae is low (i.e. clonal cercariae from an individual snail).

Somules can be cultured for several weeks with regular media changes.

IMAGE ATTRIBUTION

Images of somules taken by Dr. Gabriel Rinaldi

GUIDELINES

Media changes and opening of transformed somules to take place in tissue culture hood using sterile techniques

MATERIALS

- DMEM high glucose GlutaMAX **Gibco - Thermo Fischer Catalog #31966021**
- Lactalbumin Hydrolysate, powder (extra soluble) **Thermo Fisher Catalog #11800042**
- Hypoxanthine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H9636-1G**
- Serotonin Hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H9523-25MG**

- Insulin solution from bovine pancreas **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I0516-5ML**
- 33'5-Triiodo-L-thyronine sodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T6397**
- MEM Vitamin Solution (100×) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M6895**
- Schneiders Insect Medium **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S0146**
- HEPES solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H0887**
- Fetal Bovine Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F4135**
- Antibiotic-Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062**
- Dulbecco's Phosphate Buffered Saline 10X **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D1283**
- MilliQ water **Contributed by users**
- Sterile Graduated Transfer Pipets **Fisher Scientific Catalog #13479108**
- Falcon 15 mL Conical Centrifuge Tubes **Fisher Scientific Catalog #1077350**
- Nun Non-Treated 6-well plate **Thermo Scientific Catalog #10396482**
- 1000 mL Vacuum Filter/Storage Bottle System 0.22 µm Pore 54.5cm² PES Membrane Sterile 12 **Corning Catalog #431098**

Lamp or other light source
Chilling benchtop centrifuge with 15ml swing bucket rotor
Incubator at 37°C and 5% CO_2

Reciprocating water bath

Class 2 Microbiological Safety Cabinet

1X DPBS + 2% ANTI-ANTI

50ml 10x DPBS
10ml 100x Antibiotic-Antimycotic (-20°C)
Fill to 500ml in vacuum filter unit with MilliQ water
Sterilise media with vacuum filter unit and store at 4°C. Use within 2 weeks.

SOMULE MEDIUM – BASCH MODIFIED MEDIUM (BMM)

1. Mix the following reagents:

	A	B	C	D	E	F
	Reagent	1L	500ml	250ml	[working]	Storage
	1x DMEM high glucose	810.5 ml	405.25 ml	202.625 ml	1x	4°C

	A	B	C	D	E	F
	1g/L Lactalbumin hydrolysate	1g	0.5g	0.25g	1g/L	4°C
	1mM Hypoxanthine	500µl	250µl	125µl	0.5µM	-20°C
	1mM Serotonin	1ml	500µl	250µl	1µM	-20°C
	Insulin	1ml	500µl	250µl	8 µg/ml	4°C
	1mM Hydrocortisone	1ml	500µl	250µl	1 µM	-20°C
	0.2mM Triiodo-L-thyronine	1ml	500µl	250µl	0.2 µM	-20°C
	100x MEM Vitamins	5ml	2.5ml	1.25ml	1x	4°C
	1x Schneider's medium	50ml	25ml	12.5ml	5%	4°C
	1M HEPES	10ml	5ml	2.5ml	10mM	4°C
	1x Fetal calf serum inactivated	100ml	50ml	25ml	10%	-20°C
	100x PSF (add just before use)	20ml	10ml	5ml	2%	-20°C

2. Sterilise media with vacuum filter unit and store at 4°C. Use within 2 weeks.

SEROTONIN STOCK 80mM (17mg/ml)

25mg serotonin hydrochloride (Sigma H9523-25MG) (4°C)

1.47ml H2O

Vortex well and store at -20°C in 350µl aliquots

1mM SEROTONIN

312.5µl of 80mM stock solution

24.6875ml NFW

Filter sterilize and store at -20°C in 1ml aliquots

3,3'5-TRIIODO-L-THYRONINE STOCK 10mM

100mg T3 (Sigma T6397-100MG)

14.86ml 0.2N NaOH

Vortex well and store at -20°C in 2ml aliquots

0.2mM 3,3',5-TRIIODO-L-THYRONINE

5ml of 10mM stock solution

20ml NFW

Filter sterilize and store at -20°C in 1ml aliquots

HYPOXANTHINE STOCK 368mM (50mg/ml)

1g hypoxanthine (Sigma H9636-1G)

20ml 2:1 formic acid:H2O
Vortex well and store at -20°C in 1ml aliquots

1mM HYPOXANTHINE
34µl of 368mM stock solution
12.466ml sterile medium
Filter sterilize and store at -20°C in 500µl aliquots

HYDROCORTISONE STOCK 2.75mM (50µg/ml)
1mg hydrocortisone (H0135-1MG) (RT)
1ml absolute ethanol
Gently swirl to dissolve
19ml sterile medium
Swirl to mix and store in 9.5ml aliquots at -20°C

1mM HYDROCORTISONE
9.058ml of 2.76mM stock solution
15.942ml sterile medium
Filter sterilize and store at -20°C in 1ml aliquots

SAFETY WARNINGS

Cercariae are infectious to humans. Please use proper PPE at all times, including a lab coat, waterproof over-gown, long-cuff gloves AQL <=1.5, and face shield.

- All disposable materials should be placed in biohazardous waste bins
- Glassware should be immersed in a klorsept solution of at least 50ppm for at least 2 hours, rinsed with diH_2O and then autoclaved
- Any liquids should be treated with klorsept solution of at least 50ppm for at least 2 hours
- Liquids treated with klorsept should be diluted further and disposed in the drain

Schistosomula in suspension are not a risk to humans UNLESS they are injected directly into the blood stream.

BEFORE START INSTRUCTIONS

Place 1 sterile 15ml falcon tube on ice per snail to be shed
Pre-chill benchtop centrifuge to 4°C
Prepare 1x DPBS+2% anti-anti and place in 37°C reciprocating water bath (see recipes in "MATERIALS" section)
Prepare somule media and place in 37°C reciprocating water bath (see recipes in "MATERIALS" section)

Cercariae collection

1 Shed cercariae from snails in a 6-well plate (see protocol "*Schistosoma mansoni* cercariae shedding"). The snails can be shed for up to 2 hrs by collecting cercariae and replacing with fresh water every 30 min

2 Using a sterile transfer pipette, dispense cercariae into sterile 15ml falcon tubes on ice

Note

IMPORTANT. All the following steps are carried out in the tissue culture biosafety cabinet. Keep a beaker containing 70% ethanol in the cabinet and before discarding any aspirating pipette or serological pipette aspirate ethanol to kill any contaminating cercariae in the pipette.

3 After collecting cercariae, adjust the volume in each 15ml falcon tube to 15ml with sterile water

4 Incubate tubes containing cercariae for 00:30:00 On ice 30m

Cercariae tail removal

5 Centrifuge the cercariae 2200 rpm, 4°C, 00:30:00 , Eppendorf 5810R centrifuge 30m

6 Quickly remove the supernatant and resuspend the pellet in 10ml pre-warmed 1x PBS + 2% PSF (see "MATERIALS" section for recipes) by gently inverting the tube (do not use pipet to mix)

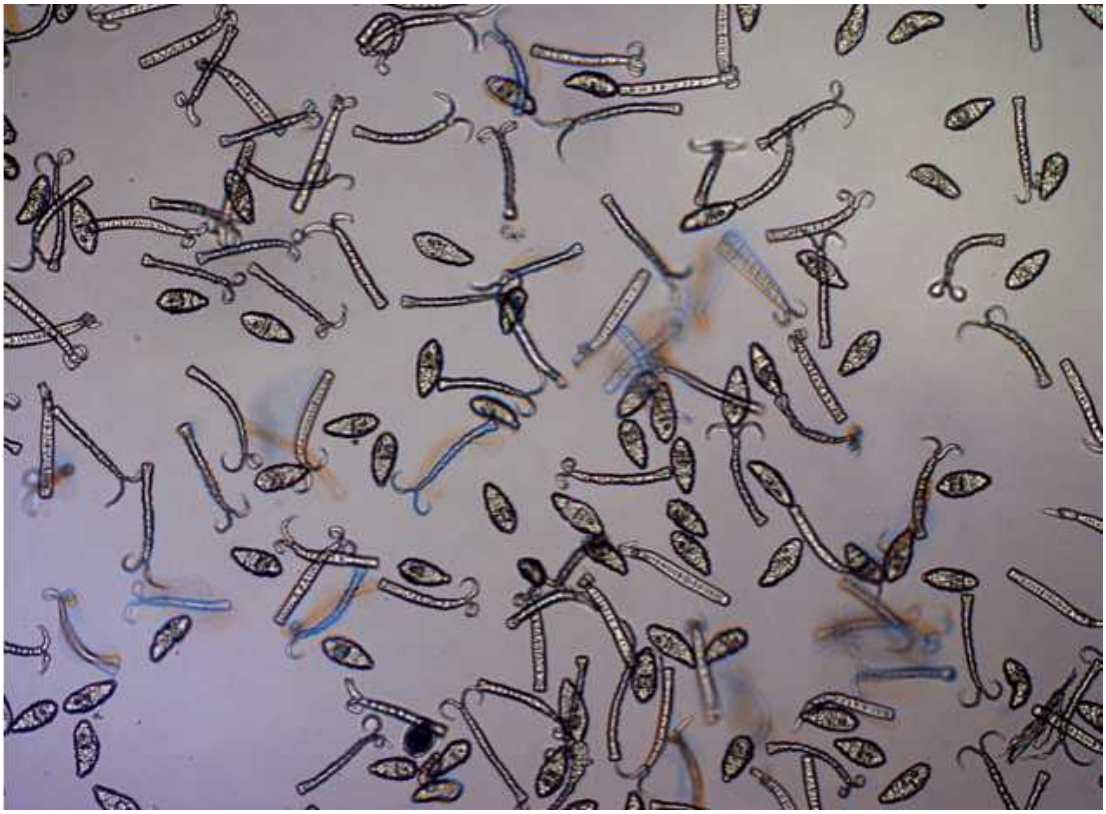
7 Centrifuge the cercariae 1500 rpm, 4°C, 00:10:00 , Eppendorf 5810R centrifuge 10m

8 Repeat steps 6 and 7 six more times

Somules in culture 30m

9 Resuspend pellet of somules in ~5ml pre-warmed 37 °C somule media (see "MATERIALS" section for recipes)

10 Place somules in a 6-well plate and top up each well with pre-warmed 37 °C somule media so each has a total of approximately 4ml media



Somules on same day of transformation (Image credit: Dr. Gabriel Rinaldi)

11 Incubate at 37 °C, 5% CO_2 Overnight 30m

12 Optional: the following day, remove tails with transfer pipet (the tails will have floated to the top of the water column in the wells)

Somules the day after transformation, after removing tails (Image credit: Dr. Gabriel Rinaldi)