Oct 25, 2022

Schistosoma mansoni cercariae sexing

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzby12vx1/v1

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Schistosoma mansoni

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Protocol Citation: Sarah Buddenborg, Matt Berriman 2022. Schistosoma mansoni cercariae sexing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzby12vx1/v1

Manuscript citation:
in preparation

Protocol status: Working
We use this protocol and it's working

Created: October 24, 2022

Last Modified: October 25, 2022

Protocol Integer ID: 71750

Keywords: dna extraction method for schistosoma, pcr primers for the w1 repeat, schistosoma mansoni, dna extraction method, dna extraction, schistosoma, bp w1 repeat, w1 repeat, pcr primer, genome, dna, w1a, schistosoma mansoni cercariae

Funders Acknowledgements:
Wellcome Trust
Grant ID: 098051
Wellcome Trust
Grant ID: 206194

Abstract

This DNA extraction method for *Schistosoma mansoni* cercariae is based on the HOTSHOT method https://health.uconn.edu/mouse-genome-modification/protocols/hotshot-method-of-dna-preparation/. DNA isolation is followed by PCR amplification of the "W1" female W chromosome repetitive region. The 476 bp W1 repeat was identified by Webster, Mansour, and Bieber (1989). PCR primers for the W1 repeat were designed by Gasser, Morahan, and Mitchell (1991). Existing primers for the *S. mansoni* actin gene are used as a positive control (Delcroix et al, 2006).

W1a - 5' CAA CAC AGT GAA ATT CTT CC 3' (positions 10-29)
W1b- 5' GAA TTC ACC ACT CGA CAT TC 3' (positions 463-482)

Citation

Philippa Webster, Tag E. Mansour, David Bieber (1989)
. Isolation of a female-specific, highly repeated Schistosoma mansoni DNA probe and its use in an assay of cercarial sex.
Molecular and Biochemical Parasitology.

10.1016/0166-6851(89)90169-2 LINK

Citation

Robin B. Gasser, Grant Morahan, Graham F. Mitchell (1991)
. Sexing single larval stages of Schistosoma mansoni by polymerase chain reaction.
Molecular and Biochemical Parasitology.

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Citation

Delcroix M, Sajid M, Caffrey CR, Lim KC, Dvorák J, Hsieh I, Bahgat M, Dissous C, McKerrow JH (2006)
. A multienzyme network functions in intestinal protein digestion by a platyhelminth parasite.
The Journal of biological chemistry.

Materials

Custom DNA oligos ordered from IDT - LabReady (Normalized to 100µM in IDTE pH 8.0)**:**
W1a - 5′ CAA CAC AGT GAA ATT CTT CC 3′
W1b - 5′ GAA TTC ACC ACT CGA CAT TC 3′

SmAct-F - 5′ CAG TGT TCC CTT CCA TCG TT 3′
SmAct-R - 5′ GGA CAG GGT GTT CTT CTG GA 3′

2x Alkaline Lysis Reagent

25ml H_2O
125µl 10N NaOH
20µl 0.5M disodium EDTA

Make fresh every 1-2 months
Store at Room temperature

1M Tris-HCl

6.057g Tris-HCl
To 50ml with nuclease-free H_2O

Store at Room temperature

2x Neutralizing Reagent

23ml H_2O
2ml 1M Tris-HCl

Store at Room temperature

MilliQ water
Costar® 6-well Clear TC-treated Multiple Well Plates Individually Wrapped Sterile **Corning** **Catalog #**3516
Featherweight forceps **BioQuip** **Catalog #**4750
Protein LoBind® 5.0 mL with snap cap **Eppendorf** **Catalog #**0030108302
Protein LoBind® 15 mL conical tube **Eppendorf** **Catalog #**0030122216
pluriStrainer Mini 100 µm **pluriSelect Life Science** **Catalog #**43-10100-40
3ml Sterile Graduated Transfer Pipets individually wrapped **Fisher Scientific** **Catalog #**13469108
Platinum II Taq Hot-Start DNA Polymerase (Invitrogen) **Thermo Fisher Scientific** **Catalog #**14966001
Sodium Hydroxide solution 10N **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**SX0607N
Ethylenediaminetetraacetic acid disodium salt solution BioUltra for molecular biology pH 8.0 ~0. **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**03690

Tris hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**10812846001

Nuclease-free Water **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W4502-1L

Equipment

Centrifuge 5810 R	NAME
Refrigerated centrifuge	TYPE
Eppendorf	BRAND
5811000065	SKU
https://www.eppendorf.com/gb-en/eShop-Products/Centrifugation/Multipurpose-Centrifuges/Centrifuge-5810-5810R-p-PF-240994	LINK

Equipment

ThermoMixer	NAME
Benchtop Incubator	TYPE
Eppendorf	BRAND
5382000023	SKU
https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html	LINK
Any heat block will suffice	SPECIFICATIONS

Equipment

Mastercycler X50	NAME
thermocycler	TYPE
Eppendorf	BRAND
6311000010	SKU
https://www.eppendorf.com/pt-en/eShop-Products/PCR/Thermocyclers/Mastercycler-X50-p-PF-217186	LINK
any thermocycler will be sufficient	SPECIFICATIONS

Safety warnings

Safety information

Biomphalaria spp. snails with a patent *Schistosoma mansoni* infection are classed as CL2/BSL2 Cercariae from shedding snails are infectious and proper PPE should be worn at all times

Before start

- Pre-heat heat block to 95 °C
- Pre-cool centrifuge to 4 °C
- Fill an ice bucket with ice

Cercariae Collection

1h

1 While holding with wide-tip featherweight forceps, rinse patent *Biomphalaria glabrata* snails, individually, with MilliQ water

Note

- Rinsing snails individually prevents cross-contamination of cercariae from another snail
- Snails should be at least 5 weeks post infection with a single miracidium

2 Place rinsed snails into individual wells of 6- or 12-well plates

3 Add 3-4ml of MilliQ water to each well

4 Induce shedding of cercariae by place snails under a direct light for up to 2 hours or until cercariae are visible in well with a naked eye

2h

4.1 After 2 hrs, remove snails that are not shedding cercariae and place into a new tank with food. These snails can be re-shed in one week. Some monomiracidium-infected snails will not begin shedding until up to 8 weeks after exposure. At this time, if they are still not shedding cercariae, it is safe to assume they were not infected and can be disposed of properly.

4.2 For snails that are shedding low amounts of cercariae (<100), place into a new tank with food and re-shed in one week

5 Put an "X" on the wells of the snails that are shedding and uniquely number each. This numbering must be preserved throughout.

6 Prepare numbered 5ml Eppendorf tubes each fitted with a 100µm pluriStrainer Mini

7 Using a new sterile transfer pipet for each snail, pipette water containing the cercariae through the pluriStrainer

7.1 Rinse the well and snails with fresh MilliQ water and collect this water as above to fill each 5ml tube to the top

Note

If you do not have time to complete the DNA isolation, PCR, and gel electrophoresis in a single day, snails can be kept in 6-well plates with a fresh water change daily. Be sure to maintain the numbering of the snails.

8 Discard pluriStrainers, close, and submerge 5ml tubes containing cercariae into ice for 00:30:00 30m

9 Centrifuge tubes at 2000 rpm, 4°C, 00:30:00 30m

10 After centrifugation, place tubes immediately back on ice and aspirate/pipette off liquid, taking care not to disrupt the cercariae pellet. Try to leave no more than 50µl of water on the cercariae pellet as the more dilute the lysate is, the more difficult amplification will be.

Cercariae and Cell Lysis

11 Add 1x volume of 2x Alkaline Lysis Reagent to each cercariae pellet and mix well (i.e. add 25µl 2x Alkaline Lysis Reagent to 25µl of cercariae in MilliQ)

Note

2x Alkaline Lysis Reagent
25ml H_2O
125µl 10N NaOH
20µl 0.5M disodium EDTA
Make fresh every 1-2 months
Store at Room temperature

12 Incubate at 95 °C for ~1 hr or until cercariae dissolve, mixing at least every 15 min. 1h
Centrifuge using a mini-centrifuge if needed

13 After tissue is dissolved, place on ice to cool 15m

Lysate Neutralization

14 Add 1x volume of 2x Neutralizing Reagent and briefly vortex to mix (i.e. add 50µl 2x Neutralizing Reagent to 50µl of lysate)

Note

1M Tris-HCl
6.057g Tris-HCl
To 50ml with nuclease-free H_2O
Store at Room temperature

2x Neutralizing Reagent
23ml H_2O
2ml 1M Tris-HCl
Store at Room temperature

PCR amplification of sex-specific region

15 Prepare two PCR master mixes on ice (for actin control and W1 primer sets) each with enough for your samples, 1 female positive control, 1 male negative control, and 1 water blank control (i.e. if you have 8 samples, you will need 11 reactions for actin primers and 11 reactions for W primers)

Note

W1a - 5′ CAA CAC AGT GAA ATT CTT CC 3′
W1b - 5′ GAA TTC ACC ACT CGA CAT TC 3′

SmAct-F - 5′ CAG TGT TCC CTT CCA TCG TT 3′
SmAct-R - 5′ GGA CAG GGT GTT CTT CTG GA 3′

ACTIN primers	
Sample 1	Female
Sample 2	Male
Sample 3	Water
Sample 4	
Sample 5	
Sample 6	
Sample 7	
Sample 8	

W1 primers	
Sample 1	Female
Sample 2	Male
Sample 3	Water
Sample 4	
Sample 5	
Sample 6	
Sample 7	
Sample 8	

Mock-up of layout for 8 samples and their controls in four 8-well PCR strips

	A	B	C
	Reagent	**Final concentration**	**Volume per reaction**
	Nuclease-free water	-	6.32µl
	Platinum II Taq	0.04U/µl	0.08µl
	5x Platinum II Buffer	1x	2µl
	10mM dNTP	0.2mM each	0.2µl
	10uM F	0.2uM	0.2µl
	10uM R	0.2uM	0.2µl
	DNA*	-	1µl
			10µl reaction

*If you think your DNA concentration is really low/high, you can measure first on Nanodrop (blank with Neutralization Buffer) and change input volume as necessary. Keep in mind that using more than 10% of the overall volume may inhibit the PCR reaction.

16 Run the PCR program as follows for both primer sets: 30m

	A	B	C	D
	Initial denaturation	94° C	2 min	
	Denature	98° C	5 sec	
	Anneal and extend	60° C	15 sec	To step 2 x25
	Hold	4°C	~	

Analyze PCR Amplicons 1h

17 Run a 1% agarose gel to analyze 5-10µl of your PCR product. Males will have PCR product band for actin only; Females will have PCR product band for actin and W1. 1h

Citations

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