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We use this protocol and it's working

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Genetic Characterization of Schistosomes

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ABSTRACT

Schistosomiasis is a parasitic disease transmitted through water by blood-fluke trematodes of the genus Schistosoma. After malaria and soil-transmitted helminthiasis, it is the third most important parasitic tropical disease. An estimated 236 million people are infected, with another 700 million at risk of infection. It is estimated that 90% of all schistosomiasis cases occur in Africa. The disease is regarded as a public health threat, with numerous debilitating effects on growth, well-being, and overall health. The importance of snail-intermediate hosts in schistosomiasis transmission cannot be overstated, and thus monitoring the prevalence and distribution of Schistosoma cercariae is critical for indirect estimation of schistosomiasis in human or animal populations. As a result, developing sensitive tools to aid in the characterization of specific schistosomes is critical, and this protocol outlines procedures ranging from DNA isolation to PCR and the expected outcomes from newly developed oligonucleotides.

GUIDELINES

Cercaria Suspension Pre-Treatment
Schistosome Vector (Snail) Pre-Treatment
Nucleic Acid Isolation
Preparation of PCR Master Mix and Reaction
Thermal Cycling Conditions
Agarose Gel Electrophoresis
Expected Results

Keywords: Schistosomes, Cercariae, PCR, Vector, Snails, Xenomonitoring

MATERIALS

Consumables

PCR tubes (0.2 ml)
1.5 ml microcentrifuge tube
Filtered tips 100 - 1000µL
Filtered tips 20 - 200µL
Filtered tips 2 - 20µL
Filtered tips 0.5 - 10µL
Autoclavable glass bottles (500 mL)

Reagents

OneTaq® 2X Master Mix
Nuclease-free water
Polyvinylpolypyrrolidone (PVPP)
Proteinase K (20 ng/mL)
Agarose Powder
TBE Buffer (1 X)
TE Buffer (1X)
6X Loading Dye
Ethidium Bromide
Molecular Weight Marker (50 bp and 100 bp)
Zymo Quick DNA Miniprep Kit (Zymo Research, USA)

Equipment and Materials

Automatic micropipettes
Thermocycler (Eppendorff)
Microcentrifuge (Eppendorff)
MagNa Lyser
Vortex Mixer
Microwave
Gel Electrophoresis tank and power supply system
Ultraviolet Light Transilluminator

SAFETY WARNINGS

1. β-mercaptoethanol has a pungent smell, it should be opened in a well-aerated space.
2. Handle all solutions as potential biohazard materials
3. Care should be taken during the electrophoresis process as it involves the use of electricity.

BEFORE START INSTRUCTIONS

Aliquot the required volume of reagents for extraction into a sterile tube for the assay.
Allow the frozen reagents to thaw completely at 4°C before use and avoid centrifuging to thaw.

Reagent Preparation for Nucleic Acid Isolation
Genomic Lysis Buffer
Add 500µL of β-mercaptoethanol to 100 mL of Genomic Lysis Buffer. Shake slightly to mix

2% PVPP in 1 X TE Buffer
Dissolve 2 g of PVPP powder in 100 mL of 1X TE Buffer

Cercariae Suspension Pre-Treatment

1 Centrifuge the cercariae suspension at 1600 rpm for 00:05:00 to concentrate the cercaria 5m

2 Pipette off the supernatant leaving 200 µL of cercariae suspension for nucleic acid isolation.

Schistosome Vector (Snail) Pre-Treatment

3 Crush each snail in 200 µL of 1XTE Buffer using a pestle in a 1.5 mL microcentrifuge tube. Ensure the end mixture is as fine as possible.

Nucleic Acid Isolation

36m

4 Transfer 200 µL of the pre-treated sample (cercariae suspension or snail) into a sterile 1.5 mL microcentrifuge tube.

5 Add 200 µL of [M] 2 Mass / % volume PVPP in 1XPBS and vortex the sample with glass beads at 3000 rpm for 00:01:00 1m

6 Add 400 µL of Genomic Lysis Buffer and 10 µL of Proteinase K ([M] 20 ng/mL) to the sample.

7 Vortex briefly and incubate the sample at 56 °C for 3 - 5 hours or Overnight 20m

8 Vortex the sample at 3000 rpm for 00:00:30 and centrifuge at 10000 rpm for 00:01:00 1m 30s

9 Transfer the supernatant into a **Zymo-Spin IIC** column in a new collection tube.

10 Centrifuge at 10000 rpm for 00:01:00 . Discard the flow-through liquid. 1m

11 Transfer the **Zymo-Spin IIC** column into a new collection tube

12 Add 200 µL of **DNA Pre-Wash Buffer** to the spin column and centrifuge at 10000 rpm for 00:01:00 . 1m

13 Add 500 µL of **gDNA Wash Buffer** to the spin column and centrifuge at 10000 rpm for 00:01:00 1m

14 Transfer the **Zymo-Spin IIC** column into the sterile 1.5 mL microcentrifuge tube.

15 Add 100 µL of **DNA Elution Buffer**to the spin column and incubate for 00:30:00 30m

16 Centrifuge at 13000 rpm for 00:00:30 to elute the DNA. 30s

17 Store the DNA at -20 °C pending further analysis.

Preparation of PCR Master Mix and Reaction

18 Prepare the master mix for each schistosome species separately following the protocol:

	A	B	C
	Concentration	Reagent	1X Volume (µL)
	2X	One Taq Mastermix	5.0
	10 µM	Forward Primer	2.0

	A	B	C
	10 µM	Reverse Primer	2.0
	NA	Nuclease-Free Water	1.6
		Template DNA	3.0
		Total Reaction Volume	10.0

	A	B	C	D	E
	Schistosome species	Forward Primer	Reverse Primer	Fragment Size	Primer Tm
	ITS - Schistosome sp.	TCT TGA CCG GGG TAC CTA	ATT AAG CCA CGA CTC GAG CAC	691 bp	60.1°C
	S. mansoni	GAG GGG TCT GGT TTT GGT GT	GCA GAT AAA GCC ACC CCT GT	659 bp	58.7°C
	S. haematobium	TTG AGC CTAT GGG TGG TGG T	ACC AGT AAC ACC ACC TAT CGT	410 bp	58.7°C
	S. bovis	TGG GCA TCC TGA GGT GTA T	CAC AGG ATC AGA CAA ACG AGT ACC	301 bp	55.6°C
	S. haematobium/S. bovis hybrid	CCT CCA TTA TCT ATA TCT GAG AAT TCT	CGA AGT CTT AAA ATC CAC ACA ACT	141 bp	55.6°C

Thermal Cycling Conditions

19

	A	B	C	D
	Step	Temperature	Time	Cycles
	Initial Denaturation	95°C		NA
	Denaturation	95°C	45 seconds	40
	Annealing	Tm	45 seconds	
	Extension	72°C	45 seconds	
	Final Extension	72°C	5 minutes	NA

Tm: Refer to the primer list for the individual annealing temperatures of the primer pairs
NA: Not applicable

Agarose Gel Electrophoresis

20m

20 To prepare [M] 1.5 Mass / % volume agarose solution, weigh 1.5 g of agarose into a glass beaker containing 100 mL 1XTBE Buffer

21 Microwave the solution until the agarose completely dissolves and forms a clear solution.

22 Allow the solution to cool at Room temperature to about 50 °C.

23 Add 5 µL of [M] 10 mg/mL Ethidium Bromide to the agarose solution. Swirl to ensure complete mixing of the stain with the agarose solution.

24 Pour the stained agarose molten solution into a casting tray (5mm deep) fitted with combs of the desired size for a well. Allow the molten solution to solidify for about 00:20:00 at Room temperature 20m

25 Gently remove the combs from the solidified agarose gel and move the casting tray into the electrophoresis tank.

Note

Ensure the agarose gel is submerged in the 1XTBE Buffer in the electrophoresis tank

26 Load 5 µL to 12 µL the Sample into each well (mix 1µL of 6X Loading with 5µL of amplicon).

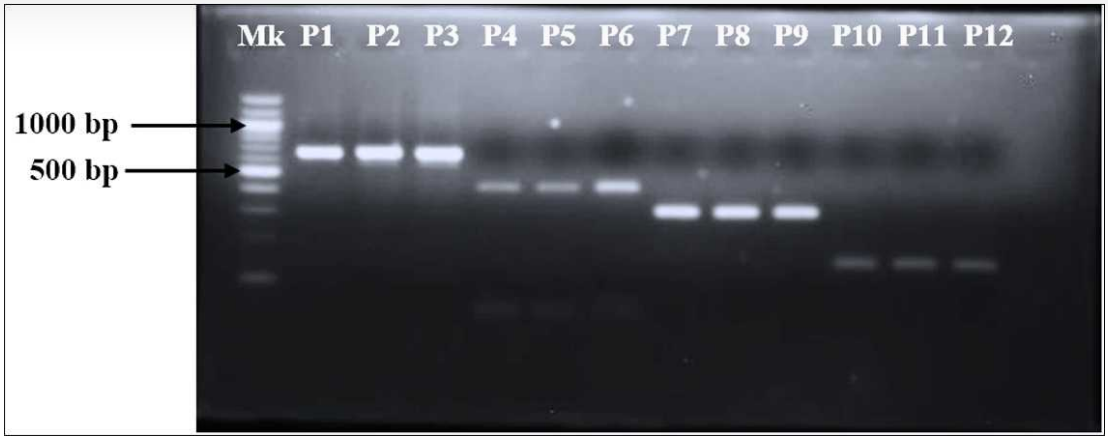
27 Load 3 µL of 100 or 50 bp (depending on the fragment size of the amplicon of interest) Molecular Weight Marker into the first well which will serve as a reference.

28 Electrophorese the amplicons at 100 volts until the molecular weight marker has travelled two-thirds of the length of the agarose gel.

29 Visualize the agarose gel under UV light using the transilluminator.

Expected Results

30



Mk: 100 bp molecular weight marker
P1 – P3: *S. mansoni* (659 bp);
P4 – P6: *S. haematobium* (410 bp);
P7 – P9: *S. bovis* (301 bp);
P10 – P12: *S. haematobium/S. bovis* (141 bp)