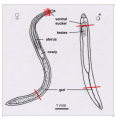

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Manual dissection of the *Schistosoma mansoni* and *S. japonicum* head and back end for transcriptomic analysis

In 1 collection

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ABSTRACT

Schistosomes are intravenous parasites with ability to survive in the mammalian host for decades, using its blood as a source of nutrients. The feeding process is multistep and takes place along the worm's alimentary tract, which comprises an (i) oral cavity opening to a short (ii) esophagus that is connected to the (iii) the gut caecum. The ultrastructureal morphology of *Schistosoma mansoni* and *S. japonicum* has revealed the existence of two secretory cell masses surrounding the esophagus tube, referred to as the anterior and posterior esophageal glands (antESO and postESO, respectively). We recently established that the esophageal glands have a pivotal role in the first steps of blood processing. For instance, erythrocytes and leucocytes are quickly processed along the esophagus before they are propelled to the lower parts of the intestines for further digestion nutrient uptake. We propose that incorrect functioning of alimentary tract is associated with worms death by starvation. This was first observed in the self-cure response of Rhesus macaque (*Macaca mulatta*), one of few known vertebrate hosts capable of combating the disease through worm elimination once the infection is stablished. Classical immunoproteomics (2D-PAGE and Western blotting) has revealed potential targets in both exposed tegument and secreted gut proteins. Recently, a more detailed investigation using *S. japonicum* in the Rhesus model shed light on the possible operating mechanisms that prevent parasite feeding on blood. Ultrastructural studies and immunocytochemistry on surviving worms indicated the esophageal lumen and the gland secretions as the primary targets of a potent and protective humoral immune response that ultimately disrupts the esophageal functions. Therefore, the molecular characterisation of the esophageal gland constituents is imperative if one intends to emulate the Rhesus self-cure response for therapeutic purposes. However, this is not a trivial task as challenges are multiple. Perhaps, the most important caveat is that both anterior and posterior parts of the oesophageal gland represent a minor fraction of the whole parasite body (or even of its head), meaning that whole worm analyses are not sufficient for identification of genuine gland products. We tackled these challenges by developing a dissection technique on worms preserved in RNAlater solution aided by an essential set of scissors and tweezers that delivers adequate precision during the procedure. The method herein described is compatible with downstream Next Generation Sequencing. This methodology can be applied in the molecular characterisation of other schistosome organs and tissues that present a well-defined anatomic location.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

The Schistosome Esophagus Is a 'Hotspot' for Microexon and Lysosomal Hydrolase Gene Expression: Implications for Blood Processing and Microexon gene transcriptional profiles and evolution provide insights into blood processing by the Schistosoma japonicum esophagus

GUIDELINES

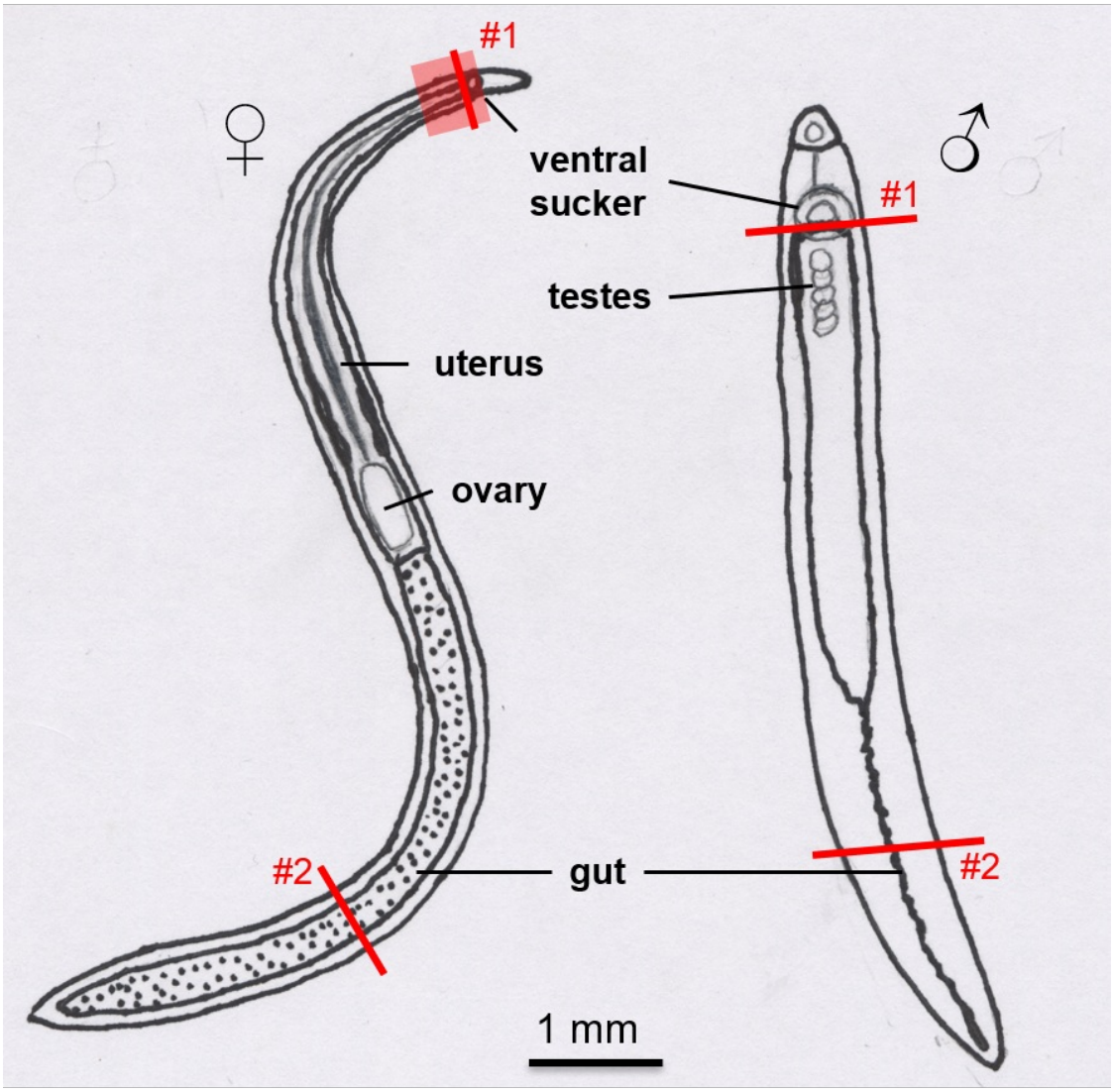
This protocol will guide you through the dissection of the head and back end of adult schistosome worms (males or females). Although the recovery of adult worms by portal perfusion of infected animals is obviously indispensable this is not addressed in this protocol. Nevertheless, worm handling and fixation are utterly important and should be performed according to the following instructions:

- Perfuse 45-days infected animals using RPMI-1640 medium (without phenol red) buffered with 10mM HEPES, pH 7.4, containing 4 UI/mL of heparin.
- Wash the parasites in the same medium (pre-warmed at 37°C) until total removal of blood and tissue debris.
  OBS.: always use pre-warmed medium to prevent getting worms too curled up as this makes the dissection procedure difficult.
- Carefully transfer the parasites, by pouring them with plenty of pre-warmed medium, into a Corning 50 mL conical tube.
- Discard the medium and add 5-6 volumes of RNAlater for instantaneous worm fixation.
- Keep the worms at 4°C until dissection.

- CRITICAL POINT *Parasites respond to the* ex vivo *environment; thus it is mandatory to avoid a long waiting between the perfusion and fixation in RNAlater. The total procedure time (perfusion, washings and fixation) must not exceed 10 min.*

Procedure overview

Once the parasites are fixed you are able to perform the dissection. In summary, the isolation of the schistosome head (HE) is achieved by cut along the line of the transverse gut. The posterior third of the parasite (BE) is also dissected and constitute a second sample for comparative analysis. This protocol will simultaneously illustrate the entire process in male and female worms however we strongly recommend to perform the dissection of different sexes separately.



Cut #1 detaches the whole worms' head

Cut #2 detaches the back end

Obs.: The shaded area in the female indicates the range of the excision point due to the small size of the female head region.

- **CRITICAL POINT** *The procedure must be entirely performed in ice-cold conditions. Keep HE and BE fragments on ice during the dissection, then store them in the fridge.*

ALTERNATIVE METHOD APPLICATION: Once the set of stereomicroscope, scissors and tweezers are available, different incision points can be used for obtaining alternative body sections. It is important to consider that the precision decreases proportionally to the fragment size (e.g. the female head is too small so that it is difficult to cut exactly in the transverse gut). In our lab we have successfully dissected the whole head, esophagus, back end, ovary and ootype on either *S. mansoni* or *S. japonicum* male and female worms. Check the protocols available at our collection.

MATERIALS

NAME	CATALOG #	VENDOR
RNAlater	R0901-100ML	Sigma-aldrich

SAFETY WARNINGS

Adult schistosomes are not an infective stage thus biological hazard is not evident. Nevertheless, follow the safety instructions of all chemicals used (*e.g.* RNAlater, heparin). Always wear gloves and lab coat.

Place the Petri dish with worms on the microscope

1 Take the Petri dish out of the ice bath, touch it against a paper to remove the excess of water, place it under the stereomicroscope and adjust the focus.

Stereo microscope EZ4, Leica 10447197
Leica EZ4 educational stereomicroscope.

Start a 10 min countdown

2 This will help you to control how long the Petri dish is out of the ice.

Zoom in at the head of a worm

3 Look for a parasite with uncurled head/esophagus. Increase the lenses magnification and use your dissecting tools (tweezers and scissors) to keep the head in the field-of-view.

Biology Tweezer 115 mm, Ideal Tak 7SG.CX.0
Biology Tweezer 115 mm (Ideal Tak Chiasso, Switzerland). Manufacturer Part Number: 7SG.CX.0

Vannas scissors, John Weiss 0103123
Vannas scissors (John Weiss, Milton Keynes, UK). Straight with sharp tips.

Make the first incision at the line of the transverse gut

4 Hold the parasite carefully with the tweezers, positioning it in an angle that allows you to clearly see the suckers (*i.e.* lateral view, or suckers facing up), then make the first incision (#1) along the transverse gut (*i.e.* where the dark pigmentation of hemozoin starts; indicated by the red arrow in the illustration below).

Collect the HE fragment using a P20 micropipette

5 Once the HE fragment is detached it floats around. Aspirate it with the RNAlater solution using the P20 micropipette then dispense it inside the HE microtube. Always keep the tube on ice.

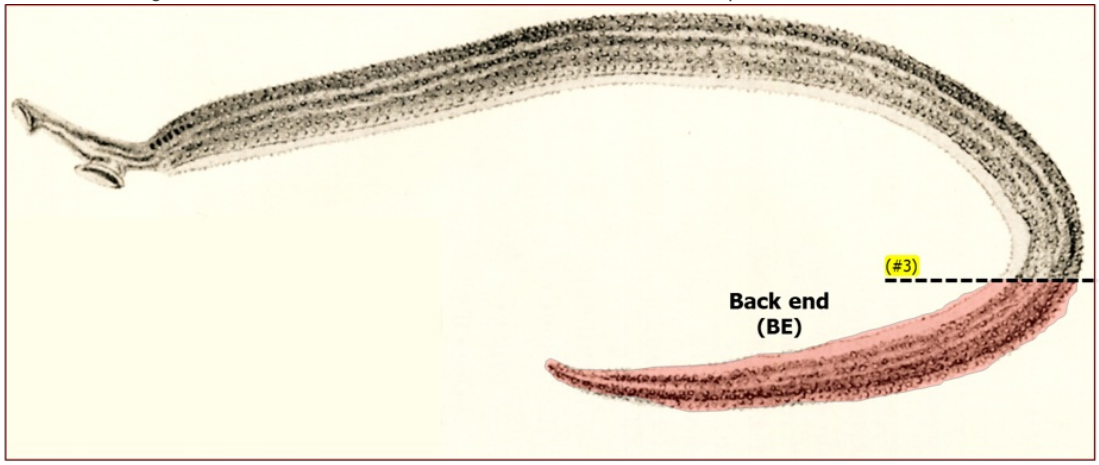
CRITICAL STEP *do not aspirate any other fragment or particulate material with the HE.*

Record your progress in the notepad

6

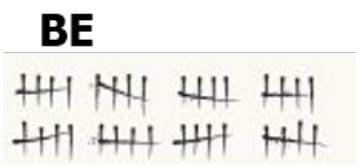
Cut off the back end

7 The final cut (#2) is considerably easier and usually does not require high magnification. Simply cut the posterior third of the parasite body. Collect the BE fragment with the tweezers, transfer it to the BE microtube and keep in the ice box.



Record your progress in the notepad

8



Find another parasite and repeat steps 3 to 9

9 If needed, you can immerse the tweezers and scissors in the beaker with deionized water to get rid of salt crystals. Dry your tools in a soft and lint-free paper before continuing the dissection.

When the 10 minutes timer runs out place the Petri dish back in the ice

10 In Step 2 you started a 10 minutes countdown. You can dissect as many worms as you can in this time, but it is important to chill the Petri dish every 10 minutes to preserve your material.

ATTENTION *This is an opportunity to transfer the dissected worms to the "dissected body" microtube then add new ones to the Petri dish for the next round. In addition, if you notice that salt crystals are accumulating in the RNAlater solution discard it, wash the Petri dish with deionized water, dry it with lint-free paper and add fresh RNAlater solution.*

PAUSE POINT *If you wish, store the dissected fragments in the fridge and continue the procedure another time.*

Start a new round of dissection

11 Restart from Step 1.