In vitro miracidia to sporocyst transformation protocol

for *Schistosoma mansoni*

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Warning:

Miracidia to sporocyst transformation is very sensitive. For a successful transformation, we need to avoid contamination by bacteria and/or fungi. All the materials need to be sterilized and all the work needs to be done in a sterilized place.

Do not forget to book the cell cultures safety cabinet.

Materials:

- 2 months old infested mice
- Sterilized mortar and pestle
- 1 sterilized trough
- 4 sterilized sieves (400, 200, 120 and 80µm)
- 2 Sterilized Corex
- 12 wells plate
- Sterilized scissors
- Sterilized Pasteur pipette
- 10mL sterilized pipette
- NaCl
- Filtered water
- Ice
- CBSS medium composition (with Penicilin-Streptomycin Sigma P4458-20mL 50x) for 1L H_2O (Ultrapure water):
  $MgSO_4$-$7H_2O$: 0.45g
  Na_2HPo_4 : 0.07g
  KCl : 0.15g
  $NaHCO_3$: 0.05g
  NaCl : 2.8g
  $CaCl_2$: 0.4g

Method:

1. Euthanize mice with CO_2

2. Recover the livers (do not take intestine, their bacteria could contaminate the *in vitro* transformation) and put in a sterilized mortar with NaCl.

3. Cut them into small pieces with sterilized scissors and crush them with a sterilized pestle.

3. Put the milling in the sieves and rinse it with NaCl.

4. At the last sieve (80µm), rinse with filtered water and recover the eggs in a sterilized trough.

5. Expose the eggs to the light at 26°C and wait for 1 hour. The miracidia will hatch gradually.

6. In a dark room, put the trough in a binocular optical and put a small light near the side of the trough. The light attracts the miracidia and it will be easy to collect them with pasteur pipette.

7. Put the miracidia into a Corex tube which is in the ice. They will fall down the Corex.

8. With the Pasteur pipette, discard carefully the water and do not touch the white sediment (miracidia). The Corex tube needs to stay in the ice.

9. Go to the cell cultures safety cabinet with the 12 wells plate and the Corex tube in the ice.

10. Transfer the miracidia in the well with a 10mL sterilized pipette and add CBSS medium. Close the plate and do not forget to write your name, the date, the strain (BRE, VEN, GH2, …).

11. Leave the plate in cell cultures safety cabinet at 26°C and in the dark during 24h.

12. Observe with the binocular microscope if they lost their cilia (do not open the plate, risk of contamination).