Detwiler Lab Chelex extraction protocol 6/22/2016

Need
Thermocycler
96-well PCR plates for a large number of extractions or 8-strip tubes
8-strip tube caps to prevent evaporation
Chelex 100 beads
ultra-pure sterile water (MilliQ) for chelex solution
Proteinase K
50 mL conical with magnetic stir bar

Method
1) Prepare and set-up beakers with 10% bleach and MilliQ water. Also need a beaker for waste.

2) Wash and rinse all tools to be used in extraction (forceps, dishes, depression slides).

3) Clean area and microscope base with 70% ethanol.

4) Use pipette and forceps to rinse 70% EtOH from tissue (do twice), and then add to PCR plate/tubes. Verify with microscope that tissue is in the well. Cap off each column or set of strip tubes to prevent placing specimens into same wells. KEEP ON ICE or IN FROZEN TUBE HOLDER.

5a) If using small amounts of tissue like cercariae or rediae, may want to add tissue into wells with 2 ul of water, and then add chelex solution. This order helps you verify visually that the tissue has been added to the wells as the beads can obscure little bits of tissue.

5b) If using more easily seen tissue like a 1 mm^2 piece of adult worm tissue, then can add chelex solution to tubes first, and then the adult tissue. Still check visually to verify that tissue ended up in the wells/strip tubes.

6) Prepare a 5% chelex solution in a 50 ml conical tube

a) 200 ul extraction for 95 samples plus a chelex neg [typically used for adult worm and rediae]
- Obtain a new 50 mL conical tube.
- Weigh out and add 1.25 chelex beads to tube.
- Add 25 mL of extraction water (MilliQ in 1000 mL beaker) to tube.
- Add 250 ul ProK to tube (stock at 20mg/ml) to mixture [1ul (or 2 ul) ProK per 200 ul 5% chelex approximate final concentration of ProK ~ 0.1mg/ml (or 0.2mg/ml)].
- Transfer magnetic stir bar from old tube of chelex to new tube.

b) 100 ul extractions for less than 95 samples plus a chelex neg [typically used for cercariae]
- Obtain a new 50 mL conical tube.
- Weigh out and add 0.75 chelex beads to tube.
- Add 15 mL of extraction water (MilliQ in 1000 mL beaker) to tube.
- Add 150 ul ProK to tube.
- Transfer magnetic stir bar from old tube of chelex to new tube.

Note: if doing only a few samples (less than ½ a plate), then add ProK to each tube. 1% = 2 ul in a 200 ul extractions, or 1 ul in 100 ul of chelex solution per sample)

7) Place conical tube with chelex solution on stir plate and keep solution stirring while aliquoting to 96-well plate/strip tubes.
Use P200 tips with end of tip cut off to make sure chelex beads are added.

Aliquot enough to ensure that you have a final volume of 200 or 100 ul of solution to each well. The exact amount to add will depend upon what was done in steps 5a or 5b.

8) Once plate is loaded place in Thermocycler, incubate samples at 56 C for 2 hours with a final 4 C forever in case you cannot get back in time. However, best to take out at right when finished incubating.

9) Vortex for 30 seconds and place back in Thermocycler to boil extractions at 100 C for 8 minutes with a final 4 C forever in case you cannot get back in time

10) Let it cool to 40 C before taking out and vortex for 30 sec while still warm.

11) Spin plate down to get moisture off lids and store at –20 C until ready for PCR.

Ready for PCR
Use anywhere from 1 to 10 ul in of extraction supernatant in PCR reactions (25 or 50 ul PCR rxns)