D. magna / P. ramosa co-extraction (Day 1)

Introduction

This is day 1 of a 2 day protocol for co-extraction of D. magna and P. ramosa DNA from a single infected D. magna.

Materials

- (n) Daphnia samples
- (n) 1.5 mL Eppendorf tubes
  - Label each with the sample number and "S". The letter "S" indicates that this is the tube which will contain the un-lysed P. ramosa spores at the end of the procedure. DO NOT DISCARD.
- (n) clean pestles
- MilliQ water (room temperature)
- Qiagen lysis buffer (room temperature)
- Proteinase K (on ice)

Procedure

Ethanol removal from preserved sample, cell lysis

1. Transfer daphnia to labled 1.5 mL tube. Pipette off any transferred ethanol remaining on preserved sample.
2. Wash sample by adding 1 mL milliQ water, inverting 3 times, waiting 1 minute, and then pipetting off water. Daphnia should remain wet, but not sitting in water.
3. Add 100 uL cell lysis solution to daphnia and grind 50 times with mortar
4. Add 200 uL cell lysis solution (300 uL total) to sample and vortex for 2-3 seconds
5. Add 1.5 uL proteinase K, invert 25 times, and incubate at 55 °C overnight with moderate shaking (~800 rpm).