D. magna / P. ramosa co-extraction (Day 2)

Introduction

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

Materials

- (n) overnight digested Daphnia samples
- (n) 1.5 mL Eppendorf tubes
  - Label each with the sample number and "W". The letter "W" indicates that this is the tube which will contain the waste ethanol and isopropanol. If DNA content is suspiciously low, a second DNA precipitation can be attempted with this waste. Otherwise, these tubes can be discarded after checking samples on the Qubit.
- (n) 1.5 mL Eppendorf tubes
  - Label each with the sample number and "D". The letter "D" indicates that this is the tube which will contain the DNA.
- RNAse A (on ice)
- Protein precipitation solution (room temperature)
- Isopropanol (100% - chilled to -20 °C)
- MilliQ water (room temperature)
- Ethanol (70% - room temperature)
- Glycogen (on ice)

Procedure

DNA purification, pasteuria re-quantification

1. Add 1.5 uL RNase A, invert 25 times, incubate at 37 °C for 30 minutes with gentle shaking (300 RPM)
2. Remove samples from shaker and cool by incubating for 1 minute on ice
3. Add 100 uL of protein precipitation solution and vortex for 20s at high speed
4. Incubate sample for 5 minutes on ice
5. Centrifuge for 5 minutes at 16,000 RCF in chilled (4 °C) rotor
6. Pipette 400 uL (1:1 ratio) of chilled (-20 °C isopropanol) into a clean 1.5 mL labled ("D") tube and pour supernatant into this tube. **Retain both tubes. DNA is in the supernatant – Pasteuria spores are in the pellet.**
7. Add 2.0 uL glycogen to each sample and mix by inverting 25 times.
8. Incubate Daphnia sample at -20 °C for 1 hour

9. **While Daphnia samples are incubating**, Add 1 mL MQ water to Pasteuria spores, vortex for 5 – 10 seconds, and place in the freezer for storage at -20 °C

10. Centrifuge sample for 15 minutes at maximum speed using chilled centrifuge

11. Discard the supernatant by pouring into a numbered "W" labled tube (KEEP the pellet)

12. Add 300 uL of room temperature 70% ethanol and wash the pellet by inverting several times

13. Centrifuge for 5 minutes at 16,000 rcf at room temperature

14. Discard the supernatant (into "W" tube) (KEEP the pellet), invert the tubes over a paper towel and allow to air dry for 5 - 10 minutes

15. Add 100 uL DNA hydration solution and vortex for 5 seconds at medium speed

16. Incubate at 65 C for 1 hour

17. Incubate at room temperature (15 – 25 C) overnight with gentle shaking(300 RPM).