PEG PCR Clean Up Protocol 18/08/2022

**Description:**

Clean PCR products for downstream use (e.g. preparing library for MinIon or Sanger sequencing). Test comparing NEB clean up kit fungal ITS (n=7) and PEG on nematode 18s products (n=8):

Measurement ng/ul 260/230 260/280

NEB (n=7) 21.7 +/- 3.6 3.02 +/- 0.38 1.73 +/- 0.03

PEG (n=8) 41.5 +/- 5.6 4.41 +/- 1.7 1.79 +/- 0.02

**Reagents (20 ul rxns X 48) (x24)**

1. 20% PEG, 15% NaCl (store @ 4C) (can mix in 1.5mL tube)
   1. 240 mg PEG 120 mg PEG (tare 1.5mL tube and add)
   2. 180 mg NaCl 90 mg NaCl (as above)
   3. 1200 uL H2O (ultra pure, DNAse free) 600 uL H2O
2. 80% ETOH (10 mL) in falcon tube
   1. 2 mL H2O (ultra pure, DNAse free) 1 mL H2O
   2. 8 mL ETOH 4 mL ETOH
3. 95% ETOH (10 mL) in falcon tube
   1. 500 uL H2O (ultra pure, DNAse free) 250 uL H2O
   2. 9.5 mL ETOH 4.75 mL ETOH or
4. Ultrapure DNAse free H2O (25 uL/ rxn) – 1200 mL 600 uL H2O

**Things**

1. Pipette tips (20-200ul)
2. Centrifuge tubes (1.5mL)

**Protocol**

1. Prepare reagents and place 80% (and 95%) ETOH on ice (or keep in -80 freezer)
2. In a 1.5mL Eppendorf centrifuge tube, mix PCR product (~20-25uL) and 25 uL PEG by pipetting
3. Incubate in heat block @ 37C (15 min)
4. Centrifuge @ top speed > 20K X G (15 min)
5. Remove supernatant
6. Clean with 80% ETOH: Add 150 uL cold 80%ETOH to each tube and centrifuge @ top speed > 20K X G (10 min)
7. Remove supernatant
8. Clean with 95% ETOH as above (see step 6) and remove supernatant
9. Dry with caps of tubes open in flow hood ~45 min
10. Warm H2O to 30C in heat block
11. Resuspend in ultrapure DNAse free H2O (25 uL)