**Mycology Molecular Barcoding Prep sheet**

**Supplies needed (quantities vary on size of class/number of samples)**

Dry Goods:

100ml media bottles

50ml falcon tubes or similar

1.5-2ml sterile Eppendorf tubes

Small beakers of toothpicks

8 strip PCR tubes

Various sizes of sterile filter-tipped pipette tips

Gloves in assorted sizes

Sharpies

Strips of parafilm

Chemicals/Solutions:

Items for Extraction Solution (ES)

1M Tris solution (pH=8.0)

KCl

EDTA

1M NaOH

For the Dilution Solution

3% BSA (Bovine Serum Albumin) Sigma A7030, 10g or similar

Other

ExTaq+buffer (Hot Start Taq DNA Polymerase, New England Biolabs #M04955 or similar)

Molecular Grade Water (Nuclease Free Water, New England Biolabs #B1500S or similar)

dNTPS (Deoxynucleotide (dNTP) Solution Mix, New England Biolabs #N04475 or similar)

Primer 1 (Forward: ITS1-F: CTTGGTCATTTAGAGGAAGTAA, IDT or similar)

Primer 2 (Reverse: ITS4: TCCTCCGCTTATTGATATGC, IDT or similar)

Dye (eg. etBr or Syber Green) and 1 kb ladder

Loading dye

Positive and negative controls

2% Agarose gel

1x TAE solution

Other Supplies:

Assorted pipettors

Racks for Eppendorf tubes and PCR tubes

Freezer storage boxes for Eppendorf tubes

Ice buckets

Gel Rigs, combs, trays, buffer, and gel reader

Vortex

Thermocycler

Biohazard/waste bags and containers

**Before preparing recipes:**

Autoclave 100ml media bottles for Extraction & Dilution Solutions

Autoclave small beakers of toothpicks

UV sterilize 50ml tubes for 30 minutes

**Recipes:**

Extraction Solution (ES) make into a sterile 100ml media bottle.

Stocks: 1 M Tris solution (pH=8.0)

1. add 10 ml of 1 M Tris stock into clean 100 ml vessel
2. add 1.86 g KCl
3. add 0.37 g EDTA
4. add 80 ml DI H2O and shake until solutes dissolve
5. titrate with 1 M NaOH to pH = ~ 9.5-10.0
6. top up to 100 ml with DI H2O
7. transfer some stock into smaller sterile container and filter sterilize into sterilized 2 ml Eppendorf tubes

Dilution Solution (BSA 3%) make into a sterile 100ml media bottle.

1. add 3 g of BSA (e.g. Sigma or Omni – 98-99% purity, heat shock fractionated) into clean vessel
2. top up to 100 ml with DI H2O
3. shake BSA into solution

4) transfer some stock into smaller sterile container and filter sterilize into sterilized 2 ml Eppendorf tubes

**Procedure - Tissue Kit (Abbreviated version)**

1. pipette out **Extraction Solution** (ES) into 8-strip tubes. For an ectomycorrhizal tip 20ul is sufficient. For larger samples use up to 100 ul.
2. Using sterile toothpicks (provide 4 beakers w/ 24+ toothpicks in each) place tissue sample (individual root, fruitbody tissue, pure culture isolate) sample into Extraction Solution. Submerge sample and smash sample if possible. Be sure not to add it any extra water or liquid (if picking roots, blot dry the forceps).
3. Incubate at room temp for 10+ minutes then incubate for 10 minutes at 95 C.
4. Add an equal volume of **Dilution Solution** (DS) (3% BSA) to the tubes so that the final Extraction:Dilution solution ratio is 1:1.
5. Samples are now ready for PCR. 1-2 ul is usually sufficient for PCR (from a 20ul ES + 20ul DS = 40ul volume extraction)
6. Store DNA extractions in freezer.

PCR

Make up Master Mix (MM) (recipe in with the Hot Start Taq), aliquot 120 ul into each 1.5 ml eppendorf tube for group of 4, an 8-strip PCR tube, give each student group a strip tube

Each student group gets: 2ul pipette, 23 ul pipette, tips, 1.5 ul eppendorf tube with MM, 8 strip tube, sharpie

Students add 23 ul of MM to each tube to be used. Then, each student adds 2 ul of their unknown’s DNA to a tube, enter their ID in sheet.

Thermocycler

Add in negative control(s) and positive controls

Use ITS protocol

Gel Electrophoresis supplies

2% agarose gel, gel combs, pipette (1-10 ul), pipette tips, 1X TAE buffer, gel rig, loading dye, 1kb ladder, DNA stain (etBr or Syber Green), parafilm for mixing PCR product and loading dye.

Sequencing

Exo Sap for clean up

Forward primer aliquot for sequencing

New strip tubes for sequencing