

Running the PCR

- 1- Label your tubes very well. PCR reactions are performed in 0.2mL strip-cap tubes.
- 2- Keep your PCRs on ice
- 3- Thermocycler should be turned ON.

Reagent	Volume
REExtract-N-Amp PCR ReadyMix	10 μ L
PCR-quality Water	4.4 μ L
ITS1F Primer	0.8 μ L
LR3 Primer*	0.8 μ L
Fungal extract	4 μ L
Total Volume	20 μL

Make up a master mix in a 1.5mL microcentrifuge tube that contains the reagent listed above (highlighted in yellow) (For 25 samples, multiply each reagent by 28 because: 25 samples +1 for negative +2 for pipet error).

Mix it carefully for 10sec.

Aliquot 16 μ L of master mix into each of your pre-labeled tubes.

Add 4 μ L of the fungal extract sample.

Spin the tubes down briefly before putting them in the thermocycler.

Use * If amplification failed with these primers, PCR should be repeated with primers ITS5 and LR3.

Cycling reactions:

95°C for 3 min; 36 cycles of 95°C for 30s, 54°C for 30s, 72°C for 1 min; and 72°C for 10 min

If you're going to be performing gel electrophoresis on your samples within a few hours, you can keep your samples in the fridge; otherwise, store them at -20°. Make sure you know where your samples are. Keep the freezer organized.