

## Quick DNA extraction from fungal tissue and bacterial cultures

Nhu Nguyen – University of Hawai'i at Mānoa

### Extraction Solution (ES)

- 1) Add 10 ml of 1 M Tris stock (pH = 8.0) into a clean 50 ml centrifuge tube [=0.2 M final conc.]
- 2) Add 1.1 ml of 0.5 M EDTA (disodium) [= 0.185 g], [= 0.011 M final conc.]
- 3) Add 0.93 g KCl [= 0.25 M final conc.]
- 4) Add 40 ml deionized H<sub>2</sub>O and shake until solutes dissolve.
- 5) Titrate with 1 M NaOH to pH = ~ 9.5-10.0 (add about 0.3 g NaOH pellet to start).  
Subset samples for titration. *Do not* submerge the pH probe into the solution!
- 6) Top up to 50 ml with molecular grade H<sub>2</sub>O.
- 7) Filter sterilize with a 0.22 µm syringe filter into clean 2 ml centrifuge tubes.
- 8) Store at -20 °C.

### Dilution Solution (BSA 3% w/v)

- 1) Add 10 ml of molecular grade H<sub>2</sub>O into a clean 50 ml centrifuge tube.
- 2) Add 1.5 g of BSA (98-99% purity, heat shock fractionated) into the tube.
- 3) Add 30 more ml of molecular grade H<sub>2</sub>O. Shake well to dissolve. Do not heat!
- 4) Add 1 ml of 50 mM MgCl<sub>2</sub> [1 µM final conc.]
- 5) Top to 50 ml with molecular grade H<sub>2</sub>O.
- 6) First, filter sterilize using 0.45 µm filters to remove large particles. Filter sterilize with a 0.22 µm syringe filter into clean 2 ml centrifuge tubes.
- 7) Store at -20 °C.

### **Extraction Procedure**

- 1) Pipette an appropriate amount of **Extraction Solution** (ES) into a 0.2 ml tube. For an ectomycorrhizal tip 50 µl is sufficient. For larger samples, or bacterial/yeast cultures, use 75 µl. For cultures grown in liquid, follow the procedure in step 2 first prior to this step.
- 2) Place a small amount of tissue sample (individual root, fruitbody tissue, pure culture isolate) sample into Extraction Solution. Submerge sample and smash sample to make sure that the liquid penetrated the tissue. Be sure not to add it any extra water or liquid (if picking roots, blot the forceps dry).

For bacteria or yeast cells on agar, scoop out cells from a single colony using a sterilized toothpick, 1 µl plastic loop, or needle. If cells don't go into solution easily, roll the loop between your fingers to help dislodge them. After several minutes in the solution, the cell mass should start to separate from each other. Flick the tubes to dissolve all of the cells into the solution. Spin down quickly.

For bacteria or yeast cells in liquid culture, mix the culture thoroughly and pipet out 200 µl of the culture into a 0.2 ml tube. Spin down for 1 minute or until the cells had been pelleted. Discard the supernatant. Add 200 µl of water, flick the tubes to mix thoroughly to wash the cells. Spin down again to pellet the cells. Discard the supernatant. Add 75 µl of Extraction Solution (ES). Mix thoroughly and proceed to Step 3.

- 3) Place into the PCR machine and run cycle "XTRACT". This will incubate the samples at 25 °C for 10 minutes, then at 95 °C for 10 minutes.
- 4) Add an equal volume of **Dilution Solution** (DS) so that the final ES:ED solution ratio is 1:1.

## SOIL MICROBIAL ECOLOGY LAB

NGUYEN LAB | MICROBIOME DIVERSITY & FUNCTION

- 5) For large tissue pieces, spin down for 1 minute in the small centrifuge. For bacteria and yeast cells, spin down at  $10,000 \times g$  for 2 minutes to pellet the cells. Some BSA from the DS will precipitate and may not spin down well. For plates, centrifuge at 4000 rpm for 3 minutes.
- 6) The final concentration is  $\sim 25 \text{ ng}/\mu\text{l}$ . Use 1-2  $\mu\text{l}$  of the clear solution for PCR (avoid the BSA precipitate). For bacteria and yeast samples, we typically use 2  $\mu\text{l}$  template in a 15  $\mu\text{l}$  reaction.

**Note:** Several anecdotal observations suggest that incubating the extracted samples in the fridge for 12 hours or more results in much better PCRs. We have consistently gotten excellent PCR results with 99-100% success in cultures that were incubated overnight. In other plates, we have added cells to extraction solution left the plate in the fridge over-night prior to 95C incubation, followed immediately by neutralization solution. The PCRs from these samples work just as well.

**Storage:** Solution can last in the fridge for a few weeks, but store the completed extractions in freezer for better preservation if you won't be using the samples within a week. We were able to obtain good PCR results for 2 96-well extraction plates that were left in the fridge for 7 months.

All of these observations suggest that this method is robust for extracting workable DNA from bacterial cells of diverse cultivatable lineages.