

Barcoding (activity)

DNA barcoding of samples

1. Place sample in a clean 1.5 mL tube
2. Add 100 μ l of nuclear lysis solution to tube.
 - Twist a clean plastic pestle against the inner surface
3. Add 500 μ l more nuclear lysis solution to tube.
4. Incubate the tube in a water bath or heat block at 65°C for 5-15 minutes.
5. [Optional] Add 200 μ l of protein precipitation solution to each tube incubate on ice for 5 minutes
6. Centrifuge for 4 minutes at maximum speed to pellet protein and cell debris
7. Transfer 600 μ l of supernatant to a clean labeled tube.
8. Add 600 μ l of isopropanol
9. Centrifuge for 2 minute at maximum speed to pellet the DNA.
10. Pour off the supernatant and add 600 μ l of 70% ethanol to wash the pellet
11. Centrifuge the tube for 2 minute at maximum speed and carefully remove the solution
12. Air dry the pellet for 10 minutes and add 100 μ l of the DNA rehydration solution (TE)
13. Incubate the DNA at 65°C for 5-10 minutes to dissolve
14. Obtain PCR tube containing Ready-To-Go PCR Bead. Label the tube with your identification number.
15. Use a micropipette with a fresh tip to add 23 μ L of one of the following primer/loading dye mixes to each tube. Allow the beads to dissolve for 1 minute.
 - Plants: rbcL primers (rbcLaF / rbcLa rev)
 - Fish: COI primers (VF2_t1/ FishF2_t1/ FishR2_t1/ FR1d_t1)
 - Insects: (LepF1_t1/ LepR1_t1)
16. Add 2 μ l of your DNA directly into the appropriate primer/loading dye mix.
17. Place tubes in a Thermal cycler
18. Pour 2% agarose into casting apparatus in refrigerator
 1. 2 gel per class need to be made ? 100ml of TBE with 2g agarose
 2. add 5 μ l SYBR safe solution into the molten agarose before casting
 3. place 2 sets of combs into the gel ? at one end and in the middle
19. Load DNA ladder and PCR samples
20. Run gel at 120V for 30 minutes
21. Visualize on UV transilluminator
22. Document with camera
23. Send amplicons of verified samples for sequencing
 - Plant rbcL gene
 - rbcLaf 5'- ATGTCACCACAAACAGAGACTAAAGC-3' (forward primer)
 - rbcLar 5'- GTAAAATCAAGTCCACCRCG-3' (reverse primer)

- Animal coi gene
 - lepF1 5'- ATTCAACCAATCATAAAGATATTGG -3' (forward primer)
 - lepR1 5'- TAAACTTCTGGATGTCCAAAAAATCA-3'(reverse primer)
 - vf1f 5'- TCTCAACCAACCACAAAGACATTGG-3' (forward primer)
 - vf1r 5'- TAGACTTCTGGGTGGCCAAAGAATCA-3' (reverse primer)