DNA Extraction/PCR Protocol

For use with Epicenter MasterPure Complete DNA Purification Kit MCD85201

Collection of buccal cells for human mitochondrial DNA

Follow this protocol to collect the cells and the proceed to the section below on DNA extraction

1. Label a 1.5ml tube with your assigned number and the letter 'm' (mitochondrial sample)
2. Take a paper cup with 10 ml of the saline solution (0.9% NaCl) and vigorously swish around in your mouth for 1 minute (do not drink/swallow).
3. Expel saline solution into a paper cup.
4. Swirl to mix cells in the cup and transfer 1 ml (1000 µl) of the liquid to 1.5 ml tube.
5. Place your sample tube, together with other student samples, in a balanced configuration in a microcentrifuge, and spin for 1.5 minute.
6. Carefully pour off supernatant into paper cup or sink. Be careful not to disturb the cell pellet at the bottom of the test tube. A small amount of saline will remain in the tube.
7. Use a pipette (yellow/p100) to remove as much of the supernatant as possible without disturbing the pellet.
8. Add 300ul of the Tissue and Cell Lysis Solution/Proteinase K (TCL+K)

Collection of plant tissue for DNA barcoding

1. Label a 1.5ml tube with your assigned number and the letter 'p' (plant sample)
2. Add about 10-20mg of plant leaf/flower bud tissue (about the size of your pinky fingernail to your tube.
3. Grind the sample in 300ul of Tissue and Cell Lysis Solution/Proteinase K (TCL+K) using the provided pestle

DNA Extraction

1. Take your 'm' and 'p' sample and incubate at 65^{o} C for 15 minutes; vortex/mix every 5 minutes.
2. Place the incubated sample on ice for 2-5 minutes.
3. Add 175ul of MPC Protein Precipitation Reagent to the both tubes and vortex for 10 seconds.
4. Pellet the debris by centrifugation at 4^{o} C at >10,000 x g for 10 minutes
5. Transfer the supernatant to a clean **labeled** microcentrifuge tube and dispose of the tube with the pelleted debris
6. Add 500l of isopropanol to the supernatant. Invert the tube several (30-40) times.
7. Pellet the DNA by centrifugation at 4^{o} C at >10,000 x g for 10 minutes
8. Carefully pour off the isopropanol without disturbing the DNA pellet
9. Rinse the pellet with 70% ethanol, without disturbing the pellet. Spin the tube for 1 min at >10,000 g. Pour off the ethanol and repeat.

    > **The pellet IS your DNA - don't loose it!**

10. After pouring of any remaining ethanol, spin the tubes (containing only the empty pellets) for 1 min. Then use a pipette to remove any remaining ethanol. Let the pellet dry 1-2 min.

11. Resuspend the pellet in 35l of TE-RNAse
12. Keep the tubes on ice or freeze until ready for PCR

prepare PCR reactions for sequencing

We will be doing two separate PCR reactions. The plant DNA PCR will amplify a few hundred basepairs for DNA Barcoding. The MT DNA will be used to amplify the entire human mitochondrial genome for Nanopore Sequencing. The MT DNA PCR products will be mixed to create a composite sequence which can be aligned to the human reference mitochondrial genome.

1. You will be given a 'p' and 'm' tube; each containing the appropriate PCR mix (enzymes, primers, dNTPs, etc.); label each of these tubes with your assigned number (e.g. 1p and 1m)
2. Add 2ul of the 'p' DNA to the 'p' master mix
3. Add 2ul of the 'm' DNA to the 'm' master mix
4. Return your two PCR tubes and your two DNA tubes to the instructor