DNA Extraction and Purification Protocol/ Mucus extraction - Baker lab (modified by R McMinds)

Notes:
If you are running multiple samples through different parts of the method, please be sure to label your racks of samples with the last step completed.

Work quickly with small batches of samples and do not interrupt process until you have a reached a step labeled below as a “potential stopping point”.

Sample processing

Set-up:
- Clean working area with EtOH and dry.
- Take 12-16 samples out of -80°C freezer so they can thaw
- Switch on water baths/heat blocks to 65°C (for SDS incubation)

Pellet isolation:

1. Top off Falcon tube with 0.2um filtered seawater *as necessary* to balance the tubes in pairs for centrifugation. With cap tightly on, invert tube several times.
2. Centrifuge at max speed at **4°C**, for 10 minutes (ensure centrifuge is balanced, and wait for centrifuge to reach full speed before leaving room). Carefully remove tubes from centrifuge and note whether you can see a pellet.
3. Carefully pour off the supernatant – make sure the pellet does not come off the side of the tube! When pouring, the pellet should be on the side of the tube closest to the ceiling.
4. Add 0.5ml of (0.2um-filtered DNAB + 1% SDS) to pellet and vortex briefly to suspend.
5. Briefly spin down all liquid in the tubes in centrifuge (5 – 10s is fine).
6. Using a new 3mL serological pipette for each sample, transfer sample into labeled 1.5mL tube (minimizing bubble creation) and close cap tightly. Do not touch the inside of the tubes or their caps AND MAKE SURE YOUR LABEL IS CLEARLY LEGIBLE TO OTHER PEOPLE!
7. Vortex again and cook for 1.5 hours at 65°C (Be sure to record the cooking start time). Vortex intermittently during the 1.5 hours of cooking. This is the SDS incubation step, which lyses cells and stabilizes your sample for storage at room temperature. Once these samples have been incubated, these samples are treated as “archives” and are stored for future use. These archives can be used for multiple attempts at DNA extraction.
8. Remember to turn the centrifuge off after your last spin for the day (as well as the heat block, when you are done using it)!

- Potential Stopping Point -
Samples can be stored at room temperature

DNA Extraction/Isolation:

- These steps should be undertaken in batches no larger than **<u>24</u>**, which is the maximum capacity of the centrifuges.
- Switch on water baths/heat blocks to 55°C (for Proteinase K incubation) and 65°C (for CTAB incubation)

NB. Half extractions which use less archive material are perfectly ok.

Proteinase K digest:

9. Defrost Proteinase K and vortex well to ensure it is fully mixed. Prepare a set of new 1.5mL microcentrifuge tubes (equal to the number of samples you intend to process) and add 10µL of Proteinase K (10mg/mL, stored in freezer) to the bottom of each tube (you can use the same pipette tip to do this).
10. Add 200uL of sample (which have previously been cooked in SDS and archived per earlier steps – vortex archives beforehand), to each of your new tubes. Use a new pipette tip for each sample. Vortex samples well. These tubes now contain 200uL of sample and 10uL of Proteinase K (210uL total). Return the SDS samples to the archives.
11. Cook for <u>3h at 55°C</u>. This is the "Proteinase K digest".

- Potential Stopping Point –
Freeze samples at –20°C if you intend to stop here

Organic Extraction:

12. If samples are frozen, allow them to defrost on the benchtop.
13. Add twice volume (420uL) of CTAB mix to each sample and vortex well. Cook at 65°C for 60 minutes.
14. Allow sample to cool. Under the fume hood add equal volume (630uL, since 210 + 420 = 630) of chloroform. Be sure to 'charge' (i.e., fill and empty pipette tip with chloroform 2 to 3 times) the pipette tip before first use, or your tip will leak chloroform. Total volume is now 630uL + 630uL = 1260uL (1.26mL). Vortex sample and invert several times by hand, but be careful that caps are on tightly – leaking chloroform will erase all your sample labels!! Put in rack on rotating platform for 3 hours. This is the "Organic extraction" step.

First ethanol Precipitation:

15. Centrifuge sample at 10,000g (RCF) for 10 minutes. Align tubes in centrifuge so that hinges are on the outside. Remove tubes from centrifuge and very carefully pipette off top ~500uL into new microcentrifuge tube. Label new tube and double-check all labeling. Dispose of old tubes and their contents into the appropriate containers in the fume hood waste container.
16. Add twice volume (1000uL, or 1.0ml) of pre-chilled (if possible) Punctilious 100% (200-proof) Ethanol, (EtOH). Ensure caps are shut tightly and invert samples in their rack several times, together with a few brief shakes to make sure samples are well mixed.
17. Put samples in freezer for <u>at least</u> 2 hours to promote DNA precipitation. If the EtOH is pre-chilled, you can leave it in the -20°C freezer for only a 1/2 hour. This is the "First Ethanol Precipitation". Note that the contents of the tube will not freeze due to the high alcohol content.

- Potential Stopping Point -
Keep in freezer to allow DNA to precipitate

Second Ethanol Precipitation:

18. Put samples in centrifuge (ensuring that the hinges of the Eppendorf are on the outside) and spin for 10 minutes at 10,000g (RCF)
19. Remove samples from centrifuge and look for a white DNA pellet at the bottom of the tube on the same side as the hinge (*though it's normal if this isn't visible*). Carefully decant off ethanol from all the tubes into a waste container. The pellet should remain in the stuck to the inside of the tube. Reseal all tubes and centrifuge at 10,000g again for about 20 seconds to bring rest of ethanol to the bottom of the tube. Remove tubes from centrifuge and carefully pipette off ethanol remainder using P200 to leave white DNA pellet adhered to tube wall. As you suck up the remaining ethanol at the bottom of the tube, aspirate around the pellet to help dry it out.
20. Put tubes, with their caps open, in the Vacufuge/Speedvac. Be careful when putting the tubes in and don't touch the inside of the caps. Speedvac on Medium heat (45°C) for 60 minutes.
21. Remove samples from centrifuge and add 100uL of 0.3M NaOAc **(BE SURE YOU HAVE A 0.3M SOLUTION: DO NOT USE THE STOCK 3M!)**. Vortex sample well to dissolve pellet. When the pellet is dissolved the sample will appear "syrupy" and will not bounce around as droplets inside the tube.
22. Once the pellet is dissolved, add 200uL of 100% Punctilious Ethanol, vortex and invert several times and put in freezer for at least 2hrs. This is the "Second Ethanol Precipitation".

- Potential Stopping Point -
Keep in freezer to allow DNA to precipitate

Ethanol Wash and Resuspension of Purified DNA:

23. Remove samples from freezer, and centrifuge for 10minutes at 10,000g (RCF). Isolate pellet as before by pipetting off supernatant with P200 pipette. Aspirate pellet as last of ethanol is sucked up, but work quickly – this time you do not want the pellets to dry out.

24. Add 100uL of 70% Ethanol, and vortex thoroughly (this is the “Ethanol Wash” step). Centrifuge for 10 minutes at 10,000g (RCF), and isolate pellet by pipetting off the supernatant and aspirating the pellet using a P200.

25. Put samples in Vacufuge with the caps open (careful!), and speedvac on Medium heat for 60 minutes to thoroughly dry the pellet.

26. Take samples out of centrifuge and add 50uL TE. Vortex briefly to mix and store at -20°C in freezer. Keep in labeled racks or boxes. Sample is now ready for PCR.

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