DNA Extraction from Blood and Tissue

(Using a Qiagen® DNeasy Kit)

Materials

- Blood or tissue sample
- Qiagen DNEasy kit for blood and tissue
- Proteinase K
- Molec µLar grade ethanol

Equipment/Labware

- Heat block
- Micro-centrifuge tubes
- Pipettes
- Vortex
- Centrifuge
- Tube racks

DNA Extraction from Blood (Takes approximately 1 hour)

Digestion

1. Preheat heating block to 56º C.
    a. Suggestion: Read through directions and label appropriate tubes prior to starting.
2. Pipette 20 µL QIAGEN Protease (Proteinase K can be substituted) into the bottom of a 1.5 mL micro-centrifuge tube.
    a. Notes: Protease should be kept at -20º C (freezer), Proteinase K at 4º C (refrigerator) or room temperature.
3. Add 200 µL sample (blood) to the micro-centrifuge tube.
4. Add 200 µL Buffer AL to the sample (micro-centrifuge) tube.
    a. If precipitates form, dissolve at 56º C)
    b. Vortex for 15 seconds.
5. Incubate in heating block at 56º C for 10 min.
6. Centrifuge sample at 8 (x 1000) rpm for 1 min.

Washing

7. Add 200 µL ethanol to the sample tube.
    a. Vortex for 15 seconds.
    b. Centrifuge again at 8 (x 1000) rpm for 1 min.
8. Carefully pipette entire sample mixture from micro-centrifuge tube into the QIAamp spin column (in a 2 mL collection tube) without wetting the rim, close the cap.
    a. Label the cap of the spin column with sample ID.
9. Centrifuge at 8 (x 1000) rpm for 1 min.
    a. Note: If there is sample left in filter column, spin again at full speed (13.2 rpm) for 1 min.
    b. Place the QIAamp spin column in a clean 2 mL collection tube and discard the previous tube containing the filtrate. (Filtrate is the liquid that ran through the column.)
10. Add 500 µL Buffer AW1 without wetting the rim and close cap.
    a. Centrifuge 8 (x 1000) rpm for 1 min.
    b. Place QIAamp spin column in another clean 2 mL collection tube and discard the previous tube containing filtrate.

Alaska BioPREP is funded by Science Education Partnership Award (SEPA) 5R25RR024282-02
from the National Institutes of Health (NIH)
BioPREP website: www.uaf.edu/bioprep

11. Add 500 µL Buffer AW2 without wetting rim and close cap.
    a. Centrifuge at f ull speed 13.2 (x 1000) rpm for 3 min.
    b. Place QIAamp spin column in another clean 2 mL collection tube and discard previous tube containing filtrate.
    c. Centrifuge again at fullspeed for 1 min.

Collection

12. Place QIAamp spin column into a new 1.5 mL micro-centrifuge tube that is labeled with sample ID, date, gDNA and species. Discard previous tube containing filtrate.
    a. Add 200 µL Buffer AE (or dH2O) to column.
    b. Let sit at room temp for 2-5 min.
    c. Centrifuge at 8 (x 1000) rpm for 1 min.
    d. Discard filter column.
    e. Keep micro-centrifuge tube which now contains your gDNA.

13. Run samples a 1% gel to verify that your DNA extraction was successf µL and you do indeed have gDNA. If bands are visible…You now have Genomic DNA (gDNA) collected in the labeled micro-centrifuge tube. This sample sho µLd be kept frozen.

DNA Extraction from Tissue (Takes approximately 30 minutes to finish after step 3)

Digestion

1. Preheat heating block to 55º C. Read through directions and label appropriate tubes prior to starting.
2. Cut up 25mg (you may need to convert this to grams in order to weigh this on your scale) tissue into small pieces (slivers).
    a. Place in a 1.5 mL micro-centrifuge tube.
    b. Think about what tissues will digest better for your tissue selection.
    c. Add 180 µL Buffer ATL.
3. Add 20 µL Proteinase K to sample (micro-centrifuge) tube.
    a. Vortex for 15 seconds.
    b. Incubate in heating block at 55º C for at least 1 hour or as long as necessary
    c. Vortex every hour (if possible) during incubation to dissolve sample.
    d. It is ok for the sample to be somewhat thickened (oil vs. water) but should not be gelatinous (like Jell-o). You should not have clumps of tissue remaining; tissue should be broken into very, very small particles. Ideally, tissue will be completely dissolved as fragments or gelatinous material will clog the filter.
    e. Tissue may require anywhere from 1 hour to over 24 hours to digest, especially for skin tissue.
    f. Vortex for 15 seconds before continuing to next step.
4. Set heating block to 70ºC.
5. Add 200 µL Buffer AL to sample tube.
    a. Buffer AL is light sensitive, keep container covered in aluminum foil.
    b. Mix thoroughly by vortexing.
6. Incubate at 70º C for 10min. Turn off block heater.**Washing**
7. Add 200 µL ethanol to sample.
    a. Mix thoroughly by vortexing.
8. Carefully pipette entire sample into a DNeasy spin column (in a 2 mL collection tube).

   a. Label the cap of the spin column with sample ID.
   b. If there is any precipitate in micro-centrifuge tube, make sure to transfer that to the spin column also.Centrifuge at 8 (x 1000) rpm for 1 min. If there is sample left in filter column, spin again at fullspeed (13.2 rpm) for 1 min.
   c. Place the DNeasy spin column in a new 2 mL collection tube and discard tube containing filtrate.
9. Add 500 µL Buffer AW1 to DNeasy spin column.
   a. Centrifuge at 8 (x 1000) rpm for 1 min.
   b. Place the DNeasy spin column in a new 2 mL collection tube and discard tube containing filtrate.
10. Add 500 µL Buffer AW2 to DNeasy spin column.
   a. Centrifuge at fullspeed (13.2 x 1000 rpm) for 3 min.
   b. Place QIAamp spin column in another clean 2 mL collection tube and discard previous tube containing filtrate.
   c. Centrifuge again at fullspeed for 1 min.

Collection

11. Place the DNeasy spin column in a clean 1.5 mL micro-centrifuge tube that is labeled with the sample ID, date, gDNA, species, and #1 (representing the 1st elution). Discard the previous tube containing the filtrate.
   a. Add 200 µL of Buffer AE (or dH2O) to spin column. (100 µL for more concentrated gDNA)
   b. Let sit at room temperature for 2-5 min.
   c. Centrifuge at 8 (x 1000) rpm for 1 min.
12. Keep micro-centrifuge tube which now contains your st elution of gDNA.
13. Place the DNeasy spin column in another clean 1.5 mL micro-centrifuge tube that is labeled with the sample ID, date, gDNA, species, and #2 (representing the 2nd elution).
   a. Add 200 µL of Buffer AE (or dH2O) to spin column. (100 µL for more concentrated gDNA)
   b. Let sit at room temperature for 2-5 min.
   c. Centrifuge at 8 (x 1000) rpm for 1 min.
14. Discard filter column. Keep micro-centrifuge tube which now contains your 2nd elution of gDNA.
   a. Elution #1 and #2 can be combined for a larger amount of concentrated gDNA (use caution as this can be a source of contamination).
15. Run a 1% gel to verify that your DNA extraction was successful and you do indeed have gDNA. If bands are visible …You now have Genomic DNA (gDNA) collected in the labeled micro-centrifuge tubes. Sample elution #1 will have a higher concentration of gDNA than sample elution #2 therefore generally giving better results. These samples should be kept frozen.

Alaska BioPREP is funded by Science Education Partnership Award (SEPA) 5R25RR024282-02
from the National Institutes of Health (NIH)
BioPREP Wiki: bioprep.wikis.uaf.edu