



DNA Extraction from Skeletal Remains

Powdering Bone Samples

Materials and Reagents Needed:

Ultraviolet Crosslinker (UVP Inc, Upland, CA)
Motor Drill with Dental Bur and Cutting Disk
SPEX 6750 Freezer/Mill (SPEX SamplePrep, Metuchen, NJ)
6751 Grinding Vial Set (SPEX SamplePrep, Metuchen, NJ)
Liquid Nitrogen

Note: All of the reagents and disposable materials should be exposed to UV light for a minimum 30 minutes prior to use.

Procedure:

1. Using a motor drill with an dental bur, sand the outer surface of the bone to remove dirt and debris.
2. Cut the bone using a motor drill with a cutting disk to small pieces.
3. Expose the pieces of bone to UV light for 15 minutes in a UV Crosslinker. After that, turn over the pieces of bone and additionally expose those to UV light for 15 minutes.
4. Assemble the center cylinder and blunt end-plug of a 6751 Vial.
5. Add three ~ five pieces of the bone samples and stainless steel impactor into polycarbonate center cylinder, and close it with the flanged end-plug. Shake the vial to make sure the impactor has room to move back and forth.
6. Keeping the top of the Freezer/Mill open, fill it with liquid nitrogen below a fill mark.
7. Insert carefully the vial in the coil of the Freezer/Mill facing out end-plug.
8. Close the lid of the Freezer/Mill and wait until the stream of vapor decreases.
9. Set pre-cooling time at 3 minutes and grinding time at 2 minutes.
10. Press the Run button to begin the grinding cycle.
11. At the end of the grinding cycle, unlatch the lid and lift it. Open the coil gate by pulling the coil gate handle to the right. Remove the vial from the coil.
12. To open the vial, slip the open end of the Extractor over the flanged end-plug, align the pegs in the end-plug with the slots in the Extractor, and turn the knob clockwise until the end-plug is drawn out. Always be careful when you do this that the vial is supported in a rack or on a table so it does not fall and spill its contents.
13. Separate the impactor from the bone powder and can be handled with gloved fingers, a strong magnet, tongs, or pliers.
14. Transfer the bone powder to 15 mL tube and store at -20°C until DNA extraction.



DNA Extraction from Skeletal Remains

Small Scale DNA Extraction

Materials and Reagents Needed:

Ultraviolet Crosslinker (UVP Inc, Upland, CA)
QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany)
Extraction Buffer; 0.5 M EDTA and 0.5% SDS
Proteinase K (Sigma, Saint Louis, MO)
Micro Centrifuge

Note: All of the reagents and disposable materials should be exposed to UV light for a minimum 30 minutes prior to use.

Procedure:

1. Carefully pour approximately 0.1 g pulverized sample onto wax paper and empty the pulverized sample into 1.5 mL micro centrifuge tube.
2. To this tube and the tube with cotton swab tip (reagent blank) add 1 mL of 0.5M EDTA, 25 uL of 20% SDS and 50 uL of 20 mg/mL proteinase K.
3. Briefly vortex-mix and centrifuge the tubes in a micro centrifuge to force the bone powder into the solution.
4. Incubate at 56°C for 48 hours.
5. Remove the tubes from the water bath and centrifuge at 5000 × g for 5 minutes in a micro centrifuge. Aliquot the supernatant to a 1.5 mL micro centrifuge tube.
6. Centrifuge the tubes at 12800 × g for 5 minutes in a micro centrifuge. Aliquot the supernatant to a 15 mL centrifuge tube.
7. Add 5 volumes of the PB buffer from the QIAquick kit and mix with sample.
8. Apply 750 uL of each sample onto QIAquick Spin column and centrifuge the columns at 12800 × g for 1 minutes in a micro centrifuge.
9. Discard flow-through and place the QIAquick Spin column into clean 2 mL collection tube.
10. Repeat the step 8 and step 9 until each sample is exhausted.
11. To wash, add 750 uL of PE buffer and centrifuge at 12800 × g for 1 minutes.
12. Discard flow-through and place the Qiagen spin column in a clean 2 mL collection tube. Centrifuge the column at maximum speed for an additional 1 minutes.
13. To elute DNA, add 50 uL deionized water to the center of the membrane in column, incubate at room temperature for 5 minutes and centrifuge at 12800 × g for 1 minutes.



DNA Extraction from Skeletal Remains

Large Scale DNA Extraction

Materials and Reagents Needed:

Ultraviolet Crosslinker (UVP Inc, Upland, CA)
QIAvac 24 Plus and QIAvac Connecting System (Qiagen, Hilden, Germany)
VacConnectors (Qiagen, Hilden, Germany)
Vacuum Pump (Qiagen, Hilden, Germany)
Extraction Buffer; 0.5 M EDTA and 0.5% SDS
(e.g. Mix 39 mL of 0.5 M EDTA and 1 mL of 20% SDS)
Proteinase K (Sigma, Saint Louis, MO)
QIAamp® DNA Blood Maxi Kit (Qiagen, Hilden, Germany)
QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany)
Buffer PB (Qiagen, Hilden, Germany)
Buffer PE (Qiagen, Hilden, Germany)

Note: All of the reagents and disposable materials should be exposed to UV light for a minimum 30 minutes prior to use.

Day 1:

1. Carefully pour approximately 0.5 g pulverized sample into 50 mL tube.
2. Add 15 mL of extraction buffer and 150 uL of 20 mg/mL Proteinase K.
3. Extensively wrap the top of the tubes with sealing film.
4. Briefly vortex-mix and centrifuge the tubes to force the bone powder into the solution.
5. Incubate the tubes in a rotary shaker at 56°C for 48 hours.



DNA Extraction from Skeletal Remains

Large Scale DNA Extraction

Day 2:

1. Before at least one hour to start DNA extraction, add additionally 150 μ L of 20 mg/mL Proteinase K to the tubes and re-incubate in a rotary shaker at 56°C.
2. Briefly centrifuge the tubes. If powder is remaining in the tube, simply transfer the supernatant.
3. Prepare two new 50 mL tube per sample and add 38 mL of buffer PB into the each tube.
4. Add each 7.5 mL of the sample mixture into each of the 50 mL tube containing buffer PB. Mix well and briefly centrifuge.
5. Insert the QIAamp Maxi column into the VacConnector on the vacuum manifold. Open the corresponding VacValve. Close the main vacuum valve and switch on the vacuum pump.
6. Carefully transfer 15 mL of the mixture from the step 4 onto QIAamp Maxi column. Open the main vacuum valve. If sample flow differ, close the VacValves where the lysate has already passed through in order to ensure a consistent vacuum over the remaining column. Load 15 mL of the remaining mixture from the step 4 and open the corresponding VacValve until the mixture is exhausted. After all lysates have been drawn through the columns, close the main valve.
7. Open the VacValve, and add 15 mL Buffer PE to the QIAamp Maxi column. Open the main vacuum valve. After all of the Buffer PE has been drawn through the columns, close the main vacuum valve and switch off the vacuum pump.
8. Place the QIAamp Maxi column in a clean 50 mL centrifuge tube, and centrifuge at 3000 x g for 5 minutes to remove the remaining Buffer PE.
9. Pipet 1 mL distilled water directly onto the membrane of the QIAamp Maxi column and close the cap. Incubate at room temperature for 5 minutes, and centrifuge at 3000 x g for 5 minutes.
10. For maximum yield, repeat step 9 so as to obtain 2 mL of eluted DNA.
11. Add 10 mL of the Buffer PB to the above in 50 mL tube containing the eluted DNA and mix well.
12. Insert QIAamp DNA Mini column into the VacConnector on the vacuum manifold. Open the corresponding VacValve. Close the main vacuum valve and switch on the vacuum pump.
13. Carefully apply 750 μ L the mixture from step 11 onto the QIAamp DNA Mini column without wetting rim. Open the main vacuum valve. After the mixture have been drawn through the columns, load 750 μ L of the remaining mixture from step 11 onto the QIAamp Mini column until the mixture is exhausted.
14. Close the main vacuum valve. Apply 750 μ L Buffer PE onto the QIAamp DNA Mini column without wetting rim. Open the main vacuum valve. After all of the Buffer PE has been drawn through the columns, close the main vacuum valve and switch off the vacuum pump.
15. Remove the QIAamp Mini column from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column into a clean 2 mL collection tube and centrifuge at 13,200 rpm for 3 minute to dry the membrane completely.
16. Place the QIAamp Mini column in a clean 1.5 mL microcentrifuge tube. Discard the collection tube containing the filtrate. Carefully open the column. and add 50 μ L distilled water. Incubate at room temperature for 5 minutes, and then centrifuge at 8000 rpm for 1 minute.
17. Store the eluted DNA at -20°C.