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Supplementary Protocol

Purification of DNA from bones and teeth using the EZ1® Advanced or EZ1 Advanced XL

This document describes automated purification of mitochondrial and total genomic DNA from large sample amounts of powdered bone or teeth using the EZ1&2® DNA Investigator® Kit in combination with EZ1 Advanced instruments and the EZ1 Advanced DNA Investigator Large-Scale Bone Card or EZ1 Advanced XL DNA Investigator Large-Scale Bone Card. Up to 6 (EZ1 Advanced) or up to 14 (EZ1 Advanced XL) samples of bone or teeth lysate can be processed per run with sample volumes of 1 ml, 1.5 ml, or 1.8 ml.

Important: Please refer to the *EZ1&2 DNA Investigator Kit Handbook* for general information on handling and storage of kit components. Please refer to the *EZ1 Advanced* or *Advanced XL User Manual* for detailed information about instrument setup.

Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

For all users:

- EZ1&2 DNA Investigator Kit (cat. no. 952034)
- Water bath or heating block
- 5 ml or 15 ml centrifuge tubes
- Centrifuge (for 5 ml or 15 ml tubes)
- Vortexer
- Microcentrifuge
- Pipettes and pipette tips
- QIAGEN Proteinase K (cat. no. 19131 or 19133)
- Buffer MTL (cat. no. 19112)
- 0.5 M EDTA, pH 8.3
- Liquid nitrogen

- 2 ml Sarstedt tubes (cat. no. 72.693.005)
- TissueLyser II, cat. no. 85300, with the Grinding Jar Set, S. Steel, cat. no. 69985, or an equivalent bead mill

For EZ1 Advanced users:

- EZ1 Advanced instrument (cat. no. 9001410)
- EZ1 Advanced DNA Investigator Large-Scale Bone Card (cat. no. 9022499)

For EZ1 Advanced XL users:

- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 Advanced XL DNA Investigator Large-Scale Bone Card (cat. no. 9022497)

Pretreatment protocol

This protocol describes the decalcification using EDTA and lysis of bone or teeth samples using proteinase K.

Important points before starting

- Make sure to use the correct sample tubes: 2 ml Sarstedt tubes (cat. no. 72.693.005). These tubes can be used in any position, including the heating block. Please note that the sample tubes provided with the EZ1&2 DNA Investigator Kit cannot be used in the heating block.
- Familiarize yourself with the TissueLyser II before grinding bone or teeth samples. See the *TissueLyser Handbook*.
- Perform the pretreatment protocol then proceed to the DNA purification protocol.
- In some steps of the procedure, one of 3 choices can be made. Choose ■ if processing 400 mg biological sample material; choose ▲ if processing 600 mg biological sample material; choose ● if processing 750 mg biological sample material. Do not exceed these sample amounts.

Things to do before starting

- Heat a water bath or heating block capable of holding 5 ml or 15 ml centrifuge tubes to 37°C for the decalcification in step 2.
- Prepare a fine powder from bone or teeth by the following procedure.
  Remove and discard the bone or teeth surfaces. Grind the remaining bone or tooth root to a fine powder using the TissueLyser II system or an equivalent bead mill.

- When using the TissueLyser II, transfer the bone or teeth sample and the ball into a grinding jar. Pour liquid nitrogen* into the grinding jar over the ball and bone or teeth fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer the grinding jar to the TissueLyser. Grind at 30 Hz for 1 min or until the bone or teeth is pulverized (grinding times depend on type, condition, and size of fragments).

Procedure

1. Place up to ■ 400 mg, ▲ 600 mg, or ● 750 mg of powdered bone or teeth into a 5 ml or 15 ml centrifuge tube. Do not exceed these sample amounts.
2. Add ■ 1.2 ml, ▲ 1.7 ml, or ● 2 ml 0.5 M EDTA (pH 8.3) and incubate the sample at 37°C for 24–48 h.

   After incubation, set the temperature to 56°C for the next incubation step.
3. Add 1 µl of carrier RNA solution (1 µg/µl).
4. Add ■ 40 µl, ▲ 60 µl, or ● 75 µl QIAGEN Proteinase K and incubate at 56°C for 3 h.
5. Centrifuge at 6000 x *g* for 4 min. Transfer ■ 1 ml or ▲ ● 1.5 ml of the supernatant into ■ two 2 ml sample tubes or ▲ ● three 2 ml sample tubes with 500 µl in each tube.
6. Add 400 µl Buffer MTL to sample tube 1 and ■ 1120 µl Buffer MTL to sample tube 2 or ▲ ● 1120 µl Buffer MTL to sample tubes 2 and 3. Vortex briefly and centrifuge briefly in a microcentrifuge to collect the liquid in the bottom of the tube.
7. Continue with "DNA purification protocol", below.

DNA purification protocol

This protocol is for isolation of mitochondrial and total genomic DNA from bone or teeth samples that have been pretreated as described in "Pretreatment protocol", page 2. The DNA purification protocol describes the simple procedure for setting up the EZ1 Advanced instrument and starting a run.

Important points before starting

- All steps of the protocol must be performed at room temperature (15–25°C), so work quickly during the setup procedure.
- The reagent cartridges and Buffer MTL contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach.
- Perform "Pretreatment protocol" before starting the DNA purification procedure.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, they must be equilibrated to operating temperature before use. Place the reagent cartridge into a shaker–incubator and incubate at 30–40°C with mild agitation for at least 2 hours before use. If precipitates are visible at the bottom of the wells, redissolve by incubating at 30–40°C with mild agitation for a further 2 hours. Do not use the reagent cartridges if the precipitates do not redissolve.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve buffer by mild agitation at 37°C, and then place at room temperature.

Procedure

1. Insert the EZ1 Advanced DNA Investigator Large-Scale Bone Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Investigator Large-Scale Bone Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL.
2. Switch on the EZ1 Advanced instrument.
3. Press "START" to start protocol setup. Follow the onscreen instructions for data tracking.
4. Press "1" (for 1.0 ml protocol), "2" (for 1.5 ml protocol), or "3" (for 1.8 ml protocol).
5. Choose the elution buffer and volume: press "1" to elute into water or "2" to elute into TE buffer.* Then press "1", "2", "3", or "4" to select the elution volume.
6. Press any key to proceed through the text shown on the display and start worktable setup.
7. Open the instrument door.
8. Invert reagent cartridges twice to mix the magnetic particles. Tap the cartridges to deposit the reagents at the bottom of their wells. Check to see that the magnetic particles have been completely resuspended.
9. Load the reagent cartridges into the cartridge rack.

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

   Note: For the 1.8 ml protocol, pierce a hole in well 10 (containing 420 µl Buffer MTL) of an EZ1&2 DNA Investigator cartridge. Transfer 300 µl of the sample supernatant and add 260 µl of Buffer MTL to well 10.
10. Load opened elution tubes into the first row of the tip rack.
11. Load tip holders containing filter-tips into the second row of the tip rack.
12. Load opened sample tubes containing digested samples. For the 1.0 ml protocol, follow step 12a; for the 1.5 ml and 1.8 ml protocols, follow step 12b.

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12a. 1.0 ml protocol: Load a 2 ml sample tube containing 500 µl of sample and 1120 µl Buffer MTL in the third row and a 2 ml sample tube with 500 µl of sample and 400 µl Buffer MTL in the fourth row. Load an empty 2 ml tube into the heating block. Proceed with step 13.

Note: Loading an empty 2 ml tube into the heating block provides increased process safety. If the wrong protocol was selected, the sample will be transferred to the 2 ml tube instead of the empty hole in the heating block.

12b. 1.5 ml and 1.8 ml protocol: Load a 2 ml sample tube with 500 µl sample and 1120 µl Buffer MTL in the third row and a 2 ml sample tube with 500 µl sample and 400 µl Buffer MTL in the fourth row. Load a 2 ml sample tube with 500 µl sample and 1120 µl Buffer MTL into the heating block. Proceed with step 13.

13. Close the instrument door.
14. Press "START" to start the purification procedure.

    The automated purification procedure takes 25 min (1.0 ml protocol), 32 min (1.5 ml protocol), or 38 min (1.8 ml protocol).
15. When the protocol ends, the display shows "Protocol finished". Press "ENT" to generate the report file.

    The EZ1 Advanced and the EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.
16. Open the instrument door.
17. Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.*

    If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.
18. **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.
19. To run another protocol, press "ESC", prepare samples as described in "Pretreatment protocol", and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 Advanced instrument.
20. Clean the EZ1 Advanced instrument.

    Follow the maintenance instructions in the user manual supplied with your EZ1 Advanced instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach.

Document Revision History

Date	Changes
09/2021	Rebranded “EZ1 DNA Investigator Kit” to “EZ1&2 DNA Investigator Kit”. Revised step 9 in the “DNA Purification Protocol”: Updated EZ1&2 DNA Investigator cartridge protocol instructions.

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