Supplementary Protocol October 2017

Purification of genomic DNA from casework samples using high-input volumes incorporating the Investigator® Lyse&Spin Basket Kit and EZ1® Advanced XL

This document describes automated purification of total genomic DNA from large sample amounts of casework samples using the EZ1 DNA Investigator Kit, in combination with the EZ1 Advanced XL instrument and the EZ1 Advanced XL DNA Investigator Large-Scale Bone Card utilizing Investigator Lyse&Spin baskets. Up to 14 casework sample lysates can be processed per run, with sample input volumes of 1 ml or 1.5 ml.

IMPORTANT: Please refer to the *EZ1 DNA Investigator Kit Handbook* for general information on handling and storage of kit components. Please refer to the *Advanced XL User Manual* for detailed information about instrument setup and the *Investigator Lyse&Spin Basket Kit Handbook* for detailed information on the spin baskets.

Equipment and reagents

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 DNA Investigator Kit (cat.no. 952034)
- Thermal shaker or heated orbital incubator
- Investigator Lyse&Spin Basket Kit (cat.no. 19597 or 19598)
- Vortexer
- Microcentrifuge
- Pipettes and pipette tips
- QIAGEN Proteinase K (cat. no. 19131 or 19133)
- Buffer MTL (cat. no. 19112)
- EZ1 Advanced XL Instrument (cat. no. 9001492)

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

Pre-treatment protocol

This protocol describes the lysing of casework samples using proteinase K and G2 lysis buffer. Buffer ATL can also be substituted for buffer G2.

Important points before starting

- Perform the pre-treatment protocol then proceed to the DNA purification protocol.
- In some steps of the procedure, one of 2 choices can be made. Choose ■ if processing 1 ml sample input volumes or choose ▬ if processing 1.5 ml sample input volume. Do not exceed these sample amounts.
- If 1ml sample input volume is required, divide the sample material into 2 equal aliquots. If 1.5 ml sample input volume is required, divide the sample material into 3 equal aliquots. Place the aliquoted material into individual QIAGEN Lyse&Spin baskets within a 2 ml microcentrifuge tube (provided) and label with the appropriate sample ID.

Procedure

1. To each Lyse&Spin basket, add 475 µl Buffer G2 or ATL

   Note: Reduce buffer volumes to 455 µl if semen stains are processed.

   Note: Avoid pipetting on the rim of the basket. For bulky substrates, do not exceed the fill line on the basket.
2. Add 25 µl proteinase K, close the lid and mix by vortexing.

   Note: If processing semen stains, add 20 µl 1 M DTT.
3. Place the tube and basket in a thermomixer or heated orbital incubator and incubate with shaking at 750 rpm at 56°C, for a minimum of 1 hr.

   Note: Incubation can be extended to up to 16 h, without affecting yield or quality of DNA.
4. Centrifuge for 5 min at a minimum of 10,000 x *g*.

   Note: Keep the lid closed during centrifugation.

   Note: Up to 20,000 x *g* can be used for centrifugation.

   Note: Make sure that no liquid remains in the basket after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane. If larger pieces of chewing gum are processed, clogging of the basket can be avoided by pressing the chewing gum against the sides of the basket.
5. Discard the basket, including the solid sample substrate.

Note: Alternatively, the basket can be stored in the second 2 ml sample tube provided (only for cat. no. 19597).

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 and centrifuge briefly in a microcentrifuge, to collect the liquid in the bottom of the tube.

DNA purification protocol

This protocol is for the isolation of total genomic DNA from casework samples that have been pre-treated, as described in the "Pre-treatment protocol", on page 2. The DNA purification protocol describes the simple procedure for setting up the EZ1 Advanced XL 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 the "Pre-treatment protocol" before starting the DNA purification procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, they must be equilibrated to the 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 h 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 h. 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 (15–25°C).

Procedure

1. Ensure that the EZ1 Advanced XL Instrument is switched off.
2. Insert the EZ1 Advanced XL DNA Investigator Large-Scale Bone Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL.
3. Switch on the EZ1 Advanced XL instrument.
4. Press "START" to start protocol setup. Follow the onscreen instructions for data tracking.

5. Press “1” (for 1.0 ml protocol) or ”2” (for 1.5 ml protocol). Do **not** use option “3”.
6. 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.
7. Press any key to proceed through the text shown on the display and start worktable setup.
8. Open the instrument door.
9. 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.
10. 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.
11. Load opened elution tubes into the first row of the tip rack.
12. Load tip holders containing filter-tips into the second row of the tip rack.
13. Load opened sample tubes containing digested samples. For the 1ml protocol, follow step 13a; for the 1.5 ml follow step 13b.

    Note: The lids of the sample tubes must be removed before loading into the instrument

13a. **1ml 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 14.

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.

13b. **1.5 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 14.

14. Close the instrument door.
15. Press “START”, to start the purification procedure.

    The automated purification procedure takes approx. 25 min for the 1ml protocol and approx. 32 min for the 1.5 ml protocol.

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

† 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.

16. When the protocol ends, the display shows "Protocol finished". Press "ENT" to generate the report file.

    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.

17. Open the instrument door.

18. 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*†.

19. **Optional:** Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

20. To run another protocol, press "ESC", prepare samples as described in "Pre-treatment 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 XL instrument.

21. Clean the EZ1 Advanced instrument.

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

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

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Troubleshooting

For general troubleshooting, please consult the "Troubleshooting Guide" in the corresponding kit handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.