QIAGEN Supplementary Protocol:

Purification of DNA from rodent tail tissue using the BioSprint 96 DNA Blood Kit

This protocol is designed for the purification of total (genomic and mitochondrial) DNA from up to 1.2 cm (~25 mg) of rodent tail tissue using the BioSprint 96 DNA Blood Kit in combination with the BioSprint 96 workstation.

Introduction

BioSprint DNA Blood Kits use MagAttract® magnetic-particle technology for DNA purification. MagAttract technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. BioSprint DNA Blood Kits provide high-quality DNA that is free of protein, nucleases, and other contaminants or inhibitors. The DNA is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions.

Note: BioSprint 96 workstations purchased before 1 June 2004 will need to have the protocol "BS96 DNA Tissue" installed. For more information, please contact one of the QIAGEN Technical Service Departments or local distributors.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *BioSprint DNA Blood Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Equipment and reagents to be supplied by user

- BioSprint 96 workstation, cat. no. 9000852
- Magnetic head for use with large 96-rod covers (supplied with the BioSprint 96)
- BioSprint 96 DNA Blood Kit (48), cat. no. 940054, or BioSprint 96 DNA Blood Kit (384), cat. no. 940057
- Microcentrifuge or centrifuge capable of holding S-blocks (e.g., Centrifuge 4-15C)
- Buffer ATL, cat. no. 19076
- QIAGEN® Proteinase K (2 ml), cat. no. 19131, or QIAGEN Proteinase K (10 ml), cat. no. 19133
- Tape Pads (5), cat. no. 19570
- Optional: DNase-free RNase A (required if purified DNA needs to be RNA-free)
- Multichannel pipettor and disposable pipet tips with aerosol barriers (20–1000 µl)
- Ethanol (96–100%)
- Isopropanol (100%)

- Vortexer
- Shaking incubator (e.g., Thermomixer from Eppendorf)
- Tween®-20

Important notes before starting

- Check that Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions in the "Preparing reagents" section of the *BioSprint DNA Blood Handbook*.
- Check that Buffer AL does not contain a white precipitate. If necessary, incubate Buffer AL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-block. If using a new packet of two, store the second 96 rod cover on another plate. It is important that the 96-rod cover does not become bent.

Things to do before starting

- Set a shaking incubator with a 96-well plate adapter to 56°C for use in step 2.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).
- Prepare a master mix according to Table 1 for use in step 5 of the protocol. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 min before using for the first time, and for 1 min before subsequent uses.

  Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed.

Table 1. Master Mix Buffer Volumes

Buffer/solution	Volume per reaction
Buffer AL	200 µl
Isopropanol	200 µl
MagAttract Suspension G	30 µl

Procedure

1. **Cut ≤1.2 cm of each rodent tail tissue sample into small pieces. Place tissue samples into the wells of an S-block or into 1.5 ml microcentrifuge tubes, and add 180 µl Buffer ATL.**

   All samples must have the same volume. If the volume of a sample needs to be increased, add the appropriate volume of Buffer ATL.

2. **Add 20 µl proteinase K, seal the S-block with a tape sheet or close the microcentrifuge tubes, and incubate the samples at 56°C in a shaking incubator until the tissue is completely lysed.**

   Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If it is more convenient, samples can be lysed overnight; this will not affect the DNA quality.

   Optional: If RNA-free genomic DNA is required, add 4 µl of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C)

3. **Towards the end of proteinase K digestion, prepare 5 S-blocks (slots 2–6) and the 96-well microplates (slots 7 and 8) according to Table 2. The S-blocks and microplates are loaded onto the worktable in step 9.**

   In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 2. BioSprint 96 Worktable Setup and Buffer Volumes

Slot	Message when loading	Plate/block	To add	Volume to add per well (µl)
8	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
7	Load Elution	96-well microplate MP	Buffer AE	200
6	Load Wash 5	S-block	RNase-free water*	500
5	Load Wash 4	S-block	Buffer AW2	500
4	Load Wash 3	S-block	Buffer AW2	500
3	Load Wash 2	S-block	Buffer AW1	500
2	Load Wash 1	S-block	Buffer AW1	650
1	Load Lysate	S-block	Lysate†	650

* Contains 0.02% (v/v) Tween-20.

† Includes volume of sample, proteinase K, Buffer ATL, Buffer AL, isopropanol, and MagAttract Suspension G; added in steps 1, 2, and 5.

4. **Briefly centrifuge the S-block or microcentrifuge tubes containing the sample to remove drops from underneath the tape or inside the lids. Remove the tape sheet from the S-block.**
5. **Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G (see "Things to do before starting") for 1 min. Add 430 µl of master mix to each sample in the S-block.**

   Note: If using an Eppendorf Multipipette®, aliquot 450 µl master mix to each sample. The starting volume of master mix should be increased accordingly.
6. **Switch on the BioSprint 96 at the power switch.**
7. **Slide open the front door of the protective cover.**
8. **Select the protocol "BS96 DNA Tissue" using the ▲ and ▼ keys on the BioSprint 96 workstation. Press "Start" to start the protocol run.**
9. **The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover (see Table 2). After loading slot 8, press "Start". The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.**

   Note: Each slot is labeled with a number. Load each 96-well plate or S-block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).
10. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

    Warning: To avoid contact with moving parts during operation of the BioSprint 96, do not insert your hands and fingers inside the workstation.
11. **Press "Start" to start sample processing.**
12. **After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.**

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, in the *BioSprint DNA Blood Handbook*).
13. **Press "Stop" after all plates and blocks are removed.**
14. **Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

    Note: See "Safety Information" in the *BioSprint DNA Blood Handbook*.

15. **Switch off the BioSprint 96 at the power switch.**
16. **Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

    Note: Do not use bleach as disinfectant. See "Safety Information" in the *BioSprint DNA Blood Handbook*.

BioSprint workstations and BioSprint DNA Blood Kits are intended as general purpose devices. No claim or representation is intended for their use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of BioSprint workstations and BioSprint DNA Blood Kits for any particular use, since their performance characteristics have not been validated for any specific organism. BioSprint workstations and BioSprint DNA Blood Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

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Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp .