

PreAnalytiX Supplementary Protocol

Purification of genomic DNA from sections of PAXgene[®] Tissue fixed, cryo-embedded (PFCE) tissue placed directly into a microcentrifuge tube

This protocol is for using the PAXgene Tissue DNA Kit to purify genomic DNA from sections of PAXgene Tissue fixed, cryo-embedded (PFCE) tissue placed directly into a microcentrifuge tube.

IMPORTANT: The tissue sample must be fixed and stabilized in PAXgene Tissue Containers (see the *PAXgene Tissue Container Product Circular* for information on tissue fixation and stabilization), washed in a sucrose solution, snap-frozen and cryo-embedded. For details, see the PreAnalytiX[®] Supplementary Protocol *Cryo-embedding tissue specimens fixed and stabilized with the PAXgene Tissue System (PX14)*.

Also read the *PAXgene Tissue DNA Kit Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.

For Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents

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.

- Ethanol (96–100%, purity grade p.a.)
- Pipets and pipet tips
- Cryostat*
- Variable-speed microcentrifuge* capable of attaining 1000–8000 x g, and equipped with a rotor for 2 ml microcentrifuge tubes
- Shaker–incubator* capable of incubating at 45–80°C and shaking at ≥400 rpm, not exceeding 1400 rpm (e.g., Eppendorf[®] Thermomixer Compact)[†]
- Vortex mixer*
- Forceps
- 1.5 ml safelock microcentrifuge tubes

* Make sure that instruments have been checked and calibrated according to the manufacturer’s recommendations.

[†] This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Starting material

Starting material for genomic DNA purification is 1–3 PFCE tissue sections with a thickness of 8–12 μm and a tissue surface area $\leq 225 \text{ mm}^2$.

Things to do before starting

- Tissue specimens must be fixed and stabilized according to the *PAXgene Tissue Container Product Circular*. Fixed and stabilized tissue must be cryo-embedded according to the Supplementary Protocol *Cryo-embedding of tissue specimens fixed and stabilized with the PAXgene Tissue System*.
- Set the temperature of the shaker–incubator to 56°C.
- Buffer TD1 and Buffer TD2 may form precipitates during storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer TD3 and Buffer TD4 are supplied as concentrates. Before using the buffers for the first time, add the amount of ethanol (96–100%, purity grade p.a.) indicated on the bottle to obtain a working solution.

Procedure

1. **Label the lid and the body of a 1.5 ml safelock microcentrifuge tube (not provided). Prepare a lysis mixture in the tube by mixing 180 μl Buffer TD1 with 20 μl Proteinase K. Mix by gently flicking the tube, and centrifuge briefly (1–2 s at 50–1000 x g) to collect residual liquid from the sides of the tube.**
2. **Using a cryostat, make a tissue section of 8–12 μm thickness from the PFCE tissue.**
3. **Transfer the PFCE tissue section into the lysis reagent using pre-cooled forceps and mix by vortexing for 5 s.**
4. **If required, repeat steps 2 and 3 for a maximum of 3 sections.**
5. **Incubate the tissue on a shaker–incubator for 1 h at 56°C and 1400 rpm.**
6. **After incubation, centrifuge briefly (1–2 s at 500–1000 x g) to remove drops from the tube lid.**

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

7. **Add 200 μl Buffer TD2 and mix by pulse-vortexing for 15 s.**

Note: It is essential that the sample and Buffer TD2 are mixed thoroughly by vortexing or pipetting to yield a homogeneous solution.

8. Continue with the addition of ethanol in step 14 of the protocol “Purification of Genomic DNA from Sections of PAXgene Treated, Paraffin-Embedded Tissue” in the *PAXgene Tissue DNA Kit Handbook* (page 17).

Note: If carrier RNA is required (see “Error! Reference source not found.”, on page 13 of the *PAXgene Tissue DNA Kit Handbook*).

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

Safety data sheets (SDS) for any QIAGEN or PreAnalytiX product can be downloaded from www.qiagen.com/safety.

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