PreAnalytiX Supplementary Protocol

Purification of genomic DNA from microdissected PAXgene® Tissue-fixed, paraffin-embedded (PFPE) and PAXgene Tissue-fixed, cryo-embedded (PFCE) tissues

This protocol is for using the PAXgene Tissue DNA Kit to purify genomic DNA from PAXgene Tissue-fixed, paraffin-embedded (PFPE) and PAXgene Tissue-fixed, cryo-embedded (PFCE) tissues that have been manually or laser microdissected (LMD) from a slide.

IMPORTANT: The tissue samples must be fixed and stabilized in PAXgene Tissue Containers. The *PAXgene Tissue Container Product Circular* includes information on tissue fixation and stabilization. For instructions on the preparation of PFCE tissue blocks, read the PreAnalytiX® Supplementary Protocol *Cryo-embedding tissue specimens fixed and stabilized with the PAXgene Tissue System* (PX14). To prepare sections from PFPE and PFCE tissue blocks for microdissection, follow instructions of the Supplementary Protocol *Preparation of sections from PAXgene Tissue fixed, paraffin-embedded (PFPE)* and *PAXgene Tissue fixed, cryo-embedded (PFCE) tissue for manual or laser microdissection* (PX20).

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

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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.

- RNase-free water
- Xylene
- Ethanol (96–100%, purity grade p.a.)
- Variable-speed microcentrifuge* capable of attaining 1000–8000 x g, and equipped with a rotor for 2 ml microcentrifuge tubes
- Microtome or cryostat*

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

- 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*
- Adhesion slides for manual microdissection (e.g., SuperFrost® Plus Slides, VWR® Cat. no. 631-0108) or frame slides for laser microdissection, (e.g., PPS-membrane 25mm x 76 mm, Leica® Cat. no. 11505273) †
- Laser Microdissection System* (e.g., *Leica LMD6500 & LMD7000*, Leica Microsystems)†
- 1.5 ml safelock microcentrifuge tubes
- 0.5 ml PCR tubes suitable for the dedicated LMD system (e.g. CORNING® AXYGEN® 0.5 ml Tube with Flat Cap, Cat. no. 10169-890† for Leica LMD6500 & LMD7000)

Carrier RNA

The PAXgene Tissue DNA Kit contains poly-A RNA for use as carrier RNA, which can be added to Buffer TD1 if required. Carrier RNA enhances binding of DNA to the PAXgene DNA spin column membrane if samples contain very few target molecules. We recommend adding carrier RNA to Buffer TD1 for purification of DNA from very small samples. If carrier RNA is used, eluates from PAXgene DNA spin columns contain both sample DNA and carrier RNA, with the amount of carrier RNA greatly exceeding the amount of DNA. Therefore, the eluate volume used in downstream amplification depends on the amount of carrier RNA added to Buffer TD1. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to Buffer TD1.

Starting material

Starting material for DNA purification is a PFPE or PFCE section mounted on a glass adhesion slide for manual microdissection (procedure A) or on a frame slide with a membrane for laser microdissection (procedure B). Sections must be prepared according to the Supplementary Protocol *Preparation of sections from PAXgene Tissue fixed, paraffin-embedded (PFPE) and PAXgene Tissue fixed, cryo-embedded (PFCE) tissue for manual or laser microdissection (LMD)*.

Detach the microdissected specimen from the slide and transfer it to a dedicated collection device for direct purification of genomic DNA.

Things to do before starting

- Buffer TD1 and Buffer TD2 may form precipitates during storage. If necessary, warm to 56°C until the precipitates have fully dissolved.

* 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.

- 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.
- Set the temperature of the shaker–incubator to 56°C for the Proteinase K digestion.
- For PFCE tissue sections only: Prepare 80% ethanol by mixing ethanol (96–100%, purity grade p.a.) and RNase-free water (supplied). Precool the ethanol in a staining dish on ice.
- When processing <5000 cells, carrier RNA may be added to the lysate (see "Carrier RNA", page 2). Before using carrier RNA for the first time, dissolve it (310 µg) in 1 ml RNase-free water. Store this stock solution at –20°C and use it to make fresh dilutions for each set of DNA preps. The concentration of this stock solution is 310 ng/µl. Prepare a working solution of 4 ng/µl using Buffer TD1. Add 5 µl of this solution to the lysate in step 1.

Procedure A: Purification of genomic DNA from manually microdissected PFPE and PFCE tissue

In this procedure, ● refers to PFPE tissue and ▲ refers to PFCE tissue

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 90 µl Buffer TD1 with 20 µl Proteinase K. Mix by gently flicking the tube and centrifuge briefly (1–2 s at 500–1000 x g) to collect residual liquid from the sides of the tube.**

   Note: When processing <5000 cells, add 20 ng of carrier RNA (5 µl of a 4 ng/µl solution) to the mixture. Prepare the carrier RNA as described in "Things to do before starting".

2. **Using a ● microtome or ▲ cryostat, make a tissue section of 6–12 µm thickness from a ● paraffin-embedded or ▲ cryo-embedded tissue block. Capture the tissue section on an adhesion slide for manual microdissection.**
3. **Remove embedding medium and stain (optional) according to the Supplementary Protocol *Preparation of sections from PAXgene Tissue fixed, paraffin-embedded (PFPE) and PAXgene Tissue fixed, cryo-embedded (PFCE) tissue for manual or Laser microdissection (LMD)*. Keep freshly prepared slides in ice-cold ethanol until microdissection.**
4. **For manual microdissection, remove the slide from the ethanol. Using an absorbent sheet, wipe away the liquid on the slide that surrounds the tissue section.**

   Note: Tissue areas of interest can be isolated by scratching away other tissue parts with a scalpel blade or cell scraper. Remove tissue portions from the slide immediately after taking the section out of the ethanol. Avoid completely drying the section because this makes it harder to dissolve the tissue from the slide.

5. **Place the slide on a horizontal working plate and overlay it with 100 µl Buffer TD1. Make sure that the whole section is covered.**

   Note: Work quickly. We recommend using a dark underlay as a work surface to make it easier to see the tissue. The volume of Buffer TR1 needed depends on the tissue surface area.

6. **Detach the tissue from the slide by pipetting the lysis mixture up and down. Transfer the tissue and all liquid to the labeled 1.5 ml safelock microcentrifuge tube from step 1 and mix by vortexing for 5 s.**

   Note: If the tissue does not dissolve easily, use the pipet tip to scrape it from the slide.

7. **Incubate the tissue on a shaker–incubator for 1 h at 56°C and 1400 rpm.**

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

8. **Optional for PFPE tissue: Increase the temperature of the shaker-incubator to 80°C and incubate for an additional 1 h at 1400 rpm.**

   Note: For some tissue types and downstream applications, an additional incubation at 80°C increases DNA yield and amplificability.

9. **After incubation, centrifuge briefly (1–2 s at 500–1000 x *g*) to remove drops from the tube lid.**

10. **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.

11. **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).**

Procedure B: Purification of genomic DNA from laser microdissected (LMD) PFPE and PFCE tissue

In this procedure, ● refers to PFPE tissue and ▲ refers to PFCE tissue.

1. **Using a ● microtome or ▲ cryostat, make a tissue section of 6–12 µm thickness from a ● paraffin-embedded or ▲ cryo-embedded tissue block. Capture the tissue section on a frame slide with membrane for laser microdissection.**

2. **Remove embedding medium and stain (optional) according to the Supplementary Protocol *Preparation of sections from PAXgene Tissue fixed, paraffin-embedded (PFPE) and PAXgene Tissue fixed, cryo-embedded (PFCE) tissue for manual or Laser microdissection (LMD)*. Keep freshly prepared slides in ice-cold ethanol until laser microdissection.**

3. **Using a Laser Microdissection System, dissect a tissue area of ≥5000 µm² (≥50 cells) from the PFPE or PFCE LMD slide.**

   Note: ≤50 cells may require pre-amplification of DNA for downstream applications (using, for example, the QIAGEN® REPLI-g® Single Cell Kit).

4. **Collect cells directly into a dedicated collection tube filled with 15 µl Buffer TD1 (e.g., use the cap of a 0.5 ml PCR tube when using a Leica LMD system).**

   Note: If not possible, add buffer TD1 immediately after collecting the cells.

5. **Add 165 µl Buffer TD1. Mix by vortexing for 30 s and centrifuge briefly (1–2 s at 500–1000 x g) to remove drops from the tube lid**.

   Note: If processing <5000 cells, 20 ng carrier RNA (5 µl of a 4 ng/µl solution) may be added to the lysate. Prepare the carrier RNA as described in "Things to do before starting".

6. **Add 20 µl Proteinase K and mix by vortexing for 5 s.**

   Note: Do not mix Buffer TD1 and Proteinase K before adding them to the sample.

7. **Incubate the tissue on a shaker–incubator for 1 h at 56°C and 1400 rpm.**

   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).

8. **Optional for PFPE tissue: Increase the temperature of the shaker-incubator to 80°C and incubate for an additional 1 h at 1400 rpm.**

   Note: For some tissue types and downstream applications, an additional incubation at 80°C increases DNA yield and amplificability.

9. **After incubation, centrifuge briefly (1–2 s at 500–1000 x g) to remove drops from the tube lid.**

10. **Transfer the sample and Buffer TD1 into a 1.5 ml safelock microcentrifuge tube (not provided).**

    Note: Lysate samples in buffer TD1 can now be frozen at –80°C. Thaw frozen lysates on ice and proceed with step 11.

11. **Add 200 µl buffer TD2 to the sample. Mix by vortexing for 15 s.**

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

12. **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: The elution volume can vary between 20–200 µl and strongly depends on the area and number of microdissected cells. Low input requires lower elution volumes for high concentrations of genomic DNA.

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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