PreAnalytiX Supplementary Protocol

Deparaffinization of PAXgene® Tissue fixed, paraffin-embedded tissue sections with Deparaffinization Solution

This protocol is for the deparaffinization of PAXgene Tissue fixed, paraffin-embedded tissue (PFPE) sections prior to DNA or RNA purification. Before starting, the tissue sample must be fixed and stabilized in a PAXgene Tissue Container (cat. no. 765112) or a PAXgene Tissue FIX Container (50 ml) (cat. no. 765112), dehydrated, and embedded in paraffin.

IMPORTANT: The method for deparaffinization described here may be used with PFPE tissue and the PAXgene Tissue RNA Kit, the PAXgene Tissue miRNA Kit, or the PAXgene Tissue DNA Kit. Please read the kit handbooks, paying careful attention to the "Safety Information" and "Important Notes" sections, before starting the deparaffinization procedure.

For research use only. Not for use in diagnostics 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 safety data sheets (SDSs), available from the product supplier.

- Deparaffinization Solution (16 ml) from QIAGEN, cat. no. 19093
- PAXgene Tissue RNA Kit (cat. no. 765134), PAXgene Tissue miRNA Kit (cat. no. 766134), or PAXgene Tissue DNA Kit (cat. no. 767134)
- Microtome
- Shaker-incubator* capable of incubating at 45°C and 65°C and shaking at ≥400 rpm, not exceeding 1400 rpm (e.g., Eppendorf® Thermomixer Compact, www.ependorf.com† or equivalent).
- Variable-speed microcentrifuge* capable of attaining 1000–8000 x *g* and equipped with a rotor for 2 ml microcentrifuge tubes
- Vortex mixer*

* Ensure 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.

Protocol: Purification of RNA or Total RNA, including miRNA, from Sections of PFPE Tissue with Deparaffinization Solution

Starting material

Starting material for purification of RNA or total RNA, including miRNA, should be 1–5 sections of PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue (see the *PAXgene Tissue Container Product Circular* and the *PAXgene Tissue FIX Container (50 ml) Product Circular* for information about tissue fixation, stabilization, and paraffin embedding). Each section should have a thickness of 5–10 µm and a tissue surface area of up to 15 x 15 mm. Thicker sections may result in lower RNA yields or copurification of miRNA.

Things to do before starting

- Refer to the *PAXgene Tissue RNA Kit Handbook* or the *PAXgene Tissue miRNA Kit Handbook*.
- Tissue specimens must be fixed, stabilized, processed, and embedded in paraffin according to the *PAXgene Tissue Container Product Circular* or the *PAXgene Tissue FIX Container (50 ml) Product Circular*.
- A shaker-incubator is required in step 4. Set the temperature of the shaker-incubator to 56°C.
- Buffer TR1 or Buffer TM1 may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer TR1 or Buffer TM1 before use. Add 10 µl β-ME per 1 ml Buffer TR1 or Buffer TM1. Dispense in a fume hood and wear appropriate protective clothing. Buffer containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.

Procedure

1. **Using a microtome, generate a minimum of 1 and a maximum of 5 tissue sections of 5–10 µm thickness from the PFPE tissue.**

   Note: If the PFPE tissue surface has been exposed to air, discard the first 2 or 3 sections.

2. **Place sections in a 1.5 ml microcentrifuge tube.**
3. **Add 160 µl (when processing 1–2 sections per sample) or 320 µl (when processing 3–5 sections per sample) Deparaffinization Solution to the sample and vortex vigorously for 10 s.**
4. **Incubate for 3 min at 56°C in a shaker-incubator, and then cool at room temperature (15–25°C) for approximately 5 min. Set the temperature of the shaker-incubator to 45°C for later use in step 7.**

5. **Prepare Proteinase K incubation mix. For each sample, mix 150 µl Buffer TR1 or Buffer TM1 and 290 µl RNase-free water. Mix briefly by vortexing, then add 10 µl Proteinase K and mix by vortexing again.**

   For example, if processing 10 samples, mix 1500 µl Buffer TR1 or Buffer TM1, 2900 µl RNase-free water, and 100 µl Proteinase K.

   Note: Do not mix Buffer TR1 or Buffer TM1 and Proteinase K directly together. Instead dilute Buffer TR1 or Buffer TM1 with RNase-free water before adding the Proteinase K.

6. **Add 450 µl Proteinase K incubation mix, and mix by vortexing for 5 s.**

7. **Incubate for 15 min at 45°C using a shaker-incubator at 1400 rpm. After incubation, centrifuge briefly (1–2 s at 500–1000 x *g*) to remove drops from the inside of the tube lid. Set the temperature of the shaker-incubator to 65°C for later use.**

   Note: For purification of RNA or total RNA, including miRNA, from fibrous tissue (e.g., skin, heart, aorta, or skeletal muscle), increase incubation time to 2 h.

8. **Centrifuge for 1 min at 11,000 x *g*.**

9. **Pipet the lower, clear phase directly into a PAXgene Shredder spin column (lilac) placed in a 2 ml processing tube, and centrifuge for 3 min at maximum speed (but do not exceed 20,000 x *g*).**

10. **Continue with step 11 of the protocol for "Purification of Total RNA from Sections of PFPE Tissue" from the *PAXgene Tissue RNA Kit Handbook* or the "Purification of Total RNA, Including miRNA, from Sections of PFPE Tissue" from the *PAXgene Tissue miRNA Kit Handbook*.**

Protocol: Purification of Genomic DNA from Sections of PFPE Tissue with Deparaffinization Solution

Starting material

Starting material for purification of genomic DNA should be 1–5 sections of PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue (see the *PAXgene Tissue Container Product Circular* or the *PAXgene Tissue FIX Container (50 ml) Product Circular* for information about tissue fixation, stabilization, and paraffin embedding). Each section should have a thickness of 5–10 µm and a tissue surface area of up to 15 x 15 mm^2. Thicker sections may result in lower DNA yields.

Things to do before starting

- Refer to the *PAXgene Tissue DNA Kit Handbook*.
- Tissue specimens must be fixed, stabilized, processed, and embedded in paraffin according to the *PAXgene Tissue Container Product Circular* or the *PAXgene Tissue FIX Container (50 ml) Product Circular*.
- A shaker-incubator is required in step 4. Set the temperature of the shaker-incubator to 56°C.
- Buffer TD1 and Buffer TD2 may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.

Procedure

1. **Using a microtome, generate a minimum of 1 and a maximum of 5 tissue sections of 5–10 µm thickness from the PFPE tissue.**

   Note: If the PFPE tissue surface has been exposed to air, discard the first 2 or 3 sections.

2. **Place sections in a 1.5 ml microcentrifuge tube.**
3. **Add 160 µl (when processing 1–2 sections per sample) or 320 µl (when processing 3–5 sections per sample) Deparaffinization Solution to the sample and vortex vigorously for 10 s.**
4. **Incubate for 3 min at 56°C, and then cool at room temperature (15–25°C) for approximately 5 min.**
5. **Prepare Proteinase K incubation mix. For each sample add 20 µl Proteinase K to 180 µl Buffer TD1 and mix by vortexing.**

   For example, if processing 10 samples, mix 1800 µl Buffer TD1 and 200 µl Proteinase K.

6. **Add 200 µl Proteinase K incubation mix, and mix by vortexing for 5 s.**
7. **Incubate for 60 min at 56°C using a shaker-incubator at 1400 rpm. After incubation, centrifuge briefly (1–2 s at 500–1000 x *g*) to remove drops from the inside of the tube lid. Set the temperature of the shaker-incubator to 80°C for later use.**
8. **Centrifuge for 1 min at 11,000 x *g*.**
9. **Pipet the lower, clear phase directly into a new 1.5 ml microcentrifuge tube.**

   Note: If RNA-free genomic DNA is required, add 4 µl RNase A (100mg/ml), mix by vortexing, and incubate for 2 min at room temperature.
10. **Continue with step 11 of the protocol for "Purification of Genomic DNA from Sections of PAXgene Treated, Paraffin-Embedded Tissue" from the *PAXgene Tissue DNA Kit Handbook*.**