# PreAnalytiX Supplementary Protocol

## PAXgene® Tissue AllPrep® DNA/RNA

## Simultaneous purification of genomic DNA and total RNA from sections of PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue

This protocol is designed for using the PAXgene Tissue RNA and the PAXgene Tissue DNA Kit for simultaneous purification of genomic DNA and total RNA from the same sections of PFPE tissue. Before beginning, the tissue sample must be fixed and stabilized in one of the PAXgene Tissue Containers, dehydrated, and embedded in paraffin.

**IMPORTANT**: Please read the *PAXgene Tissue RNA Handbook* and 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.

- Xylene
- Staining dishes
- Horizontal working plate
- Ethanol (96–100%, purity grade p.a.)
- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
- Pipets* (10 µl – 1 ml)
- Sterile, aerosol-barrier, RNase-free pipet tips
- Graduated cylinder†
- Variable-speed microcentrifuge,* capable of attaining 1000–8000 x *g*, and equipped with a rotor for 2 ml microcentrifuge tubes

* Ensure that equipment has been checked and calibrated according to the manufacturer's recommendations.

† For the addition of ethanol to Buffer TR3 concentrate and for preparation of 80% ethanol.

- 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, wwww.ependorf.com,† or equivalent)
- Vortex mixer*
- Scalpel
- Crushed ice

# Starting material

Starting material for genomic DNA and RNA purification should be 1 to 5 sections of PFPE (PAXgene Tissue fixed, paraffin-embedded) tissue (see the *PAXgene Tissue Container 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 225 $mm^2$. Thicker sections may result in lower nucleic acids yields.

# Important points before starting

- If working with RNA for the first time, see "Appendix A: General Remarks on Handling RNA" in the *PAXgene Tissue RNA Kit Handbook*.
- Ensure that the kit boxes are intact and undamaged, and that buffers have not leaked. Do not use a kit that has been damaged.
- When using a pipet, ensure that it is set to the correct volume, and that liquid is carefully and completely aspirated and dispensed.
- To avoid transferring samples to the wrong tube or spin column, ensure that all tubes and spin columns are properly labeled. Label the lid and the body of each tube. For spin columns, label the body of its processing tube.
- Close each tube or spin column after liquid is transferred to it.
- Spillages of samples and buffers during the procedure may reduce the yield and purity of RNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature (15–25°C).

* Ensure that equipment has been checked and calibrated according to the manufacturer's recommendations.

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

# Things to do before starting

- Before using the kit for the first time, prepare 80% ethanol by mixing 24 ml ethanol (96–100%) and 6 ml RNase-free water (supplied).
- A shaker–incubator is required in steps 7, 10, and 21. Set the temperature of the shaker–incubator to 37°C.
- Buffer TR1 may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer TR1 before use. Add 10 µl β-ME per 1 ml Buffer TR1. Dispense in a fume hood and wear appropriate protective clothing. Buffer TR1 containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.
- Buffers TR3, TD3, and TD4 are supplied as concentrates. Before using for the first time, add ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.

# Procedure

1. **Using a microtome, generate up to 5 tissue sections of 5–10 µm thickness from the PFPE tissue.**

   **Note**: If the sample 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 650 µl xylene to the sample. Vortex vigorously for 20 s, and incubate for 3 min on the benchtop (at 15–25°C).**
4. **Add 650 µl ethanol (96–100%, purity grade p.a.), and mix by vortexing for 20 s.**
5. **Centrifuge at maximum speed for 5 min (but do not exceed 20,000 x g).**

   To prevent damage to processing tubes, do not exceed 20,000 x g.

6. **Remove the supernatant by pipetting. Do not remove any of the pellet.**

   **Note**: In some cases the pellet may be loose. Remove the supernatant carefully.

   **Note**: It is essential to remove all residual alcohol from the pellet.

   **Note**: The pellet might contain residual paraffin; however, the paraffin will dissolve during digestion with proteinase K and will not affect the PAXgene Tissue AllPrep DNA/RNA procedure.

7. **Open the tube and incubate for 5 min at 37°C using a shaker–incubator for evaporation of residual alcohol. After incubation, set the temperature of the shaker–incubator to 45°C for use in step 10.**
8. **Add 150 µl Buffer TR1, and resuspend the pellet by vortexing for 20 s.**
9. **Add 130 µl RNase-free water to the resuspended pellet. Then add 20 µl proteinase K and mix by vortexing for 5 s.**

   **Note**: Do not mix Buffer TR1 and proteinase K together before adding them to the sample.

10. **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 use in step 21.**

    **Note**: For purification of DNA and RNA from fibrous tissue (e.g., skin, heart or skeletal muscle, aorta), incubate for 2 h at 45°C.

11. **Add 200 µl Buffer TR1, resuspend the pellet by vortexing for 5 s, and centrifuge briefly (1–2 s at 500–1000 x *g*) to remove drops from the inside of the tube lid.**

12. **Pipet the sample, including any precipitate that may have formed, into a PAXgene DNA spin column placed in a 2 ml processing tube, and centrifuge for 1 min at 6000 x *g*.**

    If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the PAXgene DNA spin column is empty.

13. **Place the PAXgene DNA spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25°C) or at 4°C for up to 24 hours for DNA purification in steps 23–29. Use the flow-through for RNA purification in steps 14–22.**

    **Note**: Do not store the PAXgene DNA spin column at room temperature or at 4°C for more than 24 hours. Do not freeze the column.

**Total RNA purification**

14. **Add 250 µl ethanol (96–100%, purity grade p.a.) to the flowthrough from step 13. Mix by vortexing for 5 s, and centrifuge briefly (1–2 s at 500–1000 x *g*) to remove drops from the inside of the tube lid.**

    **Note**: The length of the centrifugation must not exceed 1–2 s, as this may result in pelleting of nucleic acids and reduced yields of total RNA.

    **Note**: A precipitate may form after the addition of ethanol, but this will not affect the PAXgene Tissue RNA procedure.

15. **Pipet the sample, including any precipitate that may have formed, into a PAXgene RNA MinElute® spin column (red) placed in a 2 ml processing tube, and centrifuge for 1 min at 8000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

    If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the PAXgene RNA MinElute spin column is empty.

16. **Pipet 700 µl Buffer TR2 into the PAXgene RNA MinElute spin column. Centrifuge for 20 s at 8000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

* Flow-through contains Buffer TR1 or Buffer TR2 and is therefore not compatible with bleach.

17. **Pipet 500 µl Buffer TR3 into the PAXgene RNA MinElute spin column, and centrifuge for 20 s at 8000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.**

    **Note**: Buffer TR3 is supplied as a concentrate. Ensure that ethanol is added to Buffer TR3 before use (see "Things to do before starting", page 3).

18. **Pipet 500 µl 80% ethanol into the PAXgene RNA MinElute spin column, and centrifuge for 2 min at 8000 x *g*.**

19. **Discard the processing tube containing the flow-through, and place the PAXgene RNA MinElute spin column in a new 2 ml processing tube. Open the cap of the spin column, and centrifuge for 5 min at maximum speed.**

20. **Discard the processing tube containing the flow-through. Place the PAXgene RNA MinElute spin column in a 1.5 ml microcentrifuge tube, and pipet 14–40 µl Buffer TR4 directly onto the PAXgene RNA MinElute spin column membrane. Centrifuge for 1 min at maximum speed to elute the RNA.**

    **Note**: It is important to wet the entire membrane with Buffer TR4 to achieve maximum elution efficiency.

    Smaller volumes of Buffer TR4 can be used to obtain a higher total RNA concentration, but this will influence the overall yield.

    The dead volume of the PAXgene RNA MinElute spin column is 2 µl; elution with 14 µl Buffer TR4 results in an eluate with a volume of 12 µl.

21. **Incubate the eluate for 5 min at 65°C in the shaker–incubator (from step 10) without shaking. After incubation, chill immediately on ice.**

    **Note**: This incubation at 65°C denatures the RNA for downstream applications. Do not exceed the incubation time or temperature.

22. **If the RNA samples will not be used immediately, store at –15 to –30°C or –70°C. Since the RNA remains denatured after repeated freezing and thawing, it is not necessary to repeat the incubation at 65°C.**

    **Note**: For quantification in Tris buffer, use the relationship $A_{260} = 1 \Rightarrow 44$ µg/ml. See "Appendix A: General Remarks on Handling RNA" in the *PAXgene Tissue RNA Kit Handbook*.

**Genomic DNA purification**

23. **Pipet 350 µl Buffer TD3 into the PAXgene Tissue DNA spin column from step 13, and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

    **Note**: Buffer TD3 is supplied as a concentrate. Ensure that ethanol is added to Buffer TD3 before use (see "Things to do before starting", page 3).

24. **Add 25 µl proteinase K to 50 µl Buffer TD3 in a 1.5 ml microcentrifuge tube. Mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.**

    For example, if processing 10 samples, add 250 µl proteinase K to 500 µl DNA Buffer TD3. Use the 1.5 ml microcentrifuge tubes supplied with the kit.

25. **Pipet the proteinase K incubation mix (75 µl) directly onto the PAXgene DNA spin column, and incubate for 30 min at ambient temperature (20–30°C).**

26. **Pipet 350 µl Buffer TD3 into the PAXgene Tissue DNA spin column, and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

27. **Pipet 500 µl Buffer TD4 into the PAXgene DNA spin column, and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.**

28. **Centrifuge for 3 min at maximum speed (but not to exceed 20,000 x *g*) to dry the membrane completely.**

    This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

29. **Discard the processing tube containing the flow-through. Place the PAXgene DNA spin column in a 1.5 ml microcentrifuge tube, and pipet 20–200 µl Buffer TD5 directly onto the PAXgene DNA spin column membrane. Centrifuge for 1 min at maximum speed (but not to exceed 20,000 x *g*) to elute the DNA.**

    Incubating the PAXgene DNA spin column loaded with Buffer TD5 for 5 min at room temperature (15–25°C) before centrifugation generally increases DNA yield.

* Flow-through contains Buffer TD2 or Buffer TD3 and is therefore not compatible with bleach.

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX® or 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.

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



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