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Supplementary Protocol

Purification of DNA from the PAXgene® Saliva Collector Using the QIAamp® DNA Mini Kit; Spin Procedure

This protocol is for using the QIAamp DNA Mini Kit for purification of DNA from human saliva collected into PAXgene Saliva Collector.

Important: Saliva sample must be collected into PAXgene Saliva Collector. For specimen collection and stabilization, read the *PAXgene Saliva Collectors (25) Handbook*.

For preparation of DNA, read the *QIAamp DNA Mini Kit Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections before beginning this procedure.

For molecular biology applications only. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Automated purification of genomic DNA on QIAcube® instruments

Purification of genomic DNA can be fully automated on the QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN® spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the QIAamp DNA Mini Kit for purification of DNA.

The QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect

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.

- Ethanol (96–100%)
- 1.5 ml microcentrifuge tubes
- Pipets and pipet tips
- Vortexer
- Water bath or heating block at 56°C/70°C
- Standard laboratory centrifuge or microcentrifuge
- RNase A (DNase-free; optional)

Starting material

Starting material for DNA purification is human saliva collected into PAXgene Saliva Collector (see the *PAXgene Saliva Collectors (25) Handbook* for information about collection and stabilization).

Important notes

Storage of PAXgene Saliva samples

Saliva samples collected with the PAXgene Saliva Collector have stable DNA levels for at least 24 months at temperatures up to 25°C. In addition, PAXgene Saliva can be frozen long term at –20°C (–15 to –30°C) or –80°C (–65 to –90°C) when transferred into a suitable cryovial (see **Resources** section at **www.PreAnalytiX.com** or **www.qiagen.com** for latest results on long-term storage).

Yield and quality of purified DNA

Using PAXgene Saliva, the QIAamp DNA Mini procedure yields pure DNA, indicated by A_{260}/A_{280} ratios typically greater than 1.7. Depending on the quality of the starting material, the purified DNA is greater than 20 kb in size. DNA of this length and purity is suitable for archiving as well as for immediate use in all downstream applications.

Important points before starting

- Ensure that the kit boxes are intact and undamaged and that the 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.
- Spilling samples and buffers during the procedure may reduce the yield and purity of DNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature (15–25°C).

- QIAamp Mini spin columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

Things to do before starting

- Preheat a water bath or heating block to 56°C.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions in the *QIAamp DNA Mini Handbook*.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

Procedure

1. Pipet 20 µl QIAGEN Protease (or Proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.
2. Mix PAXgene Saliva sample thoroughly to yield a homogenous solution and add 200 µl PAXgene Saliva to the microcentrifuge tube.

   If the sample volume is less than 200 µl, add the appropriate volume of PBS.

   Note: For greater yield, it is possible to use up to 400 µl PAXgene Saliva.

   Note: It is possible to add QIAGEN Protease (or Proteinase K) to samples that have been dispensed already into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.
3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.

   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   If the sample volume is larger than 200 µl, increase the amount of QIAGEN Protease (or Proteinase K) and Buffer AL proportionally; for example, a 400 µl sample will require 40 µl QIAGEN Protease (or Proteinase K) and 400 µl Buffer AL. If sample volumes larger than 400 µl are required, use of QIAamp DNA Blood Midi or Maxi Kits is recommended; these can process up to 2 ml or up to 10 ml of sample, respectively.

   If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

   Note: Do not add QIAGEN Protease or Proteinase K directly to Buffer AL.

4. Incubate at 56°C for 10 min.

   DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation times have no effect on yield or quality of the purified DNA.

5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid. Heat water bath or heating block to 70°C and incubate the required volume of elution Buffer AE at 70°C for use in step 11.

   Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

   If the sample volume is greater than 200 µl, increase the amount of ethanol proportionally; for example, a 400 µl sample will require 400 µl of ethanol.

6. Carefully apply the mixture from step 5 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.*

   Close each spin column to avoid aerosol formation during centrifugation.

   Centrifugation is performed at 6000 x *g* (8000 rpm) to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty.

7. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.*

   Note: It is not necessary to increase the volume of Buffer AW1 if the original sample volume is larger than 200 µl.

8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

9. **Recommended:** Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

   This step helps to eliminate the chance of possible Buffer AW2 carryover.

10. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach.

QIAamp Mini spin column and add 50–200 µl of pre-heated Buffer AE or molecular-grade water. Incubate at 70°C for 5 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.

11. **Optional:** For greater yield, add additional 50–200 µl pre-heated Buffer AE or molecular-grade water onto QIAamp spin column. Incubate at 70°C for 5 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.

    Optional: For greater yield and concentration, take the eluate from step 10 from the collection tube and pipet it onto the QIAamp Mini spin column again. Incubate another 5 min at 70°C, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.

    A 200 µl sample of PAXgene Saliva typically yields 1–2 µg of DNA in 50 µl Buffer AE (20–40 ng/µl) with an A_{260}/A_{280} ratio of 1.7–1.9.

    Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

    For long-term storage of DNA, eluting in Buffer AE and storing at –30 to –15°C is recommended, because DNA stored in water is subject to acid hydrolysis.

Document Revision History

Date	Changes
08/2021	Initial release

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