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Supplementary Protocol

Purification of DNA from the PAXgene® Saliva Collectors using the Puregene® Cell Kit

This protocol is for using the Puregene Cell Kit (cat. nos. 158043 and 1158046, former Gentra® Puregene Cell Kit) for purification of DNA from human saliva collected into PAXgene Saliva Collector.

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

For preparation of DNA, read the *Puregene DNA 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.

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.

- 100% isopropanol
- 70% ethanol *
- 1.5 ml microcentrifuge tubes, 15 ml centrifuge tubes, or 50 ml centrifuge tubes
- Pipets and pipet tips
- Vortexer
- Standard laboratory centrifuge or microcentrifuge
- Water baths
- Crushed ice
- Glycogen Solution (500 µl; cat. no. 158930) or Glycogen Powder (e.g. Sigma-Aldrich, cat. no. G0885)
- Proteinase K (e.g., QIAGEN Proteinase K (>600 mAU/ml; 2 ml, cat. no. 19131; 10 ml, cat. no. 19133) or Puregene Proteinase K (650 µl, cat. no. 158143; 5 ml, cat. no. 158146)
- RNase A Solution (cat. no. 158153 or 158156) for the optional RNase treatment

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

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 Puregene DNA procedure yields pure DNA, indicated by A260/A280 ratios typically greater than 1.7. Depending on the quality of the saliva specimen, the purified DNA can be greater than 50 kb in size. DNA of this length and purity is suitable for archiving as well as for immediate use in all downstream applications.

Processing different volumes of PAXgene Saliva

Table 1 provides information about scaling the Puregene reagents for use with different volumes of PAXgene Saliva starting material.

Table 1. Reagent volumes for scaling PAXgene Saliva

	Volume of PAXgene Saliva (ml)						
	0.2	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
Tube size (ml)	1.5	1.5	15	15	15	15	15
Cell Lysis Solution (µl)	50	125	250	375	500	625	750
Proteinase K (µl)	10	25	50	75	100	125	150
Protein Precipitation Solution (µl)	85	213	425	638	850	1063	1275
Isopropanol (ml)	0.250	0.625	1.25	1.875	2.5	3.125	3.75
Glycogen Solution (µl)	2	5	10	15	20	25	30
70% ethanol (ml)	0.3	0.75	1.5	2	3	3.75	4
DNA Hydration Solution	100	150	200	250	300	350	400

Important points before starting

- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature (15–25°C).
- Choose ■ if processing 200 µl PAXgene Saliva samples; choose ▲ if processing 1 ml PAXgene saliva samples; choose ● if processing 3 ml PAXgene Saliva samples.
- For additional PAXgene Saliva sample volumes, see Table 1.
- For further information and troubleshooting, please refer to *Puregene DNA Handbook*.

Things to do before starting

- Preheat a water bath or heating block to 65°C for use in step 20 of the procedure.
- Frozen PAXgene Saliva samples should be thawed quickly in a 37°C water bath or heating block with mild agitation and stored on ice before beginning the procedure.
- For preparing a Glycogen Solution from powder, dissolve 10 mg Glycogen Powder in 500 µl autoclaved ultrapure water and mix by inverting 50 times. Allow Glycogen Powder to dissolve for at least 24 h at 4°C, meanwhile invert periodically. Filter Glycogen Solution and store in aliquots at –20°C.

Procedure

1. Dispense ■ 50 µl, ▲ 250 µl, or ● 750 µl Cell Lysis Solution into a ■ 1.5 ml microcentrifuge tube, ▲15 ml centrifuge tube, or ● 15 ml centrifuge tube.
2. Add ■ 200 µl, ▲1 ml, or ● 3 ml PAXgene Saliva.

   Ensure that PAXgene Saliva is thoroughly mixed to yield a homogenous solution prior to taking aliquot.
3. Incubate for 15 min at room temperature.
4. Add ■ 10 µl, ▲50 µl, or ● 150 µl Proteinase K and mix by inverting 3 times.
5. Vortex vigorously at high speed for 20 s to mix.
6. Incubate for 10 min at room temperature.
7. **Optional:** If RNA-free DNA is required, add ■ 1.25 µl, ▲ 6.25 µl, or ● 18.75 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Incubate for 1 min on ice to quickly cool the sample.

   Samples can be incubated at 37°C for up to 1 h.
8. Add ■ 85 µl, ▲ 425 µl, or ● 1.275 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
9. Incubate for 10 min on ice.

   Incubation on ice is important to ensure a tight pellet in the next step.
10. Centrifuge for 10 min at ■ 2000 x *g*, ▲ 4000 x *g*, or ● 4000 x *g*.

    The precipitated proteins should form a tight, green pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat centrifugation.
11. Pipet ■ 250 µl, ▲1.25 ml, or ● 3.75 ml isopropanol, and ■ 2 µl, ▲10 µl, or ● 30 µl Glycogen Solution into a clean ■ 1.5 ml, ▲15 ml, or ● 15 ml centrifuge tube.
12. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into the tube containing isopropanol and Glycogen Solution. Keep the remaining samples on ice meanwhile to ensure that pellets remains tight.

    Be sure that the protein pellet is not dislodged during pouring.

13. Mix by inverting gently 50 times.
14. Centrifuge for 5 min at ■ 2000 x *g*, ▲ 4000 x *g*, or ● 4000 x *g*.
15. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
16. Add ■ 300 µl, ▲1.5 ml, or ● 4 ml of 70% ethanol and invert several times to wash the DNA pellet.
17. Centrifuge for 1 min at ■ 2000 x *g*, ▲ 4000 x *g*, or ● 4000 x *g*.
18. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to airdry for 5–10 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
19. Add ■ 100 µl, ▲200 µl, or ● 400 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
20. Incubate at 65°C for 1 h to dissolve the DNA.
21. Incubate at room temperature overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Document Revision History

Date	Changes
08/2021	Initial revision
11/2022	Changed "Gentra Puregene" to "Puregene". Included Proteinase K and RNase A solution in Equipment & Reagents. Improved verbiage in procedure.

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