QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from 1–3 ml amniotic fluid using the Gentra® Puregene® Tissue Kit or Gentra Puregene Mouse Tail Kit

This protocol is designed for purification of DNA from 1–3 ml samples of amniotic fluid containing 0.5–2 x 10^6 cells using the Gentra Puregene Tissue Kit or Gentra Puregene Mouse Tail Kit.

Gentra Puregene Kits enable purification of high-molecular-weight DNA from a variety of sample sources. The convenient purification procedure removes contaminants and enzyme inhibitors, and purified DNA is ready for immediate use in sensitive downstream applications or for archiving. Purified DNA typically has an A_{260}/A_{280} ratio between 1.7 and 1.9, and is up to 200 kb in size.

IMPORTANT: Please read the *Gentra Puregene Handbook*, paying careful attention to the safety information, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, consult the appropriate material safety data sheets (MSDSs), available from the product supplier. The Gentra Puregene Tissue Kit and the Gentra Puregene Mouse Tail Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- If RNase A treatment is required: Gentra Puregene Tissue Kit (100 mg), (4 g), or (33 g), cat. nos. 158622, 158667, and 158689
- If no RNase A treatment is required: Gentra Puregene Mouse Tail Kit (100 mg) or (4 g), cat. nos. 158222 and 158267
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water baths heated to 55°C and 65°C
- Vortexer
- Crushed ice

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- Recommended: Glycogen Solution (500 μl), cat. no. 158930 (if DNA yields are expected to be <20 μg)
- Optional: Water bath heated to 37°C if RNase A treatment is required

Things to do before starting

- Heat water baths to 55°C and 65°C for use in steps 5b and 21 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Add 1–3 ml of amniotic fluid to a 1.5 ml microcentrifuge tube.**

   Note: If using 3 ml of amniotic fluid, add 1.5 ml to the microcentrifuge tube, centrifuge as indicated in step 2, add the remaining 1.5 ml to the tube, and repeat the centrifugation.

2. **Centrifuge at 13,000–16,000 x *g* for 5 s.**
3. **Remove the supernatant leaving behind 10–20 μl residual liquid, and vortex vigorously for 20 s.**
4. **Add 300 μl Cell Lysis Solution to the resuspended cells, and mix by pipetting up and down.**
5. **Complete cell lysis by following step 5a or 5b below:**

5a. **Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C until the solution is homogeneous. Proceed with step 6.**

Note: Samples are stable in Cell Lysis Solution for at least 2 years at room temperature (15–25°C).

5b. **If maximum DNA yield is required, add 1.5 μl Puregene Proteinase K (20 mg/ml), and mix by inverting 25 times. Incubate at 55°C for 3 h to overnight. If possible, invert tube periodically during the incubation. Proceed with step 6.**

Note: Samples are stable in Cell Lysis Solution for at least 2 years at room temperature (15–25°C).

6. **If you wish to include an optional RNase treatment, go to step 6a, otherwise proceed with step 6b.**

6a. **Add 1.5 μl RNase A Solution to the cell lysate, and mix by inverting the tube 25 times. Incubate at 37°C for 15 min to 1 h. Proceed with step 7.**

6b. **No RNase A treatment is required. Proceed with step 7.**

7. **Quickly cool the sample to room temperature by placing on ice for 1 min.**
8. **Add 100 μl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**

9. **Centrifuge at 13,000–16,000 x *g* for 3 min.**

   The precipitated proteins should form a tight pellet. If the protein pellet is not visible, vortex vigorously for 20 s at high speed, and then incubate on ice for 5 minutes. Centrifuge at 13,000–16,000 x *g* for 3 min.

10. **Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube.**
11. **Recommended: Add 0.5 µl Glycogen Solution (20 mg/ml).**
12. **Add the supernatant from step 9 by pouring carefully.**
13. **Mix by inverting gently 50 times.**
14. **Centrifuge for 13,000–16,000 x *g* for 5 min.**

    The DNA may be visible as a small white pellet.

15. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
16. **Add 300 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
17. **Centrifuge at 13,000–16,000 x *g* for 1 min.**
18. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**

    The pellet might be loose and easily dislodged.

19. **Allow DNA to air dry at room temperature for 10–15 min.**
20. **Add 50 µl DNA Hydration Solution to the tube containing the pellet.**
21. **Incubate at 65°C for 1 h to dissolve the DNA.**
22. **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.**

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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