QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from up to 30 *Drosophila melanogaster* using the Gentra® Puregene® Cell Kit

This protocol is designed for purification of DNA from 1, 10–15, or 20–30 *Drosophila melanogaster* using the Gentra Puregene Cell Kit.

Gentra Puregene Cell 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. Gentra Puregene Cell Kits 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 no RNase treatment is required: Gentra Puregene Cell Kit (6.7 x 10^9), cat. no. 158388
- If RNase treatment is required: Gentra Puregene Cell Kit (2 x 10^8), Gentra Puregene Cell Kit (8 x 10^8), or Gentra Puregene Cell Kit Plus (6.7 x 10^9), cat. nos. 158745, 158767, and 158788
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 1.5 ml microcentrifuge tubes
- Microfuge tube pestle
- Microcentrifuge
- Water bath heated to 65°C
- Vortexer
- Crushed ice

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

- Optional: Water bath heated to 37°C if RNase A treatment is required

Important point before starting

- In some steps of the procedure, one of three choices can be made. Choose ■ if processing 1 fly; choose ◆ if processing 10–15 flies; choose ● if processing 20–30 flies.

Things to do before starting

- Heat water bath to 65°C for use in steps 3 and 17 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Dispense ■ 100 µl, ◆ 300 µl, or ● 600 µl Cell Lysis Solution into a 1.5 ml microcentrifuge tube and place the tube on ice.**

   Note: The solution will turn cloudy.

2. **Add the appropriate number of flies to the chilled Cell Lysis Solution. Remove sample from ice and homogenize thoroughly using a microfuge tube pestle. Place sample back on ice until ready to proceed with the next step.**

3. **Incubate at 65°C for 15 min.**

4. **If you wish to include an optional RNase treatment, go to step 4a, otherwise proceed with step 4b.**

4a. **Add ■ 0.5 µl, ◆ 1.5 µl, or ● 3 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 5.**

4b. **No RNase A treatment is required. Proceed with step 5.**

5. **Quickly cool the sample to room temperature (15–25°C) by placing on ice for 1 min.**

6. **Add ■ 33 µl, ◆ 100 µl, or ● 200 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed. ■ Place samples on ice for 5 min.**

7. **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 tight, vortex vigorously for 20 s at high speed, and then incubate on ice for 5 min. Centrifuge at 13,000–16,000 x *g* for 3 min.

8. **Pipet ■ 100 µl, ◆ 300 µl, or ● 600 µl isopropanol into a clean 1.5 ml microcentrifuge tube. Add the supernatant from the previous step by pouring carefully.**

   Make sure not to dislodge the protein pellet when transferring the supernatant.

9. **Mix by inverting gently 50 times.**

10. **Centrifuge at 13,000–16,000 x *g* for ■ 5 min, ◆ 1 min, or ● 1 min.**
11. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
12. **Add ■ 100 µl, ◆ 300 µl, or ● 600 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
13. **Centrifuge at 13,000–16,000 x *g* for 1 min.**
14. **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.
15. **Allow DNA to air dry at room temperature for 10–15 min.**
16. **Add ■ 20 µl, ◆ 50 µl, or ● 100 µl of DNA Hydration Solution.**
17. **Incubate at 65°C for 1 h to dissolve the DNA.**
18. **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.**

    If particulates are present in the hydrated DNA sample, centrifuge at 13,000–16,000 x *g* for 5–10 min, and then transfer the supernatant to a clean 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.