



## QIAGEN Supplementary Protocol:

### Purification of archive-quality DNA from *Chlamydomonas* using the Gentra® Puregene® Tissue Kit or Gentra Puregene Mouse Tail Kit

This protocol is designed for purification of DNA from 1–2 ml samples of *Chlamydomonas* 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 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 65°C and 55°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

### Things to do before starting

- Heat water baths to 55°C and 65°C for use in step 4 and 19 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

### Procedure

1. **Place 7–15 mg fresh tissue (taken from a plate), or the pellet obtained from 1–2 ml of liquid culture, into a 1.5 ml microcentrifuge tube.**

**Note:** It may be necessary to vary the amount of starting material depending on the age of the culture and the growth conditions.

2. **Add 300 µl Cell Lysis Solution, and vortex vigorously for 5–10 s at high speed.**
3. **Add 1.5 µl Puregene Proteinase K (20 mg/ml), and mix by inverting the tube 25 times.**
4. **Complete cell lysis by incubating at 55°C for 60–90 min. Invert the tube 10 times every 30 min.**
5. **If you wish to include an optional RNase treatment, go to step 5a, otherwise proceed with step 5b.**

- 5a. **Add 1.5 µl RNase A Solution to the cell lysate, and mix by inverting the tube 25 times. Incubate at 37°C for 15–30 min. Proceed with step 6.**

- 5b. **No RNase A treatment is required. Proceed with step 6.**

6. **Quickly cool the sample to room temperature (15–25°C) by placing on ice for 1 min.**
7. **Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
8. **Incubate on ice for 15 min.**

9. **Centrifuge at 13,000–16,000 x g for 5 min.**

The precipitated proteins will form a tight, green pellet. If the pellet is not tight, vortex vigorously for 20 s at high speed, and then incubate on ice for 15 min. Centrifuge at 13,000–16,000 x g for 5 min.

10. **Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube.**

11. **Add the supernatant from step 9 by pouring carefully.**

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

12. **Invert the tube gently 50 times to mix the sample.**

13. **Centrifuge at 13,000–16,000 x g for 1 min.**

The DNA may be visible as a small pellet that ranges in color from off-white to light green.

14. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
15. Add 300  $\mu$ l of 70% ethanol, and invert several times to wash the DNA pellet.
16. Centrifuge at 13,000–16,000 x g for 1 min.
17. Allow DNA to air dry at room temperature for 10–15 min.
18. Add 50  $\mu$ l DNA Hydration Solution to the tube containing the pellet.
19. Incubate at 65°C for 1 h to dissolve the DNA.
20. 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.

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