



QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from nematodes or nematode suspensions using the Gentra® Puregene® Tissue Kit

This protocol is designed for purification of DNA from 40–90 mg (50–75 μ l), 80–180 mg (100–150 μ l), or 20 g samples of nematodes or nematode suspensions using the Gentra Puregene Tissue 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 is intended for molecular biology applications. This product is 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.

- Gentra Puregene Tissue Kit (100 mg) cat. no. 158622, Gentra Puregene Tissue Kit (4 g) cat. no. 158667, or Gentra Puregene Tissue Kit (33 g) cat. no. 158689
- Optional: Glycogen Solution (500 μ l) cat. no. 158930
- 1.5 ml microcentrifuge tube and 15 ml centrifuge tubes (if processing up to 180 mg nematodes or nematode suspension), or 50 ml centrifuge tubes (if processing 20 g nematodes)
- Standard laboratory centrifuge
- Microcentrifuge
- Water baths heated to 55°C and 65°C
- 70% ethanol*
- Isopropanol
- Crushed ice
- Optional: Water bath heated to 37°C if RNase A treatment is required

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

Important points before starting

- In some steps of the procedure, one of 3 choices can be made. Choose ■ if processing 40–90 mg (50–75 μ l) nematode suspension; choose ◆ if processing 80–180 mg (100–150 μ l) nematode suspension; choose ● if processing 20 g nematodes.

Things to do before starting

- Heat water baths to 55°C for use in step 10 and 65°C for use in step 24 of the procedure.
- Optional: Heat water bath to 37°C for use in step 11 of the procedure if RNase A treatment is required.

Procedure

1. Transfer nematodes (e.g., *Caenorhabditis elegans*) from 100 mm culture plates to ■ one 15 ml tube, ◆ two 15 ml tubes, or ● one 50 ml tube by rinsing 3 times with distilled water, using approximately 4 ml per rinse.
2. Centrifuge for 3 min at 2000 x g to pellet the worms.
3. Place the tube on ice for at least ■ 30 s, ◆ 30 s, or ● 3 min to slow down the worms. Discard the supernatant.
4. Resuspend the worm pellet in ■ 1 ml, ◆ 1 ml, or ● 30 ml distilled water. Transfer nematode suspension to ■ a 1.5 ml microcentrifuge tube, ◆ a 15 ml centrifuge tube, or ● a 50 ml centrifuge tube on ice.
5. Centrifuge for 3 min at ■ 13,000–16,000 x g, ◆ 2000 x g, or ● 2000 x g.
6. Place the tube on ice for at least ■ 30 s, ◆ 30 s, or ● 3 min.
7. Discard the supernatant.
8. Add ■ 600 μ l, ◆ 3 ml, or ● 18 ml Cell Lysis Solution, and mix by inverting several times.
9. Add ■ 3 μ l, ◆ 15 μ l, or ● 90 μ l Puregene Proteinase K (20 mg/ml), and mix by inverting 25 times.
10. Complete cell lysis by incubating at 55°C for 3 h to overnight, until all particulates are dissolved completely. Invert tube periodically if possible.
11. If you wish to include an optional RNase treatment, go to step 11a, otherwise proceed with step 11b.
- 11a. Add ■ 3 μ l, ◆ 15 μ l, or ● 90 μ l 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 12.
- 11b. No RNase A treatment is required. Proceed with step 12.
12. Incubate on ice for 1 min to quickly cool the sample to room temperature (15–25°C).
13. Add ■ 200 μ l, ◆ 1 ml, or ● 6 ml Protein Precipitation Solution to the cell lysate, and vortex vigorously for 20 s at high speed.

14. **Centrifuge at ■ 13,000–16,000 x g for 3 min, ◆ 2000 x g for 10 min, or ● 2000 x g for 10 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, ◆ 2000 x g for 10 min, or ● 2000 x g for 10 min.

15. **Pipet ■ 600 μ l, ◆ 3 ml, or ● 18 ml isopropanol into a clean ■ 1.5ml, ◆ 15 ml, or ● 50 ml centrifuge tube. Add the supernatant from the previous step by pouring carefully.**

Be sure the protein pellet is not dislodged during pouring.

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

17. **Centrifuge at ■ 13,000–16,000 x g for 1 min, ◆ 2000 x g for 5 min, or ● 2000 x g for 5 min.**

The DNA should be visible as a small white pellet.

18. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**

19. **Add ■ 600 μ l, ◆ 3 ml, or ● 18 ml of 70% ethanol, and invert several times to wash the DNA pellet.**

20. **Centrifuge for 1 min at ■ 13,000–16,000 x g, ◆ 2000 x g, or ● 2000 x g.**

21. **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.

22. **Allow DNA to air dry at room temperature for 10–15 min.**

23. **Add ■ 100 μ l, ◆ 200 μ l, or ● 1 ml DNA Hydration Solution.**

24. **Incubate at 65°C for 1 h to dissolve the DNA.**

25. **Incubate at room temperature (15–25°C) overnight. Ensure tube lid 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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