QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from *Eimeria* oocysts using Gentra® Puregene® Cell Kits

This protocol is designed for purification of DNA from 0.5–2 mg samples of *Eimeria* oocysts using Gentra Puregene Cell Kits.

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. 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 A treatment is required: Gentra Puregene Cell Kit (6.7×10^9) cat. no. 158388
- If RNase A treatment is required: Gentra Puregene Cell Kit (2×10^7) cat. no. 158722, Gentra Puregene Cell Kit (2×10^8) cat. no. 158745, Gentra Puregene Cell Kit (8×10^8) cat. no. 158767, Gentra Puregene Cell Kit Plus (6.7×10^9) cat. no. 158788
- Recommended: Glycogen Solution (500 µl) cat. no. 158930 (if the DNA yield is expected to be <0.3 µg)
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Microfuge tube pestle
- Water bath heated to 65°C
- 70% ethanol*
- Isopropanol

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

- Crushed ice

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

Things to do before starting

- Heat water bath to 65°C for use in steps 4 and 20 of the procedure.
- Optional: Heat water bath to 37°C for use in step 5 of the procedure if RNase A treatment is required.

Procedure

1. **Transfer purified oocyst suspension (0.5–2 mg wet weight) to a 1.5 ml microcentrifuge tube, and centrifuge at 13,000–16,000 x *g* for 20 s to pellet the tissue.**
2. **Remove as much supernatant as possible.**
3. **Add 100 µl Cell Lysis Solution, and homogenize using a microfuge tube pestle.**
4. **Incubate lysate at 65°C for 15 min.**
5. **If you wish to include an optional RNase treatment, go to step 5a, otherwise proceed with step 5b.**

5a. **Add 0.5 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.**

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

6. **Incubate on ice for 1 min to quickly cool the sample to room temperature (15–25°C).**
7. **Add 33 µl Protein Precipitation Solution to the cell lysate, and vortex vigorously for 20 s at high speed.**
8. **Incubate on ice for 5 min.**
9. **Centrifuge for 3 min at 13,000–16,000 x *g*.**

   The precipitated proteins should form a tight pellet.

10. **Pipet 100 µl isopropanol into a clean 1.5 ml microcentrifuge tube. Add the supernatant from the previous step by pouring carefully.**

    Be sure the protein pellet is not dislodged during pouring.

11. **Recommended: add 1 µl Glycogen Solution (20 mg/ml) per 100 µl isopropanol.**
12. **Mix by inverting gently 50 times.**
13. **Centrifuge for 5 min at 13,000–16,000 x *g*.**

    The DNA might be visible as a small white pellet.

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 100 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
16. **Centrifuge for 1 min at 13,000–16,000 x *g*.**
17. **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.
18. **Allow DNA to air dry at room temperature for 10–15 min.**
19. **Add 20 µl DNA Hydration Solution.**
20. **Incubate at 65°C for 1 h to dissolve the DNA.**
21. **Incubate at room temperature (15–25°C) overnight with gentle shaking. 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.