QIAGEN Supplementary Protocol

Purification of archive-quality DNA from 50–75 mg dried or 100–200 mg fresh leaf tissue using the Gentra® Puregene® Cell Kit

This protocol is designed for purification of DNA from 50–75 mg dried or 100–200 mg fresh leaf tissue using the Gentra Puregene Cell 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 Cell Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents

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 treatment is required: Gentra Puregene Cell Kit (6.7×10^9), cat. no. 158388
- If no RNase treatment is required: Gentra Puregene Cell Kit (2×10^8), Gentra Puregene Cell Kit (8×10^8), or Gentra Puregene Cell Kit Plus (6.7×10^9), cat. nos. 158745, 158767, and 158788, respectively
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- Centrifuge tubes, 15 ml
- Centrifuge, capable of attaining 2000 x g, with appropriate rotor for 15 ml tubes
- Tube pestle suitable for use with 15 ml tubes
- Water bath heated to 65°C
- Vortexer
- Crushed ice and ice bucket
- 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.*

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Sample collection and storage

After harvesting, plant tissue should be frozen in liquid nitrogen.* It can then be stored at –80°C for later processing. Ground tissue powder can also be stored at –80°C. Alternatively, tissue can be freeze-dried/lyophilized after harvesting to allow storage at room temperature (15–25°C).

If possible, it is preferable to collect young materials (e.g., leaves, needles) since they contain more cells per weight and therefore result in higher yields of DNA. In addition, young leaves and needles contain less polysaccharides and polyphenolics and are therefore easier to handle.

Things to do before starting

- Heat water bath to 65°C.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Add 50–75 mg dried tissue (finely ground), 100–200 mg frozen tissue (may be finely ground with a mortar and pestle in liquid nitrogen), or 100–200 mg fresh tissue to a 15 ml tube. Work quickly and keep tissue cold to minimize DNase activity.**

   Note: it may be necessary to use a different amount of starting material, depending upon the species, age, tissue preparation, and genome size.

2. **Add 3 ml Cell Lysis Solution to the tissue.**
3. **Vortex dried tissue 1–3 seconds to wet the tissue. Homogenize intact tissue with 30–50 strokes using a tube pestle.**
4. **Complete cell lysis by incubating cell lysate at 65°C for 60 min. After 30 and 60 min invert tube 10 times.**
5. **If you wish to include an optional RNase treatment, go to step 5a, otherwise proceed with step 5b.**

5a. **Add 15 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 6.**

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 1 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
8. **Incubate on ice for 5–15 min.**

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9. **Centrifuge for 10 min at 2000 x *g*.**

   The precipitated proteins should form a tight, green 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 for 10 min at 2000 x *g*.

10. **Pipet 3 ml 100% isopropanol into a clean 15 ml centrifuge tube. Add the supernatant from the previous step by pouring carefully.**

    Be sure the protein pellet is not dislodged during pouring.

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

12. **Centrifuge for 5 min at 2000 x *g*.**

    The DNA might be visible as a small white pellet.

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

14. **Add 3 ml 70% ethanol, and invert several times to wash the DNA pellet.**

15. **Centrifuge for 5 min at 2000 x *g*.**

16. **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.

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

18. **Add 200 µl ml DNA Hydration Solution.**

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

20. **Incubate at room temperature 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.**