Supplementary Protocol November 2018

Whole genome amplification from flash-frozen tissue sections using the REPLI-g® Advanced DNA Single Cell Kit

This protocol is optimized for whole genome amplification from flash-frozen tissue samples using REPLI-g Advanced DNA Single Cell Kit (cat. nos. 150363 and 150365). Depending on the tissue origin, potential inhibitors present in the starting material may have inhibitory effects on amplification. In these cases, we recommend upstream genomic DNA purification (e.g., using a QIAamp® Kit) if sufficient starting material is available prior to whole genome amplification using the protocol "Amplification of Purified Genomic DNA" found in the *REPLI-g Advanced DNA Single Cell Handbook*, which is for use with 1–10 ng eukaryotic DNA.

IMPORTANT: Please read the handbook of *REPLI-g Advanced DNA Single Cell Handbook*, paying attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. The REPLI-g Advanced DNA Single 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 safety data sheets (SDSs), available from the product supplier.

- Water bath or heating block
- Vortexer
- Microcentrifuge tubes
- Microcentrifuge

- Ice
- Pipets and pipet tips
- Nuclease-free water
- TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)

Important points before starting

- Avoid DNA contamination of reagents by using separate laboratory equipment (e.g., pipets, filter pipet tips, reaction vials, etc.). Set up the REPLI-g Advanced DNA Single Cell Kit reaction in a location free of DNA.
- For direct amplification of DNA from cell material, see Protocol: Amplification of Genomic DNA from Single Cells found in the *REPLI-g Advanced DNA Single Cell Handbook*.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.
- DNA yields of approximately 25-35 µg will be present in negative (no template) controls because DNA is generated during the REPLI-g Advanced DNA Single Cell reaction by random extension of primer dimers, generating high-molecular-weight product. This DNA will not affect the quality of the actual samples and will not give a positive result in downstream assays.

Things to do before starting

- Prepare Advanced Buffer DLB by adding 250 µl H_2O sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.

  Note: Reconstituted Advanced Buffer DLB can be stored for 6 months at –20°C. Advanced Buffer DLB is pH-labile.
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 15). All other components can be thawed at room temperature (15–25°C).
- All buffers and reagents should be vortexed before use to ensure thorough mixing.

- Set a water bath or heating block to 30°C.

Procedure

1. **Place 15 µl template DNA into a microcentrifuge tube**.

   The amount of template DNA should be >1 ng. A DNA control reaction can be set up using 10 ng (1 µl) control genomic DNA (e.g., REPLI-g Human Control Kit, cat. no. 150090). Adjust the volume by adding H_20 sc (provided) to the starting volume of your sample.

2. **Add 2 µl Advanced Buffer DLB to the DNA. Mix by vortexing and centrifuge briefly**.
3. **Incubate at room temperature for 3 minutes**.
4. **Add 3 µl Stop Solution to the sample. Mix by vortexing and centrifuge briefly. Store on ice**.
5. **Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex, and then centrifuge briefly**.

   The REPLI-g sc Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 seconds.

6. Prepare a master mix on ice according to Table 1. Mix and centrifuge briefly.

   Important: The master mix should be kept on ice and used immediately upon addition of the REPLI-g sc DNA Polymerase.

   Note: Scale up accordingly if performing several reactions at once by preparing a master mix sufficient for the total number of reactions.

Table 1. Preparation of master mix*

Component	Volume/reaction
REPLI-g sc Advanced Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total reaction volume	**31 µl**

* Volumes provided are sufficient for one 50 µl reaction. To prepare a master mix for multiple reactions, scale up according to the number of reactions and add 10%.

7. **Add 31 µl of master mix to 20 µl denatured DNA (from step 4)**.
8. **Incubate at 30°C for 2 hours**.

   After incubation at 30°C, heat the water bath or heating block up to 65°C if the same water bath or heating block will be used in step 9.
9. **Inactivate REPLI-g sc DNA Polymerase at 65°C for 3 minutes**.
10. **If not being used directly, store amplified DNA at 4°C for short-term storage or –20°C for long-term storage**.

    DNA amplified using the REPLI-g Advanced DNA Single Cell Kit should be treated as genomic DNA with minimal freeze-thaw cycles. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µl.
11. **Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing, array CGH, and quantitative PCR**.

    Note: Typical DNA yields are approximately 25-35 µg per 50 µl reaction and need to be diluted appropriately. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix B of the *REPLI-g Advanced DNA Single Cell Handbook* for an accurate method of quantifying REPLI-g amplified DNA.
12. **Use the correct amount of REPLI-g amplified DNA diluted in water or TE buffer according to the manufacturer's instructions. If performing PCR analysis, dilute an aliquot of amplified DNA 1:100 and use 2 µl of diluted DNA for each PCR reaction**.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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