QIAGEN Supplementary Protocol

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

This protocol is optimized for whole genome amplification from flash-frozen tissue samples using the REPLI-g Single Cell Kit (cat. nos. 150343 and 150345). 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", page 15, of the *REPLI-g Single Cell Handbook*, which is for use with 1–10 ng eukaryotic DNA.

IMPORTANT: Please read the *REPLI-g Single Cell Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. The REPLI-g 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, thermal cycler, 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

- DNA yields of approximately 40 µg will be present in negative (no template) controls because DNA is generated during the REPLI-g Single Cell reaction by primer-multimer formation, generating high-molecular weight DNA. This DNA will not affect the quality of the actual sample and will not give a positive result in downstream assays.

Things to do before starting

- Prepare Buffer DLB by adding 500 µl H_20 sc to the tube provided. Mix thoroughly and centrifuge briefly.

  Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile.
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 5). 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 flash-frozen tissue section (approximately 2 mm³) in a microcentrifuge containing 10 µl TE buffer. Incubate at room temperature (15–25°C) for 10 min, vortexing occasionally.**

   Note: Do not exceed this size of flash-frozen tissue.

2. **Prepare sufficient Buffer D2 (denaturation buffer) for the total number of whole genome amplification reactions (Table 1).**

   Note: The total volume of Buffer D2 given in Table 1 is suitable for up to 6 reactions.

Table 1. Preparation of Buffer D2

Component	Volume*
DTT, 1M	5 µl
Reconstituted Buffer DLB†	55 µl
Total volume	**60 µl**

* Volumes given are suitable for up to 6 reactions. Excess Buffer D2 can be stored at –20°C for up to 3 months.

† Reconstitution of Buffer DLB is described in "Things to do before starting".

3. **Add 10 µl Buffer D2 to each microcentrifuge tube containing flash-frozen tissue. Mix by vortexing briefly and place on ice for 30 min.**
4. **Add 10 µl Stop Solution to each microcentrifuge tube containing lysed tissue and mix briefly by vortexing. Spin down the tissue debris by pulse centrifugation.**
5. **Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex, and centrifuge briefly.**

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

6. **Prepare a master mix on ice according to Table 2. Mix and centrifuge briefly.**

   IMPORTANT: Add the master mix components in the order listed in Table 2. After addition of water and REPLI-g sc Reaction Buffer, briefly vortex and spin down the mixture before addition of REPLI-g sc DNA Polymerase. The master mix should be kept on ice and used immediately upon addition of the REPLI-g sc DNA Polymerase.

Table 2. Preparation of master mix

Component	Volume*
H_2O sc	9 µl
REPLI-g sc Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total volume	**40 µl**

7. **Add 40 µl master mix to 10 µl lysed and neutralized tissue cells (step 4). Mix well by vortexing for 10 s and centrifuge briefly.**
8. **Incubate at 30°C for 8 h.**

   After incubation, heat the water bath or heating block to 65°C if the same water bath or heating block will be used in step 9.
9. **Inactivate REPLI-g sc DNA Polymerase by heating the sample at 65°C for 3 min.**
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 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 40 µg per 50 µl reaction and should be diluted appropriately. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix B of the *REPLI-g Single Cell Kit Handbook,* page 22, for an accurate method of quantifying REPLI-g amplified DNA.

    Note: Purification of REPLI-g SC amplified DNA is only necessary when performing labeling reactions, for example, array comparative genomic hybridization (CGH). To purify REPLI-g SC amplified DNA follow the Supplementary Protocol "Purification of DNA amplified using REPLI-g Kits" (RG21).
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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