QIAGEN Supplementary Protocol

Whole genome amplification from genomic DNA using the REPLI-g® Single Cell Kit with increased sample volumes

This protocol is optimized for the amplification of 10–100 ng of purified genomic DNA template using the REPLI-g Single Cell Kit (cat. nos. 150343 and 150345). Using this protocol, up to a 15 µl sample volume can be used instead of the 2.5 µl sample volume that is used with the standard protocol for amplifying genomic DNA provided in the kit handbook. The template DNA should be stored in TE buffer. If the DNA is of sufficient quality (e.g., high-molecular-weight DNA without inhibitors such as detergents or organic solvents), then smaller amounts (1–10 ng for eukaryotic DNA or 10–100 pg for bacterial DNA) may be used.

IMPORTANT: Please refer to the *REPLI-g Single Cell Handbook* for general information on the handling and storage of kit components.

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
- 1.5 ml microcentrifuge tube
- Microcentrifuge
- Ice
- Optional: REPLI-g Human Control Kit, cat. no. 150090

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 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", page 12, of the *REPLI-g Single Cell Handbook*.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.

- 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 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 Buffer DLB by adding 500 µl H_2O sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.
  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 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 >10 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 Buffer DLB to the DNA. Mix by vortexing and centrifuge briefly.**
3. **Incubate at room temperature for 3 min.**
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 s.

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 2. Preparation of master mix*

Component	Volume/reaction
REPLI-g sc Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total 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 8 h.**

   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 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 need to be diluted appropriately. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix B of the *REPLI-g 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.**

QIAGEN Supplementary Protocol

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.

Selected handbooks can be downloaded from www.qiagen.com/literature. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

www.qiagen.com
Australia ■ 1-800-243-800
Austria ■ 0800/281010
Belgium ■ 0800-79612
Canada ■ 800-572-9613
China ■ 021-51345678
Denmark ■ 80-885945
Finland ■ 0800-914416
France ■ 01-60-920-930
Germany ■ 02103-29-12000
Hong Kong ■ 800 933 965
Ireland ■ 1800 555 049
Italy ■ 800 787980
Japan ■ 03-6890-7300
Korea (South) ■ 1544 7145
Luxembourg ■ 8002 2076
The Netherlands ■ 0800 0229592
Norway ■ 800-18859
Singapore ■ 65-67775366
Spain ■ 91-630-7050
Sweden ■ 020-790282
Switzerland ■ 055-254-22-11
UK ■ 0808-2343665
USA ■ 800-426-8157

Sample & Assay Technologies