QIAGEN Supplementary Protocol

Whole genome amplification of DNA from blood spots dried on FTA® paper using the REPLI-g® Single Cell Kit

This protocol is optimized for the amplification of DNA from blood dried on FTA paper using the REPLI-g Single Cell Kit (cat. nos. 150343 and 150345). Note that the whole genome may not be amplified with complete genome coverage, depending on the quality and storage conditions of the dried blood sample.

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, heating block, or thermocycler
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Vortexer
- Ice
- Nuclease-free water
- Potassium acetate solution (3000 mM, pH 5)*
- Tris-HCl solution (1000 mM, pH 9)*

* Potassium acetate solution and Tris-HCl solution are not provided with the REPLI-g Single Cell Kit and should be prepared in advance.

QIAGEN Supplementary Protocol

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 reaction in a location free of DNA.
- 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.
- Note that potassium acetate solution (3000 mM, pH 5) and Tris-HCl solution (1000 mM, pH 9) are not provided with the REPLI-g Single Cell Kit and should be prepared in advance.

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.
- Prepare potassium acetate solution (3000 mM, pH 5) and Tris-HCl solution (1000 mM, pH 9).
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 10). 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.

Procedure

1. **Prepare sufficient denaturation Buffer D(spot) and neutralization Buffer N(spot) for the total number of whole genome amplification reactions (see Table 1 and Table 2). Vortex Buffer D(spot) and Buffer N(spot) thoroughly and centrifuge briefly.**

   Note: The total volumes of Buffer D(spot) given in Table 1 and Buffer N(spot) given in Table 2 are suitable for 10 reactions. Buffer D(spot) should not be stored longer than 3 months.

Table 1. Preparation of denaturation Buffer D(spot)

Component	Volume*
DTT, 1 M	32 µl
Buffer DLB (reconstituted)†	351 µl
Nuclease-free water	277 µl
Total volume	**660 µl**

* Volumes given are sufficient for ten reactions.

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

Table 2. Preparation of neutralization Buffer N(spot)

Component	Volume*
Stop Solution	359 µl
Potassium acetate solution (3000 mM, pH5)†	26 µl
Total volume	**385 µl**

* Volumes given are sufficient for ten reactions.

† Not provided in the REPLI-g Single Cell Kit.

2. **Punch a small piece of FTA paper that has been soaked with blood and transfer the blood punch into a 1.5 ml microcentrifuge tube.**

   Note: The size of the blood punch should not exceed a diameter of 5 mm.

3. **Add 65 µl Buffer D(spot). Mix and centrifuge briefly.**
4. **Incubate for 10 min at room temperature.**
5. **Add 37.5 µl Buffer N(spot). Mix briefly.**

   Note: Do not centrifuge.

6. **Incubate for 5 min at room temperature.**
7. **Centrifuge for 3 min at 5600 rpm using a microcentrifuge.**
8. **Carefully transfer supernatant to a new microcentrifuge tube.**

   Note: Avoid the transfer of any pellet or precipitates.

9. **Add 10 µl Tris-HCl Solution (1000 mM, pH 9). Mix and centrifuge briefly.**

   Note: Tris-HCl Solution (1000 mM, pH 9) is not provided in the REPLI-g Single Cell Kit.

10. **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.

11. **Prepare a master mix on ice according to Table 3. Mix and centrifuge briefly.**

    Important: Add the master mix components in the order listed in Table 3. After addition of nuclease-free water and REPLI-g sc Reaction Buffer, briefly vortex and centrifuge 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 3. Preparation of master mix[*†]

Component	Volume/reaction
Nuclease-free water	11.5 µl
REPLI-g sc Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total volume	**42.5 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%.

† After the addition of nuclease-free water and REPLI-g sc Reaction Buffer, briefly vortex and centrifuge.

12. **Add 42.5 µl of the master mix to each well to a fresh microcentrifuge tube.**
13. **Add 7.5 µl DNA extracted from blood spots (step 8).**
14. **Incubate at 30°C for 8 h.**

    A thermocycler can be used for programming the incubation and reaction inactivation temperatures (see step 15).

15. **Inactivate REPLI-g sc DNA Polymerase at 65°C for 3 min.**
16. **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.

17. **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.

18. **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