Supplementary Protocol November 2018

Whole genome amplification from buccal cells using the REPLI-g® Advanced DNA Single Cell Kit

This protocol is optimized for whole genome amplification from biopsies using the REPLI-g Advanced DNA Single Cell Kit (cat. nos. 150363 and 150365). The procedure is optimized for air-dried buccal swabs obtained using cotton or Dacron® tips, and brushes or swabs with an ejectable head (e.g., Whatman® Omni Swab). Other swab types may also be used. Note that the whole genome may not be amplified with complete genome coverage, depending on the quality of swab. Freshly obtained swabs are recommended so that intact cells will be used for whole genome amplification.

Note: In rare cases, 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 of eukaryotic DNA.

IMPORTANT: Please read the *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, 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)
- Swabs, such as sterile Omni Swabs (available from Whatman) or Puritan applicators with plastic shafts and cotton or Dacron tips (available from Hardwood Products)*

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important points before starting

- To collect a sample, scrape a fresh swab firmly against the inside of each cheek 6 times. Ensure that the person providing the sample has not consumed any food or drink in the 30 minutes prior to sample collection.
- Do not store the swap due to bacterial growth and human DNA degradation.
- DNA yields of approximately 40 µg will be present in negative (no template) controls because DNA is generated during the REPLI-g Advanced DNA 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 Advanced Buffer DLB by adding 250 µl H_2O sc to the tube provided. Mix thoroughly and centrifuge briefly.

  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 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. **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 12 reactions.

Table 1. Preparation of Buffer D2

Component	Volume*
DTT, 1M	3 µl
Reconstituted Advanced Buffer DLB†	33 µl
Total volume	**36 µl**

* Volumes given are suitable for up to 12 reactions. Excess Buffer D2 can be stored at –20°C for up to 3 months.

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

2. **Place the swab in a 1.5 ml microcentrifuge tube. Add 1 ml TE buffer and vortex for 10 seconds.**

   Note: If using an Omni Swab, eject the swab head by pressing the end of the inner shaft towards the swab head. If using a cotton or Dacron swab, separate the swab head from its shaft by hand or with scissors.

3. **Remove the swab from the microcentrifuge tube using forceps. Squeeze as much liquid as possible out of the swab by pushing the swab against the side of the microcentrifuge tube.**

   IMPORTANT: The swab must be removed from the microcentrifuge tube prior to cell lysis (step 5).

4. **Centrifuge the microcentrifuge tube containing buccal cells at maximum speed for 10 seconds. Discard the supernatant and wash the buccal cells by resuspending the pellet in 1 ml TE buffer and vortexing for 1 minute**.

5. **Centrifuge the microcentrifuge tube containing buccal cells at maximum speed for 10 seconds. Discard the supernatant and resuspend the buccal cell pellet in 30 µl TE buffer**.

6. **Transfer 3 µl of resuspended buccal cells into a new microcentrifuge tube**.

7. **Add 3.5 µl Buffer D2 to the resuspended buccal cells and mix by pipetting up and down 3 times. Place the microcentrifuge tube at room temperature for 10 minutes.**

8. **Add 3.5 µl Stop Solution to each microcentrifuge tube containing lysed buccal cells and mix briefly by pipetting up and down 3 times.**

9. **Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex, and centrifuge briefly.**

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

10. **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 Advanced Reaction Buffer, briefly vortex and centrifuge the mixture before the 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 Advanced Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total volume	**40 µl**

* Volume per single sample. For more samples, simply multiply each component by the number of samples.

11. **Add 40 µl master mix to 10 µl lysed and neutralized buccal cells (step 8).**

12. **Incubate at 30°C for 2 hours.**

    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 13.

13. **Inactivate REPLI-g sc DNA Polymerase by heating the sample at 65°C for 3 minutes**.

14. **If not being used directly, store amplified DNA at 4°C for short-term storage or –20°C for long-term storage**.

15. **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.**

16. **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 Advanced DNA Single Cell Handbook* 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).

17. **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**.

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