User-Developed Protocol:

Whole genome amplification from buccal cells using the REPLI-g® Midi Kit

This procedure has been adapted by customers and is for whole genome amplification from buccal cells using the REPLI-g Midi Kit. The procedure is optimized for air-dried buccal swabs with cotton or Dacron® tips, and brushes or swabs with an ejectable head (e.g., Whatman® Omni Swab). Other swab types may also be used. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

Note: This protocol may be adapted for use with the REPLI-g Mini Kit, using the same reaction setup. In rare cases, potential inhibitors present in the starting material may have inhibitory effects on amplification when using the REPLI-g Mini Kit. In these cases, we recommend using the REPLI-g Midi Kit. Alternatively, upstream genomic DNA purification can be performed (e.g., using a QIAamp® Kit) with subsequent whole genome amplification of the purified DNA following the standard protocol in the *REPLI-g Mini/Midi Handbook*.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *REPLI-g Mini/Midi Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

Equipment and reagents to be supplied by user

- Microcentrifuge tubes
- Microcentrifuge
- Water bath or heating block
- Vortexer
- Pipets and pipet tips
- Ice
- 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.

User-developed protocol

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. Start the DNA amplification procedure within 2 hours of collection.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.
- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 7). All other components can be thawed at room temperature.
- 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).

Things to do before starting

- Prepare Buffer DLB by adding 500 µl nuclease-free water to the tube; mix thoroughly and centrifuge briefly.
- **Note**: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile. Avoid neutralization with CO_2.
- Set a water bath or heating block to 30ºC.
- All buffers and reagents should be vortexed before use to ensure thorough mixing.

Procedure

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

   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 by using scissors.

2. **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).

3. **Centrifuge the microcentrifuge tube containing buccal cells at maximum speed for 10 s. Discard the supernatant and wash the buccal cells by resuspending the pellet in 1 ml TE and vortexing for 1 min.**
4. **Centrifuge the microcentrifuge tube containing buccal cells at maximum speed for 10 s. Discard the supernatant and resuspend the buccal cell pellet in 30 µl TE.**
5. **Add 35 µl reconstituted Buffer DLB to the resuspended buccal cells and mix by pipetting up and down 3 times. Place the microcentrifuge tube on ice for 10 min.**

6. **Add 35 µl Stop Solution to the lysed buccal cells and mix by pipetting up and down 3 times.**

   Note: 10 µl lysed and neutralized buccal cells are used in a 50 µl REPLI-g reaction.

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

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

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

   IMPORTANT: Add the master mix components in the order listed in Table 1. After addition of water and REPLI-g Midi Reaction Buffer, briefly vortex and spin down the mixture before addition of REPLI-g Midi DNA Polymerase. The master mix should be kept on ice and used immediately upon addition of the REPLI-g Midi DNA Polymerase.

Table 1. Preparation of Master Mix

Component	Volume/reaction
Nuclease-free water	10 µl
REPLI-g Midi Reaction Buffer	29 µl
REPLI-g Midi DNA Polymerase	1 µl
Total volume	**40 µl**

9. **Add 40 µl master mix to 10 µl lysed and neutralized buccal cells (step 6).**
10. **Incubate at 30°C for 8–16 h.**

    Maximum DNA yield is achieved using an incubation time of 16 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 11.

11. **Inactivate REPLI-g Midi DNA Polymerase by heating the sample at 65°C for 3 min.**
12. **Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.**

    DNA amplified using the REPLI-g Midi Kit should be treated as genomic DNA with minimal freeze-thaw cycles. Storage of nucleic acids at low concentration over a long period of time may result in acid hydrolysis. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µl.