

User-Developed Protocol:

Whole genome amplification from small numbers of cells or single cells using the REPLI-g[®] Midi Kit

This procedure has been adapted by customers and is for whole genome amplification from small numbers of cells or single cells using the REPLI-g Midi Kit. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

This protocol may also be adapted for use with the REPLI-g Mini Kit, using the same reaction setup conditions. In rare cases, potential inhibitors present in the starting material may have inhibitory effects on amplification when using the REPLI-g Mini Kit. If this should occur, we recommend using the REPLI-g Midi Kit. Alternatively, upstream genomic DNA purification can be performed using, for example, 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 read the *REPLI-g Mini/Midi Handbook*, paying careful attention to the “Safety Information” section, before beginning this procedure.

Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN[®] Kit and kit component.

- REPLI-g Midi Kit (25), cat. no. 150043 or REPLI-g Midi Kit (100), cat. no. 150045
- Microcentrifuge tubes
- Microcentrifuge
- Vortexer
- Water bath(s) or heating block(s) capable of incubation at 30°C and 65°C
- Ice
- Deionized, nuclease-free water
- Pipets and pipet tips

Important points before starting

- Genomic DNA is released from the cell(s) for whole genome amplification by lysing the cells. Some cell types are efficiently lysed in Buffer D2 (Table 1, page 2), while other cell types are efficiently lysed in Buffer D3 (Table 2, page 2). We recommend performing an initial experiment to test which lysis buffer is suitable for your application.
- Avoid shearing DNA from lysed cells. If it is necessary to mix solutions of cells or DNA, mix very carefully by flicking the tube. Each break in the genomic DNA results in loss of allele information (“allele drop-out”).

- Buffer D2 and D3 should not be stored longer than 3 months at -20°C .
- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 7 of the procedure). All other components can be thawed at room temperature ($15\text{--}25^{\circ}\text{C}$).

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 .
- Vortex all buffers and reagents from the REPLI-g Midi Kit before use to ensure thorough mixing.
- Set a water bath or heating block to 30°C for use in step 10 of the procedure.
- Set a water bath or heating block to 65°C for use in step 11 of the procedure.

Procedure

1. **Prepare sufficient Buffer D2 or Buffer D3 (denaturation buffer) for the total number of whole genome amplification reactions (see Tables 1 and 2).**

The suitability of either Buffer D2 or Buffer D3 for your cells should be determined before starting this procedure (see “Things to do before starting”).

Note: The total volume of Buffer D2 and D3 given in Tables 1 and 2 is sufficient for up to 15 reactions.

Note: Once prepared, Buffer D2 and D3 should not be stored longer than 3 months at -20°C .

Table 1. Preparation of Buffer D2

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

Table 2. Preparation of Buffer D3

Component	Volume*
Nuclease-free water	5 μl
Reconstituted Buffer DLB [†]	55 μl
Total volume	60 μl

* Volumes given are sufficient for up to 15 reactions.

[†] Reconstitution of Buffer DLB is described in “Things to do before starting”, above.

2. **Pipet 3.5 µl PBS into a microcentrifuge tube.**
3. **Transfer the selected cell(s) to the microcentrifuge tube containing PBS.**
4. **Add 3.5 µl Buffer D2 or D3. Mix carefully by flicking the tube and centrifuge briefly.**
Note: Mix carefully to avoid shearing DNA from lysed cells.
5. **Incubate for 10 min on ice.**
Note: Sometimes it may be necessary to incubate at 65°C for 5 min.
6. **Add 3.5 µl Stop Solution. Mix carefully by flicking the tube and then centrifuge briefly.**
Note: Mix carefully to avoid shearing DNA from lysed cells.
7. **Thaw REPLI-g Midi DNA Polymerase on ice. Thaw all other components at room temperature (15–25°C), vortex, and centrifuge briefly.**
The REPLI-g Midi Reaction Buffer may form a precipitate after thawing. If necessary, vortex for 10 s to dissolve the precipitate.
8. **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 water and REPLI-g Midi Reaction Buffer, briefly vortex and centrifuge the mixture before addition of REPLI-g Midi DNA Polymerase. The master mix should be kept on ice and used immediately upon addition of REPLI-g Midi DNA Polymerase.

Table 3. 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 of the master mix to 10 µl of denatured DNA (from step 6). Mix carefully by flicking the tube and centrifuge briefly.**
10. **Incubate at 30°C for 8–16 h.**
11. **Inactivate REPLI-g Midi DNA Polymerase by heating the sample for 3 min at 65°C.**
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 and should not be subjected to multiple 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.

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Literature

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Further reading

Jiang, Z., Zhang, X., Deka, R., and Jin, L. (2005) Genome amplification of single sperm using multiple displacement amplification. *Nucleic Acids Res.*, **33(10)**, e91.

Handyside, A.H., et al. (2004) Isothermal whole genome amplification from single and small numbers of cells: a new era for preimplantation genetic diagnosis of inherited disease. *Mol. Hum. Reprod.*, **10**, 767.

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Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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