Quick-Start Protocol March 2015

REPLI-g® Single Cell DNA Library Kit

The REPLI-g Single Cell DNA Library Kit (cat. no. 150354) should be stored immediately upon receipt at –30°C to –15°C. If stored under these conditions, the kit is stable until the date indicated on the QC label inside the kit lid.

Further information

- *REPLI-g Single Cell DNA Library Handbook*: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

Notes before starting

- This protocol is for amplifying gDNA from single cells and constructing sequencing libraries for Illumina® NGS platforms. Refer to the kit handbook for additional protocols, required materials and kits.
- This protocol is optimized for single cell material from all species, including vertebrates, gram-positive and gram-negative bacteria, cells, sorted cells, tissue culture cells, microdissected cells from frozen sections and plants (without the cell wall). It cannot be used for cells that are treated with formalin or other cross-linking agents.
- Enzymatically or mechanically shear 5 µg amplified cDNA and start library prep with 1 µg sheared cDNA. Refer to the kit handbook for protocols and cleanup steps.
- Median fragment sizes depend on the applications and read length.
- Library enrichment is not required.

Procedure: Amplification of genomic DNA from single cells

1. Prepare sufficient Buffer D2 (denaturation buffer) for the total number of whole genome amplification reactions (Table 1). Reconstituted residual Buffer D2 can be stored at –30°C to –15°C for a maximum of 3 months.

Table 1. Preparation of Buffer D2 for 12 reactions

Component	Volume/12 reactions
DTT, 1 M	3 µl
Buffer DLB (reconstituted)	33 µl
Total reaction volume	**36 µl**

2. Place 4 µl cell material (supplied with PBS) into a microcentrifuge tube.
3. Add 3 µl Buffer D2. Mix carefully by gently flicking the tube and centrifuge briefly. Avoid any contact of pipet tips with cell material.
4. Incubate for 10 min at 65°C.
5. Add 3 µl Stop Solution. Mix carefully by flicking the tube and centrifuge briefly. Store on ice.
6. Prepare a master mix by adding the components in the order listed in Table 2. Mix and centrifuge briefly before adding REPLI-g sc DNA Polymerase.

Table 2. Preparation of master mix

Component	Volume/reaction*
H_2O sc	9 µl
REPLI-g sc Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total reaction volume	**40 µl**

* Scale up according to the number of reactions and add 10%.

7. For each amplification reaction, add 40 µl master mix to 10 µl denatured DNA (from step 5).
8. Incubate at 30°C for 3 h. Stop the reaction by incubating at 65°C for 3 min.

9. If not being used directly, store the amplified DNA (>100 ng/µl) at –30°C to –15°C until required for downstream applications.
10. Amplified DNA can be directly used for shearing and for library construction, or for target-directed amplification and library construction.
11. Amplified DNA behaves like purified genomic DNA and has an approximate length of 2000 bp up to 70,000 bp. For QC of the WGA reaction, refer to the *REPLI-g Single Cell DNA Library Handbook*.

Procedure: PCR-free library prep from REPLI-g WGA DNA

Note: High-quality DNA is essential for obtaining good sequencing results. Residual traces of contaminants (salts or proteins) will degrade the DNA or decrease the efficiency of the enzyme activities necessary for optimal library preparation.

End repair

1. Prepare a reaction mix for end-repair on ice according to Table 3, dispensing the reagents into a PCR tube or the well of a PCR plate, and mix thoroughly.

Table 3. Reaction mix for end-repair

Component	Volume/reaction
DNA	1 µg
RNase-free water	Variable
End-Repair Buffer, 10x	2.5 µl
End-Repair Enzyme Mix	2 µl
Total reaction volume	**25 µl**

2. Program a thermocycler to incubate for 30 min at 25°C, followed by 20 min at 75°C to inactivate the enzyme.

A-addition

3. Prepare a reaction mix for A-addition according to Table 4, by adding the components to the PCR tube containing the end-repaired DNA and mix.

Table 4. Reaction mix for A-addition

Component	Volume/reaction
End-repaired DNA (from step 2)	25 µl
A-Addition Buffer, 10x	3 µl
Klenow Fragment (3'→5' exo–)	3 µl
Total reaction volume	**31 µl**

4. Program a thermocycler to incubate for 30 min at 37°C, followed by 10 min at 75°C to inactivate the enzyme.

Adapter ligation

5. Prepare a reaction mix for adapter ligation according to Table 5, adding the components to the PCR tube containing DNA that has undergone end-repair and A-addition (step 4) and mix. Avoid any cross-contamination of adapters.

Table 5. Reaction setup for adapter ligation

Component	Volume/reaction
DNA from step 4 (has undergone end-repair and A-addition)	31 µl
Ligation Buffer, 2x	45 µl
GeneRead™ Adapter (ready to use)	2.5 µl*
T4 DNA Ligase	4 µl
RNase-free water	Variable
Total reaction volume	**90 µl**

* Alternatively, add the correct amount of adapter according to supplier's directions.

6. Program a thermocycler without a heated lid to incubate for 10 min at 25°C.
7. Proceed with size selection of the library for adapter removal and QC of the library as described in the kit handbook.