QIAseq FX Single Cell DNA Library Kit

The QIAseq FX Single Cell DNA Library Kit (cat. no. 180713, 180715) should be stored immediately upon receipt at –15°C to –30°C. If stored under these conditions, kits are stable until the date indicated on the QC label inside the kit lid.

Further information:

- *QIAseq FX Single Cell DNA Library Handbook*: **www.qiagen.com/HB-2138**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **www.qiagen.com/support**

Notes before starting:

- This protocol is for the generation of NGS libraries from gDNA from single cells for whole-genome or hybrid capture sequencing experiments. This protocol includes steps for gDNA amplification, fragmentation and library construction from single cells to generate NGS libraries compatible with any Illumina® sequencer. Refer to the kit handbook for additional protocols, required materials and kits.
- This protocol is optimized for eukaryotic and bacterial single cells that lack a cell wall and for microdissected cells from frozen sections. It cannot be used for cells that have been treated with formalin or other cross-linking agents.
- Library amplification is not required; this entire process is PCR-free.

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 –15°C to –30°C for a maximum of 3 months. Reconstitute DLB in 500 µl H_2O sc.

Table 1. Preparation of Buffer D2 for 12 reactions

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

2. Place 4 µl cell material (consisting of 1–1000 cells suspended in PBS) into a suitable microcentrifuge tube or plate.
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. Add 3 µl Stop Solution. Mix carefully by flicking the tube and centrifuge briefly. Store immediately on ice.
5. Prepare DTT dilution and the 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	6.5 µl
REPLI-g sc Reaction Buffer	29 µl
DTT (1:10 diluted in H_2O sc)	2.5 µl
REPLI-g sc DNA Polymerase	2 µl
Total reaction volume	**40 µl**

* Scale up according to the number of reactions.

6. For each amplification reaction, add 40 µl master mix to 10 µl denatured DNA (step 4).
7. Incubate at 30°C for 2 h. Stop the reaction by incubating at 65°C for 3 min.
8. If not being used directly, store the amplified DNA (>100 ng/µl) at –15°C to –30°C until required for downstream applications.
9. Amplified DNA can be directly used for library construction, and for other applications. For QC of the WGA reaction, refer to the *QIAseq FX Single Cell DNA Library Handbook*.

Procedure: PCR-Free Library Construction from Amplified gDNA

FX Single-Tube fragmentation, end repair and A-addition

1. Thaw all kit components on ice, program a thermocycler according to Table 3 and start the program. When the thermocycler block reaches 4°C, pause the program.

Table 3. Amplified gDNA fragmentation reaction conditions

Step	Temperature	Incubation time
1	4°C	1 min
2	32°C	15 min*
3	65°C	30 min
4	4°C	Hold

* The insert size of the completed libraries is determined by the duration of step 2. Using 200–1000 ng input WGA-DNA, 15 min fragmentation time produces a fragment distribution of around 350 bp. For detailed recommendations, please refer to the QIAseq FX Single Cell DNA Library Handbook. Use a thermocycler with a heated lid.

2. Dilute amplified gDNA to 200–1000 ng total in 10 µl H_2O sc (20–100 ng/µl).
3. Prepare the FX Reaction Mix on ice according to Table 4 and mix by pipetting.

Table 4. FX Reaction Setup for >200 ng input WGA-DNA

Component	Volume/reaction*
FX Buffer, 10x	5 µl
H_2O sc	20 µl
FX Enhancer	5 µl
FX Enzyme Mix	10 µl
Total reaction volume	**40 µl**

* Mix by pipetting and keep on ice.

4. Add 40 µl FX Reaction Mix to each diluted amplified gDNA sample on ice and gently mix.
5. Briefly spin down the PCR plate/tubes and immediately transfer to the pre-chilled thermocycler (4°C) and resume the program. Once the fragmentation program is complete, transfer samples to ice. Proceed with the next step.

Adapter Ligation

6. Mix and spin down the adapter plate. Remove the protective adapter plate lid, pierce the foil seal and transfer 5 µl from one DNA adapter well to each sample. Track the barcodes used. Freeze unused adapters.
7. Prepare the Ligation master mix on ice according to Table 5. Mix gently.

Table 5. Ligation master mix (per sample)

Component	Volume/reaction*
DNA Ligase Buffer, 5x	20 µl
H_2O sc	15 µl
DNA Ligase	10 µl
Total reaction volume	**45 µl**

8. Add 45 µl master mix to each sample. Mix well and incubate at 20°C for 15 min.
9. Proceed immediately to adapter ligation cleanup (steps 10–18)
10. Add 80 µl resuspended Agencourt AMPure XP beads to each sample and mix.
11. Incubate for 5 min at room temperature.
12. Pellet the beads on a magnetic stand and carefully discard the supernatant.
13. Add 200 µl fresh 80% ethanol to each pellet on the magnetic stand and carefully discard the supernatant.
14. Repeat step 13 for a total of 2 ethanol washes.
15. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Avoid over-drying since this may result in lower DNA recovery. Remove from the magnetic stand.
16. Elute by resuspending in 52.5 µl Buffer EB. Pellet beads on the magnetic stand. Carefully transfer 50 µl supernatant to a new PCR plate.
17. Add 50 µl of resuspended Agencourt AMPure XP beads to each sample and mix.
18. Follow steps 11–15 and continue to step 19.
19. Elute by resuspending in 26 µl Buffer EB. Pellet the beads on the magnetic stand. Carefully transfer 23.5 µl of supernatant into a new PCR plate. Store purified libraries at –20°C until ready for sequencing.

Scan QR code for handbook.