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QIAseq[®] UPX Single Cell DNA Library Kit

For ultraplex (UPX) DNA library construction from single cells or low input samples for sequencing on Illumina[®] sequencing platforms using unique dual indexes (UDIs)

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Kit Contents

Box 1

QIAseq UPX DNA Enzyme Kit	(576)
Catalog no.	1132035
No. of samples	576
QIASeq Human Control DNA	1 tube
PBS sc, 1x, pH 7.5	2 tubes
DTT, 1 M	1 tube
H ₂ O sc	4 tubes
Buffer DLB	2 tubes
Stop Solution	1 tube
REPLI-g sc Dilution Buffer	15 tubes
REPLI-g sc advanced oligo	1 tube
REPLI-g sc universal oligo	1 tube
REPLI-g SingleCellDNAPolym	6 tubes
UPX booster sc	2 tubes
EP Buffer 10x	1 tube
EP Enhancer	1 tube
EP Facilitator	1 tube
5x DNA Ligase Buffer	2 tubes
DNA Ligase	2 tubes
FX Buffer, 10x	1 tube
FERA Solution	1 tube
FX Enzyme Mix	1 tube
FG solution	1 tube
HiFi PCR Master Mix, 2x	2 tubes
Primer Mix Illumina Library Amp	2 tubes

Store at -15°C to -35°C upon arrival.

Box 2

QIAseq Beads	(25 mL)
Catalog no.	1132037
No. of preps	24 libraries

QIAseq Beads	25 mL
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Nuclease-Free Water	50 mL
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Store at 2–8°C upon arrival.

Box 3

QIAseq UPX DNA Adapter Kit	(24)
Catalog no.	1132010
No. of preps	24

Sample Barcode Plate	6 plates with 4 x 24 Sample Barcodes
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BC-i5-UDI Adapter Plate (24)	1 plate with 24 BC-i5-UDI adapters
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BC-i7-UDI Adapter Plate (24)	1 plate with 24 BC-i7-UDI adapters
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Bags 24x 8-cap strips	3 bags
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Store at –30°C to –15°C upon arrival. Bags with cap strips are stored at room temperature (15–25°C).

Shipping and Storage

The QIAseq UPX Single Cell DNA Lib Kit is shipped in 3 different types of boxes (enzyme kit, adapter kit, and QIAseq Beads) with 2 different storage conditions.

- The QIAseq UPX DNA Enzyme Kit and QIAseq UPX DNA Adapter Kit should be stored immediately upon receipt at -30°C to -15°C in a constant-temperature freezer. Bags with cap strips are stored at room temperature.
- The QIAseq Beads should be stored immediately at $2-8^{\circ}\text{C}$ in a refrigerator.

Important: QIAseq Beads should never be frozen.

Intended Use

The QIAseq UPX Single Cell DNA Lib Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAseq UPX Single Cell DNA Lib Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Single-cell genomic analysis enables researchers to gain novel insights across a diverse set of research areas – including developmental biology, tumor heterogeneity, and disease pathogenesis. Conducting single-cell genomic analysis using next-generation sequencing (NGS) methods has traditionally been challenging since the amount of genomic DNA present in a single cell is limited. Amplification methods that rely on PCR normally have high error rates, low coverage uniformity, extensive allelic drop-outs, and limited yields following extensive amplification.

The QIAseq UPX Single Cell DNA Library Kit utilizes an optimized DNA amplification and library preparation protocol to allow pooling of amplified DNA with a reduction in the total number of libraries. The key DNA amplification steps uses QIAGEN's multiple displacement amplification (MDA) technology with the REPLI-g[®] enzyme to amplify gDNA directly from single cells or previously isolated genomic DNA.

During the MDA reaction, Sample Barcodes are incorporated in the amplified DNA allowing subsequent pooling of up to 24 samples into a single library. This greatly reduces tedious library preparation steps, reducing library preparation costs and plastic consumables.

Library generation follows DNA pooling with the directed ligation of the first sample index (BC-i5-UDI) adapter. The BC-i5-UDI ligated DNA fragments are then treated in the next step with fragmentation reagents to reduce the size of fragments and to generate the second end, which is subsequently ligated to the second sample index adapter (BC-i7-UDI). The completed libraries are amplified using HiFi amplification mix and can be automatically balanced by using the QIAseq Normalizer Kit (sold separately).

This highly optimized single cell / low input protocol greatly reduces DNA library costs and plastic consumable usage while increasing the number of single cells which can be processed

for low pass sequencing or exome-enrichment workflows. When multiplexing 24 samples with 24 libraries, a total of 576 samples can be analyzed in parallel.

Principle and procedure

The QIAseq UPX Single Cell DNA Library Kit provides a fast, fully enzymatic procedure for DNA amplification, DNA end-polishing, and NGS library construction (Figure 1). The QIAseq UPX Single Cell DNA Library Kit uses MDA technology to amplify complex genomic DNA with a modified REPLI-g amplification protocol. The high-fidelity REPLI-g sc DNA Polymerase has 1000-fold higher fidelity than Taq polymerase and 3' to 5' proofreading activity to provide high yields with uniformly amplified DNA even when starting with single cells. The QIAseq UPX Single Cell DNA Library Kit has been optimized to maximize genome coverage, ensuring regions potentially containing sequence variants or features of interest are not missed.

A critical part of any single cell or low input DNA amplification procedure is limiting contaminating DNA from enzymes, buffers, and manufacturing processes. All kit components used for the MDA protocol undergo a unique, controlled decontamination procedure to ensure elimination of all amplifiable contaminating DNA. Following decontamination, the kits undergo stringent quality control to ensure complete functionality. Decontaminated buffers are filled in small volumes to reduce freeze–thaw cycles and frequent opening of tubes to exclude any subsequent environmental contamination.

The QIAseq UPX Single Cell DNA Library Kit provides a simple and reliable method to efficiently multiplex up to 24 MDA amplified samples in one library. These libraries are suitable for whole genome sequencing on any Illumina NGS instrument or can be used with hybrid-capture protocols such as the QIAseq Human Exome and QIAseq xHYB enrichment Kits, allowing researchers to focus on key regions of interest and reduce the amount of sequencing.

In the first step of the WGA procedure, the cell or DNA sample is added to the dried Sample Barcodes. The sample is then lysed and the DNA is denatured using gentle alkaline conditions. After denaturation is complete, the reaction is neutralized with stop solution and the ultraplex

master mix containing buffer, UPX Booster, and REPLI-g sc DNA Polymerase is added. The isothermal amplification reaction is started and proceeds for 12 hours at 30°C. The amplified and barcoded DNA can be stored long-term at -20°C, or it can be used directly to generate sequencing libraries. Up to 24 MDA amplified and barcoded samples can be pooled together. The pool is purified using QIAseq Beads to remove any non-incorporated Sample Barcodes and the MDA residual reaction components. For library construction, the pooled samples consisting of WGA–DNA strands are treated with EP facilitator to generate sticky ends and accept the BC-i5-UDI adapter. QIAseq Beads purification after the first adapter ligation will remove any non-ligated adapters. The ligation of BC-i5-UDI adapter is directed and ensure that the read 1 will start with the Sample Barcode sequence. In the next step the fragments are treated with fragmentation reagents to reduce the fragment size and generate suitable sticky ends for the second BC-i7-UDI adapter. In the following steps the library is purified to remove non-ligated BC-i7-UDI adapters and will be amplified with the standard P7 and P5 primers. These adapters contain sequences essential for bridge amplification and sequencing on any Illumina NGS instrument.

This kit also includes human control DNA for monitoring of the MDA amplification and library construction process.

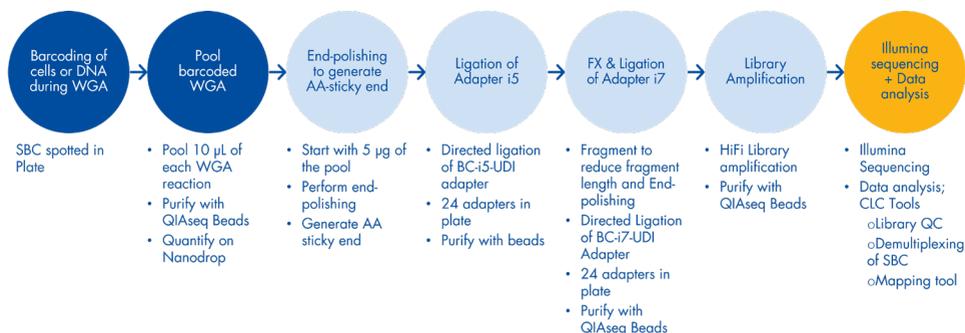


Figure 1. QIAseq UPX Single Cell DNA Library Kit workflow.

NGS adapter and index technologies

Sample multiplexing is one of the most important methods for increasing throughput and reducing costs. It works by pooling multiple samples together during a single sequencing run. This is facilitated by the integration of unique index sequences into the individual sample adapter molecules.

The QIAseq UPX Single Cell DNA Library Kit includes QIAseq Unique Dual-Index (UDI) in a 24 format. When combined with the 24 Sample Barcodes, multiplexing of 576 samples is possible in one sequencing run. Multiplexing with other QIAseq Libraries with QIAseq Unique Dual-Index is also possible.

The QIAseq UDI Adapters use a fixed combination of 2 unique barcode motifs per adapter molecule. Therefore, each single-index motif is only used once on any UDI adapter plate.

Usage of UDI adapters effectively mitigates the risk of read misassignment due to index hopping. This is enabled by filtering misassigned reads during the demultiplexing of individual samples, thus generating highly accurate output data.

Compatible sequencing platforms

- Illumina NovaSeq® 6000
- Illumina HiSeq®
- Illumina MiSeq®
- Illumina NextSeq® 550/500
- Illumina NextSeq 1000/2000
- Illumina iSeq®
- Illumina MiniSeq®

For any additional compatibility questions, please contact QIAGEN technical services.

Equipment and Reagents to be Supplied by User

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.

- PCR tubes or plates
- LoBind® tubes for storage of generated libraries (Eppendorf, cat. no. 0030108094, 0030108116 or 0030108132) or equivalent
- Pipettes and pipette tips
- Magnetic racks for magnetic bead separation (e.g., DynaMag™-2 Magnet, Thermo Fisher Scientific, cat. no. 12321D*)
- Thermocycler or heating block
- Microcentrifuge
- Vortexer
- NanoDrop® UV-Vis spectrophotometer for DNA quantification
- Ice
- 96–100% ethanol
- Buffer EB (QIAGEN, cat. no. 19086) or 10 mM Tris·Cl pH 8.0
- QIAxcel®, Agilent® 2100 Bioanalyzer, or similar, to evaluate the DNA fragmentation profile (optional) or other comparable capillary electrophoresis device or method to assess the quality of DNA library
- QIAseq Library Quant Assay Kit (QIAGEN, cat. no. 333314) for library quantification
- QIAseq Library Normalizer Kit (optional) (QIAGEN, cat. nos. 180613, 180615)
- REPLI-g Single Cell Cryo-Protect Reagent (15 mL) (optional)

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

Important Notes

Cells, DNA preparation, and quality control

High-quality DNA is essential for obtaining good amplification and sequencing results. The most important prerequisite for any DNA sequence analysis experiment is consistent, high-quality DNA from every experimental sample. Therefore, cell handling and DNA isolation procedures are critical to the success of the experiment. Low integrity DNA decreases the efficiency of amplification and the quality of the generated libraries.

General precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Avoid DNA contamination of reagents by using separate laboratory equipment (e.g., pipettes, filter pipette tips, reaction vials, etc.). Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile, DNase-free, and RNase-free.
- Set up the REPLI-g single cell reaction in a location free of DNA. Before starting, wipe down the work area and pipettes with an RNase- and DNA-cleaning product such as RNase Away® (Molecular Bio-Products) and LookOut® DNA Erase (Sigma-Aldrich), DNAZap™ (Invitrogen), DNA remover DNA-ExitusPlus™ (VWR), or bleach (10% solution of sodium hypochlorite) to remove any contaminating DNA. Moreover, UV irradiation at 254 nm can inactivate DNA contamination in disposables and reagents that are not included in the QIAseq Single Cell Library Kit UDI.
- All components labeled with sc are decontaminated and suitable for whole genome amplification (WGA) from single cells and low amount DNA.
- For consistent genome amplification, library construction, and amplification, ensure that the thermal cycler used in this protocol is in good working order and has been calibrated according to the manufacturer's specifications.
- Read the entire protocol before beginning. Take note of stopping points where samples can be frozen at -20°C and plan your workflow accordingly.

- End-polishing of the fragments and generation of sticky ends for directed adapter ligation is sensitive to many factors such as reaction temperature, time, and setup conditions, as well as the quantity of the input DNA.

Recommended library quantification method

QIAGEN's QIAseq Library Quant Assay Kit (cat. no. 333314), which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library. Alternatively, Qubit® or Agilent Bioanalyzer may be used, but they are not recommended for non-enriched libraries, and are less accurate since they measure all fragments (adapter-ligated, non-adapter-ligated, and ligated fragment with only one adapter).

Protocol: Whole Genome Amplification from Single Cells and Purified DNA Using Random Primers

This protocol is for the amplification of genomic DNA from 1–50 intact cells, or >6 pg high-molecular-weight (HMW) purified gDNA.

Important points before starting

- This protocol is optimized for single cells and isolated nuclei from all species, including vertebrates, bacteria (gram positive), flow-sorted cells, tissue culture cells, micromanipulated cells, and laser-microdissected cells from frozen sections.
Note: The protocol cannot be used with cells that have been fixed with formalin or other cross-linking agents (e.g., single-cell samples obtained by laser microdissection from formalin-fixed, paraffin-embedded tissues).
- Samples of 1–50 intact cells (e.g., eukaryotic or bacterial cells) are optimal for whole-genome amplification reactions using the QIAseq Single Cell DNA Library Kit UDI.
- If starting with purified DNA and not intact cells, ensure that the DNA is of high quality (HMW DNA and free of inhibitors such as solvents and detergents) and suspended in Buffer TE or Nuclease-Free Water. For complete genome coverage if working with eukaryotic DNA, we recommend using 1–100 ng; for bacterial gDNA and other small genomes (viral DNA, mitochondrial DNA), we recommend using 6 pg to 1 ng.
- REPLI-g advanced oligo has been specifically developed to uniformly amplify DNA from eukaryotic cells. REPLI-g universal oligo can be used for amplification of DNA from all other sources.
- REPLI-g sc DNA Polymerase should be thawed on ice. All other components can be thawed at room temperature (15–25°C).
- Buffer D2 (denaturation buffer) should not be stored for longer than 3 months.

- DNA yields of up to 10 µg may be present in negative (no template) controls, because DNA is generated during the REPLI-g single cell reaction by random extension of primer dimers – generating HMW product. This DNA will not affect the quality of the actual samples and will not give a positive result in downstream assays.

Things to do before starting

- Prepare Buffer DLB by adding 500 µL H₂O sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.
Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile.
- Vortex all buffers and reagents before use to ensure thorough mixing. Mix enzymes by flicking the tube. Collect reagents in the bottom of the tube by centrifuging briefly.
- Set a programmable thermal cycler or a heating block to 30°C.
- If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

Procedure: Whole genome amplification using random primers

1. Thaw H₂O sc, DTT, and REPLI-g sc dilution buffer at room temperature (15–25°C), vortex, and then centrifuge briefly. The REPLI-g sc Dilution Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
2. 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 sufficient for 24 reactions. If performing fewer reactions, store residual Buffer D2 at –20°C. Buffer D2 should not be stored longer than 3 months.

Table 1. Preparation of Buffer D2 for 24 reactions

Component	Volume for 24+2 reactions (µL)
DTT, 1 M	3.25
Buffer DLB (reconstituted)*	35.75
Total volume	39

* Reconstitution of Buffer DLB is described in “Things to do before starting,” page 18.

3. Place 2 µL cell material (supplied with PBS) or gDNA into each well of a Sample Barcode Plate. If not using the complete plate, cut the wells required for the corresponding number of samples on the marked line using a pair of scissors.

4. Add 1.5 µL Buffer D2. Mix carefully by gently flicking the tube and centrifuge briefly.

Note: Ensure that the cell material does not stick to the tube wall above the buffer line. During pipetting, avoid any contact of pipette tips with cell material.

5. Incubate:

a. cell preparations for 10 min at 65°C and then cool down to 4°C.

b. gDNA preparations for 5 min at 25°C, and then cool down to 4°C.

Note: Due to the small volume it is recommended to use a thermal cycler with the heating lid set at 70°C to avoid evaporation. Alternatively, incubation can be performed in a heating block. Spin down the samples after the incubation to collect all liquid on the bottom of the tubes.

Note: The denaturation temperature can be increased up to 65°C if working with organisms that have GC content higher than 50%.

6. Add 1.5 µL Stop Solution. Mix carefully by flicking the tube, and then centrifuge briefly. Immediately place on ice or ice blocks.

7. Thaw REPLI-g sc DNA Polymerase on ice. Tip gently on the tube to mix and centrifuge briefly.

8. Prepare a master mix according Table 2. Mix and centrifuge briefly.

9. Use REPLI-g advanced oligo for amplification of eukaryotic DNA or cells and universal oligo for all other types of species.

Important: Add the master mix components in the order listed in Table 2. After the addition of water, REPLI-g sc Dilution Buffer, oligos, and UPX Booster sc, briefly vortex and centrifuge the mixture before adding REPLI-g sc DNA Polymerase. After adding REPLI-g sc DNA Polymerase, flick carefully and then centrifuge briefly. The master mix should be kept on ice and used immediately upon addition of REPLI-g sc DNA Polymerase.

Note: Scale up accordingly if performing several reactions at once by preparing a Master Mix sufficient for the total number of reactions.

Table 2. Preparation of master mix*

Component	Volume for 1 reaction (µL)	Volume for 24+2 reactions (µL)
H ₂ O sc	2.5	65
REPLI-g sc Dilution Buffer	14.5	377
REPLI-g sc universal oligo or REPLI-g sc advanced oligo	0.7	18.2
UPX Booster sc	1.8	46.8
REPLI-g sc DNA Polymerase	0.5	13
Total volume	20	520

10. For each amplification reaction, add 20 µL master mix to 5 µL denatured DNA (from step 6). Mix by flicking the tube, and then centrifuge briefly.
11. Incubate at 30°C for 12 h to amplify DNA.

Note: Incubating the samples for 12 h generates 3–10 µg of amplified DNA.
12. After incubation at 30°C, heat the heating block up to 65°C if the same heating block will be used in step 13.

Note: If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.
13. Inactivate REPLI-g sc DNA Polymerase at 65°C for 5 min.
14. If the amplified DNA will not be used immediately, store it either at 4°C (for short-term storage up to 1 week) or at –20°C (for long-term storage). The completed amplification reaction can remain in the cycler overnight at 4°C.

DNA amplified using the QIAseq UPX Single Cell DNA Library Kit should be treated as genomic DNA and should undergo minimal freeze–thaw cycles. We recommend storage of nucleic acids at a concentration of at least 100 ng/μL.

15. Amplified DNA can be directly used for pooling and library construction. Additionally, extra amplified DNA can be used for PCR analysis or for targeted resequencing.

Note: If required, MDA QC can be performed prior to library generation. You may quantify the amplified DNA following the instructions in PicoGreen™ Quantification of QIAseq Single Cell Amplified DNA. Optical density (OD) measurements overestimate REPLI-g amplified DNA and should not be used.

Protocol: Pooling and Purification of MDA-Amplified and Barcoded DNA

This protocol describes the pooling and purification of the DNA that was amplified and barcoded in the previous protocol (step 15).

Important points before starting

- Equilibrate QIAseq Beads at room temperature (15–25°C) for 30 min.
- Prepare fresh 80% ethanol.

Procedure: Pooling and purification of MDA-barcoded DNA

Pooling of MDA

1. Mix amplified DNA well at full speed by repeated vortexing (5x). Spin down to collect liquid on bottom of the tube
2. Pipette 10 μ L of each MDA in a fresh 1.5 mL Tube. When pooling 24 samples this should result to 240 μ L pool. Mix well by repeated vortexing and spin down.

Purification of MDA-pool

3. Mix by repeated vortexing the equilibrated QIAseq Beads. Ensure that beads suspension is completely homogenized without any visual clumps.
4. Perform a 1.2x QIAseq Beads purification by adding 288 μ L of resuspended QIAseq Beads slurry to each MDA pool and mix well by pipetting or repeated vortexing.
5. Incubate the mixture for 5 min at room temperature (15–25°C).

6. Spin down to collect remaining droplets and pellet the beads on a magnetic stand for 5–10 min, then carefully discard the supernatant.

Note: The QIAseq Beads must be completely immobilized on the magnet.

7. Wash the beads by adding 200 μ L fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand, then carefully discard the supernatant.

8. Repeat the wash step 7 once for a total of 2 ethanol washes.

9. Incubate on the magnetic stand for 15 min or until the beads are dry. Remove from the magnetic stand.

10. Elute by resuspending in 55 μ L Nuclease-Free Water. Incubate for 5 min and then place the tube on the magnetic stand. Pellet the beads for up to 15 min until the supernatant is clear. Carefully transfer 50 μ L supernatant to a new PCR plate/tube.

Note: if the supernatant does not become clear you may add additional 25 μ L Nuclease-Free Water to improve the elution condition. In this case carefully transfer the 70 μ L in a fresh tube.

11. The purified DNA must be quantified on NanoDrop™ (Thermo Fisher Scientific). Perform 3 replicate measurements and build the average of the concentration of the measured replicates.

Protocol: Library Preparation of Pooled MDA-Barcoded DNA

The procedures describe the reactions and steps required to prepare the ends of the pooled amplified samples to accept the unique indexed adapters and the final library amplification.

Important points before starting

- This protocol is for constructing sequencing libraries for Illumina NGS platforms using the QIAseq Single Cell DNA Library Kit UDI.
- The amplified DNA should be diluted to 250 ng/ μ L Nuclease-Free Water before starting.
- Program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved on a thermal cycler in advance (Table 3).
- Prepare fresh 80% ethanol.
- Equilibrate QIAseq beads at room temperature (15–25°C).

Procedure: End-polishing of MDA pool

This protocol describes the reaction of end-polishing of the pooled MDA DNA from previous step. This reaction generates a sticky end, which allows a directed ligation of the BC-i5-UDI adapter upstream from Sample Barcode.

1. Thaw all kit components on ice. Once reagents are thawed, mix buffers thoroughly by quickly vortexing the tubes to avoid any localized concentrations.
2. Mix EP-Facilitator and EP-enhancer by tapping the tube and place on ice. Briefly centrifuge down all reagents before use.
3. Program a thermocycler according to Table 3 and start the program.
4. When the thermocycler block reaches 4°C, pause the program.

Table 3. End-polishing reaction conditions

Step	Temperature (°C)	Time (min)
1	4	1
2	37	60
3	4	Hold

- Dilute amplified pooled MDA DNA to 250 ng/μL in Nuclease-Free Water.
- Pipette 20 μL of the diluted DNA in PCR tubes or strips and place them on ice or in a cooling block.

Note: 20 μL of the diluted DNA should be 5 μg in total. Do not exceed 5 μg to not negatively affect the end-polishing efficiency.

- Prepare the end-polishing reaction mix on ice according to Table 4 and mix by pipetting. Add the components of the End-polishing Reaction Mix in the same order as stated in the table. Pipette up and down the buffer mix. You can scale up the End-polishing Reaction Mix according to the number of samples required.

Table 4. End-polishing reaction setup

Component	Volume for 1 reaction* (μL)	Volume for 24+2 reactions* (μL)
Barcoded MDA Pool	20	–
Nuclease-Free Water	4	104
EP Buffer 10x (440/1.5)	3	78
EP Facilitator	0.5	13

* Mix by pipetting and keep on ice.

- When working with a single MDA pool pipette the components as shown in Table 4 in the column “Volume for 1 reaction (μL)”, mix and place on ice.
 - When working with the mix for 24 + 2 samples add 7.5 μL of end-polishing master mix to each 20 μL of diluted barcoded MDA pool. Gently vortex to mix and place on ice.
- Add 2.5 μL EP Enhancer to the end-polishing mixes from step 8. This should give a total reaction volume of 30 μL. Mix by vortexing and spin down.
- Immediately place in the cyclor and run the program shown in Table 3.

11. Once the cycling is complete place the reaction on ice and proceed with BC-i5-UDI adapter ligation.

Procedure: BC-i5-UDI Adapter ligation and clean-up

12. Program the thermocycler as outlined in Table 5. Turn the heating lid off.

Table 5. Ligation reaction conditions

Step	Temperature (°C)	Time (min)
1	4	1
2	25	30
3	4	Hold

13. Place DNA Ligase on ice. Thaw 5x Ligation Buffer and adapter plates at room temperature (15–25°C).

14. Prepare the ligation master mix as described in Table 6.

Table 6. BC-i5-UDI ligation mix

Component	Volume for 1 reaction (µL)	Volume for 24+2 reactions (µL)
Sample from previous step	30	–
Nuclease-Free Water	31.5	819
5x DNA Ligase Buffer	18	468
DNA Ligase	4	104
BC-i5-UDI	6.5	–

Note: A different BC-i5-UDI adapter is used for each 30 µL sample.

15. a. When working with one sample prepare the ligation mix as outlined in column “Volume for 1 reaction (µL)” in Table 6. Mix and spin down.
- b. When working with 24 samples, prepare the master mix as outlined in column “Volume for 24+2 reactions” in Table 6. Add 53.5 µL of the ligation master mix and 6.5 µL of one BC-i5-UDI adapter to each 30 µL sample from the step 11 of the protocol. Take note of the adapter used for each sample. Mix and spin down.

Important: Only one single adapter should be used per ligation reaction.

16. Replace the adapter plate lid and freeze unused adapters. The adapter plate is stable for a maximum of 10 freeze–thaw cycles.
17. Place the ligation mixes in the cycler and start the Ligation cycling program from Table 5.
Important: Do not use a thermocycler with a heated lid.

Cleanup of i5-ligation product

18. Perform a 1x QIAseq Beads purification by adding 90 μL of completely resuspended QIAseq Beads slurry to each ligation reaction and mix well by pipetting or gently vortexing.
19. Incubate the mixture for 5 min at room temperature (15–25°C).
20. Spin down to collect remaining droplets and pellet the beads on a magnetic stand for 2 min, then carefully discard the supernatant.
21. Wash the beads by adding 200 μL fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand for 2 min, then carefully discard the supernatant.
22. Repeat the wash step 21 once for a total of 2 ethanol washes.
23. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Avoid over-drying, which may result in lower DNA recovery. Remove from the magnetic stand.
24. Elute by resuspending in 55 μL Nuclease-Free Water. Pellet the beads on the magnetic stand. Carefully transfer 52 μL supernatant to a new PCR plate/tube.
25. Perform a second 1x QIAseq Beads purification using 52 μL of resuspended QIAseq Beads to each sample and mix.
26. Repeat steps 19–23.
27. Elute by resuspending in 25 μL Nuclease-Free Water. Pellet the beads on the magnetic stand. Carefully transfer 22 μL of supernatant into a new PCR plate. Store ligated product libraries at -20°C .

Procedure: DNA fragmentation and end-polishing

1. Thaw all kit components on ice. Once reagents are thawed, mix buffers thoroughly by repeated vortexing to avoid any localized concentrations. Mix Fragmentation enzyme mix by flicking the tube.
2. Program a thermocycler according to Table 7 and start the program. Set the heated lid at 70°C.
3. When the thermocycler block reaches 4°C, pause the program.

Table 7. End-polishing reaction conditions

Step	Temperature (°C)	Time (min)
1	4	1
2	32	8
3	65	30
4	4	Hold

4. Prepare the fragmentation and end-polishing reaction mix on ice according to Table 8 and mix by pipetting. Add all components of the Reaction Mix in the same order as stated in the table. Pipette up and down the buffer mix. You can scale up the Reaction Mix according to the number of samples required.

Table 8. Fragmentation and end-polishing Reaction Setup

Sample protocol	Volume for 1 reaction* (µL)	Volume for 24+2 reactions* (µL)
Sample from step 27	16.75	–
FX Buffer. 10x (0.12/1.5)	2.5	65
FERA Solution	0.75	19.5
FX Enzyme Mix	5	130

* Mix by pipetting and keep on ice.

5. a. When working with a single MDA pool pipette the components as shown in Table 8 (previous page) in the column "Volume for 1 reaction (μL)", mix and place on ice.
- b. When working with 24 samples add 8.25 μL of the master mix to each 16.75 μL of the i5-ligated product from step 27. Gently vortex to mix and place on ice.
6. Place the reaction mixes in the cycler and run the program shown in Table 7.
7. Once the cycling is complete place sample on ice and proceed with BC-i7-UDI adapter ligation.

Procedure: BC-i7-UDI Adapter ligation and clean-up

1. Program the thermocycler as outlined in Table 9. Set the heating lid to off. When the thermocycler block reaches 4°C, pause the program.

Table 9. Ligation reaction conditions

Step	Temperature (°C)	Time (min)
1	4	1
2	25	15
3	4	Hold

2. Place DNA Ligase on ice. Thaw 5x Ligation Buffer and adapter plates at room temperature (15–25°C).
3. Prepare the ligation master mix as described in the Table 10.

Table 10. BC-i5-UDI ligation mix

Component	Volume for 1 reaction (µL)	Volume for 24+2 reactions (µL)
Sample from previous step	25	–
Nuclease-Free Water	0.3	7.8
FG Solution	7.2	187.2
5x DNA Ligase Buffer	10	260
DNA Ligase	5	130
BC-i7-UDI	2.5	–

Note: A separate BC-i7-UDI adapter must be used for each sample.

4. a. When working with one sample prepare the ligation mix as outlined in column “Volume for 1 reaction (µL)” in Table 10. Mix and spin down.
b. When working with 24 samples prepare the master mix as outlined in column “Volume for 24+2 reactions (µL)” in Table 10. Add 22.5 µL of the ligation master mix and 2.5 µL of one BC-i7-UDI adapter to each 25 µL sample from step 7 of the previous procedure. Protocol the adapter used for each sample. Mix and spin down. Total volume of the BC-i7-UDI ligation reaction is 50 µL.
Important: Only one single adapter should be used per ligation reaction.
5. Freeze unused adapters. The adapter plate is stable for a maximum of 10 freeze–thaw cycles.
6. Place the ligation mixes in the cycler and start the Ligation cycling program from Table 9.
Important: Do not use a thermocycler with a heated lid.

Cleanup of i7-ligation product

7. After ligation is complete add 50 µL of Nuclease-Free Water to the 50 µL sample of step 6.
8. Perform a 0.8x QIAseq Beads purification by adding 80 µL of complete resuspended QIAseq Beads slurry to each ligated sample and mix well by pipetting or gently vortexing.

9. Incubate the mixture for 5 min at room temperature.
10. Spin down to collect remaining droplets and pellet the beads on a magnetic stand, then carefully discard the supernatant.
11. Wash the beads by adding 200 μL fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand, then carefully discard the supernatant.
12. Repeat the wash step 11 once for a total of 2 ethanol washes.
13. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Avoid overdrying, which may result in lower DNA recovery. Remove from the magnetic stand.
14. Elute by resuspending in 53 μL Nuclease-Free Water. Pellet beads on the magnetic stand. Carefully transfer 50 μL supernatant to a new PCR plate/tube.
15. Perform a second 0.8x QIAseq Beads purification using 40 μL of resuspended QIAseq Beads to each sample and mix.
16. Repeat steps 9–13.
17. Elute by resuspending in 18 μL Nuclease-Free Water. Pellet the beads on the magnetic stand. Carefully transfer 15 μL of supernatant into a new PCR plate. Store ligated product libraries at -20°C .

Procedure: Final Library Amplification and clean-up

1. Thaw sample from previous step and HiFi PCR Master Mix and Primer Mix on ice. Mix by flicking the tubes and spin down.
2. Program the thermocycler as outlined in Table 11. Set heated lid on.

Table 11. Library amplification cycling conditions

Step	Temperature (°C)	Time	Cycle Number
1	98	2 min	1x
2	98	20 s	16x
	62	30 s	
	72	30 s	
3	72	1 min	1x
4	4	Hold	Hold

3. Prepare the library amplification master mix as described in the Table 12.

Table 12. Library amplification reaction mix

Component	Volume for 1 reaction (µL)	Volume for 24+2 reactions (µL)
Sample from previous step	11.5	–
HiFi PCR Master Mix, 2x	12.5	325
Primer Mix Illumina Library Amp	1	26

4. a. When working with one sample prepare the library amplification mix as outlined in column “Volume for 1 reaction (µL)” in Table 12. Mix and spin down.
- b. When working with 24 samples prepare the master mix as outlined in column “Volume for 24+2 reactions (µL)” in Table 12. Add 13.5 µL of the amplification master mix to each 11.5 µL sample from step 17 of the i7-ligated product. Mix and spin down. Total volume of the amplification reaction is 25 µL.
5. Place the HiFi reaction mixes in the cycler and start the library amplification program from Table 11.

Final Library Cleanup

6. After amplification is complete add 75 µL of Nuclease-Free Water to the 25 µL sample of step 5.

7. Perform a 1x QIAseq Beads purification by adding 100 μL of complete resuspended QIAseq Beads slurry to each ligated sample and mix well by pipetting or gently vortexing.
8. Incubate the mixture for 5 min at room temperature.
9. Spin down to collect remaining droplets and pellet the beads on a magnetic stand for 2 min, then carefully discard the supernatant.
10. Wash the beads by adding 200 μL fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand, then carefully discard the supernatant.
11. Repeat the wash step 10 once for a total of 2 ethanol washes.
12. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Remove from the magnetic stand.
13. Elute by resuspending in 23 μL Nuclease-Free Water. Pellet beads on the magnetic stand. Carefully transfer 20 μL supernatant to a new PCR plate/tube.
14. Store final libraries at -20°C .
15. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution (Figure 2) of library fragments and for the absence of adapters or adapter dimers.

Note: The median size of the DNA fragment should be shifted by the size of the adapters that were ligated to the library fragments (approx. 140 bp).

Note: The main peak maximum can be used for subsequent qPCR-based quantification methods.

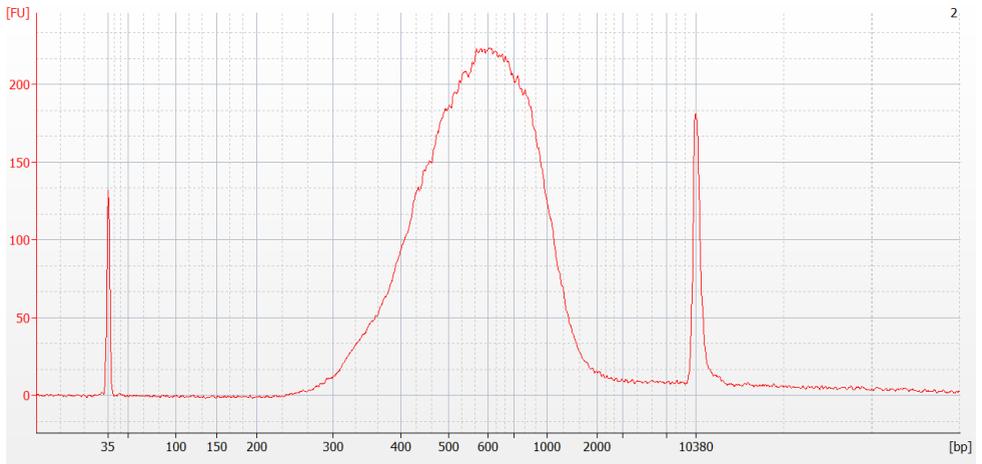


Figure 2. Capillary electrophoresis device trace of generated libraries. Capillary electrophoresis device trace data showing the correct size distribution of the library after amplification of 16 cycles. The average fragment size of these libraries is 600–700 bp.

16. Quantify the library using the QIAseq Library Quant Assay Kit (ordered separately).

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Comments and suggestions

Low library yields from library preparation protocol

- | | |
|--|---|
| a) Inefficient incorporation of Sample Barcodes during MDA | Quantify the MDA yields using PicoGreen. Yields lower than 100 ng/ μ L indicate lower Sample Barcode incorporation. Check for the DNA quality used as input for the reaction.

Yields greater than 500 ng also indicate poor incorporation of Barcodes due to poor ratio to random primers. Check for right reaction setup, the DNA input and do not increase the input of random primer in the reaction mix. |
| b) Inefficient end-polishing and adapter ligation | Quantify the pool accurately and do not exceed 5 μ g in the end-polishing reaction. |
| c) Suboptimal reaction conditions due to ethanol contamination | Ensure that ethanol residue has been fully removed during washing steps of the MDA pool. Remove carefully ethanol residues and dry the beads, since ethanol contamination inhibits the subsequent enzymatic reaction. |
| d) Suboptimal reaction conditions during end-polishing | Ensure that cycling conditions and incubation temperatures are programmed correctly. |
| e) Inefficient library amplification | Inefficient adapter–dimer removal using QIAseq Beads. Mix beads thoroughly and use the recommended amount for purification.

Beads are overdried. Make visual control during drying of beads; humidity of environment may accelerate or slow the drying procedure.

Ethanol residues inhibit the reaction. Ensure that all liquids have been removed prior to beads drying. If uncertain, perform a short spin to collect the liquid droplets and remove carefully before drying the beads. |

Comments and suggestions

Unexpected signal peaks in capillary electrophoresis device traces

- | | |
|--|---|
| a) Presence of shorter peaks between 60 bp and 120 bp | These peaks represent library adapters and adapter–dimers that occur when there is no – or insufficient – adapter depletion after library preparation. As adapter–dimers can form clusters on the flow cell and will be sequenced, this will reduce the capacity of the flow cell for the library fragments, even though a low ratio of adapter–dimers versus library will not be a problem. QIAseq Beads efficiently remove adapter–dimers, as well as free adapter molecules. |
| b) Presence of larger library fragments after library enrichment | When performing library enrichment, if the fragment population shifts higher than expected after adapter ligation and PCR enrichment (e.g., more than the expected 140 bp shift), this may be a PCR artifact due to over-amplification of the DNA library. Reduce the number of amplification cycles. |

Appendix A: Determination of Concentration and Quality of Amplified DNA

Quantification of DNA yield

A 25 μ L QIAseq UPX WGA reaction typically yields approximately 3–10 μ g of DNA depending on the quality of the input material. These yields allow direct use of the amplified DNA in most downstream genotyping experiments and library preparation for sequencing. For a more accurate quantification of the amplified DNA, it is important to utilize a DNA quantification method that is specific for double-stranded DNA, because amplification products contain unused reaction primers and barcodes. Quant-iT™ PicoGreen dsDNA reagent displays enhanced binding to double-stranded DNA and may be used in conjunction with a fluorometer to quantify the double-stranded DNA product. A protocol for the quantification of QIAseq Single Cell amplified DNA is discussed below.

PicoGreen Quantification of QIAseq Single Cell Amplified DNA

This protocol is designed for quantification of double-stranded QIAseq Single Cell amplified DNA using Quant-iT PicoGreen dsDNA reagent.

Alternatively, Qubit quantification might be also performed according to manufacturer's protocol. We recommend diluting the QIAseq single cell amplified DNA 1:100 when using the Qubit dsDNA HS Assay Kit ([thermofisher.com](https://www.thermofisher.com) > **Qubit Fluorometric Quantitation** > **Qubit Assays**).

Important: When working with hazardous chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (SDSs), available from the product supplier.

Equipment and reagents to be supplied by user

- Quant-iT PicoGreen dsDNA Reagent (Invitrogen, cat. no. P7581)
- Buffer TE (10 mM TrisCl; 1 mM EDTA, pH 8.0)
- Human Genomic DNA (e.g., Promega, cat. no. G3041)
- 2 mL microcentrifuge tube, or 15 mL Falcon® tubes (Corning, Inc.)
- 96-well plates (suitable for use in a fluorescence microplate reader)
- Fluorescence microplate reader (e.g., Tecan® ULTRA)

Dilution steps and measurement

1. Make a 1:200 dilution of PicoGreen stock solution in Buffer TE. Each quantification reaction requires 50 μ L. Depending on the final volume, use a 2 mL microcentrifuge tube or a 15 mL Falcon tube. Cover the tube in aluminum foil or place it in the dark to avoid photodegradation of the PicoGreen reagent.
2. For example, to prepare enough PicoGreen working solution for 100 samples, add 25 μ L PicoGreen to 4975 μ L Buffer TE

Important: Prepare the PicoGreen/TE solution in a plastic container as the PicoGreen reagent may adsorb to glass surfaces.

3. Prepare a 16 μ g/mL stock solution of genomic DNA in Buffer TE.
4. Make 200 μ L of 1.6, 0.8, 0.4, 0.2, and 0.1 μ g/mL DNA standards by further diluting the 16 μ g/mL genomic DNA with Buffer TE.
5. Transfer 50 μ L of each DNA standard in duplicate into 96-well plate labeled A (Figure 3).

Note: The 96-well plate must be suitable for use in a fluorescent microplate reader.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H	Blank	Blank	1.6	0.8	0.4	0.2	0.1	1.6	0.8	0.4	0.2	0.1

Figure 3. 96-well plate. Shaded squares: genomic DNA standards ($\mu\text{g}/\mu\text{L}$).

6. Mix well by pipetting up and down, place 2 μL of each QIAseq Single Cell amplified DNA sample for quantification into a new 96-well plate, and add 198 μL Buffer TE to make a 1:100 dilution. Store the remaining QIAseq Single Cell amplified DNA at -20°C .
7. Place 5 μL diluted QIAseq Single Cell amplified DNA (from step 8) into an unused well of 96-well plate A and add 45 μL Buffer TE to make a 1:1000 dilution.
8. The 1:100 dilutions from step 5 can be stored at -20°C and used for future downstream sample analysis.
9. For Blanc measurements, pipette 50 μL Buffer TE in 2 empty wells of plate A.
10. Add 50 μL PicoGreen working solution (from step 1) to each sample (amplified DNA and DNA standards) in 96-well plate A. Gently shake the plate on the bench top to mix the samples and reagent.
11. Centrifuge the 96-well plate briefly to collect residual liquid from the walls of the wells and incubate in the dark for 5 min at room temperature.

12. Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescence filters (excitation: approx. 480 nm; emission: approx. 520 nm).
13. To ensure that the sample readings remain in the detection range of the microplate reader, adjust the instrument's gain so that the sample with the highest DNA concentration yields fluorescence intensity near the fluorometer's maximum.

Calculation of DNA concentration and yield

1. Generate a standard curve by plotting the concentration ($\mu\text{g}/\text{mL}$) of DNA standards (x-axis) against the fluorescence reading generated by the microplate reader (y-axis). Plot an average of the fluorescence recorded for each DNA standard of the same concentration.
2. Use the standard curve to determine the concentration ($\mu\text{g}/\text{mL}$) of the diluted QIAseq Single Cell amplified DNA sample. This is achieved by plotting the fluorescence reading of the sample against the standard curve and reading the DNA concentration on the x-axis.

Note: The calculation of DNA concentration depends on the standard curve and the determination of the slope. For accurate results, the standard curve should be a straight line. Any deviation from this may cause inaccuracies in the measurement of QIAseq Single Cell amplified DNA concentrations.

3. Multiply the value determined in step 12 by 1000 to show the concentration of undiluted sample DNA (since the sample DNA measured by PicoGreen fluorescence had been diluted 1:1000).
4. To determine the total amount of DNA in your sample, multiply the concentration of undiluted sample DNA ($\mu\text{g}/\text{mL}$, determined in step 12) by the reaction volume in milliliters (e.g., for a 50 μL reaction, multiply by 0.05).

Quantification of locus representation

Locus representation for each sample can be quantified by real-time PCR.

If gDNA amplified via WGA is to be used in costly and labor-intensive downstream applications such as NGS, we strongly recommend controlling first the quality of the WGA samples using qPCR.

Each qPCR reaction should contain 5–10 ng of the gDNA amplified via WGA. Real-time PCR assays that recognize conservative gDNA regions are recommended. For example, QIAGEN's QuantiFast® Probe Assays, which detect exon region of the genes and therefore amplify gDNA, in combination with QuantiNova® or QuantiFast mixes, are recommended for such quality control assays.

For further information, please refer to the respective kit handbooks, which are available at www.qiagen.com, or contact QIAGEN Technical Services.

Appendix B: QIAseq UPX Single Cell Sample Barcodes

Sequences of Sample Barcodes

Sample Barcode sequences for demultiplexing of the FASTQ file generated after the Illumina sequencing run are available for download at [qiagen.com](https://www.qiagen.com).

The layout of the 24 unique Sample Barcodes is shown in Figure 4. The 24 index motifs are distributed 4 times on each of the 6 plates. Multiplexing of 24 Sample Barcoded MDAs in one Library using the 24-plex set of BC-i5-UDI and BC-i7-UDI adapter results in multiplexing of 576 samples. The 24 unique index motifs used in the QIAseq UPX Single Cell Sample Barcodes are listed in Table 14.

	1	2	3	4	5	6	7	8	9	10	11	12
A	SBC 001	SBC 009	SBC 017	SBC 001	SBC 009	SBC 017	SBC 001	SBC 009	SBC 017	SBC 001	SBC 009	SBC 017
B	SBC 002	SBC 010	SBC 018	SBC 002	SBC 010	SBC 018	SBC 002	SBC 010	SBC 018	SBC 002	SBC 010	SBC 018
C	SBC 003	SBC 011	SBC 019	SBC 003	SBC 011	SBC 019	SBC 003	SBC 011	SBC 019	SBC 003	SBC 011	SBC 019
D	SBC 004	SBC 012	SBC 020	SBC 004	SBC 012	SBC 020	SBC 004	SBC 012	SBC 020	SBC 004	SBC 012	SBC 020
E	SBC 005	SBC 013	SBC 021	SBC 005	SBC 013	SBC 021	SBC 005	SBC 013	SBC 021	SBC 005	SBC 013	SBC 021
F	SBC 006	SBC 014	SBC 022	SBC 006	SBC 014	SBC 022	SBC 006	SBC 014	SBC 022	SBC 006	SBC 014	SBC 022
G	SBC 007	SBC 015	SBC 023	SBC 007	SBC 015	SBC 023	SBC 007	SBC 015	SBC 023	SBC 007	SBC 015	SBC 023
H	SBC 008	SBC 016	SBC 024	SBC 008	SBC 016	SBC 024	SBC 008	SBC 016	SBC 024	SBC 008	SBC 016	SBC 024

Figure 4. QIAseq UPX Single Cell DNA Sample Barcodes plate layout.

Table 13. Sample Barcode motifs

Unique SBC index number	Bases to enter into CLC workflow for demultiplexing
SBC001	CGCCGCGGCG
SBC002	CGGAATTCCG
SBC003	CGGTTAACCG
SBC004	CGGCCGCGCG
SBC005	GCCGTACGGC
SBC006	CGCGTACGCG
SBC007	GCCAATTGGC
SBC008	GCCTCGAGGC
SBC009	CCGCCGGCGG
SBC010	GGCTTAAGCC
SBC011	GGCACGTGCC
SBC012	CGACTAGTCG
SBC013	GAGACAGCTC
SBC014	GAGATGTCTC
SBC015	TGACTTATCA
SBC016	CAGGAACCTG
SBC017	GGTTCCAACC
SBC018	GCTAGTGAGC
SBC019	GTCTGAGGAC
SBC020	CACATCAGTG
SBC021	CAATTCGTTG
SBC022	TGTGCTGACA
SBC023	ACATTCTTGT
SBC024	CTGCGCACAG

Appendix C: QIAseq UPX Single Cell BC-i5 and BC-i7 Unique Adapters

Generation of sample sheets for Illumina instruments

The index sequences for QIAseq UPX Single Cell BC-i5 and BC-i7 Unique Adapters and the structure is fully compatible with the QIAseq UDI Y-Adapters. All index sequences are available for download at [qiagen.com](https://www.qiagen.com). Library prep definition files allow you to use the Illumina Experiment Manager Software to create sample sheets according to your needs. Alternatively, ready-to-use sample sheets containing all QIAseq UDI Y-Adapter barcode sequences are available for MiSeq, NextSeq, MiniSeq, HiSeq, and NovaSeq instruments and can be used with QIAseq UPX Single Cell BC-i5 and BC-i7 Unique Adapters. These can be edited using the Illumina Experiment Manager Software or any text editor.

The following guide describes how to set up custom library prep kits within the Illumina Experiment Manager Software (version 1.18.1). Alternatively, refer to the Illumina Experiment Manager User Guide (support.illumina.com/downloads/illumina-experiment-manager-user-guide-15031335.html).

1. Download custom library prep definition files from www.qiagen.com.
2. Locate the installation directory of the Illumina Experiment Manager (typically, it would be in **Program Files\Illumina\Illumina Experiment Manager**).
3. Place copies of the downloaded files **QIAseq UDI Y.txt** in the "SamplePrepKits" folder.
4. Navigate to the "Applications" folder and locate the files:
 - GenerateFASTQ.txt
 - NextSeqGenerateFASTQ.txt
 - HiSeqGenerateFASTQ.txt
 - NovaSeqGenerateFASTQ.txt.

Apply steps 5–7 to each file.

5. Open each file in a text editor and locate the text block [Compatible Sample Prep Kits].
6. Generate 1 new line underneath the header, and then add the following entry:
 - QIAseq UDI-Y
7. Save and close the file when complete.
8. Restart the Illumina Experiment Manager and select **Create Sample Sheet**.
9. After selecting the instrument, navigate to the respective “FASTQ Only” workflow.
10. In the run settings for “Library Prep Workflow”, select **QIAseq UDI-Y** to generate a sample sheet for QIAseq Y-adapters.

QIAseq UPX Single Cell BC-i5-UDI and BC-i7-UDI adapters

The layout of the 24-plex single-use BC-i5-UDI and BC-i7-UDI adapter plate is shown in Figure 5 and Figure 6. The index motifs used in the QIAseq Unique Dual Index Kits are listed in Table 14.

To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments at [qiagen.com](https://www.qiagen.com).

	1	2	3	4	5	6	7	8	9	10	11	12
A	BC-i5-UDI001	BC-i5-UDI009	BC-i5-UDI017	Empty								
B	BC-i5-UDI002	BC-i5-UDI010	BC-i5-UDI018	Empty								
C	BC-i5-UDI003	BC-i5-UDI011	BC-i5-UDI019	Empty								
D	BC-i5-UDI004	BC-i5-UDI012	BC-i5-UDI020	Empty								
E	BC-i5-UDI005	BC-i5-UDI013	BC-i5-UDI021	Empty								
F	BC-i5-UDI006	BC-i5-UDI014	BC-i5-UDI022	Empty								
G	BC-i5-UDI007	BC-i5-UDI015	BC-i5-UDI023	Empty								
H	BC-i5-UDI008	BC-i5-UDI016	BC-i5-UDI024	Empty								

Figure 5. QIAseq UPX Single Cell BC-i5-UDI Adapter Plate (24) layout (BC-i5-UDI 1–24).

	1	2	3	4	5	6	7	8	9	10	11	12
A	BC-i7-UDI001	BC-i7-UDI009	BC-i7-UDI017	Empty								
B	BC-i7-UDI002	BC-i7-UDI010	BC-i7-UDI018	Empty								
C	BC-i7-UDI003	BC-i7-UDI011	BC-i7-UDI019	Empty								
D	BC-i7-UDI004	BC-i7-UDI012	BC-i7-UDI020	Empty								
E	BC-i7-UDI005	BC-i7-UDI013	BC-i7-UDI021	Empty								
F	BC-i7-UDI006	BC-i7-UDI014	BC-i7-UDI022	Empty								
G	BC-i7-UDI007	BC-i7-UDI015	BC-i7-UDI023	Empty								
H	BC-i7-UDI008	BC-i7-UDI016	BC-i7-UDI024	Empty								

Figure 6. QIAseq UPX Single Cell BC-i7-UDI Adapter Plate (24) layout (BC-i7-UDI 1–24).

Table 14. UDI motifs used in the QIAseq UPX Single Cell DNA Library Kit

Indexes for entry on sample sheet

Unique i5-index number	i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)	i5 bases for entry on sample sheet (iSeq100, MiniSeq, NextSeq, HiSeqX, HiSeq 3000/4000)*	Unique i7-index number	i7 bases for entry on sample sheet (all instruments)
BC-i5-UDI001	ATGGCCGACT	AGTCGGCCAT	BC-i7-UDI001	TGAACGTTGT
BC-15-UDI002	CGATGAGCAC	GTGCTCATCG	BC-17-UDI002	ACCAGACTTG
BC-15-UDI003	GATAAGTCGA	TCGACTTATC	BC-17-UDI003	ACTGGCGAAC
BC-15-UDI004	TCACGCCTTG	CAAGGCGTGA	BC-17-UDI004	GCGTTAGGCA
BC-15-UDI005	AGGAACACAA	TTGTGTTCT	BC-17-UDI005	TTATCGGCCT
BC-15-UDI006	CTCAGTAGGC	GCCTACTGAG	BC-17-UDI006	GAGGTATAAG
BC-15-UDI007	GAAGTGCTCG	CAGGCACCTC	BC-17-UDI007	TCAAGGATTC
BC-15-UDI008	TCTCTGCCT	AGGCGAGAGA	BC-17-UDI008	CGAACCGAGA
BC-15-UDI009	AGGCACCTTC	GAAGGTGCCT	BC-17-UDI009	GAGCCAAGTT
BC-15-UDI010	CTGTTGGTAA	TTACCAACAG	BC-17-UDI010	AAGGCCGTAG
BC-15-UDI011	GCTGGTACCT	AGGTACCAGC	BC-17-UDI011	TTAGAGAAGC
BC-15-UDI012	TAAGGAGCGG	CCGCTCCTTA	BC-17-UDI012	TCTAAGACCA
BC-15-UDI013	AATCGCTCCA	TGGAGCGATT	BC-17-UDI013	TGTAACCACT
BC-15-UDI014	CTCCTAATTG	CAATTAGGAG	BC-17-UDI014	CCGACACAAG
BC-15-UDI015	GCCTCATAAT	ATTATGAGGC	BC-17-UDI015	CTCTGATGGC
BC-15-UDI016	TGTATTGAGC	GCTCAATACA	BC-17-UDI016	CGGCCTGTTA
BC-15-UDI017	AGCCATAACA	TGTATGGCT	BC-17-UDI017	TGCATAGCTT
BC-15-UDI018	CCACAAGTGG	CCACTGTGG	BC-17-UDI018	AACCTTCTCG
BC-15-UDI019	GTTATCACAC	GTGTGATAAC	BC-17-UDI019	AAGAGATCAC
BC-15-UDI020	TACCGTTCTT	AAGAACGGTA	BC-17-UDI020	GCCTGAAGGA
BC-15-UDI021	AGGCGTTAGG	CCTAACGCCT	BC-17-UDI021	ATTGTGCCTT
BC-15-UDI022	CCGTAACGTC	GACGTTACGG	BC-17-UDI022	TCCTTACCG
BC-15-UDI023	GTAATAGCCA	TGGCTATTAC	BC-17-UDI023	TACCATGAAC
BC-15-UDI024	TAGCGCCGAT	ATCGGCGCTA	BC-17-UDI024	CATTGGCAGA

Appendix D: Sequencing Recommendations on Illumina Platforms

Sequencing recommendations for whole genome sequencing analysis are summarized in the Table 15.

Table 15. Sequencing recommendations

Instrument	Sequencing recommendation	Comments
iSeq	PE 2 x 150 bp	Small genomes
MiniSeq	PE 2 x 150 bp	Small genomes
MiSeq	PE 2 x 150 bp	Small genomes and library QC
NextSeq	PE 2 x 150 bp	All types
HiSeq	PE 2 x 125 bp	All types
NovaSeq	PE 2 x 150 bp	All types

Pool and dilute QPCR quantified Libraries to 2 nM and load 10 pM on Illumina MiSeq flow cells for optimal clustering. Load on other instruments as recommended by Illumina for each instrument.

When sequencing in 2-Channel SBS Instruments we recommend to add 10% PhiX to increase complexity of the Read 1, which is starting with the Sample Barcodes.

Appendix E: Exome Enrichment Using the QIAseq xHYB Human Hybrid Capture Panels

Exome sequencing has been widely adopted in the past 10 years as an efficient way of screening the genome for disease-associated mutations. By focusing reads on coding regions, which harbor >80% of disease-causing mutations, the probability of identifying variants associated with disease is increased. At the same time, the amount of sequencing required is reduced by 99% compared to whole genomes, significantly minimizing the cost of sequencing. The QIAseq Single Cell DNA libraries are fully compatible with the QIAseq Human Exome kits, deliver excellent coverage uniformity regardless of the target GC composition, and allow sensitive detection of pathogenic variants even from single cells, while reducing cost and increasing scalability.

Up to 8 QIAseq UPX Single Cell DNA libraries can be pooled together equimolarly to generate exome libraries for sequencing on Illumina instruments. Following the *QIAseq xHYB Human Hybrid Capture Panels Handbook* recommendations, 200 ng to 3 µg library pool can enter the hybridization protocol. It is recommended to adjust the input in QIAseq xHyb protocol by multiplexing QIAseq UPX Single Cell DNA libraries and not by increasing the number of cycles during the final library amplification outlined in page 33, as this will increase the percentage of duplicate reads. The QIAseq UPX Single Cell DNA Library quantification over QPCR is important to maintain equal library representation during the hybrid capture protocol.

For detailed protocol instructions please refer to the *QIAseq Human Exome Handbook* (qiagen.com/HB-2731).

Appendix F: Enrichment of Small Genomes Using the Target-specific Primers

Important points before starting

- This protocol is optimized to specifically boost whole-genome amplification of small genomes, starting from 500 pg to 10 ng of purified DNA template. It is recommended to use template DNA suspended in Buffer TE or H₂O. DNA of low quality or contaminated with detergents or organic solvents may compromise the amplification reaction.
- Avoid DNA contamination of reagents by using dedicated laboratory equipment (e.g., pipettes, filter pipette tips, reaction vials, etc.).
- REPLI-g sc DNA Polymerase should be thawed on ice. All other components can be thawed at room temperature.
- Specific primers can be designed using open source online primer design tools such as Primer3 (bioinfo.ut.ee/primer3-0.4.0/) or Primer-BLAST (ncbi.nlm.nih.gov/tools/primer-blast/). The Primer Blast allows specificity checking. It is recommended to set T_m of primer to 34–40°C and length between 17 bp and 21 bp. Amplicon length should be 300–4000 bp. We recommend to choose alternating primer on both strands every 2000–3000 bp to capture the total length of the genome intended to be amplified.
- Primers should be stabilized on 3' primer end with phosphorothioate modifications on the last 3'-bases as shown in the example 5'-NNNNNNNNNNN*N*N-3'. Primer mix should include 10 μM of each primer.

Things to do before starting

- Prepare Buffer DLB by adding 500 μL H₂O sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.

Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile.

- Vortex all buffers and reagents before use to ensure thorough mixing. Mix enzymes by flicking the tube. Collect reagents in the bottom of the tube by centrifuging briefly.
- Set a programmable thermal cycler or a heating block to 30°C.
- If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

Procedure: Whole genome amplification using target-specific primers

1. Thaw H₂O sc, DTT, and REPLI-g sc dilution buffer at room temperature (15–25°C), vortex, and then centrifuge briefly. The REPLI-g sc Dilution Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
2. Prepare sufficient Buffer D2 (denaturation buffer) for the total number of whole genome amplification reactions (Table 16).

Note: The total volume of Buffer D2 given in Table 16 is sufficient for 24 reactions. If performing fewer reactions, store residual Buffer D2 at –20°C. Buffer D2 should not be stored longer than 3 months.

Table 16. Preparation of Buffer D2 for 26 reactions

Component	Volume for 24+2 reactions (µL)
DTT, 1 M	3.25
Buffer DLB (reconstituted)*	35.75
Total volume	39

* Reconstitution of Buffer DLB is described in “Things to do before starting,” page 18.

3. Place 2 µL gDNA into each well of a Sample Barcode Plate. If not using the complete plate, cut the wells required for the corresponding number of samples on the marked line using a pair of scissors.
4. Add 1.5 µL Buffer D2. Mix carefully by gently flicking the tube and centrifuge briefly.
5. Incubate gDNA preparations for 5 min at 25°C, and then cool down to 4°C.
6. Add 1.5 µL Stop Solution. Mix carefully by flicking the tube, and then centrifuge briefly. Immediately place on ice or ice blocks.

7. Thaw REPLI-g sc DNA Polymerase on ice. Tip gently on the tube to mix and centrifuge briefly.
8. Prepare a master mix according to Table 17. Mix and centrifuge briefly.

Note: Scale up accordingly if performing several reactions at once by preparing a Master Mix sufficient for the total number of reactions.

Note: Use target specific primers to boost the amplification of small genomes in a mixture of DNA. Since barcoding during WGA is taking place using the random portion of the barcodes, other genomes of the mixture will be amplified as well but with less efficiency as the one addressed by the target specific primers.

Table 17. Preparation of master mix*

Component	Volume for 1 reaction (µL)	Volume for 24+2 reactions (µL)
H ₂ O sc	0.7	18.2
REPLI-g sc Dilution Buffer	14.5	377
Target-specific primers 10 µM each	2.5	65
UPX Booster sc	1.8	46.8
Total volume	19.5	507

9. For each amplification reaction, add 19.5 µL master mix to 5 µL denatured DNA (from step 6). Mix by flicking the tube, and then centrifuge briefly.
10. Incubate first at 75°C for 5 min, and then at room temperature for 5 min.
11. Add 0.5 µL REPLI-g sc DNA Polymerase to each sample, mix by flicking the tube, and then centrifuge briefly.
12. Incubate at 30°C for 12 h to amplify DNA.

Note: Incubating the samples for 12 h generates 3–10 µg of amplified DNA.
13. After incubation at 30°C, heat the heating block up to 65°C if the same heating block will be used in step 12.

Note: If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.
14. Inactivate REPLI-g sc DNA Polymerase at 65°C for 5 min.

15. If the amplified DNA will not be used immediately, store it either at 4°C (for short-term storage up to 1 week) or at –20°C (for long-term storage). The completed amplification reaction can remain in the cycler overnight at 4°C.

DNA amplified using the QIAseq UPX Single Cell DNA Library Kit should be treated as genomic DNA and should undergo minimal freeze–thaw cycles. We recommend storage of nucleic acids at a concentration of at least 100 ng/μL.

16. Amplified DNA can be directly used for pooling and library construction as described in Protocol: Library Preparation of Pooled MDA-Barcoded DNA (page 25).

Additionally, extra amplified DNA can be used for PCR analysis.

Note: If required, MDA QC can be performed prior to library generation. You may quantify the amplified DNA following the instructions in PicoGreen Quantification of QIAseq Single Cell UPX Amplified DNA. Optical density (OD) measurements overestimate REPLI-g amplified DNA and should not be used.

Ordering Information

Product	Contents	Cat. no.
QIAseq UPX Single Cell DNA Library Kit (576)	Enzymes, Buffers, Reagents for 576 MDA reactions and 24 libraries for use with Illumina Instruments	180502
Related products		
QIAseq Hyman Exome	For 3 hybridization capture reactions to process up to 24 Illumina-compatible libraries in pools of 8; contains probes, blocking oligos, and reagents for hybrid capture and post-hybrid capture amplification for whole exome enrichment	333937
QIAseq xHYB Human Hybrid Capture Panels	QIAseq xHYB panels contain materials for hybridization of targeted probes for enrichment of curated genomic regions from NGS-ready DNA libraries, such as ones generated by QIAseq FX Library Kits; includes all materials for up to 24 samples for carrier testing	333362
QIAseq Universal Normalizer Kit	Oligonucleotides, amplification reagents, and normalizer beads for Library normalization	180613, 180603
QIAseq Single Cell DNA Library Kit UDI (24)	For 24 reactions: Buffers and reagents for cell lysis, whole genome amplification, and library preparation including DNA fragmentation, end-repair, and adapter ligation. Includes QIAseq Beads and a plate containing 24 UDI barcoded adapters for use with Illumina instruments.	181703
QIAseq Single Cell DNA Library Kit UDI (96) A,B,C, or D	For 96 reactions: Buffers and reagents for cell lysis, whole genome amplification, and library preparation including DNA fragmentation, end-repair, and adapter ligation. Includes QIAseq	181705, 181725, 181765, 181785

Product	Contents	Cat. no.
QIAseq Single Cell DNA Library Kit UDI (384)	<p>beads and a plate containing 96 UDI barcoded adapters for use with Illumina instruments.</p> <p>For 384 reactions: Buffers and reagents for cell lysis, whole genome amplification, and library preparation including DNA fragmentation, end-repair, and adapter ligation. Includes QIAseq beads and 4 plates containing 96 UDI barcoded adapters (A, B, C, or D) for use with Illumina instruments.</p>	181707
QIAGEN QIAseq for next-generation sequencing applications		
QIAseq Single Cell RNA Library Kit UDI (24)	<p>For 24 reactions: Buffers and reagents for cell lysis, whole transcriptome amplification, and library preparation including DNA fragmentation, end-repair, and adapter ligation. Includes QIAseq beads and a plate containing 24 UDI barcoded adapters for use with Illumina instruments.</p>	180703
QIAseq Single Cell RNA Library Kit UDI A,B,C, or D (96)	<p>For 96 reactions: Buffers and reagents for cell lysis, whole transcriptome amplification, and library preparation including DNA fragmentation, end-repair, and adapter ligation. Includes QIAseq beads and a plate containing 96 UDI barcoded adapters (either A,B,C, or D) for use with Illumina instruments.</p>	180705, 180725, 180765, 180785
QIAseq Single Cell RNA Library Kit UDI (384)	<p>For 384 reactions: Buffers and reagents for cell lysis, whole transcriptome amplification, and library preparation including DNA fragmentation, end-repair, and adapter ligation. Includes QIAseq beads and 4 plates containing 96 UDI barcoded adapters (A, B, C, D) for use with Illumina instruments.</p>	180707

Product	Contents	Cat. no.
QIAseq Library Quant Assay Kit	Laboratory-verified forward and reverse primers for 500 x 25 µL reactions (500 µL); DNA Standard (100 µL); Dilution Buffer (30 mL); (1.35 mL x 5) GeneRead® qPCR SYBR® Green Mastermix	333314

Other kit sizes/formats available; see www.qiagen.com.

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Document Revision History

Revision	Description
01/2024	Initial release

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