October 2021

QIAseq® Single Cell DNA Library Kit with Unique Dual Indexes Handbook

For DNA-seq library construction from single cells and low-input DNA samples for next-generation sequencing with Illumina® NGS instruments

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAseq Single Cell DNA Library Kit UDI Catalog no. Number of preps	**(24) 181703 24**	**(96) 181705 96**	**(96) 181725 96**	**(96) 181765 96**	**(96) 181785 96**	**(384) 181707 384**
QIAseq Single Cell DNA Enzyme Kit	1124553	1124559	1124559	1124559	1124559	4 x 1124559
QIAseq UDI Y-Adapter Kit (24) In plate format	180310	–	–	–	–	–
QIAseq UDI Y-Adapter Kit A, B, C, or D In plate format	–	1 plate (180312)	1 plate (180314)	1 plate (180316)	1 plate (180318)	4 plates (180312, 180314, 180316, 180318)
QIAseq Beads	1107149 10 ml	1124693 1 x 25 ml	1124693 1 x 25 ml	1124693 1 x 25 ml	1124693 1 x 25 ml	1124696 2 x 50 ml

QIAseq Single Cell DNA Enzyme Kit Catalog no.	(24) 1124553 (1 x 24)	(96) 1124559 (1 x 96)	4 x (96) 1124559 (4 x 96)
REPLI-g® SC DNA Polymerase	1 tube	5 tubes	20 tubes
REPLI-g SC Dilution Buffer	1 tube	5 tubes	20 tubes
REPLI-g SC universal oligo	1 tube	3 tubes	12 tubes
REPLI-g SC advanced oligo	1 tube	3 tubes	12 tubes
Buffer DLB	1 tube	2 tubes	8 tubes
Stop Solution	1 tube	1 tube	4 tubes
PBS sc 1x	1 tube	3 tubes	12 tubes
DTT, 1 M	1 tube	1 tube	4 tubes
H2O sc	3 tubes	9 tubes	36 tubes
QIASeq Human Control DNA	1 tube	1 tube	4 tubes
HiFi PCR MMix, 2x	2 tubes	2 tubes	8 tubes
Illumina Library Ampl. Primer Mix	2 tube	1 tube	4 tubes
FX Enzyme Mix	1 tube	1 tube	4 tubes
FX Buffer, 10x	1 tube	1 tube	4 tubes
FX Enhancer	1 tube	1 tube	4 tubes
DNA Ligase	1 tube	1 tube	4 tubes
5x DNA Ligase Buffer	1 tube	2 tubes	8 tubes

Note: QIAseq single cell library UDI kits are combined with one of the four compatible unique dual-index (UDI) QIAseq Y-adapter kits. Y-adapter kits are available for 24 and 96 reactions and for multiplexing of up to 384 samples.

Important: Store QIAseq Y-adapter kits at –30 to –15°C.

QIAseq UDI Y-Adapter Kit Catalog no.	(24) 180310
QIAseq UDI Y-Adapter Plate (24)*	1
UDI Y-Adapter Plate (24) Reference Card	1

* Barcode: UDI 1–24.

QIAseq UDI Y-Adapter Kits A–D	Catalog no.	Barcodes
QIAseq UDI Y-Adapter Kit A (96)	180312	UDI 1–96
QIAseq UDI Y-Adapter Plate A (96)	1118062	
UDI Y-Adapter Plate A (96) Reference Card	1119822	
QIAseq UDI Y-Adapter Kit B (96)	180314	
QIAseq UDI Y-Adapter Plate B (96)	1118063	UDI 97–192
UDI Y-Adapter Plate B (96) Reference Card	1119823	
QIAseq UDI Y-Adapter Kit C (96)	180316	
QIAseq UDI Y-Adapter Plate C (96)	1118064	UDI 193–288
UDI Y-Adapter Plate C (96) Reference Card	1119825	
QIAseq UDI Y-Adapter Kit D (96)	180318	
QIAseq UDI Y-Adapter Plate D (96)	1118065	UDI 289–384
UDI Y-Adapter Plate D (96) Reference Card	1119827	

Storage

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

- The QIAseq Single Cell DNA Enzyme Kit and QIAseq UDI Y-Adapter Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.
- The QIAseq Beads should be stored immediately at 2–8°C in a refrigerator.

  Important: QIAseq Beads should never be frozen.

Intended Use

The QIAseq Single Cell DNA Library Kit UDI 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 Single Cell DNA Lib Kit UDI 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 reply on PCR normally have high error rates, low coverage uniformity, extensive allelic drop-outs, and can have limited amplification yields.

The QIAseq Single Cell DNA Library Kit UDI applies an optimized protocol using QIAGEN's unique multiple displacement amplification (MDA) technology (REPLI-g) to amplify gDNA directly from single cells. The amplified DNA is subsequently fragmented using the QIAGEN QIAseq FX technology, which incorporates enzymatic DNA fragmentation into a streamlined, optimized protocol with a sample cleanup between the fragmentation and adapter ligations steps. This highly optimized protocol saves time and reduces sample loss. The optimized enzyme and buffer compositions ensures high library yield while the streamlined library construction protocols enable straightforward automation of library preps on different liquid-handling platforms.

Principle and procedure

The QIAseq Single Cell DNA Library Kit UDI provides a fast, fully enzymatic procedure for DNA amplification, DNA fragmentation, and NGS library construction without a need for PCR-based library enrichment. The QIAseq Single Cell DNA Library Kit UDI uses MDA technology (REPLI-g) 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, and with the optimized buffer conditions, this will provide high yields of uniformly amplified DNA from single cells. The QIAseq 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.

All kit components used for WGA undergo a unique, controlled decontamination procedure to ensure elimination of all MDA amplifiable contaminating DNA. Buffers and reagents are treated with an innovative and standardized procedure during manufacturing to ensure the absence of any detectable residual 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 Single Cell DNA Library Kit UDI provides a simple and reliable method to efficiently generate DNA libraries in less than 3.5 hours. These libraries are suitable for sequencing on any Illumina NGS instrument. The kit provides a complete workflow for highly uniform amplification across the entire genome – with negligible sequence bias – followed by a fast, one-tube library construction including enzymatic fragmentation.

In the first step of the WGA procedure, the cell sample is lysed using gentle alkaline conditions and the DNA is denatured. After denaturation has been stopped by the addition of neutralization buffer, a master mix containing buffer and REPLI-g SC DNA Polymerase is added. The isothermal amplification reaction proceeds for 2 hours at 30°C. The REPLI-g SC amplified DNA can be stored long-term at –20°C with no negative effects, or it can be used directly to generate sequencing libraries. For library construction, the samples consisting of long WGA–DNA strands are first enzymatically sheared into smaller fragments. The median fragment size is dependent on the desired application and sequencing read length and can be adjusted by varying the QIAseq FX DNA fragmentation reaction conditions. The fragmented DNA is directly end-repaired and an A-nucleotide is added to the 3′ ends in the same tube following enzymatic shearing, making the DNA fragments ready for ligation. Following this step, unique-dual indexes adapters are ligated to both ends of the DNA fragments. These adapters contain sequences essential for bridge amplification and sequencing on any Illumina NGS instrument.

The WGA procedure when starting with single cells results in high yields of DNA so that PCR-free library preparation can be performed and subsequent PCR-based artifacts will be avoided. However, if additional library enrichment is required, as when working with enrichment of small genomes, a high-fidelity amplification step can be performed using the reagents included in the QIAseq Single Cell Library Kit UDI. The proprietary HiFi PCR Master Mix can amplify DNA regions with vastly different GC contents, minimizing sequencing bias caused by PCR.

Following library construction, the QIAseq Beads (included) are used for reaction cleanup and removal of adapter dimers.

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

NGS adapter and index technologies

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

The QIAseq Single Cell DNA Library Kit UDI includes QIAseq Unique Dual-Index (UDI) Y-Adapter plates in 24 format, 96 A/B/C/D format, or the 384 format, which includes one of the A, B, C, and D plates, thus enabling the multiplexing of up to 384 samples per sequencing run.

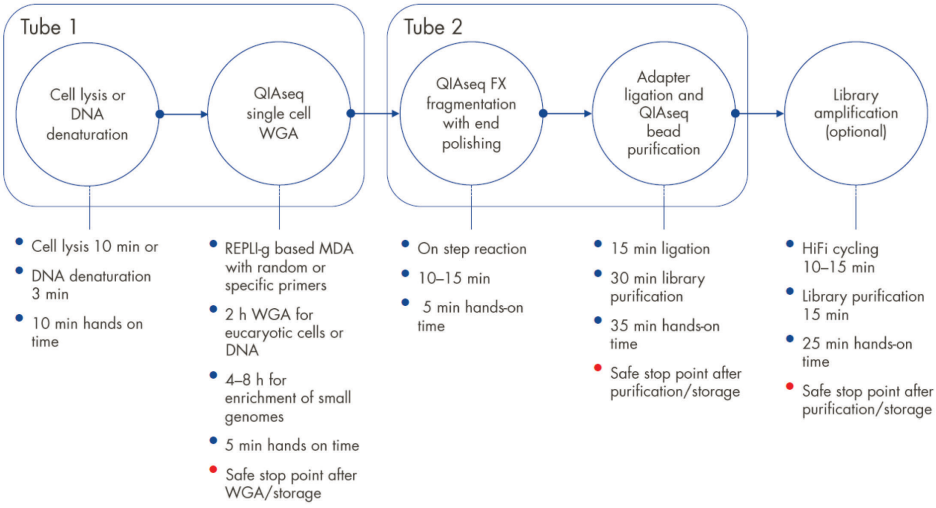
The QIAseq UDI Adapters use a fixed combination of 2 unique barcode motives per adapter molecule. Therefore, each single-index motive is only used once on any UDI adapter plate.

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®
- Illumina HiSeq®
- Illumina MiSeq®
- Illumina NextSeq®
- Illumina iSeq®
- Illumina MiniSeq®

Workflow chart



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.

- Microcentrifuge tubes
- 100% ethanol (ACS grade)
- Nuclease-free water
- Buffer EB (QIAGEN, cat. no. 19086) or 10 mM Tris·Cl pH 8.0
- PCR tubes or plates
- Low-binding tubes (e.g., from Axygen or Eppendorf®)
- Pipettes and pipette tips
- Magnetic racks for magnetic beads separation (e.g., Thermo Fisher Scientific/Life Technologies, DynaMag™-2 Magnet, cat. no. 12321D)
- Heating block
- Thermocycler
- Microcentrifuge
- Vortexer
- Ice
- 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 (cat. no. 333314)
- Quant-iT™ PicoGreen® dsDNA Assay Kit (optional)

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.
- 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.
- Enzyme-based DNA fragmentation is sensitive to many factors, such as reaction temperature, time and setup conditions, as well as the quality of the input DNA.

Recommended library quantification method

QIAGEN's QIAseq Library Quant Assay Kit, 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® may be used, but it is not recommended for non-enriched libraries.

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

This protocol is for the amplification of genomic DNA from 1–1000 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. 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 1–1000 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 1–10 ng; for bacterial gDNA and other small genomes (viral DNA, mitochondrial DNA), we recommend using 6–100 pg.
- 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.
- For whole-genome enrichment of small genomes, specific primer can be used. Specific primers can be designed according to recommendations as described in Appendix D: Design of Primer for Specific Amplification of Small Genomes.

- 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 H_2O 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 H_2O SC, DTT, and REPLI-g SC dilution buffer at room temperature, vortex, and then centrifuge briefly. The REPLI-g SC Reaction 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 12 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 12 reactions

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

* Reconstitution of Buffer DLB is described in "Things to do before starting," page 17.

3. Prepare sufficient 1:10 dilution DTT in H_2O SC for the total number of reactions (Table 2).

Table 2. Preparation of DTT 1:10 dilution for 12 reactions

Component	Volume for 12 reactions* (µl)
DTT, 1 M	3
H_2O SC	27
Total volume	**30**

* Add 10%.

4. Place 4 µl cell material (supplied with PBS) or gDNA into each well of a 96-well plate or microcentrifuge tube. If using less than 4 µl of starting material, add PBS SC to bring the volume up to 4 µl.

   Note: During pipetting, avoid any contact of pipette tips with cell material.

5. Add 3 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.

6. Incubate cell preparations for 10 min at 65°C and gDNA preparations for 3 min at 25°C, and then cool down to 4°C.

   Note: If a thermal cycler is used, the temperature of the heating lid should be set at 70°C to avoid evaporation. Alternatively, incubation can be performed in a heating block.

7. Add 3 µl Stop Solution. Mix carefully by flicking the tube, and then centrifuge briefly. Store on ice.

8. Thaw REPLI-g SC DNA Polymerase on ice. Tip gently on the tube to mix and centrifuge briefly.
9. Prepare a master mix according Table 3. Mix and centrifuge briefly.

   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 3. After the addition of water, REPLI-g SC Dilution Buffer, oligos, and DTT; 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 3. Preparation of master mix*

Component	Volume/reaction (µl)
H_2O SC	6.5
REPLI-g SC Dilution Buffer	14.5
REPLI-g SC universal oligo or REPLI-g SC advanced oligo	14.5
DTT (1:10)	2.5
REPLI-g SC DNA Polymerase	2
Total volume	**40**

* To prepare a master mix for multiple reactions, scale up according to the number of reactions and add 10%.

10. For each amplification reaction, add 40 µl master mix to 10 µl denatured DNA (from step 7). Mix by flicking the tube, and then centrifuge briefly.
11. Incubate at 30°C for 2 h if amplifying eukaryotic and bacterial cells, or DNA.

    Note: Incubating eukaryotic and bacterial DNA samples for 2 h generates sufficient DNA for PCR-free library prep using this kit. Incubation of 1 h is also possible for eucaryotic cells but leads to reduced yields and may not be appropriate for all types of cells.

    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.

12. Inactivate REPLI-g SC DNA Polymerase at 65°C for 3 min.
13. If the amplified DNA will not be used immediately, store it either at 4°C (for short-term storage, e.g., up to 1 week) or at –20°C (for long-term storage).

    DNA amplified using the QIAseq Single Cell DNA Library Kit UDI 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.
14. Amplified DNA can be directly used for library construction. Additionally, extra amplified DNA can be used for PCR analysis or for targeted resequencing.

    Note: To proceed with library preparation, you may quantify the amplified DNA following the instructions in Appendix B: PicoGreen Quantification of QIAseq Single Cell Amplified DNA. Optical density (OD) measurements overestimate REPLI-g amplified DNA and should not be used.

Protocol: Whole Genome Amplification for Specific Enrichment of Small Genomes

Important points before starting

- This protocol is optimized for whole-genome amplification of small genomes, for example, of mitochondrial DNA from 500 pg to 10 ng of purified genomic eukaryotic DNA template and >10^5 copies of viral DNA. It is recommended to use template DNA suspended in Buffer TE or H_2O. 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., pipets, filter pipet tips, reaction vials, etc.).
- REPLI-g SC DNA Polymerase should be thawed on ice. All other components can be thawed at room temperature.
- Buffer D1 (denaturation buffer) and Buffer N1 (neutralization buffer) should not be stored longer than 3 months.

Procedure – enrichment of small genomes using specific primers

1. Thaw H_2O SC, and REPLI-g SC Dilution Buffer at room temperature, 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 D1 (denaturation buffer) for the total number of whole genome amplification reactions (Table 4).

   Note: The total volume of Buffer D1 given in Table 4 is sufficient for 12 reactions. If performing fewer reactions, store residual Buffer D1 at –20°C. Buffer D1 should not be stored longer than 3 months.

Table 4. Preparation of Buffer D1 for 12 reactions

Component	Volume for 12 reactions (µl)
DTT, 1 M	6.6
Buffer DLB (reconstituted)*	23.4
Total volume	**30**

* Reconstitution of Buffer DLB is described in "Things to do before starting," page 17.

3. Prepare sufficient Buffer N1 (neutralization buffer) for the total number of whole genome amplification reactions (Table 5).

   Note: The total volume of Buffer N1 given in Table 1 is sufficient for 12 reactions. If performing fewer reactions, store residual Buffer N1 at –20°C. Buffer D1 should not be stored longer than 3 months.

Table 5. Preparation of Buffer N1 for 12 reactions

Component	Volume for 12 reactions (µl)
Stop solution	9
H_2O SC	51
Total volume	**60**

4. Place 2.5 µl DNA into each well of a 96-well plate or microcentrifuge tube.
5. Add 2.5 µl Buffer D1. Mix carefully by gently flicking the tube and centrifuge briefly.
6. Incubate for 3 min at room temperature (25°C).

   Note: If a thermal cycler is used, the temperature of the heating lid should be set at 70°C to avoid evaporation. Alternatively, incubation can be performed in a heating block.
7. Add 5 µl Stop Solution. Mix carefully by flicking the tube, and then centrifuge briefly and place immediately on ice. Proceed immediately by adding the master mix.

8. Prepare a master mix according Table 6. 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.

Table 6. Preparation of master mix

Component	Volume/reaction (µl)*
H_2O SC	7.5
REPLI-g SC dilution Buffer	29
Specific oligo mix (10µM each)	1.5
Total volume	**38**

* To prepare a master mix for multiple reactions, scale up according to the number of reactions and add 10%.

9. Add 38µl of master mix to the 10µl DNA from step 7. Mix by flicking the tube and spin down to collect liquid at the bottom of the tube. Place in a cycler or preheated heating block and incubate at 75°C for 5 min, then cool down to RT for 5 min to allow annealing of the primers on the template DNA.
10. Thaw REPLI-g SC DNA Polymerase on ice. Tip gently on the tube to mix and centrifuge briefly.
11. For each amplification reaction, add 2 µl of REPLI-g SC DNA Polymerase to the 48 µl DNA mix (from step 9). Mix by flicking the tube, and then centrifuge briefly.
12. Incubate at 33°C for 8 h.

    Note: Incubation time required for sufficient amplification is dependent on the input amount and size of the target DNA. The time may be reduced to 4 hours if sufficient DNA is present for NGS library construction.

13. Inactivate REPLI-g SC DNA Polymerase at 65°C for 3 min.

    Note: After incubation at 33°C, heat the heating block up to 65°C if the same heating block will be used in step 12.

    If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

14. If the amplified DNA will not be used immediately, store it either at 4°C (for short-term storage, e.g., up to 1 week) or at –20°C (for long-term storage).

    DNA amplified using the QIAseq Single Cell DNA Library Kit UDI 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 library construction. Additionally, extra amplified DNA can be used for PCR analysis or for targeted resequencing.

    Note: To proceed with library preparation, quantify the amplified DNA following the instructions in Appendix B: PicoGreen Quantification of QIAseq Single Cell Amplified DNA. OD measurements overestimate REPLI-g amplified DNA and should not be used. The input in the FX reaction will indicate if library amplification is required.

Protocol: Enzymatic Fragmentation and Library Preparation of QIAseq Single Cell Amplified DNA

This protocol describes the FX reaction for single-tube fragmentation, end-repair, A-addition, and size selection of QIAseq Single Cell amplified DNA. The libraries will be ready for quantification and use in next-generation sequencing on NGS instruments from Illumina.

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 in $H2O_{SC}$ or nuclease-free water before starting.

Things to do 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 7).

  Refer to Table 7 to determine the time and protocol required to fragment input DNA to the desired size.
- Prepare fresh 80% ethanol.
- Prepare Buffer 10 mM Tris·Cl, pH 8.0.

Procedure: enzymatic fragmentation and library preparation

FX single-tube fragmentation, end repair, and A-addition

1. Thaw all kit components on ice. Once reagents are thawed, mix buffers thoroughly by quickly vortexing the tubes to avoid any localized concentrations. Briefly centrifuge down reagents before use.

   Program a thermocycler according to Table 7 and start the program. If possible, set the temperature of the heated lid to approximately 70°C.

2. When the thermocycler block reaches 4°C, pause the program.

Table 7. Amplified gDNA fragmentation reaction conditions

		Incubation time*	
Step	**Temperature (°C)**	**(Fragment size 400 bp)**	**(Fragment size 500 bp)**
1	4	1 min	1 min
2	32	15 min	10 min
3	65	30 min	30 min
4	4	Hold	Hold

* The fragment size of the completed libraries is determined by the duration of step 2 using 200–1000 ng input DNA and the FX enhancer. Fragmentation time can be increased or decreased to generate shorter or longer inserts, respectively. Use a thermocycler with a heated lid set at 70°C. If heated lid temperature cannot be adjusted, leave the lid open during the incubation at 32°C and close during incubation at 65°C.

3. Dilute amplified gDNA 1:10 in H_2O SC. This step should give 200–1000 ng total amplified DNA in 10 µl H_2O SC (20–100 ng/µl). If the amplified DNA is below this concentration, up to 5 µl of the undiluted amplified DNA can be used or the DNA can be concentrated by using the QIAseq Beads according to the protocol described in Appendix E: Purification of Amplified DNA after Whole Genome Amplification.

   Pipet 10 µl of the diluted DNA in PCR tubes or strips, and place them on ice or in a cooling block.

   Important: Do not exceed 5 µl of undiluted DNA input into the FX reaction.

4. Prepare the FX Reaction Mix on ice according to Table 8 and mix by pipetting. Add the components of the FX Reaction Mix in the same order as stated in the table. Before adding the FX Enzyme Mix, pipet up and down the buffer mix. You can scale up the FX Reaction Mix according to the number of samples required.

Table 8. FX Reaction Setup

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

* Mix by pipetting and keep on ice.

5. Add 40 µl FX Reaction Mix to each diluted amplified gDNA sample on ice and gently vortex to mix.
6. Briefly spin down the PCR plate/tubes, immediately transfer to the prechilled thermocycler (4°C) and resume the program. Once the fragmentation program is complete, transfer samples to ice.
7. Immediately proceed with adapter ligation as described in the next protocol.

Adapter ligation

8. Equilibrate the QIAseq Beads to room temperature for 20–30 min before use and mix them thoroughly.
9. Vortex and spin down the adapter plate. Remove the protective adapter plate lid, carefully pierce the foil seal, and transfer 5 µl from one DNA adapter well to each 50 µl sample from the previous protocol. Track the barcodes used for each sample.
10. Replace the adapter plate lid and freeze unused adapters. The adapter plate is stable for a minimum of 10 freeze–thaw cycles.

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

11. Prepare the ligation master mix (per DNA sample) in a separate tube on ice according to Table 9. Mix well by gently vortexing at low rpm.

Table 9. Ligation master mix (per sample)

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

* Mix by pipetting and keep on ice.

12. Add 45 µl of the ligation master mix to each sample. Close the tubes or seal the plate, mix well by pulse short vortexing, spin down and incubate at 20°C for 15 min.

    Important: Do not use a thermocycler with a heated lid.

13. Perform a 0.6x QIAseq Beads purification by adding 60 µl of resuspended QIAseq Beads slurry to each ligated sample and mix well by pipetting or gently vortexing.
14. Incubate the mixture for 5 min at room temperature.
15. Spin down to collect remaining droplets and pellet the beads on a magnetic stand for 2 min, then carefully discard the supernatant.
16. 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.
17. Repeat the wash step 16 once for a total of 2 ethanol washes.
18. 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.
19. Elute by resuspending in 52.5 µl 10 mM Tris·Cl, pH 8.0. Pellet beads on the magnetic stand. Carefully transfer 50 µl supernatant to a new PCR plate/tube.
20. Perform a second 1.1x QIAseq Beads purification using 55 µl of resuspended QIAseq Beads to each sample and mix.

21. Repeat steps 14–18.
22. Elute by resuspending in 26 µl 10 mM Tris·Cl, pH 8.0. 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.
23. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution (Figure 1) 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. 120 bp).

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

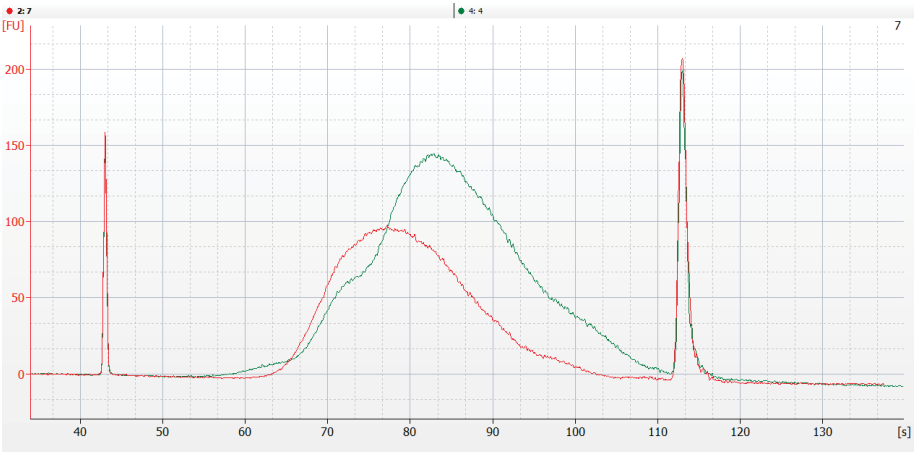


Figure 1. Capillary electrophoresis device trace of generated libraries. Capillary electrophoresis device trace data showing the correct size distribution of the same library before and after amplification of 6 cycles. The non-amplified library (green trace) show a wide fragment size distribution and shoulders with smaller and higher size. This is expected since these libraries are a mixture of ligated and non-ligated fragments, which will differ in size by 60 or 120 bp the size of the adapter and both adapters together. Moreover, the non-amplified libraries contain Y-shaped ends that may hybridize with each other and form long fragments when analyzed by electrophoresis under native conditions.

24. Quantify the library using the QIAseq Library Quant Assay Kit (ordered separately) or other comparable method.

    Note: Library quantitation with qPCR is strongly recommended to ensure accurate library dilution and flow-cell loading. Inaccurate library quantitation may cause under or over clustering of flow cell and affect sequencing quality and yield. Capillary electrophoresis or Qubit methods can overestimate library quantity because they cannot identify DNA fragments missing adapters.

    Qubit quantification can be performed when libraries are amplified. For library amplification follow the recommendations as described in "Protocol: Amplification of Library DNA".

    With 200 ng – 1 µg WGA DNA input, sufficient library should be generated for clustering on Illumina platforms without further PCR amplification (Figure 2).

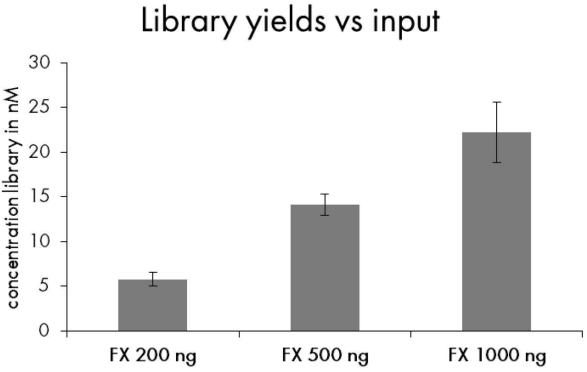


Figure 2. Library yields vs input of WGA DNA. Plotted are means of triplicate reactions with SD.

25. The purified library can be safely stored at –20°C in low-binding tubes.

Protocol: Amplification of Library DNA

PCR-based library amplification is not normally required for the QIAseq Single Cell DNA Library Kit UDI, because the WGA reaction typically generates more than sufficient material for PCR-free library construction. However, if insufficient library is available from precious samples, libraries may be amplified using HiFi polymerase included in the PCR master mix with this kit. The proprietary HiFi PCR Master Mix can evenly amplify DNA regions with vastly different GC content, minimizing sequencing bias caused by PCR.

Things to do before starting

- Thaw all reagents on ice. Once reagents are thawed, mix them thoroughly by vortexing to avoid any localized concentrations
- Equilibrate the QIAseq Beads to room temperature.

Procedure: Library amplification using Illumina primer mix

1. Prepare a reaction mix according to Table 10.

Table 10. Reaction mix for library enrichment

Component	Volume/reaction (µl)
HiFi PCR Master Mix, 2x	25
Illumina Library Ampl. Primer Mix (10 µM each)	1.5
Library DNA (from step 25, page 30)	Variable
RNase-free water	Variable
Total reaction volume	**50**

2. Program a thermocycler according to Table 11. Place the samples in the cycler and start the cycling program.

Table 11. Thermal cycling parameters

Time	Temperature (°C)	Number of cycles per input 100–200 ng	Number of cycles per input 40–100 ng	Number of cycles per input 10–40 ng	Number of cycles per input <10 ng
2 min	98	1	1	1	1
20 s	98				
30 s	60	4	6	8	10
30 s	72				
1 min	72	1	1	1	1
∞	4°	Hold	Hold	Hold	Hold

Note: Following PCR amplification, the library can be stored at –15 to –35°C.

3. After cycling completion proceed with 1.0x QIAseq Beads purification by adding 50 µl resuspended QIAseq Beads slurry to each amplified library and mix well by pipetting or gently vortexing.
4. Incubate the mixture for 5 min at room temperature.
5. Spin down to collect remaining droplets and pellet the beads on a magnetic stand for 2 min and carefully discard the supernatant.
6. 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.
7. Repeat the wash steps 5–6 once for a total of 2 ethanol washes.
8. 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.
9. Elute by resuspending in 26 µl 10 mM Tris·Cl, pH 8.0. Pellet beads on the magnetic stand. Carefully transfer 23,5 µl supernatant to a new low-binding tube.
10. Store purified libraries at –15 to –35°C until ready for sequencing or further other applications.
11. Assess the quality of the library using a capillary electrophoresis device or other comparable method. Check for the correct size distribution (Figure 1, red line) of library fragments and for the absence of adapters or adapter-dimers.

Note: The median fragment size can be used for subsequent qPCR-based quantification methods.

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

12. Quantify the library using the QIAseq Library Quant Assay Kit (not provided) or other comparable method.

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
REPLI-g DNA amplification	
Reduced yields and no HMW product in agarose gel in some samples, but DNA yield in other samples is approx. 40 µg	
a) Cells are not suitable for whole genome amplification	DNA within the cells is degraded (e.g., inappropriate storage of cells, use of fixed or apoptotic cells). If plant or fungal cells were used, lysis may not be complete due to the presence of cell walls. Generate protoplasts before starting cell lysis and WGA.
b) gDNA not suitable for whole-genome amplification	Purified gDNA is degraded (e.g., inappropriate storage, long storage).
c) Reaction failed – possible inhibitor in the genomic DNA template	Clean up or dilute the purified genomic DNA and reamplify.
d) Carryover of alcohol in isolated DNA sample	Residual alcohol in the DNA sample may reduce the yield of REPLI-g reactions. When using column-based purification procedures, ensure that the duration of the drying step prior to elution of DNA from the column is sufficient to evaporate residual ethanol.
e) Reaction time is too short	Two hours is sufficient to amplify gDNA from bacterial and eukaryotic cells. While sufficient yield is possible with shorter incubation in some experimental systems, this may not be appropriate for all cell types and should be confirmed experimentally.

	Comments and suggestions
Reduced yields	
f) Reaction temperature is too high	Check the incubator for correct reaction temperature (30°C) during the whole genome amplification reaction. If cycler with heated lid is used, set temperature to 70°C. As a control, the whole genome amplification reaction can be performed at a lower temperature (e.g., 25–28°C), which should give the appropriate yield.
The negative (no template) controls have DNA yields of up to 20 µg but no positive result in downstream assay (e.g., PCR)	
DNA is generated during the whole-genome amplification reaction by random extension of primer–dimers or amplification of contaminating DNA	HMW product can be generated by random extension of primer–dimers. This DNA will not affect the quality of actual samples or specific downstream genetic assays. Decontaminate all laboratory equipment and take all necessary precautions to avoid contamination of reagents and samples with extraneous DNA. If possible, work in a laminar flow hood. Use sterile equipment and barrier pipette tips only, and keep amplification chemistry and DNA templates in separate storage locations.
Single cell protocol	
Reduced/no locus representation or allele dropout in downstream analysis, but DNA yield is approx. 40 µg	
a) Cells are not suitable for whole-genome amplification	DNA within the cells is degraded (e.g., inappropriate storage of cells, use of fixed or apoptotic cells). Cells were lost during procedure, or insufficient mixing of lysis of cells occurred. Perform mixing by gently flicking the tube, and avoid contact of pipette tips with the cell material.
b) DNA degraded after cell lysis	Perform cell lysis carefully according to the protocol, and avoid vigorous vortexing. Do not store DNA after cell lysis.
c) Genomic DNA template is degraded	Use intact genomic DNA template and avoid repeated pipetting of DNA. Use a larger amount of genomic DNA.
d) Microdissected material does not contain the whole nucleus	When carrying out microdissection, ensure that the section thickness allows the capturing of the whole nucleus and that the nucleus is not damaged
Genome is not amplified at all, but DNA yield is up to 40 µg	
a) Cells were not lysed. DNA is generated during the whole-genome amplification reaction by random extension of primer–dimers	Additional cell envelope breakdown is necessary for cells that have strong cell walls (e.g., plant cells and cells in dormant stages, such as spores and cysts).
b) Sample is contaminated	Contaminating DNA may out compete the amplification of single cell genome.

	Comments and suggestions
A higher than expected proportion of reads map to the mitochondrial genome	
Cell lysis was incomplete, exposing mtDNA but leaving the nucleus partially intact	During cell lysis, ensure the lysis buffer and cell are mixed gently but thoroughly.
Using cells isolated with laser-capture microdissection, coverage of a particular chromosome is absent, or a complete loss of heterozygosity is observed	
Chromosomes were damaged during laser capture	During microdissection, take extra care not to damage the nucleus.
Library preparation protocol	
Low library yields	
a) WGA yields were lower than expected	Quantify the yield of WGA using PicoGreen Reagent. Typically, 100 ng of WGA DNA generates enough Illumina-compatible library to use directly for sequencing without amplification. If the final library yield is not sufficient for the expected number of sequencing runs, a library amplification step can be performed following the adapter ligation step.
b) Suboptimal reaction conditions due to low DNA quality	Follow the protocol recommendation; do not exceed 10 µl DNA input in the FX reaction.
Unexpected signal peaks in capillary electrophoresis device traces	
a) Presence of shorter peaks between 60 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. Perform an additional library cleanup using the QIAseq Beads.
b) Presence of larger library fragments after library enrichment	If performing library enrichment and the fragment population shifts higher than expected after adapter ligation and PCR enrichment (e.g., more than the expected 120 bp shift), a PCR artifact may be the cause, due to overamplification of the DNA library. Make sure to use as few amplification cycles as possible (8–10) to avoid this effect or avoid PCR enrichment completely.

		Comments and suggestions
c)	Incorrect library fragment size after adapter ligation	During library preparation, adapters of approx. 60 bp are ligated to both ends of the DNA library fragments. This ligation should be reflected on a capillary electrophoresis device by a shift in size of all library fragments of 120 bp. If using library adapters from other suppliers, please refer to the size information given in the respective documentation. The fragment distribution of nonamplified libraries may differ from the distribution of amplified libraries. The absence of a clear size shift may indicate no, or only low, adapter ligation efficiency. Make sure to use the correct amount of starting DNA and the parameters and incubation times described in the handbook for end repair, A-addition, and ligation. Also insufficient bead mixing or inaccurate pipetting of beads will lead to shifts in the size distribution of the library.
d)	Mean fragment size is smaller than expected	The temperature of the fragmentation reaction is higher than 32°C. Check for the correct temperature, and calibrate the thermocycler if necessary. Alternatively, to increase fragment size, reduce incubation time to 10–12 min.

Appendix A: Determination of Concentration and Quality of Amplified DNA

Quantification of DNA yield

A 50 µl QIAseq WGA reaction typically yields approximately 40 µg of DNA, allowing direct use of the amplified DNA in most downstream genotyping experiments and library preparation for sequencing. Depending on the quality of the input material, the resulting amount of DNA may be less (dead or apoptotic cells with fragmented or damaged DNA should not be used). 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. 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 can be found in Appendix B.

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 first controlling 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: 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** > 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; 1mM EDTA, pH 8.0)
- Human genomic DNA (e.g., Promega, cat. no. G3041)
- 2 ml microcentrifuge tube, or 15 ml Falcon tubes
- 96-well plates (suitable for use in a fluorescence microplate reader)
- Fluorescence microplate reader (e.g., TECAN® Ultra)

Procedure

1. Make a 1:200 dilution of PicoGreen stock solution in Buffer TE. Each quantification reaction requires 50 µl. 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.

For example, to prepare enough PicoGreen working solution for 100 samples, add 25 µl PicoGreen to 4975 µl Buffer TE (check this IA).

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

2. Prepare a 16 µg/ml stock solution of genomic DNA in Buffer TE.
3. Make 200 µl of 1.6, 0.8, 0.4, 0.2, and 0.1 µg/ml DNA standards by further diluting the 16 µg/ml genomic DNA with Buffer TE.
4. Transfer 50 µl 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. Gray squares: genomic DNA standards (µg/µl).

5. 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.
6. Place 5 µl diluted QIAseq Single Cell amplified DNA (from step 5) into an unused well of 96-well plate A and add 45 µl Buffer TE to make a 1:1000 dilution.

The 1:100 dilutions from step 5 can be stored at –20°C and used for future downstream sample analysis.

7. For Blanc measurements, pipette 50 µl Buffer TE in two empty wells of plate A.
8. 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.
9. 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.
10. Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescence filters (excitation: approx. 480 nm; emission: approx. 520 nm).

    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

11. Generate a standard curve by plotting the concentration (µg/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.
12. Use the standard curve to determine the concentration (µg/ml) of the diluted QIAseq Single Cell amplified DNA sample. This determination 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.

13. Multiply the value determined in step 11 by 1000 to show the concentration of undiluted sample DNA (since the sample DNA measured by PicoGreen fluorescence had been diluted 1:1000).
14. To determine the total amount of DNA in your sample, multiply the concentration of undiluted sample DNA (µg/ml, determined in step 12) by the reaction volume in milliliters (i.e., for a 50 µl reaction, multiply by 0.05).

Appendix C: QIAseq Dual-Index Y-Adapters

Generation of sample sheets for Illumina instruments

Index sequences for QIAseq Unique and Combinatorial Dual-Index Y-Adapters are available for download at **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. 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 the actions in the next steps to each file.
5. Open each file in a text editor and locate the text block **[Compatible Sample Prep Kits]**.

6. Generate 1 new lines 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.

Unique Dual-Index Y-Adapters

The layout of the 24-plex and 96-plex (A/B/C/D) single-use UDI adapter plate is shown in Figure 4 to Figure 8. The index motives used in the QIAseq Unique Dual Index Kits are listed in Table 12.

To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments at **www.qiagen.com**.

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

Figure 4. QIAseq UDI Y-Adapter Plate (24) layout (UDI 1–24).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 001	UDI 009	UDI 017	UDI 025	UDI 033	UDI 041	UDI 049	UDI 057	UDI 065	UDI 073	UDI 081	UDI 089
B	UDI 002	UDI 010	UDI 018	UDI 026	UDI 034	UDI 042	UDI 050	UDI 058	UDI 066	UDI 074	UDI 082	UDI 090
C	UDI 003	UDI 011	UDI 019	UDI 027	UDI 035	UDI 043	UDI 051	UDI 059	UDI 067	UDI 075	UDI 083	UDI 091
D	UDI 004	UDI 012	UDI 020	UDI 028	UDI 036	UDI 044	UDI 052	UDI 060	UDI 068	UDI 076	UDI 084	UDI 092
E	UDI 005	UDI 013	UDI 021	UDI 029	UDI 037	UDI 045	UDI 053	UDI 061	UDI 069	UDI 077	UDI 085	UDI 093
F	UDI 006	UDI 014	UDI 022	UDI 030	UDI 038	UDI 046	UDI 054	UDI 062	UDI 070	UDI 078	UDI 086	UDI 094
G	UDI 007	UDI 015	UDI 023	UDI 031	UDI 039	UDI 047	UDI 055	UDI 063	UDI 071	UDI 079	UDI 087	UDI 095
H	UDI 008	UDI 016	UDI 024	UDI 032	UDI 040	UDI 048	UDI 056	UDI 064	UDI 072	UDI 080	UDI 088	UDI 096

Figure 5. QIAseq UDI Y-Adapter Plate A (96) layout (UDI 1–96).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 097	UDI 105	UDI 113	UDI 121	UDI 129	UDI 137	UDI 145	UDI 153	UDI 161	UDI 169	UDI 177	UDI 185
B	UDI 098	UDI 106	UDI 114	UDI 122	UDI 130	UDI 138	UDI 146	UDI 154	UDI 162	UDI 170	UDI 178	UDI 186
C	UDI 099	UDI 107	UDI 115	UDI 123	UDI 131	UDI 139	UDI 147	UDI 155	UDI 163	UDI 171	UDI 179	UDI 187
D	UDI 100	UDI 108	UDI 116	UDI 124	UDI 132	UDI 140	UDI 148	UDI 156	UDI 164	UDI 172	UDI 180	UDI 188
E	UDI 101	UDI 109	UDI 117	UDI 125	UDI 133	UDI 141	UDI 149	UDI 157	UDI 165	UDI 173	UDI 181	UDI 189
F	UDI 102	UDI 110	UDI 118	UDI 126	UDI 134	UDI 142	UDI 150	UDI 158	UDI 166	UDI 174	UDI 182	UDI 190
G	UDI 103	UDI 111	UDI 119	UDI 127	UDI 135	UDI 143	UDI 151	UDI 159	UDI 167	UDI 175	UDI 183	UDI 191
H	UDI 104	UDI 112	UDI 120	UDI 128	UDI 136	UDI 144	UDI 152	UDI 160	UDI 168	UDI 176	UDI 184	UDI 192

Figure 6. QIAseq UDI Y-Adapter Plate B (96) layout (UDI 97–192).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 193	UDI 201	UDI 209	UDI 217	UDI 225	UDI 233	UDI 241	UDI 249	UDI 257	UDI 265	UDI 273	UDI 281
B	UDI 194	UDI 202	UDI 210	UDI 218	UDI 226	UDI 234	UDI 242	UDI 250	UDI 258	UDI 266	UDI 274	UDI 282
C	UDI 195	UDI 203	UDI 211	UDI 219	UDI 227	UDI 235	UDI 243	UDI 251	UDI 259	UDI 267	UDI 275	UDI 283
D	UDI 196	UDI 204	UDI 212	UDI 220	UDI 228	UDI 236	UDI 244	UDI 252	UDI 260	UDI 268	UDI 276	UDI 284
E	UDI 197	UDI 205	UDI 213	UDI 221	UDI 229	UDI 237	UDI 245	UDI 253	UDI 261	UDI 269	UDI 277	UDI 285
F	UDI 198	UDI 206	UDI 214	UDI 222	UDI 230	UDI 238	UDI 246	UDI 254	UDI 262	UDI 270	UDI 278	UDI 286
G	UDI 199	UDI 207	UDI 215	UDI 223	UDI 231	UDI 239	UDI 247	UDI 255	UDI 263	UDI 271	UDI 279	UDI 287
H	UDI 200	UDI 208	UDI 216	UDI 224	UDI 232	UDI 240	UDI 248	UDI 256	UDI 264	UDI 272	UDI 280	UDI 288

Figure 7. QIAseq UDI Y-Adapter Plate C (96) layout (UDI 193–288).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 289	UDI 297	UDI 305	UDI 313	UDI 321	UDI 329	UDI 337	UDI 345	UDI 353	UDI 361	UDI 369	UDI 377
B	UDI 290	UDI 298	UDI 306	UDI 314	UDI 322	UDI 330	UDI 338	UDI 346	UDI 354	UDI 362	UDI 370	UDI 378
C	UDI 291	UDI 299	UDI 307	UDI 315	UDI 323	UDI 331	UDI 339	UDI 347	UDI 355	UDI 363	UDI 371	UDI 379
D	UDI 292	UDI 300	UDI 308	UDI 316	UDI 324	UDI 332	UDI 340	UDI 348	UDI 356	UDI 364	UDI 372	UDI 380
E	UDI 293	UDI 301	UDI 309	UDI 317	UDI 325	UDI 333	UDI 341	UDI 349	UDI 357	UDI 365	UDI 373	UDI 381
F	UDI 294	UDI 302	UDI 310	UDI 318	UDI 326	UDI 334	UDI 342	UDI 350	UDI 358	UDI 366	UDI 374	UDI 382
G	UDI 295	UDI 303	UDI 311	UDI 319	UDI 327	UDI 335	UDI 343	UDI 351	UDI 359	UDI 367	UDI 375	UDI 383
H	UDI 296	UDI 304	UDI 312	UDI 320	UDI 328	UDI 336	UDI 344	UDI 352	UDI 360	UDI 368	UDI 376	UDI 384

Figure 8. QIAseq UDI Y-Adapter Plate D (96) layout (UDI 289–384).

Table 12. UDI motives used in the QIAseq UDI Y-Adapter Kits (24 and 96 A/B/C/D)

Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-001	ATGGCCGACT	AGTCGGCCAT	TGAACGTTGT
QDIY-002	CGATGAGCAC	GTGCTCATCG	ACCAGACTTG
QDIY-003	GATAAGTCGA	TCGACTTATC	ACTGGCGAAC
QDIY-004	TCACGCCTTG	CAAGGCGTGA	GCGTTAGGCA
QDIY-005	AGGAACACAA	TTGTGTTCCT	TTATCGGCCT
QDIY-006	CTCAGTAGGC	GCCTACTGAG	GAGGTATAAG
QDIY-007	GAAGTGCCTG	CAGGCACTTC	TCAAGGATTC
QDIY-008	TCTCTCGCCT	AGGCGAGAGA	CGAACCGAGA
QDIY-009	AGGCACCTTC	GAAGGTGCCT	GAGCCAAGTT
QDIY-010	CTGTTGGTAA	TTACCAACAG	AAGGCCGTAG
QDIY-011	GCTGGTACCT	AGGTACCAGC	TTAGAGAAGC
QDIY-012	TAAGGAGCGG	CCGCTCCTTA	TCTAAGACCA
QDIY-013	AATCGCTCCA	TGGAGCGATT	TGTAACCACT
QDIY-014	CTCCTAATTG	CAATTAGGAG	CCGACACAAG
QDIY-015	GCCTCATAAT	ATTATGAGGC	CTCTGATGGC
QDIY-016	TGTATTGAGC	GCTCAATACA	CGGCCTGTTA
QDIY-017	AGCCATAACA	TGTTATGGCT	TGCATAGCTT
QDIY-018	CCACAAGTGG	CCACTTGTGG	AACCTTCTCG
QDIY-019	GTTATCACAC	GTGTGATAAC	AAGAGATCAC
QDIY-020	TACCGTTCTT	AAGAACGGTA	GCCTGAAGGA

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-021	AGGCGTTAGG	CCTAACGCCT	ATTGTGCCTT
QDIY-022	CCGTAACGTC	GACGTTACGG	TCCTCTACCG
QDIY-023	GTAATAGCCA	TGGCTATTAC	TACCATGAAC
QDIY-024	TAGCGCCGAT	ATCGGCGCTA	CATTGGCAGA
QDIY-025	CATTCTTGGA	TCCAAGAATG	CACTGCTATT
QDIY-026	ATGCAAGGTT	AACCTTGCAT	AATGGTAGGT
QDIY-027	CGCCAGACAA	TTGTCTGGCG	GATACCTATG
QDIY-028	GAAGGTTGGC	GCCAACCTTC	CACTAGGTAC
QDIY-029	TCGCATCACG	CGTGATGCGA	AGCTCGTTCA
QDIY-030	CCGGTCATGA	TCATGACCGG	TGTCAGTCTT
QDIY-031	ATTCACAAGC	GCTTGTGAAT	GATGAACAGT
QDIY-032	CAACCTGTAA	TTACAGGTTG	ACAATCGGCG
QDIY-033	GCCAGTCGTT	AACGACTGGC	GATTGAGTTC
QDIY-034	TGCCTTGTCG	CGACAAGGCA	GTAATGCCAA
QDIY-035	CTATCCGCTG	CAGCGGATAG	TCGTTGCGCT
QDIY-036	AATGCCGGAA	TTCCGGCATT	AGGTGAGTAT
QDIY-037	CGGTTATCCG	CGGATAACCG	TCGATAATGG
QDIY-038	GCGGAAGAGT	ACTCTTCCGC	GCGTCTCTTC
QDIY-039	TTGGTTAGTC	GACTAACCAA	GTCTCCTGCA
QDIY-040	TTCAGTGTGA	TCACACTGAA	GAGCTTCATT

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-041	AGAATTCTGG	CCAGAATTCT	AGGCCTACAT
QDIY-042	CATTGACTCT	AGAGTCAATG	TGTGGAACCG
QDIY-043	GCGGCTTCAA	TTGAAGCCGC	CGTATTAAGC
QDIY-044	TTATGGTCTC	GAGACCATAA	CCAGTGGTTA
QDIY-045	CGTAACCAGG	CCTGGTTACG	GCGTTCGAGT
QDIY-046	AGCTCAGATA	TATCTGAGCT	CCTTCCGGTT
QDIY-047	CCGGTGTTAC	GTAACACCGG	CACAAGACGG
QDIY-048	GACCTAACCT	AGGTTAGGTC	GCTTACACAC
QDIY-049	TTGTAGAAGG	CCTTCTACAA	AGGATGTCCA
QDIY-050	CCTAGCACTA	TAGTGCTAGG	CACCTTATGT
QDIY-051	ATCGTGTTCT	AGAACACGAT	AAGCGGCTGT
QDIY-052	CCAACTTATC	GATAAGTTGG	TTCCTGTGAG
QDIY-053	GAAGCCAAGG	CCTTGGCTTC	AGTACAGTTC
QDIY-054	TGGAGTTCAA	TTGAACTCCA	TACAGCCTCA
QDIY-055	CTTCAATCCT	AGGATTGAAG	GTTCTATTGG
QDIY-056	ATCTTGCGTG	CACGCAAGAT	ATATACCGGT
QDIY-057	CGTCTAAGGT	ACCTTAGACG	CCTCGGAATG
QDIY-058	GAGGTGAACA	TGTTCACCTC	GTTCTGGAAC
QDIY-059	TCAGAACTAC	GTAGTTCTGA	AGATTCACCA
QDIY-060	CGGATATTGA	TCAATATCCG	TCGGTCAGAT

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Table continued from previous page

Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-061	AGGAGTAGAT	ATCTACTCCT	CACTCTCGCT
QDIY-062	CCGCCGAATA	TATTCGGCGG	GTTGGTCCAG
QDIY-063	GAGTCTATAC	GTATAGACTC	AGCTCGAAGC
QDIY-064	TTATTACCGG	CCGGTAATAA	AGAGGTTCTA
QDIY-065	CGCTCGTTAG	CTAACGAGCG	ATGACTCGAA
QDIY-066	AACAACGCTG	CAGCGTTGTT	GAACAATCCT
QDIY-067	CGCGGCTATT	AATAGCCGCG	TGGCAAGGAG
QDIY-068	GCTCGACACA	TGTGTCGAGC	GAATATTGGC
QDIY-069	TTCTTCCAAC	GTTGGAAGAA	CCGGAACCTA
QDIY-070	TTGGCGGTTG	CAACCGCCAA	ACTTGTTCGG
QDIY-071	AACAGGCAAT	ATTGCCTGTT	CAAGTCCAAT
QDIY-072	CAGAATGGCG	CGCCATTCTG	AACCGCAAGG
QDIY-073	GTTGAGATTC	GAATCTCAAC	ACGTTGACTC
QDIY-074	TGTGTGCGGA	TCCGCACACA	CCACTTAACA
QDIY-075	GTTCGGCGAA	TTCGCCGAAC	AGCAGTTCCT
QDIY-076	AGCTGTATTG	CAATACAGCT	TCGCCTTCGT
QDIY-077	CAGCGGATGA	TCATCCGCTG	TAGGACTGCG
QDIY-078	GTCCTTGGAT	ATCCAAGGAC	TCCGAGCGAA
QDIY-079	TCTAGATGCT	AGCATCTAGA	TTCGGTTGTT
QDIY-080	CGAGCCACAT	ATGTGGCTCG	ACAGGAGGAA

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-081	ATGGAATGGA	TCCATTCCAT	CCTCCATTAA
QDIY-082	CATTCCTCAC	GTGAGGAATG	AGTCGCGGTT
QDIY-083	GCATAGGAAG	CTTCCTATGC	CTCATCCAGG
QDIY-084	TGTTCGTGTT	AACACGAACA	TGTGGTTGAA
QDIY-085	TAAGACCGTT	AACGGTCTTA	TTATGCGTGG
QDIY-086	ATGGTACCAG	CTGGTACCAT	GCGAATGTAT
QDIY-087	CCGACAGCTT	AAGCTGTCGG	GTCAAGCTCG
QDIY-088	GACGATATGA	TCATATCGTC	TAGAGTTGGA
QDIY-089	TTGTACTCCA	TGGAGTACAA	CTGATGATCT
QDIY-090	GTGCACATAA	TTATGTGCAC	ACTAGGTGTT
QDIY-091	AGGACAAGTA	TACTTGTCCT	CTGTTAGCGG
QDIY-092	CCGATTCGAG	CTCGAATCGG	ATCGCACCAA
QDIY-093	GTAGGAACTT	AAGTTCCTAC	CTTACTTGGT
QDIY-094	TACACTACGA	TCGTAGTGTA	CCTTAATGCG
QDIY-095	ATGACCTTGA	TCAAGGTCAT	TCTCGCCTAG
QDIY-096	CTACGTGACG	CGTCACGTAG	TCTTCAGAGA
QDIY-097	AACAATCAGG	CCTGATTGTT	TACCGGTGGT
QDIY-098	CTGGTGTGCA	TGCACACCAG	AGGTGTTACG
QDIY-099	GCATATCCTT	AAGGATATGC	ACAGACCGAC
QDIY-100	TGTCCTGTAC	GTACAGGACA	CGAATACGTA

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-101	AGAACGTCGC	GCGACGTTCT	TAGCATCGAT
QDIY-102	CACGGACTAG	CTAGTCCGTG	CCATGAGTCG
QDIY-103	GTTGAACACT	AGTGTTCAAC	ACTAACATGC
QDIY-104	TCGCGTGGTA	TACCACGCGA	ACACTCTCTA
QDIY-105	AGCCACTATG	CATAGTGGCT	GCTCTTGCCT
QDIY-106	CCACCTACCA	TGGTAGGTGG	AATCTTGAGG
QDIY-107	GTTCCGGTGT	ACACCGGAAC	CTTAACGGTC
QDIY-108	TAGGTCTGAC	GTCAGACCTA	TTGTGACCAA
QDIY-109	AGGAAGCATT	AATGCTTCCT	TCACACACCT
QDIY-110	CCTTAGTTGG	CCAACTAAGG	CTGCAATTAG
QDIY-111	GTCCTATTCA	TGAATAGGAC	CTCCTTACTC
QDIY-112	TAAGATGGAC	GTCCATCTTA	GCAACGCAGA
QDIY-113	AGGCCATGGT	ACCATGGCCT	CCTTACCAAT
QDIY-114	CATTGGCCAA	TTGGCCAATG	TTAATCCTCG
QDIY-115	GCTATGAATC	GATTCATAGC	TTCCGAGTTC
QDIY-116	TTGGTCCTCG	CGAGGACCAA	CTCGAGAGGA
QDIY-117	AGCGACATAC	GTATGTCGCT	TGTTGGCTGT
QDIY-118	CAAGTAGTCT	AGACTACTTG	CGTATCTGCG
QDIY-119	GTCAAGAAGA	TCTTCTTGAC	CCATAGTATC
QDIY-120	TCCTGTTATG	CATAACAGGA	TGGACAGTAA

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-121	AAGTGCGATA	TATCGCACTT	GTACCTTGTT
QDIY-122	AGGCTACACG	CGTGTAGCCT	GAGTGCCTCT
QDIY-123	CTATATCGGC	GCCGATATAG	TAAGTAGCGG
QDIY-124	GCTAAGGTAA	TTACCTTAGC	CGTGGTGTTC
QDIY-125	TAACCTGGTT	AACCAGGTTA	CATTCCTGAA
QDIY-126	AGTTGGTCTA	TAGACCAACT	AAGATGCATG
QDIY-127	ATGCAGCTGG	CCAGCTGCAT	CCTTGGAGCT
QDIY-128	CGTTGCCTTC	GAAGGCAACG	ACCGGAACAG
QDIY-129	GCGTGGAGAA	TTCTCCACGC	GAATGGAAGC
QDIY-130	TACGCCTCCT	AGGAGGCGTA	GTTCTCCATA
QDIY-131	AATTCGGTAG	CTACCGAATT	GTCACTATGT
QDIY-132	ATTGTCGAAC	GTTCGACAAT	TGGTAGAACT
QDIY-133	CAACCTTGCG	CGCAAGGTTG	ACGCCTATGG
QDIY-134	GCACTGCGTA	TACGCAGTGC	AATCCGTTAC
QDIY-135	TGCTAGTAGT	ACTACTAGCA	GTTGAGGCTA
QDIY-136	AAGTCACGGA	TCCGTGACTT	TATCAACTGG
QDIY-137	AGCGATTGAA	TTCAATCGCT	AAGAGGAGAT
QDIY-138	CTACCTCTCT	AGAGAGGTAG	GTCTTCTCGG
QDIY-139	GACAACTGTC	GACAGTTGTC	GAAGCCACTC
QDIY-140	TCCATTGCGG	CCGCAATGGA	GTAGGACACA

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-141	AGCCTCGCAA	TTGCGAGGCT	CTCCTCGTAT
QDIY-142	AATACAGGCT	AGCCTGTATT	CCACATGATT
QDIY-143	CGGACCGTTA	TAACGGTCCG	AGACGGTTGG
QDIY-144	GCGCTTATGC	GCATAAGCGC	CTAGGTTGAC
QDIY-145	TTAACACGAG	CTCGTGTTAA	AAGCGTACCA
QDIY-146	CGCCTCTAGA	TCTAGAGGCG	TCATGTTGGT
QDIY-147	AATCGACCTT	AAGGTCGATT	TTGGAATGGT
QDIY-148	CCGCAATAAC	GTTATTGCGG	GTGTATGTTG
QDIY-149	GTTCCAACGA	TCGTTGGAAC	TCCTGTCAAC
QDIY-150	TGTTAGACCG	CGGTCTAACA	TAATCAGGCA
QDIY-151	AACCTCATAG	CTATGAGGTT	GTAGTGGATT
QDIY-152	ATGAATCCAC	GTGGATTCAT	AATTGCGCAT
QDIY-153	CGGCTTAATT	AATTAAGCCG	GACAATAACG
QDIY-154	GAGTTGCAGG	CCTGCAACTC	ACAGTTAAGC
QDIY-155	TCCACGAACA	TGTTCGTGGA	AGCCACACTA
QDIY-156	TGACGGAGGA	TCCTCCGTCA	CAATCGTCTT
QDIY-157	AATGAGTACG	CGTACTCATT	AGGAGCTTGT
QDIY-158	CGTCTTCCGA	TCGGAAGACG	TTGAGCGGAG
QDIY-159	GACAGAGATT	AATCTCTGTC	AGTAGCTCTC
QDIY-160	TTACGCTAAC	GTTAGCGTAA	CACGCTGTCA

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-161	CTCCTCGAAG	CTTCGAGGAG	AAGACCTCTT
QDIY-162	ATACCGCAGA	TCTGCGGTAT	GACCTCTTCT
QDIY-163	CCTATCTGAT	ATCAGATAGG	TACTTCCTTG
QDIY-164	GATCGGTTAC	GTAACCGATC	TGCGATACGC
QDIY-165	TGGTGAGGTG	CACCTCACCA	GCAGGCTTAA
QDIY-166	AACCGGCGTA	TACGCCGGTT	TAAGCTTGTG
QDIY-167	AATACCGATC	GATCGGTATT	ATGGTCCGCT
QDIY-168	CGATACTCAA	TTGAGTATCG	ATGTCAGAAG
QDIY-169	GTAAGGCGGT	ACCGCCTTAC	GACGAAGGTC
QDIY-170	TTCAAGGTCG	CGACCTTGAA	ATCACCGTGA
QDIY-171	TATCCGAGTA	TACTCGGATA	GCTACAGTGT
QDIY-172	AGCGCGCTTA	TAAGCGCGCT	CGTCGAATAT
QDIY-173	CCGGAGACAT	ATGTCTCCGG	CAACCATCGG
QDIY-174	GAGATAACTG	CAGTTATCTC	CGGTCCATTC
QDIY-175	TTGTAAGCGC	GCGCTTACAA	AGAAGAGCCA
QDIY-176	CAAGAGGAGG	CCTCCTCTTG	CTATGCAATG
QDIY-177	AACCTTAGGA	TCCTAAGGTT	CACTGAACCG
QDIY-178	CTGGCAACTC	GAGTTGCCAG	TACTGTGTGA
QDIY-179	GAACTTGTTG	CAACAAGTTC	GCATTCTGTT
QDIY-180	TGTGCAAGAT	ATCTTGCACA	CTCCGCTAAG

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Table continued from previous page

Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-181	AATCGAGAGA	TCTCTCGATT	TCGCTTGAGA
QDIY-182	AGCGTGTCAG	CTGACACGCT	AACTAGCCTT
QDIY-183	CTTGGTGATT	AATCACCAAG	TTCGCTCAGG
QDIY-184	GAAGCAGCAA	TTGCTGCTTC	CTCTACAACA
QDIY-185	TTCCGTCGAC	GTCGACGGAA	TGAGTGTGTT
QDIY-186	CGAGATGCCA	TGGCATCTCG	TAGTTAGTCG
QDIY-187	AAGTTCGTGC	GCACGAACTT	GCCTGATCCT
QDIY-188	CGTCCATAAG	CTTATGGACG	CGAGTACAGG
QDIY-189	TTGTGGCATA	TATGCCACAA	GCCTAGATTA
QDIY-190	AGATCGGAAT	ATTCCGATCT	TCGGCACTGT
QDIY-191	CATTCTACTG	CAGTAGAATG	CCGTGCAAGA
QDIY-192	ATCGCCGTAG	CTACGGCGAT	CTGGCTGGTT
QDIY-193	ATCCTTACAC	GTGTAAGGAT	CGTTAGGATT
QDIY-194	CGCAAGGACT	AGTCCTTGCG	TTCCATTACG
QDIY-195	GCTGGCGTTA	TAACGCCAGC	TAGCGGTAAC
QDIY-196	TACTTAGAGG	CCTCTAAGTA	GTAGCCAGGA
QDIY-197	ATGGCGATGC	GCATCGCCAT	AGGATACTCT
QDIY-198	CATTGGTGCG	CGCACCAATG	TATCCTCCAG
QDIY-199	GCGAGATATA	TATATCTCGC	TAAGTCGTTC
QDIY-200	TGACTGCTAT	ATAGCAGTCA	TCCGGATTGA

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Table continued from previous page

Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-201	AACGTCCGCT	AGCGGACGTT	ACGTCTTGTT
QDIY-202	CGCACATGTC	GACATGTGCG	ATGAAGTGCG
QDIY-203	GCACACCTGA	TCAGGTGTGC	CGATCACTGC
QDIY-204	TTGTCCAGAG	CTCTGGACAA	CCTATCGGAA
QDIY-205	AGCCTTCCTG	CAGGAAGGCT	CAGAGAGCTT
QDIY-206	CCTTACGCCA	TGGCGTAAGG	GCAACTTGCG
QDIY-207	GAATACGTAC	GTACGTATTC	TATGGAGGAC
QDIY-208	TTGGCACCGT	ACGGTGCCAA	TGAGATCAGA
QDIY-209	ATTAGGTGGC	GCCACCTAAT	TCAGCCTATT
QDIY-210	CGATCAAGAA	TTCTTGATCG	GTTGTGAGCG
QDIY-211	GCTGTCTTCT	AGAAGACAGC	TCAGTAACAC
QDIY-212	TACATGTCTG	CAGACATGTA	AAGGCTCAGA
QDIY-213	AACCAGTTGA	TCAACTGGTT	GTGTGGTGGT
QDIY-214	CCGGTAAGCT	AGCTTACCGG	CCGAGCTTAG
QDIY-215	GTTCGAATAG	CTATTCGAAC	ATCACGCTTC
QDIY-216	TGTCAGGCTC	GAGCCTGACA	TAGCTATGCA
QDIY-217	CAACAGTGTT	AACACTGTTG	TGTTCCTCAT
QDIY-218	AAGAGAGGAA	TTCCTCTCTT	CATACCTTCT
QDIY-219	CGGTTGTAGC	GCTACAACCG	GCCTTCAATG
QDIY-220	GCCTGAAGTG	CACTTCAGGC	CTTGACCAGC

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-221	TTACGACACT	AGTGTCGTAA	CTACACACAA
QDIY-222	CGCCTAGATC	GATCTAGGCG	TAGGCTGAAT
QDIY-223	AATCTGGATG	CATCCAGATT	TCGGAGTCCT
QDIY-224	CGACGGTACA	TGTACCGTCG	AACATCGCGG
QDIY-225	GTAGTATTGC	GCAATACTAC	GTTGTCTTAC
QDIY-226	TCCAGCGGAT	ATCCGCTGGA	GTGGCAACTA
QDIY-227	CAACCACCTC	GAGGTGGTTG	GAGCAGGCAT
QDIY-228	AGCTTAGGCG	CGCCTAAGCT	AACGGCACCT
QDIY-229	CCGGTTCCTT	AAGGAACCGG	AGTAACCTTG
QDIY-230	GACATTGAAC	GTTCAATGTC	TCTCATAAGC
QDIY-231	TTAGAGGCGA	TCGCCTCTAA	TGCTTGCCAA
QDIY-232	CAAGCCGAAC	GTTCGGCTTG	CGGTTCCTGT
QDIY-233	AGGAGAACGG	CCGTTCTCCT	CCAAGTAGAT
QDIY-234	CCTGTTAGAC	GTCTAACAGG	AAGGTTGGCG
QDIY-235	GTTCTACGTT	AACGTAGAAC	TGCTCTGGTC
QDIY-236	TAAGTCCACA	TGTGGACTTA	ACTGTAACGA
QDIY-237	CAAGAACCAT	ATGGTTCTTG	GATTCCAGGT
QDIY-238	AGTTGATGAC	GTCATCAACT	TTCACCAGAT
QDIY-239	CCTACTCTTG	CAAGAGTAGG	ACTTCCAAGG
QDIY-240	GAACAATCCA	TGGATTGTTC	CCGAATATTC

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-241	TTCTGTTGGT	ACCAACAGAA	CTCTTATCCA
QDIY-242	CATCGTCAGG	CCTGACGATG	TCACAGCGGT
QDIY-243	ATGCATGAAG	CTTCATGCAT	CCTCTGTCGT
QDIY-244	CGTGAATCGC	GCGATTCACG	TCTGTTCTCG
QDIY-245	GAGCAGCCTT	AAGGCTGCTC	GATACTTCAC
QDIY-246	TCGATTACCA	TGGTAATCGA	AGTGCTGATA
QDIY-247	CAGTCCAATT	AATTGGACTG	ATCCTTCGGT
QDIY-248	AGAGGCTTGG	CCAAGCCTCT	GACAACGATT
QDIY-249	CAGGCTCTCA	TGAGAGCCTG	GAACCGGTAG
QDIY-250	GTTCGCTCTC	GAGAGCGAAC	AGCAATGAGC
QDIY-251	TCGGACTAAT	ATTAGTCCGA	CAAGACTCCA
QDIY-252	CGAGATCTTC	GAAGATCTCG	ACCGTGTAGG
QDIY-253	ATAACCGGAC	GTCCGGTTAT	AGGCACAGGT
QDIY-254	CGTGTAGTTA	TAACTACACG	CGACAGATCG
QDIY-255	GAACATAGGT	ACCTATGTTC	ACGCGACAAC
QDIY-256	TCTAACATCG	CGATGTTAGA	ACTTGCGTTA
QDIY-257	AACGGTGGCA	TGCCACCGTT	CACCACTCAT
QDIY-258	AGGACGGTGT	ACACCGTCCT	CTTCGTAACT
QDIY-259	CTGTGACCTG	CAGGTCACAG	CAGTATTCGG
QDIY-260	GCTGTAACAA	TTGTTACAGC	CAGTCTGGAC
QDIY-261	TACGGACGTC	GACGTCCGTA	TACCGTTCTA
QDIY-262	CCTAAGGAGC	GCTCCTTAGG	GTGTCCACAG

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Table continued from previous page

Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-263	ATAAGGCCAG	CTGGCCTTAT	TTACGACTGT
QDIY-264	CTCATCTGTA	TACAGATGAG	GACGCGAATG
QDIY-265	GAAGGCATCT	AGATGCCTTC	CAACGTACGC
QDIY-266	TCTCTACTGC	GCAGTAGAGA	AGCTCAGGAA
QDIY-267	AACCGAACAA	TTGTTCGGTT	GATAGGCGGT
QDIY-268	ATCTCGCCAC	GTGGCGAGAT	AGTAGGAAGT
QDIY-269	CCATGCAACG	CGTTGCATGG	CATGTTGTAG
QDIY-270	GAATGGTGTA	TACACCATTC	CACATTCTTC
QDIY-271	TATATGCCGT	ACGGCATATA	GCAGCTCGTA
QDIY-272	CTCGATAGAT	ATCTATCGAG	GTTCAGACGG
QDIY-273	AACACAAGAG	CTCTTGTGTT	TCCTGGAAGT
QDIY-274	CGCAATCGGT	ACCGATTGCG	GCATTGTTAG
QDIY-275	GTTGCGTAGA	TCTACGCAAC	GACCTACAGC
QDIY-276	TAGAGTGATC	GATCACTCTA	CACCGACGTA
QDIY-277	AAGACGCAGC	GCTGCGTCTT	CTCTCACCTT
QDIY-278	AACTTCTCGA	TCGAGAAGTT	CTCGTTCATT
QDIY-279	CGCAACTGAG	CTCAGTTGCG	TGGTGGCAAG
QDIY-280	GCTCCGCAAT	ATTGCGGAGC	GATTGCTTGA
QDIY-281	GTAACTTCCG	CGGAAGTTAC	CCGTTAAGGT
QDIY-282	CTCACGACTA	TAGTCGTGAG	TGCTGAGAGG
QDIY-283	AACCAACGGC	GCCGTTGGTT	TTGTCACTTG
QDIY-284	CCTGCCTGTA	TACAGGCAGG	GCTGTTATGT

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-285	TACGCTGCAG	CTGCAGCGTA	GCAGCAGTTG
QDIY-286	AATGTTGCGA	TCGCAACATT	GCAGATCAAT
QDIY-287	CGACGTTCTG	CAGAACGTCG	TGGTTCACGG
QDIY-288	AATAGGACAC	GTGTCCTATT	TCGACCGCAT
QDIY-289	ATGTGCCTCA	TGAGGCACAT	TAACCTAGGT
QDIY-290	CGACTCCGTT	AACGGAGTCG	AACTCATGCG
QDIY-291	GCTGTTGTGG	CCACAACAGC	CCGGATGAAC
QDIY-292	TACCAATCAC	GTGATTGGTA	CGTTGCCGTA
QDIY-293	ATGTCTTACG	CGTAAGACAT	GCTCTACGGT
QDIY-294	CGCAACAATA	TATTGTTGCG	TGCATTGGCG
QDIY-295	GAACGAAGAC	GTCTTCGTTC	CGATTGTGAC
QDIY-296	TCGAGGACGT	ACGTCCTCGA	GACTGCACTA
QDIY-297	ATTATGAGCG	CGCTCATAAT	GTTAACTGCT
QDIY-298	CGCGTTATAA	TTATAACGCG	TCGGACCTTG
QDIY-299	GCGTGCATGT	ACATGCACGC	TGCAGCAAGC
QDIY-300	TAAGCGGCTC	GAGCCGCTTA	CACATGCGAA
QDIY-301	AACATGGAGA	TCTCCATGTT	CAGACGTAAT
QDIY-302	CCGAGTCTCT	AGAGACTCGG	ATTCGGTACG
QDIY-303	GTACTTCTAC	GTAGAAGTAC	TTAGCACGGC
QDIY-304	TGTTCACATG	CATGTGAACA	GAGGATAGTA
QDIY-305	AAGGTAACGC	GCGTTACCTT	AACTGTGGTT
QDIY-306	CCGCCTTACT	AGTAAGGCGG	ATTACCTCGG

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-307	GTTGAGGCAG	CTGCCTCAAC	CGCTGTATAC
QDIY-308	TGGCGACCTA	TAGGTCGCCA	CTTGCTCACA
QDIY-309	AGAAGCGACA	TGTCGCTTCT	CAACACCTGT
QDIY-310	CAGGATAATC	GATTATCCTG	CAATTGCTCG
QDIY-311	GCTCCTACAG	CTGTAGGAGC	CATAGACAAC
QDIY-312	TTCAACAGGT	ACCTGTTGAA	TTGGTGTCTA
QDIY-313	CCTCGTCCAT	ATGGACGAGG	TATGTCCTGT
QDIY-314	AGCGTTGGTT	AACCAACGCT	GCCAATTCGT
QDIY-315	CATTCGAACA	TGTTCGAATG	TAGGCGATCG
QDIY-316	GCTTACCGAC	GTCGGTAAGC	ATGAGTGTAC
QDIY-317	TTAGCTTAGG	CCTAAGCTAA	CCGAAGGATA
QDIY-318	CCGACACACA	TGTGTGTCGG	AGTCCACTGT
QDIY-319	ATTCGCTGAT	ATCAGCGAAT	GCGGCTAATT
QDIY-320	CCAAGAGGCA	TGCCTCTTGG	TCTAACTCAG
QDIY-321	GACGCAGTTC	GAACTGCGTC	CAAGCTGAGC
QDIY-322	TGGAACTCGG	CCGAGTTCCA	CCAGAGCACA
QDIY-323	CCACACCAAT	ATTGGTGTGG	TGTACAAGGT
QDIY-324	AGTTCTCGGC	GCCGAGAACT	TAGAATGCCT
QDIY-325	CTTGACGACG	CGTCGTCAAG	TGTCTTACTG
QDIY-326	GAGGTCGCTA	TAGCGACCTC	ATGACTAAGC
QDIY-327	TCAGTAGCAT	ATGCTACTGA	ATGTAGGCAA
QDIY-328	CTAACGTGGA	TCCACGTTAG	GCGAAGAGGT

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-329	ATGCCAACCG	CGGTTGGCAT	CGGTGGTTCT
QDIY-330	CGGTCGATTC	GAATCGACCG	CTGTCGTTGG
QDIY-331	GAAGTACAGT	ACTGTACTTC	TGATCGACAC
QDIY-332	TCTGCAGTAA	TTACTGCAGA	CCACCAGCTA
QDIY-333	CTATCCTAGC	GCTAGGATAG	CACGGTTCGT
QDIY-334	AACACTCCTT	AAGGAGTGTT	AGTGAGAGCT
QDIY-335	CCGAACCTAA	TTAGGTTCGG	TTGCATGCGG
QDIY-336	GTCTAGTCGC	GCGACTAGAC	TATACGTGTC
QDIY-337	TGGATGTACG	CGTACATCCA	TGACGCGTTA
QDIY-338	CTACCAGCGT	ACGCTGGTAG	TACAGAACGT
QDIY-339	AAGGATTCAG	CTGAATCCTT	CTTGTCAGGT
QDIY-340	CGAGGTGTGT	ACACACCTCG	ATCCACAGCG
QDIY-341	GTAGACGCTC	GAGCGTCTAC	CCTATCCATC
QDIY-342	TCGTCCGTCA	TGACGGACGA	ACCGCGAGTA
QDIY-343	CCGTGATAGG	CCTATCACGG	AAGTTCTGGT
QDIY-344	AGGATGACCT	AGGTCATCCT	ACAGGTATCG
QDIY-345	CCTCGAGTAC	GTACTCGAGG	ATGACGGATT
QDIY-346	GTCACTGAGG	CCTCAGTGAC	GTCTGAGTAG
QDIY-347	TACGGTTAGA	TCTAACCGTA	TGCCAGATGT
QDIY-348	CAACGAGAAT	ATTCTCGTTG	GCTAAGCATT
QDIY-349	AATACACCGG	CCGGTGTATT	ACAGCATGGT
QDIY-350	CCGATCCATC	GATGGATCGG	ATAGAGACCG

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Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-351	GAATCTCGCT	AGCGAGATTC	ATATCGCGTA
QDIY-352	TGACCGGCAA	TTGCCGGTCA	TTAAGGAGGT
QDIY-353	CATGATAGCA	TGCTATCATG	CTGTGCGACT
QDIY-354	AACAGCTTCG	CGAAGCTGTT	TCCGTATGCT
QDIY-355	CTAGTGCTTA	TAAGCACTAG	CCATCGATGT
QDIY-356	TGTGATACGT	ACGTATCACA	GTGAGCCGTT
QDIY-357	ATGAGCGTAT	ATACGCTCAT	TGCCGTTAAT
QDIY-358	CTAGATATGG	CCATATCTAG	CGGATGTGGT
QDIY-359	CGCTATGCTG	CAGCATAGCG	TCGCGTGTTG
QDIY-360	TACTACGTGA	TCACGTAGTA	CCGCGATCAT
QDIY-361	ATGTGGAGGT	ACCTCCACAT	CGCGTTATCG
QDIY-362	CCATGGCTCA	TGAGCCATGG	GTAGCCTCCT
QDIY-363	CCAATCACGC	GCGTGATTGG	ACTAGACACT
QDIY-364	TTAGATCCAG	CTGGATCTAA	CGATTCGTTG
QDIY-365	AGGAATATCG	CGATATTCCT	GAAGAGATGT
QDIY-366	CCTCCTATGT	ACATAGGAGG	AGATCCGACG
QDIY-367	TAGAGACACG	CGTGTCTCTA	CCAGGACATT
QDIY-368	CCAGCTCAGT	ACTGAGCTGG	ACGTGGCATT
QDIY-369	ATGGCTCATA	TATGAGCCAT	AAGCAGGACG
QDIY-370	CGGAGTGAAG	CTTCACTCCG	ACGAGTCGGT
QDIY-371	TACCTATGGT	ACCATAGGTA	AGTGTACGCG
QDIY-372	ATGAGACAGT	ACTGTCTCAT	ACCGACCATT

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Table continued from previous page

Indices for entry on sample sheet			
Unique dual-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)***	**i7 bases for entry on sample sheet (all instruments)**
QDIY-373	CTAAGAGTTG	CAACTCTTAG	TTGCTAACGT
QDIY-374	TAACCGTATG	CATACGGTTA	CTTGATACTG
QDIY-375	AGAGTCCATG	CATGGACTCT	CTGGATAAGT
QDIY-376	CTAGACCGCA	TGCGGTCTAG	ATAGCTTACG
QDIY-377	TATGGCTTGT	ACAAGCCATA	GTCCATGAGT
QDIY-378	CGTTGTTCCT	AGGAACAACG	ACTCCAGTCG
QDIY-379	CCGACATTAG	CTAATGTCGG	TCTCAGCACG
QDIY-380	TGTGAAGGCA	TGCCTTCACA	ATCGTGATGT
QDIY-381	AGCATCGTCT	AGACGATGCT	ACGCAATCCG
QDIY-382	CCGACTAGGA	TCCTAGTCGG	GAGATCGGCT
QDIY-383	AACATTACCG	CGGTAATGTT	CTACGTCTCG
QDIY-384	CCTAATTCGT	ACGAATTAGG	CTCAGGCTGT

Unique Dual-Index adapters 1–24 are identical on the adapter plates of the QIAseq UDI Y-Adapter Kit (24) and QIAseq UDI Kit A (96).

Note: Sequencing on the MiniSeq, NextSeq, HiSeqX, and HiSeq 3000/4000 systems follow a different dual-indexing workflow than other Illumina systems, which requires the reverse complement of the i5 index adapter sequence.

Appendix D: Design of Primer for Specific Amplification of Small Genomes

Specific primers can be designed using open source online primer design tools such as Primer3 (**https://bioinfo.ut.ee/primer3-0.4.0/**) or Primer-BLAST (**https://www.ncbi.nlm.nih.gov/tools/primer-blast/**). The Primer Blast allows specificity checking. With this option on, the program will search the primers against the selected database and determine whether a primer pair can generate an amplification product on any targets in the database based on their matches to the targets and their orientations. The program will return, if possible, only primers that do not generate a valid PCR product on unintended sequences and are therefore specific to the intended template.

It is recommended to set T_m of primer to 34–40°C and length between 17 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 as shown in Figure 9.

Figure 9. Schema of specific primer design.

Primers should be stabilized on 3′ primer end with phosphorothioate modifications on the last 3′-bases as shown in the example 5′-NNNNNNNNNN*N*N-3′.

The primer mix should include primers in a concentration of 10 nM each.

Appendix E: Purification of Amplified DNA after Whole Genome Amplification

If purification of the amplified DNA is required for additional downstream applications not described in this handbook, the following purification protocol may be used.

Procedure

1. Dilute amplified DNA from step 13 (page 20) 1:2 with H_2O SC.
2. Add 50 µl resuspended QIAseq Beads slurry to 50 µl diluted WGA sample, and mix well by pipetting.
3. Incubate the mixture for 5 min at room temperature.
4. Pellet the beads on a magnetic stand for 2–5 min and carefully discard the supernatant.
5. Wash the beads by adding 200 µl fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand for 2–5 min, and then carefully discard the supernatant.
6. Repeat the wash step 5 once for a total of two ethanol washes.
7. 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.
8. Elute by resuspending in 20 µl 10 mM Tris·Cl, pH 8.0. Pellet beads on the magnetic stand. Carefully transfer 17 µl supernatant to a new PCR plate.
9. Store purified amplified DNA at –20°C until further processing.

Appendix F: Exome Enrichment using the QIAseq Human Exome Kit

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 and 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.

Eight to sixteen QIAseq Single Cell DNA libraries can be pooled together equimolar to generate exome libraries for sequencing on Illumina instruments. Following the *QIAseq Human Exome Handbook* recommendations up to 4 µg library pool can enter the hybridization protocol. The amplified or PCR-free QIAseq Single Cell DNA libraries can be handled as any other QIAseq FX DNA library. The QIAseq 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*: **https://www.qiagen.com/us/products/discovery-and-translational-research/next-generation-sequencing/dna-sequencing/qiaseq-human-exome-kits/**.

Appendix G: Sequencing Recommendations

Sequencing recommendations for whole genome sequencing analysis is summarized in the Table 13.

Table 13. 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	All types
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 2nM and load 10pM on Illumina MiSeq flow cells for optimal clustering. Load on other instruments as recommended by Illumina for each instrument.

Appendix H: Real Time WGA

This protocol is optional and can be used if WGA has to be monitored in real time. This procedure used EvaGreen® Dye and will not affect the amplification of the DNA or the subsequent library generation.

Procedure

1. Dilute EvaGreen Dye, 2000x (cat no. 31019, Biotium) down to 25x by mixing 2 µl EvaGreen and 158 µl H2O SC. Mix and centrifuge briefly. Avoid light exposure of the mixture.
2. Lyse cell or denature DNA as described in the Protocol: Whole Genome Amplification from Single Cells and Purified DNA using Random Primers.
3. Prepare a master mix according to Table 14. IUse 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 14. After the addition of water, REPLI-g SC Dilution Buffer, oligos, DTT, and EvaGreen Dye 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 14. Preparation of master mix*

Component	Volume/reaction (µl)
H_2O SC	4.5
REPLI-g SC Dilution Buffer	14.5
REPLI-g SC universal oligo or REPLI-g SC advanced oligo	14.5
DTT (1:10)	2.5
EvaGreen Dye 25x	2
REPLI-g SC DNA Polymerase	2
Total volume	**40**

* To prepare a master mix for multiple reactions, scale up according to the number of reactions and add 10%.

4. For each amplification reaction, add 40 µl master mix to 10 µl denatured DNA (from step 7, page 22). Mix by flicking the tube, and then centrifuge briefly.
5. Incubate at 30°C for 2 h in a real-time cycler while acquiring data at FAM/Green Channel every minute.

   The following table describe the cycling conditions for this incubation and data acquisition.

Table 15. Cycling condition for real-time WGA

Step	Temperature (°C)	Duration	Cycle (comments)
1	30	1 min	120x (data acquisition at FAM/Green channel)
2	65	3 min	1x (inactivation of Phi29-Polymerase)
3	4	Hold	1x

Note: If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

Note: Incubating eukaryotic and bacterial DNA samples for 2 h generates sufficient DNA for PCR-free library prep using this kit. Incubation of 1 h is also possible but leads to reduced yields and may not be appropriate for all types of cells.

6. Inactivate REPLI-g SC DNA Polymerase at 65°C for 3 min.

7. Amplified DNA can be stored at 4°C (for short-term storage, e.g., up to 1 week) or at –15 to –35°C (for long-term storage).

   The following Image shows typical traces of amplification.

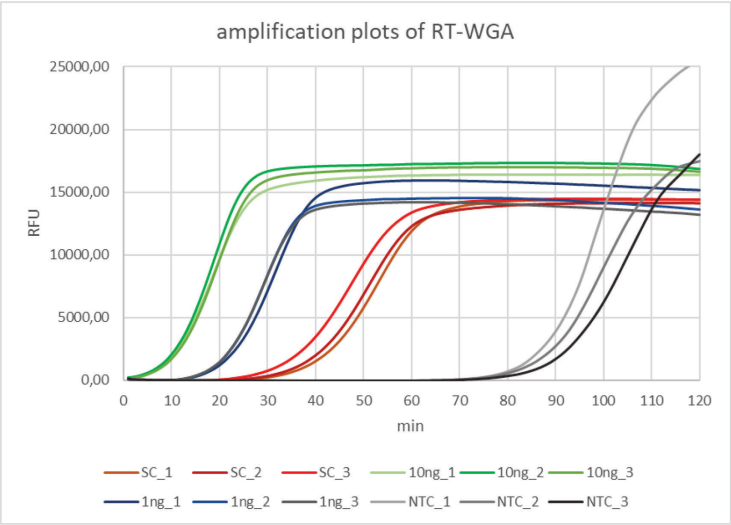

Figure 10. Amplification plots of real time WGA. The image show typical amplification traces when amplifying 10 ng, 1 ng, single cells, and no template controls (NTCs).

Ordering Information

Product	Contents	Cat. no.
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 beads and a plate containing 96 UDI barcoded adapters for use with Illumina instruments.	181705, 181725, 181765, 181785
QIAseq Single Cell DNA Library Kit UDI (384)	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.	181707

Product	Contents	Cat. no.
Related products		
QIAGEN QIAseq for next-generation sequencing applications		
QIAseq Single Cell RNA Library Kit UDI (24)	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.	180703
QIAseq Single Cell RNA Library Kit UDI A,B,C, or D (96)	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.	180705, 180725, 180765, 180785
QIAseq Single Cell RNA Library Kit UDI (384)	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.	180707

Product	Contents	Cat. no.
QIAseq Library Quant Assay Kit	The QIAseq Library Quant System provides a simple, out-of-the-box solution to quantify NGS libraries, enabling consistent results with every NGS run.	333314

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Document Revision History

Date	Changes
10/2021	Initial release

Notes

Notes

Limited License Agreement for QIAseq Single Cell DNA Library Kit UDI

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