October 2023

QIAseq® FX DNA Library Kit Handbook

For combined DNA fragmentation and preparation of DNA libraries for next-generation sequencing (NGS) applications that use Illumina® instruments

Sample to Insight

Contents

Kit Contents

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Component Part Number	**Component Name**	**QIAseq FX DNA Library UDI-A Kit HT (96)**	**QIAseq FX DNA Library UDI-B Kit HT (96)**	**QIAseq FX DNA Library UDI-C Kit HT (96)**	**QIAseq FX DNA Library UDI-D Kit HT (96)**	**QIAseq FX DNA Library Kit HT A-D (384)**
1120146	QIAseq FX DNA Library Core Kit (96)	1 pc	1 pc	1 pc	1 pc	4 pc
180312	QIAseq UDI Y-Adapter Kit A (96)	1 pc	–	–	–	1 pc
180314	QIAseq UDI Y-Adapter Kit B (96)	–	1 pc	–	–	1 pc
180316	QIAseq UDI Y-Adapter Kit C (96)	–	–	1 pc	–	1 pc
180318	QIAseq UDI Y-Adapter Kit D (96)	–	–	–	1 pc	1 pc
1129599	QIAseq Normalizer Reagent Kit (96)	1 pc	1 pc	1 pc	1 pc	4 pc
1129601	QIAseq Normalizer Primer Kit (96)	1 pc	1 pc	1 pc	1 pc	4 pc

Component Part Number	Component Name	180483 QIAseq FX DNA Library CDI Kit (24)	180477 QIAseq FX DNA Library UDI Kit (24)	180479 QIAseq FX DNA Library UDI-A Kit (96)	180480 QIAseq FX DNA Library UDI-B Kit (96)	180481 QIAseq FX DNA Library UDI-C Kit (96)	180482 QIAseq FX DNA Library UDI-D Kit (96)	180484 QIAseq FX DNA Library CDI Kit (96)
1120145	QIAseq FX DNA Library Core Kit (24)	1 pc	1 pc	–	–	–	–	–
1120146	QIAseq FX DNA Library Core Kit (96)	–	–	1 pc	1 pc	1 pc	1 pc	1 pc
180301	QIAseq CDI Y-Adapter Kit (24)	1 pc	–	–	–	–	–	–
180310	QIAseq UDI Y-Adapter Kit (24)	–	1 pc	–	–	–	–	–
180312	QIAseq UDI Y-Adapter Kit A (96)	–	–	1 pc	–	–	–	–
180314	QIAseq UDI Y-Adapter Kit B (96)	–	–	–	1 pc	–	–	–
180316	QIAseq UDI Y-Adapter Kit C (96)	–	–	–	–	1 pc	–	–
180318	QIAseq UDI Y-Adapter Kit D (96)	–	–	–	–	–	1 pc	–
180303	QIAseq CDI Y-Adapter Kit (96)	–	–	–	–	–	–	1 pc

QIAseq FX DNA Library Core Kit	(24)	(96)
Catalog no.	1120145	1120146
Number of reactions	24	96
FX Enzyme Mix (violet cap)	1 tube	1 tube
FX Buffer, 10x (blue cap)	1 tube	1 tube
FX Enhancer (black cap)	1 tube	1 tube
DNA Ligase (red cap)	1 tube	1 tube
DNA Ligase Buffer, 5x (yellow cap)	1 tube	2 tubes
RNase-Free Water (clear cap)	2 tubes	3 tubes
HiFi PCR Master Mix, 2x (green cap)	2 tubes	2 tubes
Primer Mix Illumina Library Amp, 10 µM (clear cap)	2 tubes	1 tube

QIAseq CDI/UDI Y-Adapter Kit	CDI (24)	CDI (96)	UDI (24)	UDI A (96)	UDI B (96)	UDI C (96)	UDI D (96)
Catalog no.	180301	180303	180310	180312	180314	180316	180318
Number of reactions	24	96	24	96	96	96	96
Adapter plate	1	1	1	1	1	1	1
Reference card	1	1	1	1	1	1	1

QIAseq Library Normalizer Kit	(96)	(24)
Catalog no.	180605	180603
Number of reactions	96	24
QIAseq Normalizer Reagent Kit		
Normalizer Reagent	1 x 580 µL	1 x 135 µL
Normalizer Wash Buffer	4 x 14 mL	1 x 14 mL
Normalizer Elution Buffer	2 x 1.9 mL	1 x 1.9 mL
RNase-free Water	1 x 1.9 mL	1 x 1.9 mL
QIAseq Normalizer Primer Kit		
Normalizer Primer Mix	1 x 165 µL	1 x 40 µL

The QIAseq FX DNA Library Kits contain a QIAseq Y-Adapter plate with either combinatorial dual-index adapters (CDI) or unique dual-index adapters (UDI). To multiplex more than 96 libraries in a single sequencing run, combine kits with different UDI Y-adapter plates. For example, combining the QIAseq FX DNA Library UDI-A (or B or C or D) Kit (96) Kit will allow the generation of 384 libraries with different sample indexes for 384-plex sequencing. For more information on QIAseq Y-Adapter Plates, please refer to Appendix D, page 46.

Shipping and Storage

The QIAseq FX DNA Library Kits are shipped in 2 boxes (Library Core Kit and Adapter Kit). Store both at –30°C to –15°C upon receipt. When stored correctly, all reagents are stable for at least 6 months after delivery if not otherwise stated on the label.

Intended Use

QIAseq FX DNA Library Kits are 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 the QIAseq FX DNA Library Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

Next-generation sequencing (NGS) is a driving force for numerous new and exciting applications, including cancer research, stem cell research, metagenomics, population genetics, and medical research. While NGS technology is continuously improving, library preparation remains one of the biggest bottlenecks in the NGS workflow and includes several time-consuming steps that can result in considerable sample loss and the potential to introduce handling errors. QIAGEN's QIAseq FX technology incorporates enzymatic DNA fragmentation into a streamlined, optimized protocol that does not require sample cleanup between fragmentation and adapter ligation, saving time and preventing errors. Optimized enzyme and buffer compositions ensure high sequencing library yield. Streamlined library construction protocols also enable straightforward automation of library prep on different liquid-handling platforms

Principle and procedure

The QIAseq FX DNA Library Kit provides a fast, fully enzymatic procedure from DNA fragmentation to NGS library with no cleanup steps until after adapters have been ligated to the sample DNA. The kit allows library preparation from input DNA amounts ranging from as little as 20 pg up to 1 µg.

Samples consisting of longer DNA fragments, such as genomic DNA or amplicons from long-range PCR, are first enzymatically sheared into smaller fragments. The median fragment size is dependent on the applications 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" is added to the 3' ends during the FX reaction, making the DNA fragments ready for adapter ligation. Following this step, platform-specific adapters are ligated to both ends of the DNA fragments. These adapters contain sequences essential for binding dual-barcoded libraries to a flow cell for sequencing, allowing for PCR amplification of adapter-ligated fragments, and binding standard Illumina sequencing primers.

To ensure maximum yields from limited amounts of starting material, a high-fidelity amplification step can be performed using the reagents included in the QIAseq FX DNA Library Kit. The proprietary HiFi PCR Master Mix can evenly amplify DNA regions with vastly different GC contents, minimizing sequencing bias caused by PCR.

Following library construction, the reaction cleanup and removal of adapter-dimers can be achieved by using either QIAseq Beads or Agencourt® AMPure® XP Beads (Beckman Coulter, cat. no. A63880), which enables easy automation on various high throughput automation platforms.

NGS adapter and index technologies

Sample multiplexing is one of the most important NGS tools for increasing throughput and reducing costs. It works by combining multiple samples to be processed together in a single sequencing run; as a consequence, sequencing reads need to be demultiplexed by reassigning each single read to its original source library. This is facilitated by the integration of index sequences into the individual adapter molecules.

QIAseq FX DNA Library CDI/UDI Kits include a fully compatible indexing solution. We recommend using the QIAseq Dual-Index Y-Adapter Plates delivered with the kit. Each QIAseq FX DNA Library CDI/UDI Kit includes one of the following:

- QIAseq Combinatorial Dual-Index (CDI) Y-Adapter Plate (24)
- QIAseq Combinatorial Dual-Index (CDI) Y-Adapter Plate (96)
- QIAseq Unique Dual-Index (UDI) Y-Adapter Plate (24)
- QIAseq Unique Dual-Index (UDI) Y-Adapter Plate A, B, C, or D (96)

Combining QIAseq FX DNA Library UDI-A/B/C/D (96) Kits enables multiplexing of up to 384 samples per sequencing run. For more information on QIAseq Dual-Index Y-Adapters and index sequence motifs, see “Appendix C: QIAseq Dual-Index Y-Adapters”, page 44, and “Ordering Information”, page 76.

CDI adapters use twelve i7 and eight i5 barcode motifs that can be combined to form up to 96 combinatory dual indexes. In contrast, QIAseq UDI Adapters use a fixed combination of 2 unique barcode motifs per adapter molecule. Therefore, each single-index motif is only used once on any UDI adapter plate.

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

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.

- QIAseq Beads (cat. nos. 333923, 333903, 333927) or Agencourt AMPure XP Beads (cat. no. A63880, A63881) for bead-based library purification
- 100% ethanol (ACS grade)
- Nuclease-free Water
- Buffer EB (cat. no. 19086)
- PCR tubes or plates
- Pipette tips and pipettes
- DNA LoBind® tubes (from Axygen or Eppendorf)
- Vortexer
- Microcentrifuge
- Thermocycler
- Magnetic racks for magnetic beads separation (e.g., Thermo Fisher Scientific DynaMag™ Magnet)
- Capillary electrophoresis device, e.g., QIAGEN QIAxcel, Agilent® Bioanalyzer® or similar, to evaluate the DNA fragmentation profile (optional)

Important Notes

General precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile, DNase-free, and RNase-free.
- Before starting, wipe down work area and pipettes with an RNase- and DNA-cleaning product
- For consistent library construction and amplification, ensure that the thermocycler used in this protocol is in good working order and has been calibrated within 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.

DNA preparation and quality control

High-quality DNA is essential for obtaining reliable sequencing results. The most important prerequisite for any DNA sequence analysis experiment is consistent, high-quality DNA from every experimental sample. Therefore, sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, or other contaminants will degrade the DNA or decrease the efficiency of the enzymatic activities necessary for optimal library preparation.

It is important to remove all cations and chelators from DNA preparations, therefore, make sure DNA is eluted in QIAGEN's Buffer EB or H_2O, not 1x TE buffer containing 1 mM EDTA. If the DNA was eluted or dissolved in 1x TE, or if you are not certain about the EDTA

concentration in the input DNA, we strongly recommend purifying the input DNA using either QIAseq Beads or Agencourt AMPure XP Beads, following the instructions in "Appendix B: Removal of Divalent Cations and EDTA from Input Nucleic Acid", page 43. Alternatively, we recommend setting up the FX reaction using the FX Enhancer as described in "Appendix C: Fragmentation, End-Repair, and A-Addition of DNA in 1x TE", page 44.

Recommended genomic DNA preparation method

To prepare purified DNA, we recommend using an appropriate QIAGEN DNA purification kit that supports DNA elution in Buffer EB or 10 mM Tris pH 8.0. For the best FX fragmentation performance, do not elute samples in a buffer containing >0.1 mM EDTA.

- QIAamp® DNA Mini Kit (cat. no. 51304) for the preparation of genomic DNA samples from fresh tissues and cells
- GeneRead™ DNA FFPE Kit (cat. no. 180134) for efficient recovery of high-quality gDNA from FFPE tissue
- MagAttract® HMW DNA Kit (cat. no. 67563) for isolation of high-molecular-weight genomic DNA
- QIAamp DNA Microbiome Kit (cat. no. 51704) for isolation of bacterial microbiome DNA from mixed samples

It is critical to accurately determine the input DNA concentration, especially when the input amount is below 100 ng. We recommend using Qubit®, PicoGreen®, or another fluorometric method to accurately quantify DNA with a concentration below 1.5 ng/µL.

Protocol: QIAseq FX DNA Library Preparation

Fragmentation, End-Repair, A–addition, and Adapter Ligation

This protocol describes the FX reaction for single-tube fragmentation, end-repair, and A–addition with subsequent adapter ligation

Important points before starting

- Before setting up the reaction, it is critical to accurately determine the amount of the input DNA.
- Ensure input DNA is in water, 10 mM Tris, QIAGEN's Buffer EB or low TE (0.1x TE, 0.1 mM EDTA). If input DNA is in 1x TE, please set up the FX reaction according to the protocol in Appendix C.
- If using the QIAseq Library Normalizer Kits, refer to Appendix A.

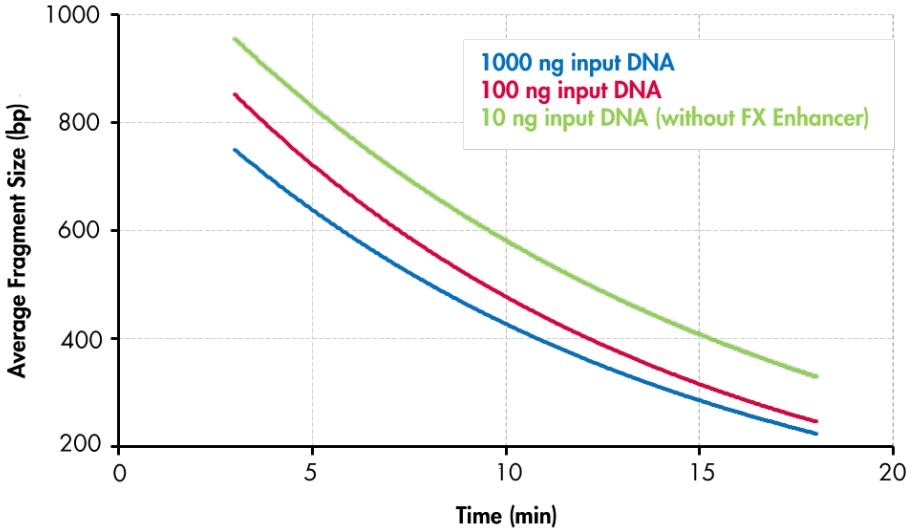

Figure 1. Fragmentation profile of different amounts of input DNA.

Table 1. Guideline for choosing the initial fragmentation time

Fragment peak size	250 bp	350 bp	450 bp	550 bp
	Fragmentation time (min) at 32°C			
50 pg – 1 ng input DNA*	14	4	1	–
10 ng input DNA†	24	16	14	10
100 ng input DNA	16	10	8	6
1000 ng input DNA	14	8	6	4

Note: The same FX fragmentation time will produce a consistent fragment size within an approximately 5-fold range of input DNA amounts. The exact reaction time may need to be optimized for DNA samples of variable quality.

* For input DNA amounts between 20 and 50 pg, incubate the FX reaction including the FX Enhancer for 25 min to produce a fragment distribution centered around 250 bp.

† For input DNA <10 ng, FX Enhancer is required for optimal performance (Table 4). To produce a fragment distribution centered around 300 bp from 1 ng input, incubate the FX reaction including FX Enhancer for 10 min.

Things to do before starting

- Refer to Figure 1 and Table 1 to determine the time required to fragment input DNA to the desired size. If input DNA is less than 10 ng, add FX Enhancer according to the protocol and use half the reaction time listed for 10 ng input DNA. For example, to produce a fragment distribution centered around 300 bp from 1 ng input, incubate the FX reaction including FX Enhancer for 10 min.
- Make fresh 80% ethanol.
- Thaw reagents on ice. Once reagents are thawed, mix buffers thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.
- Program thermocyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advance.
- Equilibrate QIAseq Beads or Agencourt AMPure XP Beads or to room temperature (15–25°C) for 20–30 min before use.
- Vortex and spin down the thawed adapter plate before use.

Procedure

1. Program a thermocycler according to Table 2 using the predetermined FX fragmentation time for step 2. Be sure to use the instrument's heated lid, and if possible, set the temperature of the heated lid to 70°C.

Table 2. Input DNA (20 pg –1000 ng) free of EDTA, Buffer EB, or in 0.1x TE

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	32°C	1–30 min*
3	65°C	30 min
4	4°C	Hold

* To determine the reaction time for step 2, refer to Figure 1 and Table 1.

2. Start the program. When the thermocycler block reaches 4°C, pause the program.
3. Prepare the FX reaction mix in a PCR plate or tube on ice according to Table 3 for >10 ng input DNA or Table 4 for <10 ng input DNA. Mix well by gently pipetting (do not vortex to mix).

Table 3. FX reaction mix setup (per sample) for >10 ng input DNA

Component	Volume (µL)
FX Buffer, 10x	5
Purified DNA	Variable
Nuclease-free Water	Variable
Total without FX Enzyme Mix	**40**

Table 4. FX reaction mix setup (per sample) for <10 ng input DNA

Component	Volume (µL)
FX Buffer, 10x	5
Purified DNA	Variable
FX Enhancer	2.5
Nuclease-free water	Variable
Total without FX Enzyme Mix	**40**

4. Add 10 µL FX Enzyme Mix to each reaction and mix well by pipetting up and down 20 times. It is critical to keep the reactions on ice for the entire time during reaction setup.
5. Briefly spin down the PCR plate/tubes and immediately transfer to the prechilled thermocycler (4°C). Resume the cycling program.
6. When the thermocycler program is complete and the sample block has returned to 4°C, remove samples and place them on ice.
7. Immediately proceed with adapter ligation.
8. Pierce the foil seal for each adapter well to be used, and transfer 5 µL from one DNA adapter well to each 50 µL sample from the previous protocol. Track the barcodes from each adapter well used for each sample.

   Note: If your DNA input is <10 ng, dilute the adapters according to Table 5.

Table 5. Adapter dilution factors

Sample DNA amount	Adapter dilution
20–99 pg	1:1000
100–999 pg	1:100
1–9 ng	1:10

9. Freeze the adapter plate containing unused adapters. QIAseq adapters are stable for a minimum of 10 freeze-thaw cycles.

   Important: Only 1 single adapter should be used per ligation reaction. If adapters from another supplier are used, follow the manufacturer's instructions. Do not reuse adapter wells once the foil seal has been pierced.

10. Prepare the ligation Master Mix (per DNA sample, Table 6) in a separate PCR plate or tube on ice, and mix well by pipetting.

Table 6. Ligation master mix setup (per sample)

Component	Volume (µL)
Ligation buffer, 5x	20
DNA ligase	10
Nuclease-free water	15
Total	**45**

11. Add 45 µL of the ligation Master Mix to each sample, for a total of 100 µL, and mix well by pipetting. Incubate the ligation reaction at 20°C for 15 min.

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

12. Proceed immediately to adapter ligation cleanup using 0.8x (80 µL) Agencourt AMPure XP beads or QIAseq Beads.
13. Add 80 µL of resuspended Agencourt AMPure XP beads or QIAseq Beads to each ligated sample and mix well by pipetting.
14. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) for 2 min, then carefully discard the supernatant.
15. Wash the beads by adding 200 µL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
16. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry.

    16a. Overdrying of Ampure XP beads may result in lower DNA recovery.

    16b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Over drying QIAseq Beads will not affect the DNA elution.

17. Remove from the magnetic stand. Elute by resuspending in 52.5 µL of Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 50 µL of supernatant into a new plate or tube.

18. Perform a second purification using 1x (50 µL) Agencourt AMPure XP beads or 1.1x (55 µL) of QIAseq Beads following steps 14–16 for DNA binding and washing. Elute DNA by adding 26 µL Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads and carefully collect 23.5 µL of purified DNA sample in a DNA LoBind tube for library amplification. If not proceeding immediately, the sample can be stored at –30°C to –15°C.

Amplification of Library DNA

PCR-based library amplification is normally required if the input DNA amount is below 100 ng or if large amounts of libraries are required for downstream hybrid capture. This protocol is for high-fidelity amplification of the DNA library using the amplification reagents provided in the QIAseq FX DNA Library Kit.

Things to do before starting

- Thaw QIAseq HiFi PCR Master Mix and Primer Mix on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations.
- Always start with the cycling conditions specified in this protocol. The cycling has been optimized for use with QIAseq HiFi PCR Master Mix for even and high-fidelity amplification of sequencing libraries.
- Equilibrate Agencourt AMPure XP or QIAseq Beads to room temperature (15–25°C) for 20–30 min before use.

Procedure

1. Program a thermocycler with a heated lid according to Table 7.

Table 7. Library amplification cycling conditions

Time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	
30 s	60°C	6 (100 ng input DNA) 10 (10 ng input DNA) 12 (1 ng input DNA) 14 (100 pg input DNA) 16 (20 pg input DNA)
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

Note: 6–16 amplification cycles are recommended based on the input DNA amount and quality.

2. Prepare a reaction mix on ice according to Table 8. Mix the components in a PCR tube or 96-well PCR plate.

Table 8. Reaction mix for library enrichment

Component	Volume (µL)
HiFi PCR Master Mix, 2x	25
Primer Mix (10 µM each)*	1.5
Library DNA	23.5
Total reaction volume	**50**

* If using QIAseq Library Normalizer, refer to "Appendix A: QIAseq FX DNA Library Preparation and Normalization using QIAseq Library Normalizer Kits", page 28, and use Normalization Primer Mix from QIAseq Library Normalizer Kit.

3. Transfer the PCR tube or plate to the thermocycler and start the program.

4. Once PCR is complete, add 50 µL of resuspended Agencourt AMPure XP Beads or 60 µL of resuspended QIAseq Beads to each reaction (50 µL) and pipette up and down thoroughly to mix.
5. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
6. Wash the beads by adding 200 µL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
7. Incubate on the magnetic stand for 5–10 min or until the beads are dry.
   7a. Overdrying of Ampure XP beads may result in lower DNA recovery.
   7b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Overdrying QIAseq Beads will not affect the DNA elution.
8. Remove from the magnetic stand. Elute by resuspending in 25 µL of Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 23 µL of the supernatant into a new tube.
   8a. Overdrying of Ampure XP beads may result in lower DNA recovery. Remove from the magnetic stand.
   8b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Overdrying QIAseq Beads will not affect the DNA elution.
9. Assess the quality of the library using a capillary electrophoresis device such as QIAGEN QIAxcel or Agilent BioAnalyzer. Check for the expected size distribution (see Figure 2) of library fragments and for the absence of an adapter-dimer peak around 120 bp.

   Note: The library should show a distribution centered around the size of the fragmented DNA plus 120 bp (see Figure 3, next page). The increase in library length reflects the addition of sequencing adapters to the DNA fragments.

   Note: The median fragment size can be used in subsequent qPCR-based quantification methods to calculate library concentration (step 10).
10. Quantify the library using a qPCR-based method such as the QIAseq Library Quant Assay Kit (cat. no. 333314; not provided), or a comparable method.

11. The purified library can be safely stored at –30°C to –15°C in a DNA LoBind tube until ready to use for sequencing or other applications.

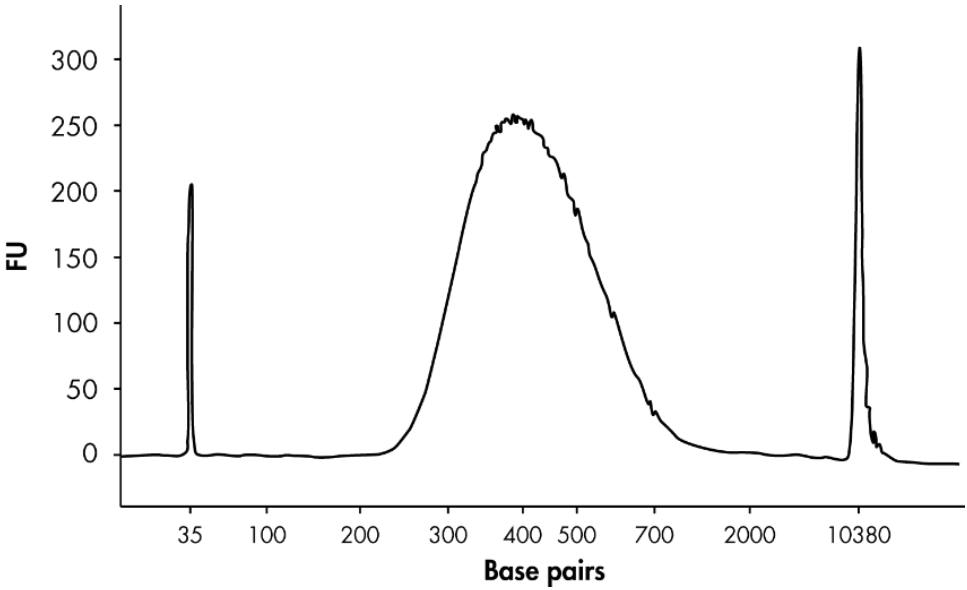

Figure 2. Capillary electrophoresis device trace data.

Troubleshooting Guide

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

	Comments and suggestions
Low library yields	
a) Suboptimal reaction conditions due to low DNA quality	Make sure to use high-quality DNA to ensure optimal activity of the library enzymes.
b) Insufficient amount of starting DNA for direct sequencing without library amplification	Typically, 100 ng of sheared genomic 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.
c) Inaccurate quantification of starting DNA due to RNA contamination.	RNA from the sample material can be co-purified with genomic DNA. This contaminating RNA will affect the accuracy of DNA quantification. To remove RNA during the sample preparation protocol, we recommend to perform RNase A treatment of the DNA.
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. QIAseq Beads or Agencourt AMPure XP Beads efficiently remove adapter-dimers, as well as free adapter molecules.

		Comments and suggestions
b)	Presence of larger library fragments after library enrichment	If the fragment population shifts higher than expected after adapter ligation and PCR enrichment (e.g., more than the expected 120 bp shift), this can be a PCR artifact due to over-amplification of the DNA library. Ensure that you use as few amplification cycles as possible to avoid this effect.
c)	Incorrect library fragment size after adapter ligation	During library preparation, adapters of approximately 60 bp are ligated to both ends of the DNA library fragments. This 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, refer to the size information given in the respective documentation. The absence of a clear size shift may indicate no, or only low, adapter ligation efficiency. Ensure that you use the parameters and incubation times described in the handbook for end-repair, A–addition and ligation, as well as the correct amount of starting DNA.
d)	Incorrect DNA fragment size prior to adapter ligation	The wrong DNA fragment size prior to adapter ligation can be due to the wrong conditions used for enzymatic DNA fragmentation. The reaction time should be optimized for different amount of input DNA. For input DNA >10 ng, we recommend 12 min as a starting point as it produces fragmentation size centers around 300 to 500 bp. Depending on the size requirement and type of input DNA, either increase or decrease reaction time by 2–4 min incrementally until expected size range is achieved.

Appendix A: QIAseq FX DNA Library Preparation and Normalization Using QIAseq Library Normalizer Kits

QIAseq Normalizer Kits provide a fast and easy way to normalize and pool NGS libraries for Illumina, while skipping library qualification and quantification steps. QIAseq Normalizer will accurately normalize over a broad range of library concentrations, but it cannot fully compensate for libraries of poor quality or libraries at lower concentrations of less than 15 nmol/L. Therefore, we recommend to adhere to the below guidelines and to use a minimum gDNA input of 5 ng for library preparation.

> **Note**: Higher input amounts will generally enable more complex libraries. While 5 ng input comprise a high number of copies for small genomes such as many microbes, larger genomes such as human or many plant genomes will benefit from higher input amounts.

- Make sure the used input material for library preparation is of good quality and/or is accurately quantified.
- The laboratory staff should be experienced with performing QIAseq FX library preparation.
- The conditions for the library preparation should be well established in your laboratory (e.g., input amount, adapter concentrations/dilution, PCR settings, cleanup procedures and other workflow parameters) so your library yields are consistent with concentrations well above 15–20 nmol/L in a minimal volume of 15 µL.
- Do not use QIAseq Normalizer Kits, if using libraries for downstream hybridization capture.

This modified QIAseq FX DNA Library protocol is optimized for subsequent library normalization using QIAseq Library Normalizer Kits. Normalizing libraries after preparation

allows pooling and sequencing without the need for library quantification by qPCR or alternative methods.

Note: Performing the QIAseq FX library preparation with subsequent normalization requires a QIAseq Library Normalizer Kit in addition to the QIAseq FX DNA Library Kit.

QIAseq Library Normalizer Kit	(96)	(24)
Catalog no.	180605	180603
Number of reactions	96	24
QIAseq Normalizer Reagent Kit		
Normalizer Reagent	1 x 580 µL	1 x 135 µL
Normalizer Wash Buffer	4 x 14 mL	1 x 14 mL
Normalizer Elution Buffer	2 x 1.9 mL	1 x 1.9 mL
RNase-free Water	1 x 1.9 mL	1 x 1.9 mL
QIAseq Normalizer Primer Kit		
Normalizer Primer Mix	1 x 165 µL	1 x 40 µL

Further reading on library normalization:

- QIAseq Normalizer Kits (**qiagen.com/qiaseq-normalizer**)
- QIAseq Normalizer Kit Handbook (**qiagen.com/HB-2015**)

Fragmentation, End-repair, A–addition, and Adapter ligation

This protocol describes the FX reaction for single-tube fragmentation, end-repair, and A–addition.

Important points before starting

- Before setting up the reaction, it is critical to accurately determine the amount of the input DNA.
- Do not use less than 5 ng of input DNA if using QIAseq Normalizer Kits.

- Ensure input DNA is in water, 10 mM Tris, QIAGEN's Buffer EB, or low TE (0.1x TE, 0.1 mM EDTA). If input DNA is in 1x TE, please set up the FX reaction according to the protocol in Appendix B.

Table 9. Guideline for choosing the initial fragmentation time

Fragment peak size	250 bp	350 bp	450 bp	550 bp
	Fragmentation time (min) at 32°C			
10 ng input DNA†	24	16	14	10
100 ng input DNA	16	10	8	6
1000 ng input DNA	14	8	6	4

Note: The same FX fragmentation time will produce a consistent fragment size within an approximately 5-fold range of input DNA amounts. The exact reaction time may need to be optimized for DNA samples of variable quality.
† For input DNA <10 ng, FX Enhancer is required for optimal performance (Table 12). To produce a fragment distribution centered around 300 bp from 1 ng input, incubate the FX reaction including FX Enhancer for 10 min.

Things to do before starting

- Refer to Figure 1and Table 9 to determine the time required to fragment input DNA to the desired size. If input DNA is less than 10 ng, add FX Enhancer according to the protocol and use half the reaction time listed for 10 ng input DNA. For example, to produce a fragment distribution centered around 300 bp from 1 ng input, incubate the FX reaction including FX Enhancer for 10 min.
- Make fresh 80% ethanol.
- Thaw reagents on ice. Once reagents are thawed, mix buffers thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.
- Program thermocyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advance.
- Equilibrate Agencourt AMPure XP beads or QIAseq Beads to room temperature (15–25°C) for 20–30 min before use.
- Vortex and spin down the thawed adapter plate before use.

Procedure

1. Program a thermocycler according to Table 10 using the predetermined FX fragmentation time for step 2. Be sure to use the instrument's heated lid, and if possible, set the temperature of the heated lid to 70°C.

Table 10. Input DNA (5 ng – 1000 ng) free of EDTA, Buffer EB, or in 0.1x TE

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	32°C	1–30 min*
3	65°C	30 min
4	4°C	Hold

* To determine the reaction time for step 2, refer to Figure 1 (page 17) and Table 9 (previous page).

2. Start the program. When the thermocycler block reaches 4°C, pause the program.
3. Prepare the FX reaction mix in a PCR plate or tube on ice according to Table 11 for >10 ng input DNA or Table 12 (next page) for <10 ng input DNA. Mix well by gently pipetting (do not vortex to mix).

Table 11. FX reaction mix setup (per sample) for >10 ng input DNA

Component	Volume (µL)
FX Buffer, 10x	5
Purified DNA	Variable
Nuclease-free water	Variable
Total without FX Enzyme Mix	**40**

Table 12. FX reaction mix setup (per sample) for <10 ng input DNA

Component	Volume (µL)
FX Buffer, 10x	5
Purified DNA (≥5 ng)	Variable
FX Enhancer	2.5
Nuclease-free water	Variable
Total without FX Enzyme Mix	**40**

4. Add 10 µL FX Enzyme Mix to each reaction and mix well by pipetting up and down 20 times. It is critical to keep the reactions on ice for the entire time during reaction setup.
5. Briefly spin down the PCR plate/tubes and immediately transfer to the prechilled thermocycler (4°C). Resume the cycling program.
6. When the thermocycler program is complete and the sample block has returned to 4°C, remove samples and place them on ice.
7. Immediately proceed with adapter ligation as described in the next protocol.
8. Pierce the foil seal for each adapter well to be used, and transfer 5 µL from one DNA adapter well to each 50 µL sample from the previous protocol. Track the barcodes from each adapter well used for each sample.

   Note: If your DNA input is <10 ng, dilute the adapters according to Table 13.

Table 13. Adapter dilution factors

Sample DNA amount	Adapter dilution
5–9 ng	1:10
≥10 ng	undiluted

9. Freeze the adapter plate containing unused adapters. The QIAseq adapters are stable for a minimum of 10 freeze-thaw cycles.

   Important: Only 1 single adapter should be used per ligation reaction. If adapters from another supplier are used, follow the manufacturer's instructions. Do not reuse adapter wells once the foil seal has been pierced.

10. Prepare the ligation Master Mix (per DNA sample, Table 14) in a separate PCR plate or tube on ice, and mix well by pipetting.

Table 14. Ligation master mix setup (per sample)

Component	Volume (µL)
Ligation buffer, 5x	20
DNA ligase	10
Nuclease-free water	15
Total	**45**

11. Add 45 µL of the ligation Master Mix to each sample, for a total of 100 µL, and mix well by pipetting. Incubate the ligation reaction at 20°C for 15 min.

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

12. Proceed immediately to adapter ligation cleanup using 0.8x (80 µL) Agencourt AMPure XP beads or QIAseq Beads.
13. Add 80 µL of resuspended Agencourt AMPure XP beads or QIAseq Beads to each ligated sample and mix well by pipetting.
14. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) for 2 min, then carefully discard the supernatant.
15. Wash the beads by adding 200 µL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
16. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry.

    16a. Overdrying of AMPureXP beads may result in lower DNA recovery. Remove from the magnetic stand.

    16b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Over drying QIAseq Beads will not affect the DNA elution.

17. Elute by resuspending in 52.5 µL of Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 50 µL of supernatant into a new plate or tube.

17a. Overdrying of AMPureXP beads may result in lower DNA recovery. Remove from the magnetic stand.

17b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Over drying QIAseq Beads will not affect the DNA elution.

18. Perform a second purification using 1x (50 µL) Agencourt AMPure XP beads or 1.1x (55 µL) of QIAseq Beads following steps 14–16 for DNA binding and washing. Elute DNA by adding 26 µL Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads and carefully collect 23.5 µL of purified DNA sample in a DNA LoBind tube for library amplification. If not proceeding immediately, the sample can be stored at –30°C to –15°C.

Amplification of Library DNA

This protocol requires library amplification primers provided in the QIAseq Normalizer Kits. Replace the amplification primer mix provided with the QIAseq FX DNA Library Kit by the Normalizer Primer Mix.

Always perform PCR-based library amplification before normalizing libraries using the QIAseq Normalizer Kits. Do not use PCR-free libraries together with QIAseq Normalizer Kits. This protocol is for high-fidelity amplification of the DNA library using the HiFi PCR Master Mix provided in the QIAseq FX DNA Library Kit together with the Normalizer Primer Mix from the QIAseq Library Normalizer Kit.

Things to do before starting

- Thaw QIAseq HiFi PCR Master Mix from the QIAseq FX DNA Library Kit and the Normalizer Primer Mix from the QIAseq Library Normalizer Kit on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations.
- Always start with the cycling conditions specified in this protocol. The cycling has been optimized for use with QIAseq HiFi PCR Master Mix for even and high-fidelity amplification of sequencing libraries.

Procedure

1. Program a thermocycler with a heated lid according to Table 15.

Table 15. Library amplification cycling conditions

Time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	
30 s	60°C	3 (1 µg input DNA) 4 (500 ng input DNA) 5 (200 ng input DNA) 6 (100 ng input DNA) 7 (50 ng input DNA) 8 (20 ng input DNA) 9 (10 ng input DNA) 11 (5 ng iput DNA)
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

2. Prepare a reaction mix on ice according to Table 16. Mix the components in a PCR tube or 96-well PCR plate.

   Important: Make sure to use the Normalizer Primer Mix to amplify the libraries. Using the library amplification primers included in the QIAseq FX DNA Library Kits will lead to normalization failure.

Table 16. Reaction mix for library enrichment

Component	Volume (µL)
HiFi PCR Master Mix, 2x	25
Normalizer Primer Mix	1.5
Library DNA	23.5
Total reaction volume	**50**

3. Transfer the PCR tube or plate to the thermocycler and start the program.
4. Once PCR is complete, add 50 µL of resuspended Agencourt AMPure XP Beads or 60 µL of resuspended QIAseq Beads to each reaction (50 µL) and pipette up and down thoroughly to mix.
5. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
6. Wash the beads by adding 200 µL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
7. Incubate on the magnetic stand for 5–10 min or until the beads are dry.
   7a. Overdrying of Ampure XP beads may result in lower DNA recovery.
   7b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Over drying QIAseq Beads will not affect the DNA elution.
8. Remove the beads from the magnetic stand. Elute by resuspending in 32.5 µL of Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 30 µL of the supernatant into a new tube.

   Note: If required, the elution volume can be reduced to 20 µL to increase the library concentration. The QIAseq Normalizer procedure will require 15 µL of modified library. Library concentrations should consistently be greater than 15 nmol/L.
9. Proceed to protocol Library Normalization. Alternatively, the purified libraries can be safely stored at –20°C.

   Note: Optionally, the libraries can be analyzed using a capillary electrophoresis device such as QIAGEN QIAxcel or Agilent BioAnalyzer. Quantification may be performed using qPCR (e.g., using the QIAseq Library Quant Assay Kit). Analysis by electrophoresis is recommended when using the QIAseq FX DNA Library Kit for the first time or while optimizing conditions, but is generally not required prior to library normalization.

Library normalization and pooling

This protocol describes the normalization of previously modified Illumina libraries. Only use libraries that have been amplified in presence of the Normalizer Primer Mix. Using non-modified libraries will lead to normalization failure. QIAseq Normalization will yield double-stranded libraries at a concentration of approximately 4 nmol/L. Normalized libraries can be pooled for sequencing at equal volumes without further quantification.

To perform QIAseq Normalization in 96-well format instead of tubes, please refer to the plate protocol included in the *QIAseq Normalizer Kit Handbook* (**www.qiagen.com/HB-3361**).

Things to do before starting

- Thoroughly mix the Normalizer Reagent by vortexing for 60 s. Make sure the pellet of beads is complete dissolved and the solution is well homogenized.
- Homogenize the bottle(s) of Normalizer Wash Buffer by vortexing and preheat the whole reagent bottle(s) to 55°C in a water bath. Alternatively, use a heating block for 1.5 mL or 2.0 mL tubes or Falcon tubes. Fill the homogenized wash buffer into compatible receptacles to preheat. Prepare 450 µL wash buffer (400 µL are required) per normalization reaction.
- Preheat a heating block for 1.5 mL tubes to 55°C.
- It is not required to equilibrate the Normalizer Reagent and Normalizer Elution Buffer to room temperature before use.

Procedure

1. Homogenize the bottle(s) of Normalizer Wash Buffer by vortexing and preheat the whole reagent bottle(s) to 55°C in a water bath. Alternatively, use a heating block for 1.5 mL or 2.0 mL tubes or Falcon tubes. Fill the homogenized wash buffer into compatible receptacles to preheat. Prepare 450 µL wash buffer (400 µL are required) per normalization reaction. Leave the wash buffer at 55°C until use.
2. Thoroughly mix the Normalizer Reagent by vortexing for 60 s. Make sure the pellet of beads is completely dissolved and the solution is well homogenized.
3. Directly before use, pulse spin the Normalizer Reagent at low force to collect all liquid at the bottom. Then use a 200 µL pipette to homogenize the reagent. Be sure to disperse a sediment of beads that may have formed during pulse spin.
4. For every library to be normalized, pipette 5 µL of homogenized Normalizer Reagent to a 1.5 mL tube. Leave the tubes at room temperature.
5. Add 15 µL of the modified library to be normalized to a 1.5 mL tube containing Normalizer Reagent. Mix well by pipetting or vortexing.
6. Incubate for 10 min at room temperature.
7. Add 200 µL pre-warmed Normalizer Wash Buffer (55°C) to each tube.
8. Pellet the beads on a magnetic stand for 2 min and wait until the solution is clear.

   Important: Make sure all beads have pelletized. If you are uncertain, leave the tubes on the magnet for 5 min.
9. Carefully discard the supernatant without disturbing the pellet.
10. With the tube on the magnet add 200 µL pre-warmed Normalizer Wash Buffer (55°C) to each pellet.
11. Switch the tube position on the magnet to wash the beads. Then wait until all beads have pelletized. Alternatively, turn the tube by 180° to force the beads to opposite side of the tube.

12. Carefully discard the supernatant without disturbing the pellet. Remove as much remaining liquid as possible, then proceed to the next step immediately.

    Note: Drying the beads is not required.

13. Add 26 µL Normalizer Elution Buffer to each pellet and mix well by vortexing. Make sure the pellet is completely dissolved.
14. Tap the tube on the benchtop to collect the liquid at the bottom. If there is liquid remaining in the lid, pulse spin the tube at low force. Do not spin to form a compact bead pellet.
15. Incubate for 5 min at 55°C in a heating block.
16. Pulse spin to collect all liquid at the bottom, then pellet the beads on a magnetic stand for 2 min and wait until the solution is clear.
17. Carefully transfer 25 µL of the supernatant to a new tube.

    Important: Do not discard the supernatant. The supernatant contains the ready-to-sequence library.

18. Pool the normalized libraries for sequencing following the below guidelines. Alternatively, the normalized libraries can be stored at –20°C for up to 3 months.

Pooling guidelines

- If normalized libraries were frozen, thaw them completely, mix by pulse vortexing, and spin down to avoid localized concentrations.
- For a homogeneous distribution of sequence reads, combine equal volumes of normalized libraries into a pool. For example, combine 5 µL of each normalized library. The pool will have an overall concentration of approximately 4 nmol/L.
- For joint sequencing, only combine libraries that have different indexes to allow complete demultiplexing of sequence data.

Library pool quantification (optional)

- Quantifying the library pool is optional, but may be considered if using QIAseq Normalizer for the first time.
- QIAseq normalized libraries are double-stranded and can be quantified using gel electrophoresis systems such as the QIAxcel or the Agilent 2100 Bioanalyzer. To prevent interference caused by the Normalizer Elution Buffer, dilute 5 µL of the library pool with 5 µL of nuclease-free water prior to electrophoresis.
- Quantification using colorimetric assays (e.g., Qubit) or a photospectrometer (e.g., Nanodrop) can be performed without diluting the normalized library pool. Colorimetric or photospectrometric assays require knowledge about the average library fragment size to calculate the approximate molarity. Table 17 lists the expected concentration for typical library fragment sizes.

Table 17. Theoretical library concentrations of normalized libraries for typical library fragment sizes

Ave. library fragment size (bp)	Expected concentration (ng/µL)
250	0.66
300	0.79
350	0.92
400	1.06
450	1.19
500	1.32

Sequencing

- Libraries normalized using the QIAseq Normalizer Kits are double-stranded and require denaturation prior to sequencing.
- For complete instructions on how to denature sequencing libraries, prepare custom sequencing primers, and to set up a sequencing run, please refer to the system-specific Illumina documents.
- QIAseq normalized libraries have a concentration of approximately 4 nmol/L. Follow the system-specific Illumina documents for denaturing and loading – starting from a concentration of 4 nmol/L.

Appendix B: Removal of Divalent Cations and EDTA from Input Nucleic Acid

Refer to manufacturer's protocol for details on methods of purification.

Procedure

1. If DNA is in a volume of less than 50 µL, adjust the volume to 50 µL with nuclease-free water.
2. Add 90 µL of resuspended QIAseq Beads or Agencourt AMPure XP beads to the reaction for a ratio of 1.8x and mix well by pipetting. If DNA is in a volume greater than 50 µL, scale the volume of magnetic beads appropriately such that the ratio of beads to DNA is 1.8x.
3. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand for 2–4 min and carefully discard the supernatant without disturbing the beads.
4. Wash the beads with 200 µL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
5. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Remove from the magnetic stand.
6. Elute by resuspending in 45 µL of QIAGEN's Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand for 2 min. Carefully transfer 42.5 µL of supernatant into a new tube.
7. Determine the concentration of the purified DNA using Qubit, PicoGreen, or another fluorometric method.

Appendix C: Fragmentation, End-Repair, and A–Addition of DNA in 1x TE

Follow the instructions below for input DNA in 1X TE buffer.

Procedure

1. Enter the following program into a thermocycler (Table 18). Ensure that you use the instrument's heated lid, and if possible, set the temperature of the heated lid to ~70°C.

Table 18. Input DNA (20 pg – 1000 ng) in 1x TE

Step	Incubation temperature (°C)	Incubation time
1	4	1 min
2	32	1–30 min*
3	65	30 min
4	4	Hold

* To determine the reaction time for step 2, please refer to Figure 1 and Table 1.

2. Prepare the FX reaction mix in a PCR plate on ice according to Table 19 for >10 ng input DNA or Table 20 for <10 ng input DNA. Mix well by gently pipetting (do not vortex). The reaction can be scaled as needed for the desired number of samples.

Table 19. Input DNA (10–1000 ng) in 1x TE

Component	Volume (µL)
FX Buffer, 10x	5
DNA in 1x TE	Variable
FX Enhancer	2.5
Nuclease-free water	Variable
Total	**40**

Table 20. Input DNA 20 pg – 10 ng in 1x TE

Component	Volume (µL)
FX Buffer, 10x	5
DNA in 1x TE	Variable
FX Enhancer	5
Nuclease-free water	Variable
Total	**40**

3. Add 10 µL FX Enzyme Mix to reach reaction and mix well by pipetting up and down 20 times. It is critical to keep the PCR tube on ice for the entire time during reaction setup.
4. Briefly spin down the PCR plate/tubes and immediately transfer to the prechilled thermocycler (4°C). Resume the cycling program.
5. When thermocycler program is complete and sample block has returned to 4°C, remove samples and place on ice.
6. Immediately proceed to adapter ligation as described in the adapter ligation protocol.

Appendix D: 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 **www.qiagen.com**

Sequencing on the NextSeq, HiSeq X™, or HiSeq 3000/4000 system follows a dual-indexing workflow different from other Illumina systems. If you are manually creating sample sheets for these instruments, enter the reverse complement of the i5 index adapter sequence. If you are using Illumina Experiment Manager, BaseSpace, or Local Run Manager for run planning, the software will automatically reverse complement index sequences when necessary.

Ready-to-use sample sheets containing all QIAseq CDI and UDI Y-Adapter barcode sequences are available for MiSeq, NextSeq, MiniSeq, HiSeq, and NovaSeq instruments. These can be imported and edited using the Illumina Experiment Manager Software, Illumina Local Run Manager, or any text editor. Make sure to download the appropriate sample sheet for NextSeq, HiSeq X, or HiSeq 3000/4000 systems depending on whether you are using Local Run Manager or manually configuring the sequencing run.

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 motifs used in the QIAseq Unique Dual-Index Kits are listed in Table 18. 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 3. 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 4. 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 5. 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 6. 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 7. QIAseq UDI Y-Adapter Plate D (96) layout (UDI 289–384).

Table 21. UDI motifs used in the QIAseq UDI Y-Adapter Kits (24 and 96 A/B/C/D)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Indexes 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)
UDI 241	TTCTGTTGGT	ACCAACAGAA	CTCTTATCCA
UDI 242	CATCGTCAGG	CCTGACGATG	TCACAGCGGT
UDI 243	ATGCATGAAG	CTTCATGCAT	CCTCTGTCGT
UDI 244	CGTGAATCGC	GCGATTCACG	TCTGTTCTCG
UDI 245	GAGCAGCCTT	AAGGCTGCTC	GATACTTCAC
UDI 246	TCGATTACCA	TGGTAATCGA	AGTGCTGATA
UDI 247	CAGTCCAATT	AATTGGACTG	ATCCTTCGGT
UDI 248	AGAGGCTTGG	CCAAGCCTCT	GACAACGATT
UDI 249	CAGGCTCTCA	TGAGAGCCTG	GAACCGGTAG
UDI 250	GTTCGCTCTC	GAGAGCGAAC	AGCAATGAGC
UDI 251	TCGGACTAAT	ATTAGTCCGA	CAAGACTCCA
UDI 252	CGAGATCTTC	GAAGATCTCG	ACCGTGTAGG
UDI 253	ATAACCGGAC	GTCCGGTTAT	AGGCACAGGT
UDI 254	CGTGTAGTTA	TAACTACACG	CGACAGATCG
UDI 255	GAACATAGGT	ACCTATGTTC	ACGCGACAAC
UDI 256	TCTAACATCG	CGATGTTAGA	ACTTGCGTTA
UDI 257	AACGGTGGCA	TGCCACCGTT	CACCACTCAT
UDI 258	AGGACGGTGT	ACACCGTCCT	CTTCGTAACT
UDI 259	CTGTGACCTG	CAGGTCACAG	CAGTATTCGG
UDI 260	GCTGTAACAA	TTGTTACAGC	CAGTCTGGAC

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Indexes 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)**
UDI 261	TACGGACGTC	GACGTCCGTA	TACCGTTCTA
UDI 262	CCTAAGGAGC	GCTCCTTAGG	GTGTCCACAG
UDI 263	ATAAGGCCAG	CTGGCCTTAT	TTACGACTGT
UDI 264	CTCATCTGTA	TACAGATGAG	GACGCGAATG
UDI 265	GAAGGCATCT	AGATGCCTTC	CAACGTACGC
UDI 266	TCTCTACTGC	GCAGTAGAGA	AGCTCAGGAA
UDI 267	AACCGAACAA	TTGTTCGGTT	GATAGGCGGT
UDI 268	ATCTCGCCAC	GTGGCGAGAT	AGTAGGAAGT
UDI 269	CCATGCAACG	CGTTGCATGG	CATGTTGTAG
UDI 270	GAATGGTGTA	TACACCATTC	CACATTCTTC
UDI 271	TATATGCCGT	ACGGCATATA	GCAGCTCGTA
UDI 272	CTCGATAGAT	ATCTATCGAG	GTTCAGACGG
UDI 273	AACACAAGAG	CTCTTGTGTT	TCCTGGAAGT
UDI 274	CGCAATCGGT	ACCGATTGCG	GCATTGTTAG
UDI 275	GTTGCGTAGA	TCTACGCAAC	GACCTACAGC
UDI 276	TAGAGTGATC	GATCACTCTA	CACCGACGTA
UDI 277	AAGACGCAGC	GCTGCGTCTT	CTCTCACCTT
UDI 278	AACTTCTCGA	TCGAGAAGTT	CTCGTTCATT
UDI 279	CGCAACTGAG	CTCAGTTGCG	TGGTGGCAAG
UDI 280	GCTCCGCAAT	ATTGCGGAGC	GATTGCTTGA

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Indexes 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)
UDI 281	GTAACTTCCG	CGGAAGTTAC	CCGTTAAGGT
UDI 282	CTCACGACTA	TAGTCGTGAG	TGCTGAGAGG
UDI 283	AACCAACGGC	GCCGTTGGTT	TTGTCACTTG
UDI 284	CCTGCCTGTA	TACAGGCAGG	GCTGTTATGT
UDI 285	TACGCTGCAG	CTGCAGCGTA	GCAGCAGTTG
UDI 286	AATGTTGCGA	TCGCAACATT	GCAGATCAAT
UDI 287	CGACGTTCTG	CAGAACGTCG	TGGTTCACGG
UDI 288	AATAGGACAC	GTGTCCTATT	TCGACCGCAT
UDI 289	ATGTGCCTCA	TGAGGCACAT	TAACCTAGGT
UDI 290	CGACTCCGTT	AACGGAGTCG	AACTCATGCG
UDI 291	GCTGTTGTGG	CCACAACAGC	CCGGATGAAC
UDI 292	TACCAATCAC	GTGATTGGTA	CGTTGCCGTA
UDI 293	ATGTCTTACG	CGTAAGACAT	GCTCTACGGT
UDI 294	CGCAACAATA	TATTGTTGCG	TGCATTGGCG
UDI 295	GAACGAAGAC	GTCTTCGTTC	CGATTGTGAC
UDI 296	TCGAGGACGT	ACGTCCTCGA	GACTGCACTA
UDI 297	ATTATGAGCG	CGCTCATAAT	GTTAACTGCT
UDI 298	CGCGTTATAA	TTATAACGCG	TCGGACCTTG
UDI 299	GCGTGCATGT	ACATGCACGC	TGCAGCAAGC
UDI 300	TAAGCGGCTC	GAGCCGCTTA	CACATGCGAA

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Indexes 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)
UDI 301	AACATGGAGA	TCTCCATGTT	CAGACGTAAT
UDI 302	CCGAGTCTCT	AGAGACTCGG	ATTCGGTACG
UDI 303	GTACTTCTAC	GTAGAAGTAC	TTAGCACGGC
UDI 304	TGTTCACATG	CATGTGAACA	GAGGATAGTA
UDI 305	AAGGTAACGC	GCGTTACCTT	AACTGTGGTT
UDI 306	CCGCCTTACT	AGTAAGGCGG	ATTACCTCGG
UDI 307	GTTGAGGCAG	CTGCCTCAAC	CGCTGTATAC
UDI 308	TGGCGACCTA	TAGGTCGCCA	CTTGCTCACA
UDI 309	AGAAGCGACA	TGTCGCTTCT	CAACACCTGT
UDI 310	CAGGATAATC	GATTATCCTG	CAATTGCTCG
UDI 311	GCTCCTACAG	CTGTAGGAGC	CATAGACAAC
UDI 312	TTCAACAGGT	ACCTGTTGAA	TTGGTGTCTA
UDI 313	CCTCGTCCAT	ATGGACGAGG	TATGTCCTGT
UDI 314	AGCGTTGGTT	AACCAACGCT	GCCAATTCGT
UDI 315	CATTCGAACA	TGTTCGAATG	TAGGCGATCG
UDI 316	GCTTACCGAC	GTCGGTAAGC	ATGAGTGTAC
UDI 317	TTAGCTTAGG	CCTAAGCTAA	CCGAAGGATA
UDI 318	CCGACACACA	TGTGTGTCGG	AGTCCACTGT
UDI 319	ATTCGCTGAT	ATCAGCGAAT	GCGGCTAATT
UDI 320	CCAAGAGGCA	TGCCTCTTGG	TCTAACTCAG

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Indexes 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)**
UDI 321	GACGCAGTTC	GAACTGCGTC	CAAGCTGAGC
UDI 322	TGGAACTCGG	CCGAGTTCCA	CCAGAGCACA
UDI 323	CCACACCAAT	ATTGGTGTGG	TGTACAAGGT
UDI 324	AGTTCTCGGC	GCCGAGAACT	TAGAATGCCT
UDI 325	CTTGACGACG	CGTCGTCAAG	TGTCTTACTG
UDI 326	GAGGTCGCTA	TAGCGACCTC	ATGACTAAGC
UDI 327	TCAGTAGCAT	ATGCTACTGA	ATGTAGGCAA
UDI 328	CTAACGTGGA	TCCACGTTAG	GCGAAGAGGT
UDI 329	ATGCCAACCG	CGGTTGGCAT	CGGTGGTTCT
UDI 330	CGGTCGATTC	GAATCGACCG	CTGTCGTTGG
UDI 331	GAAGTACAGT	ACTGTACTTC	TGATCGACAC
UDI 332	TCTGCAGTAA	TTACTGCAGA	CCACCAGCTA
UDI 333	CTATCCTAGC	GCTAGGATAG	CACGGTTCGT
UDI 334	AACACTCCTT	AAGGAGTGTT	AGTGAGAGCT
UDI 335	CCGAACCTAA	TTAGGTTCGG	TTGCATGCGG
UDI 336	GTCTAGTCGC	GCGACTAGAC	TATACGTGTC
UDI 337	TGGATGTACG	CGTACATCCA	TGACGCGTTA
UDI 338	CTACCAGCGT	ACGCTGGTAG	TACAGAACGT
UDI 339	AAGGATTCAG	CTGAATCCTT	CTTGTCAGGT
UDI 340	CGAGGTGTGT	ACACACCTCG	ATCCACAGCG

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Indexes 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)**
UDI 341	GTAGACGCTC	GAGCGTCTAC	CCTATCCATC
UDI 342	TCGTCCGTCA	TGACGGACGA	ACCGCGAGTA
UDI 343	CCGTGATAGG	CCTATCACGG	AAGTTCTGGT
UDI 344	AGGATGACCT	AGGTCATCCT	ACAGGTATCG
UDI 345	CCTCGAGTAC	GTACTCGAGG	ATGACGGATT
UDI 346	GTCACTGAGG	CCTCAGTGAC	GTCTGAGTAG
UDI 347	TACGGTTAGA	TCTAACCGTA	TGCCAGATGT
UDI 348	CAACGAGAAT	ATTCTCGTTG	GCTAAGCATT
UDI 349	AATACACCGG	CCGGTGTATT	ACAGCATGGT
UDI 350	CCGATCCATC	GATGGATCGG	ATAGAGACCG
UDI 351	GAATCTCGCT	AGCGAGATTC	ATATCGCGTA
UDI 352	TGACCGGCAA	TTGCCGGTCA	TTAAGGAGGT
UDI 353	CATGATAGCA	TGCTATCATG	CTGTGCGACT
UDI 354	AACAGCTTCG	CGAAGCTGTT	TCCGTATGCT
UDI 355	CTAGTGCTTA	TAAGCACTAG	CCATCGATGT
UDI 356	TGTGATACGT	ACGTATCACA	GTGAGCCGTT
UDI 357	ATGAGCGTAT	ATACGCTCAT	TGCCGTTAAT
UDI 358	CTAGATATGG	CCATATCTAG	CGGATGTGGT
UDI 359	CGCTATGCTG	CAGCATAGCG	TCGCGTGTTG
UDI 360	TACTACGTGA	TCACGTAGTA	CCGCGATCAT

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Indexes 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)
UDI 361	ATGTGGAGGT	ACCTCCACAT	CGCGTTATCG
UDI 362	CCATGGCTCA	TGAGCCATGG	GTAGCCTCCT
UDI 363	CCAATCACGC	GCGTGATTGG	ACTAGACACT
UDI 364	TTAGATCCAG	CTGGATCTAA	CGATTCGTTG
UDI 365	AGGAATATCG	CGATATTCCT	GAAGAGATGT
UDI 366	CCTCCTATGT	ACATAGGAGG	AGATCCGACG
UDI 367	TAGAGACACG	CGTGTCTCTA	CCAGGACATT
UDI 368	CCAGCTCAGT	ACTGAGCTGG	ACGTGGCATT
UDI 369	ATGGCTCATA	TATGAGCCAT	AAGCAGGACG
UDI 370	CGGAGTGAAG	CTTCACTCCG	ACGAGTCGGT
UDI 371	TACCTATGGT	ACCATAGGTA	AGTGTACGCG
UDI 372	ATGAGACAGT	ACTGTCTCAT	ACCGACCATT
UDI 373	CTAAGAGTTG	CAACTCTTAG	TTGCTAACGT
UDI 374	TAACCGTATG	CATACGGTTA	CTTGATACTG
UDI 375	AGAGTCCATG	CATGGACTCT	CTGGATAAGT
UDI 376	CTAGACCGCA	TGCGGTCTAG	ATAGCTTACG
UDI 377	TATGGCTTGT	ACAAGCCATA	GTCCATGAGT
UDI 378	CGTTGTTCCT	AGGAACAACG	ACTCCAGTCG
UDI 379	CCGACATTAG	CTAATGTCGG	TCTCAGCACG
UDI 380	TGTGAAGGCA	TGCCTTCACA	ATCGTGATGT
UDI 381	AGCATCGTCT	AGACGATGCT	ACGCAATCCG
UDI 382	CCGACTAGGA	TCCTAGTCGG	GAGATCGGCT
UDI 383	AACATTACCG	CGGTAATGTT	CTACGTCTCG
UDI 384	CCTAATTCGT	ACGAATTAGG	CTCAGGCTGT

Combinatorial Dual-Index Y-Adapters

The layout of the 24-plex and 96-plex single-use CDI adapter plate is shown in Figure 9 and Figure 10. The index motifs used in the QIAseq Combinatorial Dual-Index Kits are listed in Table 19. 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	501/ 701	501/ 702	501/ 703	501/ 704	501/ 705	501/ 706	501/ 707	501/ 708	501/ 709	501/ 710	501/ 711	501/ 712
B	502/ 701	502/ 702	502/ 703	502/ 704	502/ 705	502/ 706	502/ 707	502/ 708	502/ 709	502/ 710	502/ 711	502/ 712
C	503/ 701	503/ 702	503/ 703	503/ 704	503/ 705	503/ 706	503/ 707	503/ 708	503/ 709	503/ 710	503/ 711	503/ 712
D	504/ 701	504/ 702	504/ 703	504/ 704	504/ 705	504/ 706	504/ 707	504/ 708	504/ 709	504/ 710	504/ 711	504/ 712
E	505/ 701	505/ 702	505/ 703	505/ 704	505/ 705	505/ 706	505/ 707	505/ 708	505/ 709	505/ 710	505/ 711	505/ 712
F	506/ 701	506/ 702	506/ 703	506/ 704	506/ 705	506/ 706	506/ 707	506/ 708	506/ 709	506/ 710	506/ 711	506/ 712
G	507/ 701	507/ 702	507/ 703	507/ 704	507/ 705	507/ 706	507/ 707	507/ 708	507/ 709	507/ 710	507/ 711	507/ 712
H	508/ 701	508/ 702	508/ 703	508/ 704	508/ 705	508/ 706	508/ 707	508/ 708	508/ 709	508/ 710	508/ 711	508/ 712

Figure 8. QIAseq CDI Y-Adapter Plate (96) layout (CDI 1–96).

	1	2	3	4	5	6	7	8	9	10	11	12
A	501/ 701	501/ 702	501/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty
B	502/ 701	502/ 702	502/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty
C	503/ 701	503/ 702	503/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty
D	504/ 701	504/ 702	504/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty
E	505/ 701	505/ 702	505/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty
F	506/ 701	506/ 702	506/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty
G	507/ 701	507/ 702	507/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty
H	508/ 701	508/ 702	508/ 703	empty	empty	empty	empty	empty	empty	empty	empty	empty

Figure 9. QIAseq CDI Y-Adapter Plate (24) layout (CDI 1–24).

Table 22. CDI motifs used in the QIAseq CDI Y-Adapter Kits (24 and 96)

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

Indexes for entry on sample sheet				
D50X barcode name	**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)**	**D50X barcode name**	**i7 bases for entry on sample sheet**
D501	TATAGCCT	AGGCTATA	D701	ATTACTCG
D502	ATAGAGGC	GCCTCTAT	D702	TCCGGAGA
D503	CCTATCCT	AGGATAGG	D703	CGCTCATT
D504	GGCTCTGA	TCAGAGCC	D704	GAGATTCC
D505	AGGCGAAG	CTTCGCCT	D705	ATTCAGAA
D506	TAATCTTA	TAAGATTA	D706	GAATTCGT
D507	CAGGACGT	ACGTCCTG	D707	CTGAAGCT
D508	GTACTGAC	GTCAGTAC	D708	TAATGCGC
			D709	CGGCTATG
			D710	TCCGCGAA
			D711	TCTCGCGC
			D712	AGCGATAG

Appendix E: Column-based Reaction Cleanup with the GeneRead Size Selection Kit

This protocol is optimized for the removal of primers and adapter-dimers from DNA libraries prepared from at least 100 ng of DNA using the QIAseq FX DNA Library Kits. This protocol may not remove all adapter dimer from libraries prepared from less than 100 ng of DNA.

Notes before starting

- All centrifugation steps should be performed at full speed (maximum 20,000 x *g*) in a conventional, table-top centrifuge.
- Wash steps should be performed using 80% ethanol prepared from 96–100% ethanol.

Procedure

1. Add 4 volumes of Buffer SB1 to 1 volume of adapter-ligated DNA library prepared using the QIAseq FX DNA Library Kit, and mix. For example, add 360 µL Buffer SB1 to a 90 µL sample.
2. To bind DNA, apply the sample to the MinElute® spin column, and then centrifuge for 1 min. Discard the flow-through. Place the MinElute spin column back into the same tube.
3. To wash, add 700 µL of 80% ethanol to the MinElute spin column and centrifuge for 1 min. Discard the flow-through. Place the MinElute spin column back into the same tube.
4. Repeat step 3.
5. Centrifuge the MinElute spin column for an additional 1 min at maximum speed.
6. Place the MinElute spin column into a clean 1.5 mL microcentrifuge tube (provided).

7. Add 90 µL Buffer TE to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.

   Important: Ensure that the buffer is dispensed directly onto the center of the membrane.

   Important: Keep the spin column and the flow-through.

8. Place the MinElute spin column from step 7 into a new 2 mL collection tube (provided). Add 4 volumes of Buffer SB1 (~360 µL) to 1 volume of the flow-through, and mix.
9. Reapply the mixture to the MinElute spin column and centrifuge for 1 min. Discard the flow-through.
10. To wash, add 700 µL of 80% ethanol to the MinElute spin column and centrifuge for 1 min. Discard the flow-through. Place the MinElute spin column back into the same tube.
11. Repeat step 10.
12. Centrifuge the MinElute spin column for an additional 1 min at maximum speed.
13. Place the MinElute spin column in a clean 1.5 mL microcentrifuge tube (provided).
14. For elution, add 17 µL Buffer EB to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

    Important: Ensure that the buffer is dispensed directly onto the center of the membrane, for complete elution of the bound DNA.

Appendix F: Library Quantification and Quality Control

Quality control for the library construction process can be performed using QIAGEN's QIAseq Library Quant Assay Kit (cat. no. 333314). With this assay, the correct dilution of the library can also be determined for sequencing. Refer to the corresponding handbook for library quantification and quality control.

Ordering Information

Product	Contents	Cat. no.
QIAseq FX DNA Library UDI-A Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180479
QIAseq FX DNA Library UDI-B Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180480
QIAseq FX DNA Library UDI-C Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180481
QIAseq FX DNA Library UDI-D Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180482
QIAseq FX DNA Library CDI Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing	180484

Product	Contents	Cat. no.
	96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	
QIAseq FX DNA Library UDI Kit (24)	For 24 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 24 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180477
Related products		
QIAseq Y-Adapter Kits for Illumina		
QIAseq CDI Y-Adapter Kit (24)	Combinatorial Dual-Index Adapters for Illumina	180301
QIAseq CDI Y-Adapter Kit (96)	Combinatorial Dual-Index Adapters for Illumina	180303
QIAseq UDI Y-Adapter Kit (24)	Unique Dual-Index Adapters for Illumina (1–24)	180310
QIAseq UDI Y-Adapter Kit A (96)	Unique Dual-Index Adapters for Illumina (1–96)	180312
QIAseq UDI Y-Adapter Kit B (96)	Unique Dual-Index Adapters for Illumina (97–192)	180314
QIAseq UDI Y-Adapter Kit C (96)	Unique Dual-Index Adapters for Illumina (193–288)	180316
QIAseq UDI Y-Adapter Kit D (96)	Unique Dual-Index Adapters for Illumina (289–384)	180318

Product	Contents	Cat. no.
QIAseq Normalizer Kits for use with Illumina instruments		
QIAseq Library Normalizer Kit (96)	For 96 reactions: Reagents for the normalization of libraries using QIAseq CDI/UDI Y-Adapters or compatible.	180605
QIAseq Library Normalizer Kit (24)	For 24 reactions: Reagents for the normalization of libraries using QIAseq CDI/UDI Y-Adapters or compatible.	180603
QIAseq Universal Normalizer Kit (96)	For 96 reactions: Reagents for the normalization of libraries using indexed library amplification primers.	180615
QIAseq Universal Normalizer Kit (24)	For 24 reactions: Reagents for the normalization of libraries using indexed library amplification primers.	180613
QIAseq Beads (10mL)	10 mL of QIAseq Beads for library clean-up and size selection.	333923
QIAseq Beads (55mL)	55 mL of QIAseq Beads for library clean-up and size selection.	333903
QIAseq Beads (400mL)	400 mL of QIAseq Beads for library clean-up and size selection.	333927
QIAseq Library Quantification Kits for use with Illumina instruments		
QIAseq Library Quant Assay Kit	Laboratory-verified forward and reverse primers for 500 x 25 µL reactions (500 µL); DNA Standard (100 µL); Dilution Buffer (30 mL); (1.35 mL x 5) GeneRead qPCR SYBR® Green Mastermix	333314

Product	Contents	Cat. no.
QIAamp Kits – for genomic DNA purification		
QIAamp DNA Mini Kit	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 mL)	51304
QIAamp DNA Microbiome Kit	For 50 DNA preps: 50 QIAamp UCP Mini Columns, 50 Pathogen Lysis Tubes L, buffers, reagents, Collection Tubes (2 mL)	51704
MagAttract Kits – for high-molecular-weight genomic DNA purification		
MagAttract HMW DNA Kit (48)	For 48 DNA preps: MagAttract Suspension G, Buffer ATL, Buffer AL, Buffer MB, Buffer MW1, Buffer PE, Proteinase K, RNase A, Buffer AE, Nuclease-Free Water	67563
MagAttract Magnetic Rack	Magnetic rack for convenient processing of up to 12 samples	19606
REPLI-g® Kits – for MDA-based whole genome amplification		
REPLI-g Mini Kit (100)*	DNA Polymerase, Buffers, and Reagents for 100 x 50 µL whole genome amplification reactions (typical yield 10 µg per reaction)	150025
REPLI-g Single Cell Kit (96)*	REPLI-g SC Polymerase, Buffers, and Reagents for 96 whole genome amplification reactions (yields up to 40 µg/reaction)	150345

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Document Revision History

Date	Changes
01/2021	Fixed a typo in Figure 9. Changed precise storage temperatures to range temperatures. Added a statement in the Principle and Procedure section to clarify input range. Corrected the DNA input range in for adapter dilution 1:1000 in Table 5. Increased the number of times to pipette up and down from "6–8" to "20" times in step 4 of Protocol: Fragmentation, End-Repair, and A-addition, and step 3 of Appendix B.
10/2023	Updated handbook to new design. Added Appendix protocol for the use of QIAseq FX DNA Library Preparation together with QIAseq Normalizer Kits. Changed designations of other appendices. Updated Ordering Information. Updated the organization of the main protocol.

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