August 2021

QIAseq® 1-Step Amplicon Library Preparation Handbook

For the construction of Illumina®-compatible libraries from multiplexed PCR amplicons

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAseq 1-Step Amplicon Library Kit	**(12)**
Catalog no.	**180412**
No. of reactions	**12**
4x 1-Step Amplicon Buffer	150 µl
1-Step Amplicon Enzyme Mix	24 µl
HiFi PCR Master Mix, 2x	300 µl
Primer Mix Illumina Library Amp	20 µl
RNase-free Water	1.9 ml
Quick-Start Protocol	1

QIAseq 1-Step Amplicon Lib UDI/CDI Kit	**UDI-A (96)**	**UDI-B (96)**	**UDI-C (96)**	**UDI-D (96)**	**CDI (96)**
Catalog no.	**180419**	**180420**	**180421**	**180425**	**180423**
No. of reactions	**96**	**96**	**96**	**96**	**96**
4x 1-Step Amplicon Buffer	1.2 ml	1.2 ml	1.2 ml	1.2 ml	1.2 ml
1-Step Amplicon Enzyme Mix	192 µl	192 µl	192 µl	192 µl	192 µl
HiFi PCR Master Mix, 2x	1.25 ml	1.25 ml	1.25 ml	1.25 ml	1.25 ml
Primer Mix Illumina Library Amp	150 µl	150 µl	150 µl	150 µl	150 µl
RNase-free Water	5 x 1.9 ml	5 x 1.9 ml	5 x 1.9 ml	5 x 1.9 ml	5 x 1.9 ml
QIAseq UDI Y-Adapter Plate A/B/C/D (96)	1	1	1	1	–
QIAseq CDI Y-Adapter Plate (96)	–	–	–	–	1
QIAseq Y-Adapter Reference Card	1	1	1	1	1
Quick-Start Protocol	1	1	1	1	1

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

Note: The QIAseq 1-Step Amplicon Lib CDI/UDI Kits (96) 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 1-Step Amplicon Lib UDI-A (or B or C or D) Kit (96) 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 28.

The QIAseq 1-Step Amplicon Library Kit (12) adapters are available in tube format. The GeneRead Adapter I Set A/B 12-plex (cat. 180985, 180986) can be ordered separately. The QIAseq 1-Step Amplicon Library Kit (12) is also fully compatible with all plate format QIAseq Y-Adapter Kits (24/96).

Shipping and Storage

The QIAseq 1-Step Amplicon Library Kit is shipped on dry ice and should be stored at –30 to –15°C upon arrival. When stored correctly, all reagents are stable for at least 6 months after delivery if not otherwise stated on the label.

QIAseq 1-Step Amplicon Lib CDI/UDI Kits (96) each contain an adapter plate shipped in a separate box. Store the adapter plate at –30 to –15°C upon arrival.

Intended Use

The QIAseq 1-Step Amplicon Library Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Safety Information

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq 1-Step Amplicon Library Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

While next-generation sequencing (NGS) has become a vital tool for life sciences and medical research, library preparation remains a key bottleneck in the NGS workflow. The QIAseq 1-Step Amplicon Library Kit is designed for the preparation of Illumina-compatible NGS libraries from multiplexed PCR or gene panel products, and employs an optimized 30-minute one-step library preparation protocol that reduces workflow duration, sample loss, and the potential for handling errors and cross-contamination. The kit accepts multiplexed PCR products from a variety of sources including the QIAGEN GeneRead® v2 panels, custom or lab-developed panels or multiplexed PCR assays, as well as other commercial panels. Optimized enzyme and buffer compositions ensure efficient library construction with a wide range of input amounts, and the entire protocol can optionally be performed at room temperature (15–25°C), enabling easy automation.

Principle and procedure

Purified amplicons from gene panels or multiplex PCR are converted to Illumina-compatible NGS libraries using a single enzymatic library construction step. During this reaction, amplicons are simultaneously prepared for ligation and barcoded adapters are ligated to both ends of the DNA inserts. The adapters contain sequences required for the PCR enrichment of the subsequent library, for flow-cell-binding during bridge amplification, and for sequencing primer binding sites for paired-end and multiplexed sequencing.

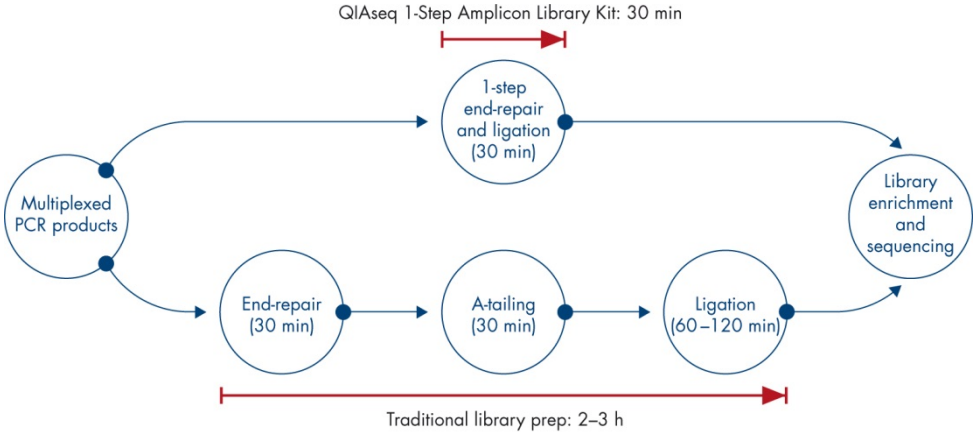

Figure 1. Scheme of optimized one-step amplicon library construction. Purified amplicons from multiplexed PCR or gene panels are converted to sequencing libraries by employing a 30-minute, one-tube library construction step. The libraries are purified with an easy and automatable size selection protocol, and the entire procedure can be performed at room temperature. For low-input applications, libraries can be amplified using the included HiFi PCR Master Mix.

Following library construction, excess adapters, adapter-dimers, and other reaction components are removed via precipitation onto Agencourt® AMPure® XP beads. This procedure is carried out at room temperature and can be easily automated on various liquid-handling platforms for high-throughput applications.

Following library purification, a high-fidelity library enrichment step can be performed to generate sufficient library from low amounts of starting material. This reaction relies on a high-fidelity DNA polymerase and optimized buffer conditions that ensure minimum GC bias and extremely low error rates.

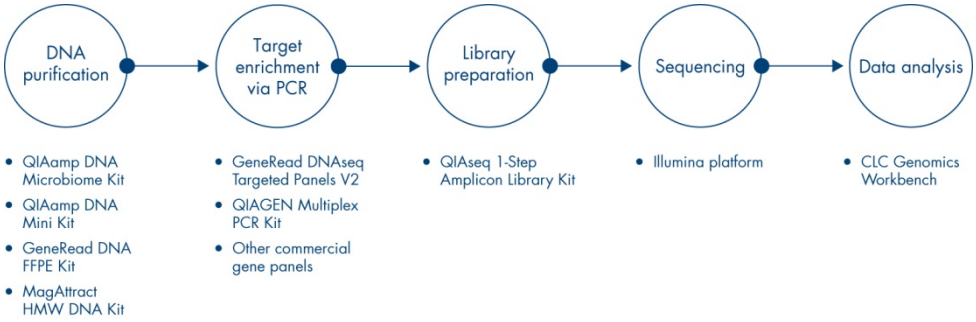

Figure 2. Overview of a complete targeted resequencing NGS workflow including the QIAseq 1-Step Amplicon Library Kit. After DNA extraction and purification with an appropriate kit, target enrichment is performed with the QIAGEN Multiplex PCR Kit, GeneRead™ DNAseq Targeted Panels v2 or other gene panels or PCR products. NGS library construction is performed with the QIAseq 1-Step Amplicon Library Kit and data is analyzed with the CLC Genomics or Biomedical Workbench.

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 1-Step Amplicon Lib CDI/UDI Kits (96) include a fully compatible indexing solution. We recommend using the QIAseq Dual-Index Y-Adapter Plates delivered with the kit. Each QIAseq 1-Step Amplicon Library Kit (96) includes one of the following:

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

Combining QIAseq 1-Step Amplicon Lib UDI-A/B/C/D Kit (96) enables multiplexing of up to 384 samples per sequencing run. For more information on QIAseq Dual-Index Y-Adapters and index sequence motives, see Appendix D, page 28, and "Ordering Information", page 56.

Adapter kits compatible with the QIAseq 1-Step Amplicon Library Kit (12) have to be purchased separately:

- GeneRead Adapter I Set A/B 12-plex (single-index adapters) (cat. no. 180985 or 180986)

GeneRead Single-Index adapter sets A and B are available in 12-plex format and can be combined with the QIAseq 1-Step Amplicon Library Kit (12). For more information on GeneRead Adapters and index sequence motives, see Appendix E, page 53, and "Ordering Information", page 56.

CDI adapters use twelve i7 and eight i5 barcode motives that can be combined to form up to 96 combinatory dual indices. In contrast, QIAseq UDI Adapters use a fixed combination of 2 unique barcode motives per adapter molecule. Therefore, each single-index motive is only used exactly 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 MiniSeq®

Starting materials

- PCR products generated with the GeneRead v2 DNAseq Targeted Panels
- PCR products generated with other custom or commercial gene panels
- PCR products generated with the QIAGEN Multiplex PCR Kit or other QIAGEN PCR reagents
- Multiplexed PCR amplicons generated with *Taq* or *Taq* derivatives
- Multiplexed PCR amplicons generated with Family B polymerases (see Appendix C)

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.

For NGS library construction

- Only for 12-reaction kits: GeneRead Adapters (cat. no. 180985 or 180986; can be used with QIAseq 1-Step Amplicon Library Kit [12] for single-indexed libraries)
- Agencourt AMPure XP beads (cat. no. A63880 or A63881)
- 80% ethanol
- PCR tubes or plates
- Pipette tips and pipettes
- DNA LoBind tubes (from Axygen or Eppendorf)
- Vortexer
- Microcentrifuge
- Thermal cycler
- Magnetic racks for magnetic beads separation (e.g., Thermo Fisher Scientific/Life Technologies, DynaMag™-2 magnet, cat. no. 12321D)

For NGS library QC

- QIAxcel® Advanced Instrument (cat. no. 9001941) or similar capillary electrophoresis instrument
- GeneRead Library Quant Kit (cat. no. 180612)
- Real-time PCR machine for library quantification

Important Notes

PCR products should be free of contaminating primers, primer-dimers, or other PCR artifacts prior to library preparation.

Recommended library quantification method

QIAGEN's GeneRead Library Quant Kit (cat. no. 180612), which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library.

We recommend purification of the PCR products prior to library preparation with Agencourt AMPure XP beads (see Appendix B).

Protocol: Library Construction Using the QIAseq 1-Step Amplicon Library Kit for Illumina Sequencing

This protocol describes library construction for sequencing on Illumina platforms.

Procedure

One-step end-repair and adapter ligation

1. Prepare a reaction mix for adapter ligation according to Table 1, adding the components to the PCR tube or plate containing purified PCR products.

   Note: When using barcode adapters, open one adapter tube at a time and change gloves between pipetting the different barcode adapters to avoid cross-contamination.

Table 1. Reagents for adapter ligation of PCR product

Component	Volume/reaction (µl)
Purified PCR amplicons from gene panel or multiplex PCR product	Variable; 10–100 ng*
4x 1-Step Amplicon Library Buffer	12.5
QIAseq CDI or UDI Y-Adapter Plate (96) *or* GeneRead 12-plex adapter (cat. nos. 180985 or 180986)†	4 2
1-Step Amplicon Enzyme Mix	2
DNase-free water	Variable
Total	**50**

* We recommend the quantification of PCR products via a microfluidics or capillary electrophoresis platform.
† If using adapters from another supplier, add the adapter to 1 µM final concentration or follow the supplier's directions.

2. Mix the components by pipetting up and down several times.

3. Program a thermal cycler to incubate at 25°C for 30 min. Optionally, the reaction can be incubated at room temperature.

   IMPORTANT: Do not use a thermal cycler with a heated lid.

4. After the reaction is complete, place the reactions on ice and proceed with purification using Agencourt AMPure XP beads.

Cleanup of adapter-ligated DNA with Agencourt AMPure XP beads

5. Prepare 1.5 ml LoBind tubes for each ligation reaction and label tubes or prepare a 96-well plate (depending on availability of magnetic rack and individual preferences).
6. Transfer the 50 µl ligation reaction from step 4 to the prepared 1.5 ml LoBind tube or 96-well plate and add 50 µl nuclease-free water.
7. **For PCR products up to 350 bp in size**:

   Add 40 µl (0.4x volume) Agencourt AMPure XP beads to each ligation reaction, mix well by pipetting, and then proceed to step 8.

   For PCR products 350–1000 bp in size:

   Add 60 µl (0.6x volume) Agencourt AMPure XP beads to each ligation reaction, mix well by pipetting, and then proceed to step 12 **(skip steps 8–11)**.

8. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) for 5 min.
9. Prepare a new LoBind tube for each ligation reaction or a new 96-well plate.
10. Carefully transfer 133 µl supernatant to the new tubes without disturbing the beads. This will leave behind about 7 µl supernatant. Discard the beads, which contain unwanted large DNA fragments. The large DNA fragments are generated by ligation of the adapter to nonspecific multiplex PCR products.

    Note: Do not discard the supernatant.

11. Add 40 µl of resuspended Agencourt AMPure XP Beads slurry to the supernatant and mix well by pipetting.
12. Incubate the mixture for 5 min at room temperature.

13. Pulse-spin the tube or plate. Pellet the beads on a magnetic stand (e.g., DynaMag) for 5 min, then carefully remove and discard the supernatant. Be careful not to disturb the beads, because they contain the library.

    Note: Do not discard the beads.

14. 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.
15. Try to remove the residual ethanol as much as possible without disturbing the beads. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. Remove from the magnetic stand.
16. Elute by resuspending in 26 µl of nuclease-free water. Mix well by pipetting and incubate the tube or plate at room temperature for 2 min to elute the DNA from the beads.
17. Place the tube or plate back on the magnetic rack to pellet the beads. Incubate until the liquid is clear.
18. Use 23.5 µl of the eluate in the library amplification procedure, or quantify and sequence directly if the amount of input amplicon was sufficient.

PCR amplification of purified library

19. Program a thermal cycler with a heated lid according to Table 2.

Table 2. Cycling conditions for the amplification of the DNA library

Time	Temperature	Number of cycles
Initial denaturation		
2 min	98°C	1
Annealing		
20 s	98°C	
30 s	60°C	4–10*
30 s	72°C	
Final extension		
1 min	72°C	1
∞	4°C	Hold

* **Note**: Cycle number depends on the amount and quality of input amplicon. In general, 4 cycles are sufficient for 20–500 ng of input PCR product and 10 cycles are sufficient for 1–20 ng of input PCR product. If input DNA is sufficient (>500 ng), library amplification can be omitted.

20. Mix the components in Table 3 in a 0.2 ml PCR tube or 96-well PCR plate.

Table 3. Reaction components for PCR amplification

Component	Volume (µl)
HiFi PCR Master Mix, 2x	25
Primer Mix (10 µM each)	1.5
Library DNA (from previous step)	23.5
Total	**50**

21. Transfer the PCR plate to the thermal cycler and start the program.
22. Once PCR is complete, add 50 µl of resuspended Agencourt AMPure XP Beads to each reaction (50 µl) and pipet up and down thoroughly to mix the beads and PCR mix.
23. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
24. 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 two ethanol washes. Remove from the magnetic stand.
25. Incubate on the magnetic stand for 5–10 min of until the beads are dry. Over-drying of beads may result in lower DNA recovery. Remove from the magnetic stand.
26. Elute by resuspending in 30 µl nuclease-free water. Mix well by pipetting. Pellet the beads on the magnetic stand. Carefully transfer 28 µl supernatant to a clean LoBind 1.5 ml tube or PCR plate.
27. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the expected size distribution of library fragments, for the absence of adapters, amplification primers, adapter-dimers or high-molecular-weight overamplification artifacts.

    Note: The library should show a distribution reflecting the size of the input PCR amplicons plus 120 bp. The increase in library length reflects the addition of the sequencing adapters to the PCR amplicons.

    Note: The median fragment size can be used in subsequent qPCR-based quantification methods to calculate library concentration.

28. Quantify the library using the GeneRead Library Quant Kit (cat. no. 180612 [not provided]) or a comparable method.
29. The purified library can be safely stored at –20°C in a DNA LoBind tube until ready to sequence.

Typical results

When high-quality, artifact-free PCR products are used; QIAseq 1-Step Amplicon libraries are typically free of adapter dimers, library amplification primers, excess adapters and high-molecular-weight amplification artifacts. When an appropriate cycle number for the input amount is chosen, library yield should be approximately 5 nM after purification, and the volume should be sufficient for quality control, library quantification and sequencing on most NGS platforms.

In the example experiment, as shown in Figure 3, reference DNA was amplified with the GeneRead DNAseq V2 Human Comprehensive Cancer Panel (06/2015 version). This panel consists of 4 separate PCR reactions, each including 1988 primer pairs. Amplicons are designed to capture 160 cancer-related genes comprising approximately 745 kb of the genome. After PCR, products were pooled and analyzed for yield and length distribution (Figure 3).

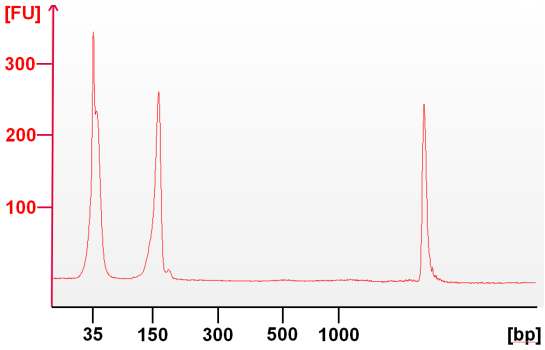

Figure 3. Electrophoresis trace of pooled multiplexed PCR amplicons. After amplification and pooling, a peak of expected size (160 bp), which represents the mean amplicon size of this set of PCR primers is seen. A second large peak centered at 35 bp is comprised of the electrophoresis marker, unextended primers and extended primer-dimers. During purification, these shorter products are removed, leaving only the desired PCR amplicons.

PCR products were purified with Agencourt AMPure XP Beads according to the *GeneRead DNAseq Targeted Panels V2 Handbook*, and 25 ng was used to generate libraries with the QIAseq 1-Step Amplicon Library Kit. Libraries were amplified with 4 cycles of PCR, and yielded a total of 28 µl of 5 nM library after purification (Figure 4, 1:10 dilution shown). While sequencing quality, read length and the number of reads obtained will vary, depending on the sequencing platform, using a library quantification method such as the QIAGEN GeneRead DNAseq Library Quant Array enables more accurate clustering, optimizing data yield and quality.

Typical data generated with the QIAseq 1-Step Amplicon Library Kit contains minimal reads arising from adapter dimers, and when paired with a high-quality panel, often has >95% on-target reads, with even read distributions over target amplicons.

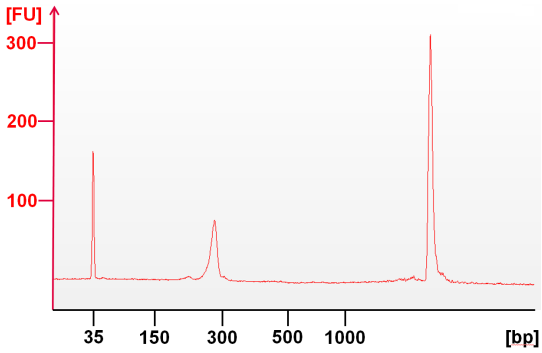

Figure 4. Electrophoresis trace of a purified library prepared using the QIAseq 1-Step Amplicon Library Kit. A characteristic size shift of 120 bp is observed due to the additional sequence added by the adapters. Typical libraries should be free of shorter peaks composed of excess primers, adapters or adapter-dimers and high-molecular-weight peaks, which can be introduced by the gene panel or through overamplification of the completed libraries.

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 impurity with multiple PCR products	Make sure to use PCR purification methods that effectively remove impurities that could potentially inhibit QIAseq 1-Step Amplicon Library Kit enzymes.
b)	Insufficient amount of starting DNA	Make sure at least 2 ng multiplex PCR products and correct cycle numbers for library amplification PCR are used for library prep. Consider increasing the amount of PCR products used or the number of cycles of library amplification
c)	Over-drying of Agencourt AMPure XP beads	Over-drying Agencourt AMPure XP beads could decrease elution efficiency. Ensure that the beads are not dried for more than 10 min at room temperature.
Unexpected signal peaks in capillary electrophoresis device traces		
a)	Presence of shorter peaks between 60 and 160 bp	These peaks represent library adapters (about 60 bp), adapter dimers (about 120 bp) or adapter-ligated PCR primer dimers (about 150–160 bp) 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. If necessary, repeat the protocol "Cleanup of adapter-ligated DNA with Agencourt AMPure XP beads", page 15, to remove the residual adapters and adapter-dimers.
b)	Presence of larger library fragments	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 due to insufficient depletion of the larger nonspecific fragments. If necessary, repeat the protocol "Cleanup of adapter-ligated DNA with Agencourt AMPure XP beads", page 15, to remove the residual large fragments.

		Comments and suggestions
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, please 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. Make sure to use the parameters and incubation times described in the handbook, as well as the correct amount of starting DNA.

Appendix A: DNA Isolation and Quality Control

High-quality DNA is essential for obtaining reliable sequencing results, and proper sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, extraction reagents or other contaminants can degrade DNA, interfere with DNA quantification or inhibit downstream PCR.

For the isolation and purification of high-quality genomic DNA, we recommend the following QIAGEN kits:

- QIAamp® DNA Mini Kit (cat. no. 51304) for the preparation of genomic DNA samples from fresh tissues
- GeneRead DNA FFPE Kit (cat. no. 180134) for the preparation of NGS-ready genomic DNA FFPE tissue samples
- 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
- QIAamp Circulating Nucleic Acid Kit (cat. no. 55114)

For accurate DNA quantification, we recommend the QIAxpert® (cat. no. 9002340).

Note: When using a UV-vis spectrophotometer to quantify DNA, ensure that samples have been treated to remove RNA, because the absorption spectra of RNA and DNA overlap significantly. For best results, DNA should be resuspended in DNase-free water or DNase-free 10 mM Tris buffer pH 8.0. Do not use DEPC-treated water.

For best results, all DNA samples should also demonstrate consistent quality according to the following criteria:

Concentration and purity determined by UV spectrophotometry

The concentration and purity of DNA should be determined with a UV-vis spectrophotometer. Prepare dilutions and measure absorbance in 10 mM Tris buffer pH 8.0, since the absorption spectra of nucleic acids is dependent on pH.

The A_{260}:A_{280} ratio should be greater than 1.8.

The concentration determined by A_{260} should ideally be >2.5 µg/ml DNA.

For accurate DNA quantification, we recommend the QIAxpert.

DNA integrity

For best results, genomic DNA should be greater than 2 kb in length, with many fragments greater than 10 kb. This can be checked by running a fraction of each DNA sample on a 1% agarose gel.

FFPE DNA

If FFPE DNA will be used, PCR conditions and cycle number for target enrichment may need to be optimized. This can be done with standard qPCR reagents, or for the GeneRead V2 DNAseq panels, the QIAGEN GeneRead DNA QuantiMIZE assay.

Appendix B: Amplicon Preparation and Quality Control

Amplicon preparation and quality control

The QIAseq 1-Step Amplicon Library Kit accepts amplicons generated from a wide variety of multiplex PCR reactions or gene panels. Regardless of the PCR system used, high-quality amplicons are key to the production of high-quality NGS libraries.

When conducting target enrichment via multiplex PCR, avoid overamplification into the plateau phase. When overamplified, amplicons produced in earlier cycles of PCR serve as both primer and template for further amplification, generating long, chimeric molecules. These are visible as high-molecular-weight products with a broad range of lengths and can be visible on the QIAxcel or Agilent® Bioanalyzer trace as a second peak much larger than the amplicons of interest, or as a high-molecular-weight smear sometimes extending into the wells on an agarose gel. These chimeric products can interfere with library preparation and sequencing, and if present, cycle number should be decreased appropriately.

Following PCR, amplicons should be purified with Agencourt AMPure XP Beads or a similar cleanup technology to remove excess primers, primer dimers and buffer components. As the QIAseq 1-Step Amplicon Library Kit accepts a wide range of DNA inputs, amplicon quantification after purification is not strictly necessary if a robust DNA extraction and PCR system is available, but is recommended. Amplicons can be quantified on a QIAxcel, agarose gel, Agilent Bioanalyzer, Qubit®, or NanoDrop®.

Low-diversity libraries

In contrast to a standard whole genome library, the base composition at the beginning of the reads in an amplicon-sequencing library reflects the base composition of the primers used to produce the PCR amplicons. In experiments involving hundreds or thousands of primer pairs,

the sequence diversity at the beginning of each read is high; however in low-multiplexing experiments, where only 8–12 primer pairs are used, sequence diversity is low.

Low sequence diversity can interfere with normal cluster calling, phasing and quality matrix calculation on Illumina platforms. We recommend users to follow all guidelines set forth by the instrument manufacturer for the sequencing of low-diversity libraries if lower-complexity amplicons are used. These may include the intentional underclustering of the flow cell or mixing amplicon libraries with the PhiX standard or barcoded high-diversity whole genome or RNAseq libraries.

Appendix C: Optional A-Tailing Protocol

While the QIAseq 1-step Amplicon Library Kit accepts PCR products from a wide range of sources, some care must be taken to confirm polymerase compatibility. The novel one-step reaction requires that PCR amplicons contain 3′ A-overhangs for efficient ligation. *Taq* polymerase, the most commonly used thermostable DNA polymerase, and its derivatives, by default carry out this non-templated A-addition during the PCR reaction. *Taq* and *Taq*-derivatives have attributes that make them amenable to multiplex PCR, and many commercial gene panels employ a *Taq*-based enzyme.

In contrast to *Taq*, other polymerases with strong 3′–*5′* exonuclease activities do not carry out this reaction. While these enzymes are not commonly used for multiplexed PCR, amplicons produced with such enzymes are still compatible with the QIAseq 1-Step Amplicon Library Kit, but require A-tailing prior to ligation. A suggested A-tailing protocol is given below.

Materials required for A-tailing

- MinElute® PCR Purification Kit (cat. no. 28004 or 28006), or Agencourt AMPure XP Beads (Beckman Coulter, cat. no. A63880)
- Klenow (3′→ *5′* exo-) Low Concentration (Enzymatics, P7010-LC-L)
- 10X Blue Buffer (Enzymatics, B0110)
- 100 mM dATP Solution (Enzymatics, N2010-A-L)
- Pipette tips and pipettes

Procedure

1. Mix the components in Table 4 and add to the PCR tube or plate containing purified PCR products.

Table 4. Reaction components for A-tailing

Component	Volume (µl)
Purified PCR product	20
10x Blue Buffer	5
100 mM dATP	1
Klenow (3'→ 5' exo-) low concentration	1
DNase-free water	23
Total	**50**

2. Incubate in a thermal cycler or heating block for 30 minutes at 37°C.
3. Purify with the MinElute PCR Purification Kit or Agencourt AMPure XP Beads as per the manufacturer's directions.

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 follow a different dual-indexing workflow than 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 layouts of the 24-plex and 96-plex (A/B/C/D) single-use UDI adapter plates are shown from Figure 5 to Figure 9. The index motives used in the QIAseq Unique Dual-Index Kits are listed in Table 5. 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
A	UDI 001	UDI 009	UDI 017	empty	empty	empty	empty	empty	empty	empty	empty
B	UDI 002	UDI 010	UDI 018	empty	empty	empty	empty	empty	empty	empty	empty
C	UDI 003	UDI 011	UDI 019	empty	empty	empty	empty	empty	empty	empty	empty
D	UDI 004	UDI 012	UDI 020	empty	empty	empty	empty	empty	empty	empty	empty
E	UDI 005	UDI 013	UDI 021	empty	empty	empty	empty	empty	empty	empty	empty
F	UDI 006	UDI 014	UDI 022	empty	empty	empty	empty	empty	empty	empty	empty
G	UDI 007	UDI 015	UDI 023	empty	empty	empty	empty	empty	empty	empty	empty
H	UDI 008	UDI 016	UDI 024	empty	empty	empty	empty	empty	empty	empty	empty

Figure 5. 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 6. 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 7. 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 8. 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 9. QIAseq UDI Y-Adapter Plate D (96) layout (UDI 289–384).

Table 5. UDI motives 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 different dual-indexing workflow than other Illumina systems, which requires the reverse complement of the i5 index adapter sequence.

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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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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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)**
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 10 and Figure 11. The index motives used in the QIAseq Combinatorial Dual-Index Kits are listed in Table 6. 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 10. 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 11. QIAseq CDI Y-Adapter Plate (24) layout (CDI 1–24).

Table 6. CDI motives 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 different dual-indexing workflow than other Illumina systems, which requires the reverse complement of the i5 index adapter sequence.

Indices 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: Adapter Indices for GeneRead Adapter I Sets A and B

The index sequences used in the GeneRead Adapter I Set A 12-plex are listed in Table 7. Indices 1–12 correspond to the respective Illumina adapter indices.

Table 7. Adapter indices

Adapter name	Indices
Adapter Bc1 Illumina	ATCACG
Adapter Bc2 Illumina	CGATGT
Adapter Bc3 Illumina	TTAGGC
Adapter Bc4 Illumina	TGACCA
Adapter Bc5 Illumina	ACAGTG
Adapter Bc6 Illumina	GCCAAT
Adapter Bc7 Illumina	CAGATC
Adapter Bc8 Illumina	ACTTGA
Adapter Bc9 Illumina	GATCAG
Adapter Bc10 Illumina	TAGCTT
Adapter Bc11 Illumina	GGCTAC
Adapter Bc12 Illumina	CTTGTA

The index sequences used in the GeneRead Adapter I Set B 12-plex are listed in Table 8. Indices 13–16, 18–23, 25, and 27 correspond to their respective Illumina adapter indices.

Table 8. Adapter indices

Adapter name	Indices
Adapter Bc13 Illumina	AGTCAA
Adapter Bc14 Illumina	AGTTCC
Adapter Bc15 Illumina	ATGTCA
Adapter Bc16 Illumina	CCGTCC
Adapter Bc18 Illumina	GTCCGC
Adapter Bc19 Illumina	GTGAAA
Adapter Bc20 Illumina	GTGGCC
Adapter Bc21 Illumina	GTTTCG
Adapter Bc22 Illumina	CGTACG
Adapter Bc23 Illumina	GAGTGG
Adapter Bc25 Illumina	ACTGAT
Adapter Bc27 Illumina	ATTCCT

Appendix F: Data Analysis

After sequencing, multiplex data can be analyzed using QIAGEN's CLC Genomics or Biomedical Workbench or the cloud-based GeneRead DNAseq Sequence Variant Analysis Software. Our data analysis software will perform quality control, read trimming (removing primer sequences), mapping to a reference genome, and variant identification. Please refer to the corresponding documentation for data analysis.

Ordering Information

Product	Contents	Cat. no.
QIAseq 1-Step Amplicon Library Kit (12)	Reagents for Illumina Amplicon Seq library preparation, and library amplification	180412
QIAseq 1-Step Amplicon Lib UDI-A Kit (96)	Reagents for Illumina Amplicon Seq library preparation and library amplification, including 96-plex Illumina Adapters	180419
QIAseq 1-Step Amplicon Lib UDI-B Kit (96)	Reagents for Illumina Amplicon Seq library preparation and library amplification, including 96-plex Illumina Adapters	180420
QIAseq 1-Step Amplicon Lib UDI-C Kit (96)	Reagents for Illumina Amplicon Seq library preparation and library amplification, including 96-plex Illumina Adapters	180421
QIAseq 1-Step Amplicon Lib UDI-D Kit (96)	Reagents for Illumina Amplicon Seq library preparation and library amplification, including 96-plex Illumina Adapters	180425
QIAseq 1-Step Amplicon Lib CDI Kit (96)	Reagents for Illumina Amplicon Seq library preparation and library amplification, including 96-plex Illumina Adapters	180423
GeneRead Adapter I Set A 12-plex (144)	12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180985
GeneRead Adapter I Set B 12-plex (144)	12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180986
Related products		
GeneRead DNAseq Library Quant Array	Reagents for NGS sample library quantification following targeted enrichment	180601

Product	Contents	Cat. no.
GeneRead qPCR SYBR® Green Mastermix	Master mix for use with the GeneRead Library Quant Arrays and Kit	Varies*
QIAquick® PCR Purification Kit (50)	QIAquick Spin Columns, buffers, collection tubes (2 ml) for purification of PCR products <150 bp	28104
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
QIAGEN panels for target enrichment		
GeneRead Custom DNAseq Gene Panels	Pools containing primer sets for targeted enrichment of a customized panel of genes or genomic regions	181902
GeneRead DNAseq Panel PCR Kit V2 (12)	PCR chemistry for use with the GeneRead DNAseq Panel V2 System	181940
GeneRead DNAseq Panel PCR Kit V2 (96)	PCR chemistry for use with the GeneRead DNAseq Panel V2 System	181942

* See **www.qiagen.com**.

Product	Contents	Cat. no.
GeneRead DNA QuantiMIZE Array Kit	qPCR arrays for optimizing the amount of input DNA and PCR cycling conditions for targeted enrichment of FFPE DNA	180642
QIAGEN PCR reagents for target enrichment		
QIAGEN Multiplex PCR Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 x 0.85 ml), 5x Q-Solution® (1 x 2.0 ml), RNase-free water (2 x 1.7 ml)	206143
QIAGEN Multiplex PCR *Plus* Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x Multiplex PCR Master Mix (3 x 0.85 ml), 5x Q-Solution (1 x 2 ml), RNase-free water (2 x 1.9 ml), 10x CoralLoad® Dye (1 x 1.2 ml)	206152
REPLI-g® whole genome amplification for sensitive applications		
REPLI-g Single Cell Kit (96)	Polymerase, buffers, and reagents for whole genome amplification from limited input materials or single cells	150345
REPLI-g FFPE Kit (100)	Polymerase, buffers, and reagents for whole genome amplification from FFPE samples	150245
QIAGEN kits for genomic DNA isolation and purification		
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, collection tubes (2 ml), reagents, and buffers	51304
GeneRead DNA FFPE Kit	QIAamp MinElute columns, Proteinase K, UNG, collection tubes (2 ml), buffers, deparaffinization solution, RNase A	180134

Product	Contents	Cat. no.
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
QIAamp DNA Microbiome Kit (50)	For 50 DNA preps: 50 QIAamp UCP Mini Columns, 50 Pathogen Lysis Tubes L, buffers, reagents, collection tubes (2 ml)	51704

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Document Revision History

Date	Changes
08/2021	Removed the QIAseq 1-Step Amplicon Library Kit (96) (cat. no. 180415) product for discontinuation. Added information about QIAseq Y-adapter kits and NGS adapter and index technologies. Removed information exclusive to formerly recommended adapters. Updated information related to Illumina instruments.

Notes

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