July 2024

QIAseq® Targeted DNA Pro Handbook

For ultrasensitive targeted next-generation sequencing (NGS) of DNA for Illumina® NGS systems

Sample to Insight

Contents

Kit Contents

QIAseq Targeted DNA Pro Catalog no. No. of samples	333651 12	HC 333661 12	333655 96	HC 333665 96	Custom 333675 96
One pool of region-specific primers	90 µL	90 µL	700 µL	700 µL	700 µL
FFPE repair reagent	15 µL	15 µL	80 µL	80 µL	80 µL
FFPE repair enzyme	17 µL	17 µL	120 µL	120 µL	120 µL
FX Buffer, 10x	23 µL	23 µL	170 µL	170 µL	170 µL
5X WGS FX Mix	42 µL	42 µL	330 µL	330 µL	330 µL
FG Solution	170 µL	170 µL	170 µL	170 µL	170 µL
UPH Ligation Buffer, 2.5x	288 µL	288 µL	1152 µL	1152 µL	1152 µL
DNA Ligase	75 µL	75 µL	330 µL	330 µL	330 µL
Ligation Cleanup Reagent	30 µL	30 µL	240 µL	240 µL	240 µL
TEPCR Buffer, 5x	3 x 60 µL	3 x 60 µL	3 x 500 µL	3 x 500 µL	3 x 500 µL
TEPCR modifier	30 µL	30 µL	230 µL	230 µL	230 µL
TEPCR Cleanup Reagent	80 µL	80 µL	600 µL	600 µL	600 µL
UPCR Buffer, 5x	500 µL	500 µL	4 x 500 µL	4 x 500 µL	4 x 500 µL
QN Taq Polymerase	2 x 60 µL	2 x 60 µL	2 x 450 µL	2 x 450 µL	2 x 450 µL
Nuclease-free water	1.5 mL	1.5 mL	10 mL	10 mL	10 mL
One bottle containing QIAseq Bead Binding Buffer	10.2 mL	10.2 mL	10.2 mL	10.2 mL	10.2 mL
One bottle containing QIAseq Beads	10 mL	10 mL	10 mL	10 mL	10 mL

QIAseq Targeted DNA Pro Booster (96) Catalog no. No. of samples	333685 96
One pool of region-specific primers	80 µL

Cat. no.	Product name	Total number of primers*	Panel size (bases)
PHS-3000Z	Comprehensive Cancer Research Panel	12,264	997,783
PHS-001Z	Breast Cancer Research Panel	2534	203,082
PHS-002Z	Colorectal Cancer Research Panel	3430	282,246
PHS-003Z	Myeloid Neoplasms Research Panel	6848	574,579
PHS-004Z	Brain Cancer Research Panel	2742	199,053
PHS-005Z	Lung Cancer Research Panel	2571	207,663
PHS-3100Z	Comprehensive Cancer Focus Panel	2990	102,488
PHS-101Z	Breast Cancer Focus Panel	633	30,391
PHS-102Z	Colorectal Cancer Focus Panel	1000	38,219
PHS-103Z	Myeloid Neoplasms Focus Panel	1680	65,577
PHS-104Z	Brain Cancer Focus Panel	649	8398
PHS-105Z	Lung Cancer Focus Panel	759	34,958
PHS-3200Z	Comprehensive Hereditary Cancer Research Panel	15,370	1,311,241
PHS-201Z	Hereditary Breast and Ovarian Cancer Panel	2508	206,234
PHS-202Z	Hereditary Colorectal Cancer Panel	2226	184,420
PHS-203Z	Hematologic Malignancy Panel	1276	103,992
PHS-204Z	Hereditary Prostate Cancer Panel	1135	92,257
PHS-205Z	Hereditary Pancreatic Cancer Panel	1595	129,279

* The number of primers in Custom and Booster panels is represented by the last digits of the catalog number. For example, a custom panel with catalog number CPHS-00100Z-1256 has 1256 primers.

QIAseq Targeted DNA Pro 96-Unique Dual Indices

QIAseq Targeted DNA Pro UDI Set* Catalog no. No. of samples	Set A 333455 96	Set B 333465 96	Set C 333475 96	Set D 333485 96	Set E 334015 96	Set F 334025 96	Set G 334035 96	Set H 334045 96
QUDI-96AA †	9 µL	N/A	N/A	N/A	N/A	N/A	N/A	N/A
QUDI-96BA †	N/A	9 µL	N/A	N/A	N/A	N/A	N/A	N/A
QUDI-96CA †	N/A	N/A	9 µL	N/A	N/A	N/A	N/A	N/A
QUDI-96DA †	N/A	N/A	N/A	9 µL	N/A	N/A	N/A	N/A
QUDI-96EA †	N/A	N/A	N/A	N/A	9 µL	N/A	N/A	N/A
QUDI-96FA †	N/A	N/A	N/A	N/A	N/A	9 µL	N/A	N/A
QUDI-96GA †	N/A	N/A	N/A	N/A	N/A	N/A	9 µL	N/A
QUDI-96HA †	N/A	N/A	N/A	N/A	N/A	N/A	N/A	9 µL
AdP-IL5-Phased Adapter	180 µL	180 µL	180 µL	180 µL	180 µL	180 µL	180 µL	180 µL
SmP-IL5 TEPCR-F Primer	240 µL	240 µL	240 µL	240 µL	240 µL	240 µL	240 µL	240 µL

* 10 bp dual indices.

† Index Primer Plate (QUDI-96AA, QUDI-96BA, QUDI-96CA, QUDI-96DA QUDI-96EA, QUDI-96FA, QUDI-96GA, QUDI-96HA); each plate contains 96 pairs of sample index primers plus universal primers, with each well corresponding to one pair of UDI sample index; each index is single use.

QIAseq Targeted DNA Pro 12-Unique Dual Indices

QIAseq Targeted DNA Pro UDI (12)* Catalog no. No. of samples	333441 12 (µL)
QUDI-12A†	9
AdP-IL5-Phased Adapter	25
SmP-IL5 TEPCR-F Primer	32

* 10 bp dual indices.

† Index Primer Plate (QUDI-12A); each plate contains 12 pairs of sample index primers plus universal primers, with each well corresponding to one pair of UDI sample index; each index is single use.

Shipping and Storage

The QIAseq Targeted DNA Pro Kits (except QIAseq Beads and QIAseq Bead Binding Buffer) are shipped on dry ice and should be stored at –30°C to –15°C in a constant-temperature freezer upon arrival.

The QIAseq Beads and QIAseq Bead Binding Buffer are shipped on cold packs and should be stored at 2–8°C upon arrival.

The QIAseq Targeted DNA Pro Index Kits are shipped on dry ice and should be stored at –30°C to –15°C upon arrival.

When stored correctly, the QIAseq Targeted DNA Pro Kits are good until the expiration date printed on the kit label.

Intended Use

The QIAseq Targeted DNA Pro Kits are intended for molecular biology applications. These products are 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 Targeted DNA Pro is tested against predetermined specifications, to ensure consistent product quality.

Introduction

The QIAseq Targeted DNA Pro enable streamlined Sample to Insight®, targeted next-generation sequencing (NGS) of DNA. This highly optimized, automation-friendly solution facilitates ultrasensitive variant detection using integrated unique molecular indices (UMIs) from cells, tissue, and biofluids within 6 hours. The required amount of template for a single QIAseq Targeted DNA Pro sequencing reaction ranges from 10–80 ng for fresh DNA or 100–250 ng for formalin-fixed paraffin-embedded (FFPE) DNA.

The NGS of DNA is a powerful tool for the detection of genetic variations, including somatic mutations, single nucleotide polymorphisms, copy number variation, and small insertions/deletions. Target enrichment technology enhances DNA NGS by enabling users to sequence specific regions of interest – instead of the entire genome – which effectively increases sequencing depth and sample throughput while minimizing cost. Many commercially available target enrichment, library preparation, and sequencing methods use DNA polymerase and amplification processes that introduce substantial bias and artifacts. This results in artifactual errors that greatly limit the detection of true low-frequency variants in heterogeneous samples, such as tumors. The QIAseq Targeted DNA Pro overcomes these biases/artifacts by utilizing a highly optimized reaction chemistry by incorporating UMIs into a single gene-specific, primer-based targeted enrichment process.

Many targeted DNA library construction workflows have multiple bead cleanup steps that often lead to lengthy and inconsistent library construction. In addition, bead cleanups after ligation and target enrichment can significantly reduce the recovery of the original DNA molecules. By replacing the bead cleanups with enzymatic cleanups after the ligation and target enrichment steps, the QIAseq Targeted DNA Pro enables a more efficient, quick, consistent, and automation-friendly workflow.

Due to the fragmented and modified nature of FFPE DNA samples, many NGS library construction workflows have a low recovery rate from FFPE DNA. The QIAseq Targeted DNA Pro incorporates a seamless FFPE DNA repair step before library construction. This repair step, together with the single primer extension technology used in target enrichment, results in great improvements on the recovery of FFPE DNA samples.

The QIAseq Targeted DNA Pro has also been optimized in combination with a specially formulated enrichment chemistry to achieve highly efficient enrichment on both regular and GC-rich regions at high multiplex levels. Additionally, the QIAseq Targeted DNA Pro library can be sequenced with Illumina default sequencing primers, and is compatible with most medium- and high-throughput Illumina sequencers.

Data analysis tools have been developed to perform all steps necessary to generate a DNA sequence variant report from NGS data. Collectively, the QIAseq Targeted DNA Pro is a Sample to Insight solution for precision variant detection of targeted genomic regions using NGS (Figure 1).

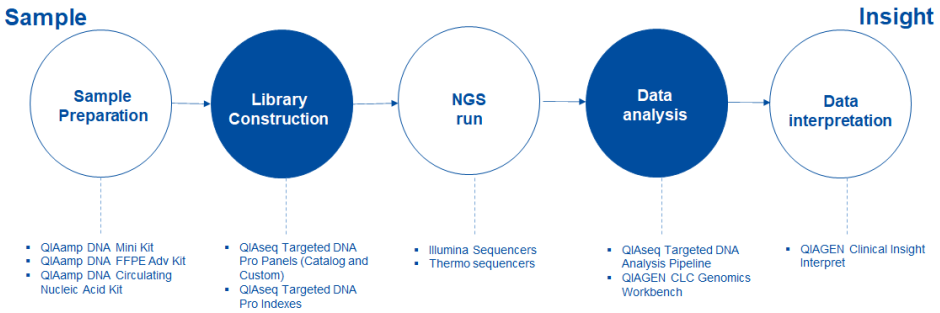
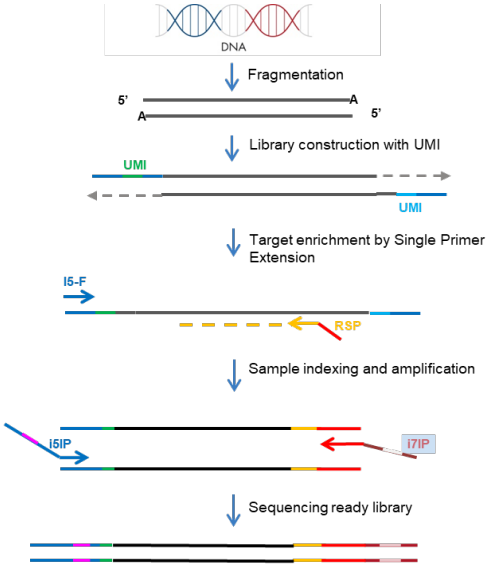


Figure 1. Overview of the Sample to Insight NGS workflow with the QIAseq Targeted DNA Pro. The complete Sample to Insight procedure begins with DNA extraction. Next is library construction and target enrichment with the QIAseq Targeted DNA Pro. Following NGS, data analysis is performed using the QIAseq Targeted DNA Pro Analysis Software pipeline or QIAGEN CLC Genomics Workbench. Ultimately, detected variants can be interpreted with the QIAGEN Clinical Insight Interpret for QIAseq.

Principle and procedure

The QIAseq Targeted DNA Pro Kits are provided as single-tube primer mixes, with up to 20,000 primers per panel. The QIAseq Targeted DNA Pro is designed to enrich selected genes and regions using 10–80 ng fresh DNA or 100–250 ng FFPE DNA (Figure 2). Lower input amounts are possible; however, this will lead to fewer sequenced UMI and reduced variant detection sensitivity.



UMI: Unique molecular indices
RSP: Region specific primer
I5-F: TEPCR-F primer
i5IP and I7IP: sample index primers

Figure 2. QIAseq Targeted DNA Pro workflow.

Fragmentation

Genomic DNA samples are first fragmented, end repaired, and A tailed within a single, controlled multienzyme reaction. The prepared DNA fragments are then ligated at their 5′ ends with a sequencing platform-specific adapter containing UMI.

For FFPE DNA samples, a repair step is carried out first to make more FFPE DNA molecules suitable for library construction. The repaired FFPE DNA can then go directly into the fragmentation reaction in the same tube.

UMI assignment

Prior to target enrichment and library amplification, each original DNA molecule is assigned a unique sequence or index, commonly referred to as a UMI. This assignment is accomplished by ligating fragmented DNA with an adapter containing a 12-base fully random sequence (i.e., the UMI). Statistically, this process provides 4^{12} possible indices per adapter, and each DNA molecule in the sample receives a unique UMI sequence.

Target enrichment and final library construction

Target enrichment is performed after UMI assignment to ensure that DNA molecules containing UMIs are sufficiently enriched in the sequenced library. For enrichment, ligated DNA molecules are subject to several cycles of targeted PCR using one region-specific primer and one universal primer complementary to the adapter. A universal PCR is ultimately carried out to amplify the library, and add platform-specific adapter sequences and sample indices.

Enzymatic cleanup after ligation and target enrichment PCR

After ligation and target enrichment PCR reactions, cleanup is carried out with an enzymatic reaction instead of beads. This greatly reduces both hands-on and total time involved in the reaction cleanups. Since there are no more highly variable bead cleanups after the ligation

and target enrichment PCR reactions, more consistent library construction can be achieved with the enzymatic cleanups. This also makes the workflow more amiable to automation.

NGS adapter and index technologies

The QIAseq Targeted DNA Pro Library Kits use unique dual index (UDI) primers for sample indexing. The UDI primers significantly reduce the risk of index-bleeding issues associated with different Illumina sequencing instruments. They also reduce the impact of low-level contamination during oligo synthesis and kit manufacturing, and carry over on the Illumina sequencing instrument itself. Hence, each sample is assigned two unique indices to overcome the error introduced by image analysis, sequencing error, demultiplexing, and oligo synthesis contamination which reduces the mis-assignment of reads to wrong samples.

Next-generation sequencing

The QIAseq Targeted DNA Pro is compatible with most medium- and high-throughput sequencers including Illumina NGS systems (MiniSeq®, MiSeq®, NextSeq® 500/550, NextSeq 1000/2000, HiSeq® 2500, HiSeq 3000/4000, and NovaSeq® 6000).

Principle of variant detection with UMIs

The principle of variant detection with UMIs is described in Figure 3, next page. Due to intrinsic noise and sequence-dependent bias, indexed molecules may be amplified unevenly across the target regions. However, target region coverage can be better achieved by counting the number of UMIs rather than counting the number of total reads for each region. Sequence reads having different UMIs represent different original molecules, while sequence reads having the same UMIs are the result of PCR duplication from one original molecule. Errors from PCR amplification and from the sequencing process may also be present in final reads that lead to false positive variants in sequencing results. These artifactual variants can be greatly reduced by calling variants across all reads within a unique UMI instead of picking up variants at the original read level.

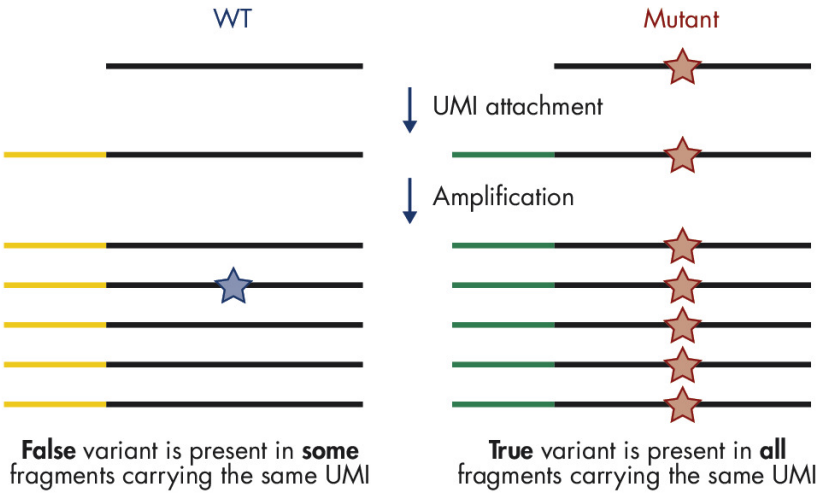


Figure 3. Principle of variant detection with UMIs. Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while false positives are mutations present in only one or a few reads within a UMI.

Data analysis

The data for the QIAseq Targeted DNA Pro can be analyzed using the Biomedical Genomics Analysis plugin to the QIAGEN CLC Genomics Workbench. The plugin provides workflows and tools for all steps from the initial data processing and quality assurance, through data analyses, annotation, and reporting. A detailed guide to UMI-directed variant detection in CLC Genomics Workbench can be found in Biomedical Genomics Analysis Plugin User Manual. Alternatively, you can access **https://geneglobe.qiagen.com/us/analyze** for the QIAseq Targeted DNA Panel Analysis pipeline – which automatically performs all steps necessary to generate a DNA sequence variant report from your NGS data. An explanation of the principles of UMI-directed variant detection and the features of the primary sequence analysis output can be found at Xu et al (1).

All detected variants can be further interpreted using QIAGEN's Clinical Insight (QCI®) Interpret.

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.

In addition to the QIAseq Targeted DNA Pro and Index Kit, the following are required:

- Ethanol, 80% (made fresh daily)*
- Nuclease-free pipette tips and tubes
- DNA LoBind® tubes, 1.5 mL (Eppendorf, cat. no. 022431021)
- PCR tubes and caps (0.2 mL individual PCR tubes [VWR, cat. no. 20170-012], or 8-well tube strips [VWR, cat. no. 93001 118]) or 96-well PCR plates and caps
- Ice
- Microcentrifuge
- Thermal cycler
- Multichannel pipettes
- Single-channel pipettes
- QIAxcel® Connect System (cat. no. 9003110) or QIAxcel Advanced System (cat. no. 9001941) or Agilent® 2100 Bioanalyzer® (Agilent, cat. no. G2939BA) or Agilent TapeStation® (Agilent, cat. no. G2991AA)
- QIAxcel DNA High Sensitivity Kit (cat. No. 929012), QIAxcel DNA High Resolution Kit (QIAGEN, cat. no. 929002) or Agilent High Sensitivity DNA Kit (Agilent cat. no. 5067-4626)
- DynaMag®-96 Side Magnet (Thermo Fisher Scientific Inc., cat. no. 12331D)
- QIAseq DNA QuantiMIZE kits, if using FFPE samples (cat. no. 333414)
- QIAxpert® Instrument (cat. no. 9002340) or Thermo Fisher Scientific Qubit Fluorometer

* Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.

Important Notes

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

DNA isolation and quality check

The most important prerequisite for DNA sequence analysis 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 may either degrade the DNA or decrease the efficiency of, if not block completely, the enzymatic activity necessary for optimal targeted enrichment. Sample purity can be checked with the QIAxpert.

The QIAGEN kits listed in Table 1 are recommended for the preparation of genomic DNA samples from cells, tissues, FFPE tissues, and serum/plasma samples. For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer pH 8.0.

Important: Do not use DEPC-treated water.

Important: Ensure that samples have been treated to remove RNA. RNA contamination will cause inaccuracies in DNA concentration measurements. Follow the recommended RNase treatment step in removing RNA.

Note: If genomic DNA samples must be harvested from biological samples for which kits are not available, please contact QIAGEN Technical Support representatives for suggestions.

Table 1. Recommended kits for purification of genomic DNA

Kit	Starting material	Cat. no.
QIAamp® DNA Mini Kit	Small amounts of cells and tissue	51304
QIAamp DNA FFPE Advanced UNG Kit	Animal/human tissues and cells	56704
QIAamp Circulating Nucleic Acid Kit	Animal and human plasma and serum	55114

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

DNA quantification

The concentration and purity can be determined by measuring the absorbance in a spectrophotometer such as a QIAxpert®. As the spectral properties of nucleic acids are highly dependent on pH, we recommend preparing dilutions and measure absorbance in 10 mM Tris·Cl, pH 8.0. The A_{260}/A_{280} ratio should be >1.8.

DNA integrity

DNA integrity can be checked using the QIAxcel, Agilent Bioanalyzer, or Agilent TapeStation. Although DNA is enzymatically fragmented before target enrichment PCR, intact DNA generally yields better results than fragmented DNA due to tiling space between primers. Intact DNA usually has better coverage uniformity, more UMIs captured, and more sensitive variant detection.

Specific recommendations for FFPE DNA

If FFPE DNA is used for the QIAseq Targeted DNA Panels, the QIAseq DNA QuantiMIZE Array or Assay Kit is strongly recommended for determining the optimal DNA amount for each FFPE DNA sample. Quantification based on mass calculations (OD, NanoDrop®) cannot reliably measure the amplifiable amounts of DNA that are important for multiplex PCR-based targeted

enrichment NGS workflow, such as the QIAseq Targeted DNA Pro. Appendix B (page 56) provides detailed information for FFPE DNA quality assessment and input amount.

DNA input amount and sequencing depth

The number of UMIs captured from the original DNA sample correlates with the DNA input amount and sequencing depth. Adequate sequencing of captured UMIs is necessary for UMI-based variant detection and requires relatively deep sequencing coverage. Table 2 provides guidance on variant detection with fresh DNA amounts at different depths of coverage.

Table 2. Suggested fresh DNA input amount and sequencing depth for variant detection*

Variant frequency (%)	Input (ng)	Read pairs/UMI	Mean read
5	10	4	7200
1	40	4	25,600
0.5	80	4	48,000

* Variant detection is based on 90% sensitivity on the entire panel region of the QIAseq Targeted DNA Pro.

Variant detection

The number of UMIs sequenced directly impacts the variant detection sensitivity. Therefore, low-frequency mutation detection usually requires more DNA input and sequencing at deeper coverage (i.e., more reads/UMI) to generate a sufficient amount of UMIs.

Sequencing capacity and sample multiplex level

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 result, sequencing reads need to be demultiplexed by reassigning each single read to its original source library. This is facilitated by the integration of sample index sequences into the individual library molecules.

The QIAseq Targeted DNA Pro Library Kits include a fully compatible UDI sample indexing solution. Each QIAseq Targeted DNA Pro Library Index Kit includes one of the following:

- QIAseq Targeted DNA Pro UDI Set A (96): DNA Pro UDI Set A index primer plate
- QIAseq Targeted DNA Pro UDI Set B (96): DNA Pro UDI Set B index primer plate
- QIAseq Targeted DNA Pro UDI Set C (96): DNA Pro UDI Set C index primer plate
- QIAseq Targeted DNA Pro UDI Set D (96): DNA Pro UDI Set D index primer plate
- QIAseq Targeted DNA Pro UDI Set E (96): DNA Pro UDI Set E index primer plate
- QIAseq Targeted DNA Pro UDI Set F (96): DNA Pro UDI Set F index primer plate
- QIAseq Targeted DNA Pro UDI Set G (96): DNA Pro UDI Set G index primer plate
- QIAseq Targeted DNA Pro UDI Set H (96): DNA Pro UDI Set H index primer plate
- QIAseq Targeted DNA Pro UDI (12): DNA Pro UDI 12 Index primer plate

The QIAseq Pro UDI Kits use a fixed combination of two unique barcode motives per sample index primer pair. Therefore, each single-index motive is only used once on any UDI index primer.

Usage of UDI indexes effectively mitigates the risk of read misassignment due to index hopping. By filtering misassigned reads during the demultiplexing of individual samples, highly accurate output data is generated.

To multiplex more than 96 libraries in a single sequencing run, combine kits with different sets – QIAseq Targeted DNA Pro UDI Set A, B, C, or D. For example, combining the unique dual QIAseq Targeted DNA Pro UDI Set A and B (96) kits will allow the generation of 192 libraries with different unique dual sample indexes for 192-plex sequencing.

Sample multiplexing level is determined by the size of the panel, required depth of coverage, and sequencing platform read capacity. General guidelines are provided for the number of samples that can be multiplexed with different sequencing platforms, based on panel size and read depth (Table 3, Table 4, and Table 5). Read depth can be fine-tuned after the first run.

Table 3. Number of multiplexed samples based on panel size with 500x mean raw read coverage*

Instrument	Version	Capacity (paired-end reads)	1000 primers	2500 primers	5000 primers	12,000 primers
iSeq	v2 reagent	8 M	16	6	3	1
MiniSeq	Mid output	16 M	32	12	6	2
MiniSeq	High output	50 M	100	40	20	8
MiSeq	v2 reagents	30 M	60	24	12	5
MiSeq	v3 reagents	50 M	100	40	20	8
NextSeq 500	Mid output	260 M	520	208	104	43
NextSeq 500	High output	800 M	1600	640	320	133
NextSeq 1000/2000	P1 flow cell	200 M	400	160	80	33
NextSeq 1000/2000	P2 flow cell	800 M	1600	640	320	133
NextSeq 2000	P3 flow cell	2.4 B	4800	1920	960	399
NovaSeq 6000	SP (2 lanes per flow cell)	1.6 B	3200	1280	640	266
NovaSeq 6000	S1 (2 lanes per flow cell)	3.2 B	6400	2560	1280	532
NovaSeq 6000	S2 (2 lanes per flow cell)	8.2 B	16,400	6560	3280	1366
NovaSeq 6000	S4 (4 lanes per flow cell)	20 B	40,000	16,000	8000	3332
NovaSeq X	1.5B flow cell	3.2 B	6400	2560	1280	532
NovaSeq X	10B flow cell	20 B	40,000	16,000	8000	3332
NovaSeq X	25B flow cell	52 B	104,000	41,600	20,800	8663

* Based on 2 × 149 bp paired-end reads for 12- and 96-UDIs.

Table 4. Number of multiplexed samples based on panel size with 2500x mean raw read coverage*

Instrument	Version	Capacity (paired-ends reads)	1000 primers	2500 primers	5000 primers	12,000 primers
iSeq	V2 reagent	8 M	3	1	N/A	N/A
MiniSeq	Mid output	16 M	6	2	1	N/A
MiniSeq	High output	50 M	20	8	4	1
MiSeq	v2 reagents	30 M	12	4	2	1
MiSeq	v3 reagents	50 M	20	8	4	1
NextSeq 500	Mid output	260 M	104	41	20	8
NextSeq 500	High output	800 M	320	128	64	26
NextSeq 1000/2000	P1 flow cell	200 M	80	32	16	6
NextSeq 1000/2000	P2 flow cell	800 M	320	128	64	26
NextSeq 2000	P3 flow cell	2.4 B	960	384	192	78
NovaSeq 6000	SP (2 lanes per flow cell)	1.6 B	640	256	128	53
NovaSeq 6000	S1 (2 lanes per flow cell)	3.2 B	1280	512	256	106
NovaSeq 6000	S2 (2 lanes per flow cell)	8.2 B	3280	1312	656	273
NovaSeq 6000	S4 (4 lanes per flow cell)	20 B	8000	3200	1600	666
NovaSeq X	1.5B flow cell	3.2 B	1280	512	256	106
NovaSeq X	10B flow cell	20 B	8000	3200	1600	666
NovaSeq X	25B flow cell	52 B	20,800	8320	4160	1731

* Based on 2 x 149 bp paired-end reads for 12- and 96-UDIs.
N/A: Not applicable, no samples can be run.

Table 5. Number of multiplexed samples based on panel size with 20,000x mean raw read coverage*

Instrument	Version	Capacity (paired-ends reads)	1000 primers	2500 primers	5000 primers	12,000 primers
iSeq	v2 reagent	8 M	N/A	N/A	N/A	N/A
MiniSeq	Mid output	16 M	N/A	N/A	N/A	N/A
MiniSeq	High output	50 M	2	1	N/A	N/A
MiSeq	v2 reagents	30 M	1	N/A	N/A	N/A
MiSeq	v3 reagents	50 M	2	1	N/A	N/A
NextSeq 500	Mid output	260 M	13	5	2	1
NextSeq 500	High output	800 M	40	16	8	3
NextSeq 1000/2000	P1 flow cell	200 M	10	4	2	N/A
NextSeq 1000/2000	P2 flow cell	800 M	40	16	8	3
NextSeq 2000	P3 flow cell	2.4 B	120	48	24	9
NovaSeq 6000	SP (2 lanes per flow cell)	1.6 B	80	32	16	6
NovaSeq 6000	S1 (2 lanes per flow cell)	3.2 B	160	64	32	12
NovaSeq 6000	S2 (2 lanes per flow cell)	8.2 B	410	164	82	34
NovaSeq 6000	S4 (4 lanes per flow cell)	20 B	1000	400	200	80
NovaSeq X	1.5B flow cell	3.2 B	160	64	32	12
NovaSeq X	10B flow cell	20 B	1000	400	200	80
NovaSeq X	25B flow cell	52 B	2600	1040	520	208

* Based on 2 x 149 bp paired-end reads for 12- and 96-UDIs.
N/A: Not applicable, no samples can be run.

NGS read-length recommendations

When using Illumina NGS systems, the QIAseq Targeted DNA Pro UDI libraries require 149 bp paired-end reads and dual 10 bp indices.

Protocol: FFPE DNA Repair, DNA Fragmentation, and End Prepare

Important points before starting

- This protocol covers all procedures required for the preparation of libraries for Illumina sequencers from "standard DNA" (i.e., cells or tissues), FFPE DNA, and cfDNA.
- Before setting up the reaction, it is critical to accurately determine the amount of the input DNA (10–80 ng for standard DNA or cfDNA; up to 250 ng of FFPE DNA can be used if QIAseq QuantiMIZE kits have been used. If an alternative method was used to determine the concentration of FFPE DNA, then up to 100 ng DNA can be used). Lower input amounts are possible; however, this will lead to fewer sequenced UMIs and reduced variant detection sensitivity.
- Reaction and cleanup procedures can be performed in either PCR tubes or 96-well plate.
- Set up reactions on ice, mixing reactions by pipetting at close to maximum volume of reaction on ice.
- Do not vortex any reagents or reactions.

Procedure: FFPE DNA repair

Note: For FFPE DNA, set up a repair reaction first before doing "Procedure: fragmentation and end prepare". For standard and cfDNA, go directly to "Procedure: Fragmentation and End Prepare" step.

1. Prepare the reagents required for FFPE DNA repair.
    1a. Thaw 10x FX Buffer and FFPE repair reagent on ice or, if needed, at room temperature (15–25°C) but place on ice immediately after being thawed. Keep FFPE repair enzyme on ice.
    1b. Mix all reagents by flicking the tube, and centrifuge briefly.

2. On ice, prepare the FFPE DNA repair reaction mix according to Table 6. Briefly centrifuge, mix by pipetting up and down 7–8 times, and briefly centrifuge again.

   Note: In general, increasing the amount of FFPE DNA input will improve variant detection sensitivity.

   Note: If setting up more than one reaction, prepare a master mix with a volume 10% greater than what is required for the total number of reactions.

   Important: Keep the reaction tubes/plate on ice during the entire reaction setup.

Table 6. Reaction mix for FFPE DNA repair

Component	Volume/reaction
FFPE DNA*	Variable
10x FX Buffer	1.4 µL
FFPE repair reagent	0.6 µL
FFPE repair enzyme	1 µL
Nuclease-free water	Variable
Total	**12 µL**

* Use up to 250 ng of FFPE DNA if QIAseq QuantiMIZE kits were used or up to 100 ng of FFPE DNA if an alternative method was used.

3. Program the thermal cycler according to Table 7. Use the instrument's heated lid (>100°C).
4. Before adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.

   Important: The thermal cycler must be prechilled and paused at 4°C.
5. Transfer the tubes/plate prepared in step 2 to the prechilled thermal cycler and resume the cycling program.

Table 7. Incubation conditions for FFPE DNA Repair reaction

Step	Incubation temperature (°C)	Incubation time (thermal cycler without ramping control)	Incubation time (thermal cycler with ramping control)
1	4	2 min	2 min
2	15	30 s	0.1°C/s from 4 to 37°C
3	25	30 s	
4	37	30 min	30 min
5	4	Hold	Hold

6. Upon completion, allow the thermal cycler to return to 4°C.
7. Place the samples on ice and immediately proceed to "Procedure: fragmentation and end prepare" below.

Procedure: fragmentation and end prepare

1. Thaw nucleic acid samples on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and then return to ice.
2. Prepare the reagents required for fragmentation and end prepare.
   2a. Thaw 10x FX Buffer and FG Solution on ice or, if required, at room temperature but immediately place on ice after being thawed. Keep 5X WGS FX Mix on ice.
   2b. Mix all reagents by flicking the tube, and centrifuge briefly.
3. On ice, prepare the fragmentation and end prepare mix according to Table 8. Briefly centrifuge, mix by pipetting up and down at least12 times with pipetting volume close to 14 µL, and briefly centrifuge again.

   Note: In general, increasing the amount of DNA input will improve variant detection sensitivity.

   Important: Keep the reaction tubes/plate on ice during the entire reaction setup.

   Note: If setting up more than one reaction, prepare a master mix with a volume 10% greater than what is required for the total number of reactions.

Table 8. Reaction mix for fragmentation and end prepare

Component	Volume/reaction (standard DNA)	Volume/reaction (FFPE DNA)	Volume/reaction (cfDNA)
DNA*	Variable	–	Variable
FFPE repair reaction (already in tube)	–	12 µL	–
10x FX Buffer	1.4 µL	–	1.4 µL
5X WGS FX Mix	2.8 µL	2.8 µL	2.8 µL
FG Solution	–	–	1.4 µL
Nuclease-free water	Variable	Variable	Variable
Total	**14 µL**	**14.8 µL**	**14 µL**

* For standard DNA or cfDNA, 10–80 ng.

4. Program the thermal cycler according to Table 9. Use the instrument's heated lid (>100°C).
5. Before adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.

   Important: The thermal cycler must be prechilled and paused at 4°C.
6. Transfer the tubes/plate prepared in step 3 to the prechilled thermal cycler and resume the cycling program.

Table 9. Incubation conditions for fragmentation and end prepare

Step	Incubation temperature (°C)	Incubation time
1	4	1 min
2	32	10 min
3	65	15 min
4	4	Hold

7. Upon completion, allow the thermal cycler to return to 4°C.
8. Place the samples on ice and immediately proceed to "Protocol: Adapter Ligation", page 29.

Protocol: Adapter Ligation

Important points before starting

- The roughly 14 µL product from "Procedure: fragmentation and end prepare", page 27, is the starting material for this protocol.
- Set up reactions on ice, mixing reactions by pipetting at close to maximum reaction volume on ice.
- Do not vortex any reagents or reactions.

Procedure: adapter ligation

1. Prepare the reagents required for the DNA ligation.
    1a. Thaw AdP-IL5-Phased Adapter on ice. Thaw UPH Ligation Buffer, 2.5x, on ice or at room temperature but immediately place on ice after being thawed. Keep DNA Ligase on ice.
    1b. Mix by flicking the tube, and then centrifuge briefly.
2. Prepare the adapter ligation mix according to Table 10. Briefly centrifuge, mix by pipetting up and down at least 12 times at pipetting volume close to 28 µL and briefly centrifuge again.

    Important: The AdP-IL5-Phased Adapter does not contain any sample index; hence, one single adapter is used for all samples.

    Note: If setting up more than one reaction, prepare a master mix with a volume 10% greater than what is required for the total number of reactions.

Table 10. Reaction mix for adapter ligation

Component	Volume/reaction (µL)
Fragmentation and end prepare reaction (already in tube)	14
UPH Ligation Buffer, 2.5x	10
AdP-IL5-Phased Adapter	1.5
DNA Ligase	2.5
Nuclease-free water	–
Total	**28**

3. Program the thermal cycler according to Table 11.

   Important: Do not use the heated lid during the 20°C incubation step. Alternatively, the lid temperature can be set at 65°C.

4. Before adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.
5. Transfer the tubes/plate prepared in step 2 to the prechilled thermal cycler and resume the cycling program.

Table 11. Incubation conditions for DNA ligation

Step	Incubation temperature (°C)	Incubation time
1	4	1 min
2	20	15 min
3	65	20 min
4	4	Hold

6. Upon completion, allow the thermal cycler to return to 4°C.
7. Place the samples on ice and immediately proceed to “Procedure: ligation cleanup reaction”.

Procedure: ligation cleanup reaction

1. After ligation, transfer the sample to ice and add 2 µL of Ligation Cleanup Reagent to each sample. Briefly centrifuge, mix by pipetting up and down at least 12 times with pipetting volume close to 30 µL and briefly centrifuge again.
2. Program the thermal cycler according to Table 12. Use the instrument's heated lid (>100°C).
3. Before adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.
4. Transfer the tubes/plate prepared in step 1 to the prechilled thermal cycler and resume the cycling program.

Table 12. Incubation conditions for ligation cleanup reaction

Step	Incubation temperature (°C)	Incubation time
1	4	1 min
2	37	15 min
3	95	10 min
4	4	Hold

5. Upon completion, allow the thermal cycler to return to 4°C.
6. Place the samples on ice and immediately proceed to "Protocol: Target Enrichment", page 32.

   Note: Some precipitation in the reaction is normal at this step and will not affect the next target enrichment reaction.

Protocol: Target Enrichment

Important points before starting

- The 30 µL product from "Protocol: Adapter Ligation", page 29, is the starting material for this protocol.
- Set up reactions on ice, mixing reactions by pipetting at close to maximum volume of reaction on ice.
- Do not vortex any reagents or reactions.

Procedure: target enrichment

1. Prepare the reagents required for the Target Enrichment PCR (TEPCR).
    1a. Thaw TEPCR Buffer, 5x; QIAseq Targeted DNA Pro Panel; SmP-IL5 TEPCR-F Primer; and TEPCR modifier on ice (or at room temperature, but immediately place on ice after being thawed).
    1b. Keep QN Taq Polymerase on ice.
    1c. Mix all reagents by flicking the tube, and then centrifuge briefly
2. Prepare the target enrichment mix according to Table 13. Briefly centrifuge, mix by pipetting up and down at least 12 times with pipetting volume close to 50 µL, and briefly centrifuge again.

    Note: If setting up more than one reaction, prepare a master mix with a volume 10% greater than what is required for the total number of reactions.

Table 13. Reaction mix for Target Enrichment

Component	Volume/reaction (µL)
Cleaned Adapter-ligated DNA from "Ligation Cleanup Reaction" (already in tube)	30
TEPCR Buffer, 5x	10
QIAseq Targeted DNA Pro Panel	6.3
SmP-IL5 TEPCR-F Primer	2
TEPCR modifier	1.95
QN Taq Polymerase	2.4
Total	**52.65**

3. Program a thermal cycler using the cycling condition in Table 14 (panel with <12,000 primers/tube) or Table 15 (panel with ≥12,000 primers/tube). Use the instrument's heated lid (>100°C), set up ramping rate at 2°C/S for all steps if the default ramping rate is more than 2°C/S.

Table 14. Cycling conditions for target enrichment if number of primers <12,000/tube

Step	Time	Temperature (°C) (<2000 primers)	Temperature (°C) (>=2000 primers)
Initial denaturation	2 min	98	98
8 cycles	15 s 2 min	98 67	98 65
1 cycle	3 min	72	72
Hold	∞	4	4

*

Table 15. Cycling conditions for target enrichment if number of primers ≥12,000/tube

Step	Time	Temperature (°C)
Initial denaturation	2 min	98
8 cycles	15 s 5 min	98 65
1 cycle	3 min	72
Hold	∞	4

4. Place the target enrichment reaction in the thermal cycler and start the run.
5. After the reaction is complete, place the reactions on ice and proceed to "Procedure: TEPCR cleanup reaction" below. For best result with FFPE sample or panel has less than 500 primers, a bead clean up instead of an enzyme clean up is recommended in Appendix D.
6. Alternatively, the samples can be stored at –20°C in a constant-temperature freezer for up to 3 days.

Procedure: TEPCR cleanup reaction

1. After TEPCR, transfer the samples to ice, mix each TEPCR reaction by pipetting, and transfer 20 µL from each reaction to a new PCR tube/plate on ice.

   Note: Taking only 20 µL of TEPCR reaction is sufficient since DNA molecules were amplified multiple cycles during TEPCR. The rest of the TEPCR reaction can be stored at –20°C if needed.

2. Prepare the TEPCR cleanup mix according to Table 16. Briefly centrifuge, mix by pipetting up and down at least 12 times with pipetting volume close to 25 µL and briefly centrifuge again.

Table 16. Reaction mix for TEPCR cleanup

Component	Volume/reaction (µL)
Target enriched DNA from "Target Enrichment"	20
TEPCR Cleanup Reagent	5
Total	**25**

3. Program the thermal cycler according to Table 17. Use the instrument's heated lid (>100°C).
4. Before adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.

   Important: The thermal cycler must be prechilled and paused at 4°C.

5. Transfer the tubes/plate prepared in step 2 to the prechilled thermal cycler and resume the cycling program.

Table 17. Incubation conditions for TEPCR cleanup reaction

Step	Incubation temperature (°C)	Incubation time
1	4	1 min
2	37	15 min
3	95	10 min
4	4	Hold

6. Upon completion, allow the thermal cycler to return to 4°C.
7. Place the samples on ice and immediately proceed to “Protocol: Universal PCR”, page 36.

Note: Some precipitation in the reaction is normal at this step and will not affect the next protocol.

Important: Keep samples on ice.

Protocol: Universal PCR

Important points before starting

- The 25 µL product from "Protocol: Target Enrichment", page 32, is the starting material for this protocol.
- Set up reactions on ice, mixing reactions by pipetting at close to maximum volume of reaction on ice.
- Do not vortex any reagents or reactions.
- The QIAseq Targeted DNA Pro UDI (12) or QIAseq Targeted DNA Pro UDI Set A (96), Set B (96), Set C (96), Set D (96), Set E (96), Set F (96), Set G (96), or Set H (96) is used for sample indexing.
    - The Index Primer Plate contains predispensed index primer pairs, and the universal PCR primer, and is sealed with pierceable aluminum heat sealing film.
    - Puncture the film using standard 200 µL pipet tips to transfer the appropriate amount of index primer to tube/plate for the universal PCR reaction.
- The QIAseq Beads are used for universal PCR reaction cleanup. Keep the bead and QIAseq Bead Binding Buffer on ice. Also, there is no need to bring beads and buffer to room temperature before use.
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

Procedure: universal PCR

1. Prepare the reagents required for the universal PCR.

1a. Thaw UPCR Buffer, 5x; DNA Pro UDI 12; and 96-Index Plate on ice (or at room temperature, but immediately place on ice after being thawed).

1b. Keep QN Taq Polymerase on ice.

1c. Mix by flicking the tube, and then centrifuge briefly.

2. Prepare the universal PCR in cleaned target-enriched DNA tube/plate from TEPCR cleanup reaction according to Table 18.

   Note: If setting up more than one reaction, a master mix with a volume 10% greater than what is required for the total number of reactions.

3. Prepare the QIAseq Targeted DNA Pro UDI plates:

   3a. Pierce the foil seal associated with each well that will be used.

   3b. Transfer 5 µL to the cleaned target-enriched DNA from "TEPCR cleanup reaction" sample tube/plate according to Table 18 (each well contains a forward primer and a reverse primer, each with a unique index).

   3c. Mix by pipetting up and down at least 12 times with pipetting volume close to 100 µL, and briefly centrifuge again.

   Important: Only one UDI pair should be used per universal PCR reaction.

   Important: The QIAseq Targeted DNA Pro UDI index plates are stable for a maximum of 10 freeze-thaw cycles. If all 96 wells have not been used at one time, cover the used wells with foil and return to the freezer. Do not reuse wells from the QIAseq Targeted DNA Pro UDI index plates once the foil seals have been pierced. Reusing wells would risk significant cross-contamination.

Table 18. Reaction components for universal PCR if using QIAseq Targeted DNA Pro UDI (12) or QIAseq Targeted DNA Pro UDI Set A, B, C, D, E, F, G, and H (96)

Component	Volume/reaction (µL)
Cleaned target-enriched DNA from "TEPCR Cleanup Reaction"	25
UPCR Buffer, 5x	20
Index primers from QIAseq Targeted DNA Pro UDI index plate*	5
QN Taq Polymerase	4.8
Nuclease-free water	45.2
Total	**100**

* Applies to QIAseq Targeted DNA Pro UDI (12) or QIAseq Targeted DNA Pro UDI Set A, B, C, D, E, F, G, and H (96).

DNA Pro UDI 12 Index Plate (QUDI-12A) in QIAseq Targeted DNA Pro UDI (12)

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL5-QUDI-1 IL7-QUDI-1	IL5-QUDI-9 IL7-QUDI-9										
B	IL5-QUDI-2 IL7-QUDI-2	IL5-QUDI-10 IL7-QUDI-10										
C	IL5-QUDI-3 IL7-QUDI-3	IL5-QUDI-11 IL7-QUDI-11										
D	IL5-QUDI-4 IL7-QUDI-4	IL5-QUDI-12 IL7-QUDI-12										
E	IL5-QUDI-5 IL7-QUDI-5											
F	IL5-QUDI-6 IL7-QUDI-6											
G	IL5-QUDI-7 IL7-QUDI-7											
H	IL5-QUDI-8 IL7-QUDI-8											

DNA Pro UDI Set A Plate (QUDI-AA) in QIAseq Targeted DNA Pro UDI Set A (96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL5-QUDI-1 IL7-QUDI-1	IL5-QUDI-9 IL7-QUDI-9	IL5-QUDI-17 IL7-QUDI-17	IL5-QUDI-25 IL7-QUDI-25	IL5-QUDI-33 IL7-QUDI-33	IL5-QUDI-41 IL7-QUDI-41	IL5-QUDI-49 IL7-QUDI-49	IL5-QUDI-57 IL7-QUDI-57	IL5-QUDI-65 IL7-QUDI-65	IL5-QUDI-73 IL7-QUDI-73	IL5-QUDI-81 IL7-QUDI-81	IL5-QUDI-89 IL7-QUDI-89
B	IL5-QUDI-2 IL7-QUDI-2	IL5-QUDI-10 IL7-QUDI-10	IL5-QUDI-18 IL7-QUDI-18	IL5-QUDI-26 IL7-QUDI-26	IL5-QUDI-34 IL7-QUDI-34	IL5-QUDI-42 IL7-QUDI-42	IL5-QUDI-50 IL7-QUDI-50	IL5-QUDI-58 IL7-QUDI-58	IL5-QUDI-66 IL7-QUDI-66	IL5-QUDI-74 IL7-QUDI-74	IL5-QUDI-82 IL7-QUDI-82	IL5-QUDI-90 IL7-QUDI-90
C	IL5-QUDI-3 IL7-QUDI-3	IL5-QUDI-11 IL7-QUDI-11	IL5-QUDI-19 IL7-QUDI-19	IL5-QUDI-27 IL7-QUDI-27	IL5-QUDI-35 IL7-QUDI-35	IL5-QUDI-43 IL7-QUDI-43	IL5-QUDI-51 IL7-QUDI-51	IL5-QUDI-59 IL7-QUDI-59	IL5-QUDI-67 IL7-QUDI-67	IL5-QUDI-75 IL7-QUDI-75	IL5-QUDI-83 IL7-QUDI-83	IL5-QUDI-91 IL7-QUDI-91
D	IL5-QUDI-4 IL7-QUDI-4	IL5-QUDI-12 IL7-QUDI-12	IL5-QUDI-20 IL7-QUDI-20	IL5-QUDI-28 IL7-QUDI-28	IL5-QUDI-36 IL7-QUDI-36	IL5-QUDI-44 IL7-QUDI-44	IL5-QUDI-52 IL7-QUDI-52	IL5-QUDI-60 IL7-QUDI-60	IL5-QUDI-68 IL7-QUDI-68	IL5-QUDI-76 IL7-QUDI-76	IL5-QUDI-84 IL7-QUDI-84	IL5-QUDI-92 IL7-QUDI-92
E	IL5-QUDI-5 IL7-QUDI-5	IL5-QUDI-13 IL7-QUDI-13	IL5-QUDI-21 IL7-QUDI-21	IL5-QUDI-29 IL7-QUDI-29	IL5-QUDI-37 IL7-QUDI-37	IL5-QUDI-45 IL7-QUDI-45	IL5-QUDI-53 IL7-QUDI-53	IL5-QUDI-61 IL7-QUDI-61	IL5-QUDI-69 IL7-QUDI-69	IL5-QUDI-77 IL7-QUDI-77	IL5-QUDI-85 IL7-QUDI-85	IL5-QUDI-93 IL7-QUDI-93
F	IL5-QUDI-6 IL7-QUDI-6	IL5-QUDI-14 IL7-QUDI-14	IL5-QUDI-22 IL7-QUDI-22	IL5-QUDI-30 IL7-QUDI-30	IL5-QUDI-38 IL7-QUDI-38	IL5-QUDI-46 IL7-QUDI-46	IL5-QUDI-54 IL7-QUDI-54	IL5-QUDI-62 IL7-QUDI-62	IL5-QUDI-70 IL7-QUDI-70	IL5-QUDI-78 IL7-QUDI-78	IL5-QUDI-86 IL7-QUDI-86	IL5-QUDI-94 IL7-QUDI-94
G	IL5-QUDI-7 IL7-QUDI-7	IL5-QUDI-15 IL7-QUDI-15	IL5-QUDI-23 IL7-QUDI-23	IL5-QUDI-31 IL7-QUDI-31	IL5-QUDI-39 IL7-QUDI-39	IL5-QUDI-47 IL7-QUDI-47	IL5-QUDI-55 IL7-QUDI-55	IL5-QUDI-63 IL7-QUDI-63	IL5-QUDI-71 IL7-QUDI-71	IL5-QUDI-79 IL7-QUDI-79	IL5-QUDI-87 IL7-QUDI-87	IL5-QUDI-95 IL7-QUDI-95
H	IL5-QUDI-8 IL7-QUDI-8	IL5-QUDI-16 IL7-QUDI-16	IL5-QUDI-24 IL7-QUDI-24	IL5-QUDI-32 IL7-QUDI-32	IL5-QUDI-40 IL7-QUDI-40	IL5-QUDI-48 IL7-QUDI-48	IL5-QUDI-56 IL7-QUDI-56	IL5-QUDI-64 IL7-QUDI-64	IL5-QUDI-72 IL7-QUDI-72	IL5-QUDI-80 IL7-QUDI-80	IL5-QUDI-88 IL7-QUDI-88	IL5-QUDI-96 IL7-QUDI-96

DNA Pro UDI Set B Plate (QUDI-BA) in QIAseq Targeted DNA Pro UDI Set B (96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL5-QUDI-97 IL7-QUDI-97	IL5-QUDI-105 IL7-QUDI-105	IL5-QUDI-113 IL7-QUDI-113	IL5-QUDI-121 IL7-QUDI-121	IL5-QUDI-129 IL7-QUDI-129	IL5-QUDI-137 IL7-QUDI-137	IL5-QUDI-145 IL7-QUDI-145	IL5-QUDI-153 IL7-QUDI-153	IL5-QUDI-161 IL7-QUDI-161	IL5-QUDI-169 IL7-QUDI-169	IL5-QUDI-177 IL7-QUDI-177	IL5-QUDI-185 IL7-QUDI-185
B	IL5-QUDI-98 IL7-QUDI-98	IL5-QUDI-106 IL7-QUDI-106	IL5-QUDI-114 IL7-QUDI-114	IL5-QUDI-122 IL7-QUDI-122	IL5-QUDI-130 IL7-QUDI-130	IL5-QUDI-138 IL7-QUDI-138	IL5-QUDI-146 IL7-QUDI-146	IL5-QUDI-154 IL7-QUDI-154	IL5-QUDI-162 IL7-QUDI-162	IL5-QUDI-170 IL7-QUDI-170	IL5-QUDI-178 IL7-QUDI-178	IL5-QUDI-186 IL7-QUDI-186
C	IL5-QUDI-99 IL7-QUDI-99	IL5-QUDI-107 IL7-QUDI-107	IL5-QUDI-115 IL7-QUDI-115	IL5-QUDI-123 IL7-QUDI-123	IL5-QUDI-131 IL7-QUDI-131	IL5-QUDI-139 IL7-QUDI-139	IL5-QUDI-147 IL7-QUDI-147	IL5-QUDI-155 IL7-QUDI-155	IL5-QUDI-163 IL7-QUDI-163	IL5-QUDI-171 IL7-QUDI-171	IL5-QUDI-179 IL7-QUDI-179	IL5-QUDI-187 IL7-QUDI-187
D	IL5-QUDI-100 IL7-QUDI-100	IL5-QUDI-108 IL7-QUDI-108	IL5-QUDI-116 IL7-QUDI-116	IL5-QUDI-124 IL7-QUDI-124	IL5-QUDI-132 IL7-QUDI-132	IL5-QUDI-140 IL7-QUDI-140	IL5-QUDI-148 IL7-QUDI-148	IL5-QUDI-156 IL7-QUDI-156	IL5-QUDI-164 IL7-QUDI-164	IL5-QUDI-172 IL7-QUDI-172	IL5-QUDI-180 IL7-QUDI-180	IL5-QUDI-188 IL7-QUDI-188
E	IL5-QUDI-101 IL7-QUDI-101	IL5-QUDI-109 IL7-QUDI-109	IL5-QUDI-117 IL7-QUDI-117	IL5-QUDI-125 IL7-QUDI-125	IL5-QUDI-133 IL7-QUDI-133	IL5-QUDI-141 IL7-QUDI-141	IL5-QUDI-149 IL7-QUDI-149	IL5-QUDI-157 IL7-QUDI-157	IL5-QUDI-165 IL7-QUDI-165	IL5-QUDI-173 IL7-QUDI-173	IL5-QUDI-181 IL7-QUDI-181	IL5-QUDI-189 IL7-QUDI-189
F	IL5-QUDI-102 IL7-QUDI-102	IL5-QUDI-110 IL7-QUDI-110	IL5-QUDI-118 IL7-QUDI-118	IL5-QUDI-126 IL7-QUDI-126	IL5-QUDI-134 IL7-QUDI-134	IL5-QUDI-142 IL7-QUDI-142	IL5-QUDI-150 IL7-QUDI-150	IL5-QUDI-158 IL7-QUDI-158	IL5-QUDI-166 IL7-QUDI-166	IL5-QUDI-174 IL7-QUDI-174	IL5-QUDI-182 IL7-QUDI-182	IL5-QUDI-190 IL7-QUDI-190
G	IL5-QUDI-103 IL7-QUDI-103	IL5-QUDI-111 IL7-QUDI-111	IL5-QUDI-119 IL7-QUDI-119	IL5-QUDI-127 IL7-QUDI-127	IL5-QUDI-135 IL7-QUDI-135	IL5-QUDI-143 IL7-QUDI-143	IL5-QUDI-151 IL7-QUDI-151	IL5-QUDI-159 IL7-QUDI-159	IL5-QUDI-167 IL7-QUDI-167	IL5-QUDI-175 IL7-QUDI-175	IL5-QUDI-183 IL7-QUDI-183	IL5-QUDI-191 IL7-QUDI-191
H	IL5-QUDI-104 IL7-QUDI-104	IL5-QUDI-112 IL7-QUDI-112	IL5-QUDI-120 IL7-QUDI-120	IL5-QUDI-128 IL7-QUDI-128	IL5-QUDI-136 IL7-QUDI-136	IL5-QUDI-144 IL7-QUDI-144	IL5-QUDI-152 IL7-QUDI-152	IL5-QUDI-160 IL7-QUDI-160	IL5-QUDI-168 IL7-QUDI-168	IL5-QUDI-176 IL7-QUDI-176	IL5-QUDI-184 IL7-QUDI-184	IL5-QUDI-192 IL7-QUDI-192

Figure 4A. Layout of DNA Pro UDI Index Primer Plate in QIAseq Targeted DNA Pro UDI (12) and QIAseq Targeted DNA Pro UDI Sets A and B (96). Each well contains one pair of predispensed sample index primers plus universal primers for a single reaction in the Protocol: Universal PCR.

DNA Pro UDI Set C Plate (QUDI-CA) in QIAseq Targeted DNA Pro UDI Set C (96)

	1	2	3	4	5	6	7	8	9	10	11	12
	IL5-QUDI-193	IL5-QUDI-201	IL5-QUDI-209	IL5-QUDI-217	IL5-QUDI-225	IL5-QUDI-233	IL5-QUDI-241	IL5-QUDI-249	IL5-QUDI-257	IL5-QUDI-265	IL5-QUDI-273	IL5-QUDI-281
A	IL7-QUDI-193	IL7-QUDI-201	IL7-QUDI-209	IL7-QUDI-217	IL7-QUDI-225	IL7-QUDI-233	IL7-QUDI-241	IL7-QUDI-249	IL7-QUDI-257	IL7-QUDI-265	IL7-QUDI-273	IL7-QUDI-281
	IL5-QUDI-194	IL5-QUDI-202	IL5-QUDI-210	IL5-QUDI-218	IL5-QUDI-226	IL5-QUDI-234	IL5-QUDI-242	IL5-QUDI-250	IL5-QUDI-258	IL5-QUDI-266	IL5-QUDI-274	IL5-QUDI-282
B	IL7-QUDI-194	IL7-QUDI-202	IL7-QUDI-210	IL7-QUDI-218	IL7-QUDI-226	IL7-QUDI-234	IL7-QUDI-242	IL7-QUDI-250	IL7-QUDI-258	IL7-QUDI-266	IL7-QUDI-274	IL7-QUDI-282
	IL5-QUDI-195	IL5-QUDI-203	IL5-QUDI-211	IL5-QUDI-219	IL5-QUDI-227	IL5-QUDI-235	IL5-QUDI-243	IL5-QUDI-251	IL5-QUDI-259	IL5-QUDI-267	IL5-QUDI-275	IL5-QUDI-283
C	IL7-QUDI-195	IL7-QUDI-203	IL7-QUDI-211	IL7-QUDI-219	IL7-QUDI-227	IL7-QUDI-235	IL7-QUDI-243	IL7-QUDI-251	IL7-QUDI-259	IL7-QUDI-267	IL7-QUDI-275	IL7-QUDI-283
	IL5-QUDI-196	IL5-QUDI-204	IL5-QUDI-212	IL5-QUDI-220	IL5-QUDI-228	IL5-QUDI-236	IL5-QUDI-244	IL5-QUDI-252	IL5-QUDI-260	IL5-QUDI-268	IL5-QUDI-276	IL5-QUDI-284
D	IL7-QUDI-196	IL7-QUDI-204	IL7-QUDI-212	IL7-QUDI-220	IL7-QUDI-228	IL7-QUDI-236	IL7-QUDI-244	IL7-QUDI-252	IL7-QUDI-260	IL7-QUDI-268	IL7-QUDI-276	IL7-QUDI-284
	IL5-QUDI-197	IL5-QUDI-205	IL5-QUDI-213	IL5-QUDI-221	IL5-QUDI-229	IL5-QUDI-237	IL5-QUDI-245	IL5-QUDI-253	IL5-QUDI-261	IL5-QUDI-269	IL5-QUDI-277	IL5-QUDI-285
E	IL7-QUDI-197	IL7-QUDI-205	IL7-QUDI-213	IL7-QUDI-221	IL7-QUDI-229	IL7-QUDI-237	IL7-QUDI-245	IL7-QUDI-253	IL7-QUDI-261	IL7-QUDI-269	IL7-QUDI-277	IL7-QUDI-285
	IL5-QUDI-198	IL5-QUDI-206	IL5-QUDI-214	IL5-QUDI-222	IL5-QUDI-230	IL5-QUDI-238	IL5-QUDI-246	IL5-QUDI-254	IL5-QUDI-262	IL5-QUDI-270	IL5-QUDI-278	IL5-QUDI-286
F	IL7-QUDI-198	IL7-QUDI-206	IL7-QUDI-214	IL7-QUDI-222	IL7-QUDI-230	IL7-QUDI-238	IL7-QUDI-246	IL7-QUDI-254	IL7-QUDI-262	IL7-QUDI-270	IL7-QUDI-278	IL7-QUDI-286
	IL5-QUDI-199	IL5-QUDI-207	IL5-QUDI-215	IL5-QUDI-223	IL5-QUDI-231	IL5-QUDI-239	IL5-QUDI-247	IL5-QUDI-255	IL5-QUDI-263	IL5-QUDI-271	IL5-QUDI-279	IL5-QUDI-287
G	IL7-QUDI-199	IL7-QUDI-207	IL7-QUDI-215	IL7-QUDI-223	IL7-QUDI-231	IL7-QUDI-239	IL7-QUDI-247	IL7-QUDI-255	IL7-QUDI-263	IL7-QUDI-271	IL7-QUDI-279	IL7-QUDI-287
	IL5-QUDI-200	IL5-QUDI-208	IL5-QUDI-216	IL5-QUDI-224	IL5-QUDI-232	IL5-QUDI-240	IL5-QUDI-248	IL5-QUDI-256	IL5-QUDI-264	IL5-QUDI-272	IL5-QUDI-280	IL5-QUDI-288
H	IL7-QUDI-200	IL7-QUDI-208	IL7-QUDI-216	IL7-QUDI-224	IL7-QUDI-232	IL7-QUDI-240	IL7-QUDI-248	IL7-QUDI-256	IL7-QUDI-264	IL7-QUDI-272	IL7-QUDI-280	IL7-QUDI-288

DNA Pro UDI Set D Plate (QUIDI-DA) in QIAseq Targeted DNA Pro UDI Set D (96)

	1	2	3	4	5	6	7	8	9	10	11	12
	IL5-QUDI-289	IL5-QUDI-297	IL5-QUDI-305	IL5-QUDI-313	IL5-QUDI-321	IL5-QUDI-329	IL5-QUDI-337	IL5-QUDI-345	IL5-QUDI-353	IL5-QUDI-361	IL5-QUDI-369	IL5-QUDI-377
A	IL7-QUDI-289	IL7-QUDI-297	IL7-QUDI-305	IL7-QUDI-313	IL7-QUDI-321	IL7-QUDI-329	IL7-QUDI-337	IL7-QUDI-345	IL7-QUDI-353	IL7-QUDI-361	IL7-QUDI-369	IL7-QUDI-377
	IL5-QUDI-290	IL5-QUDI-298	IL5-QUDI-306	IL5-QUDI-314	IL5-QUDI-322	IL5-QUDI-330	IL5-QUDI-338	IL5-QUDI-346	IL5-QUDI-354	IL5-QUDI-362	IL5-QUDI-370	IL5-QUDI-378
B	IL7-QUDI-290	IL7-QUDI-298	IL7-QUDI-306	IL7-QUDI-314	IL7-QUDI-322	IL7-QUDI-330	IL7-QUDI-338	IL7-QUDI-346	IL7-QUDI-354	IL7-QUDI-362	IL7-QUDI-370	IL7-QUDI-378
	IL5-QUDI-291	IL5-QUDI-299	IL5-QUDI-307	IL5-QUDI-315	IL5-QUDI-323	IL5-QUDI-331	IL5-QUDI-339	IL5-QUDI-347	IL5-QUDI-355	IL5-QUDI-363	IL5-QUDI-371	IL5-QUDI-379
C	IL7-QUDI-291	IL7-QUDI-299	IL7-QUDI-307	IL7-QUDI-315	IL7-QUDI-323	IL7-QUDI-331	IL7-QUDI-339	IL7-QUDI-347	IL7-QUDI-355	IL7-QUDI-363	IL7-QUDI-371	IL7-QUDI-379
	IL5-QUDI-292	IL5-QUDI-300	IL5-QUDI-308	IL5-QUDI-316	IL5-QUDI-324	IL5-QUDI-332	IL5-QUDI-340	IL5-QUDI-348	IL5-QUDI-356	IL5-QUDI-364	IL5-QUDI-372	IL5-QUDI-380
D	IL7-QUDI-292	IL7-QUDI-300	IL7-QUDI-308	IL7-QUDI-316	IL7-QUDI-324	IL7-QUDI-332	IL7-QUDI-340	IL7-QUDI-348	IL7-QUDI-356	IL7-QUDI-364	IL7-QUDI-372	IL7-QUDI-380
	IL5-QUDI-293	IL5-QUDI-301	IL5-QUDI-309	IL5-QUDI-317	IL5-QUDI-325	IL5-QUDI-333	IL5-QUDI-341	IL5-QUDI-349	IL5-QUDI-357	IL5-QUDI-365	IL5-QUDI-373	IL5-QUDI-381
E	IL7-QUDI-293	IL7-QUDI-301	IL7-QUDI-309	IL7-QUDI-317	IL7-QUDI-325	IL7-QUDI-333	IL7-QUDI-341	IL7-QUDI-349	IL7-QUDI-357	IL7-QUDI-365	IL7-QUDI-373	IL7-QUDI-381
	IL5-QUDI-294	IL5-QUDI-302	IL5-QUDI-310	IL5-QUDI-318	IL5-QUDI-326	IL5-QUDI-334	IL5-QUDI-342	IL5-QUDI-350	IL5-QUDI-358	IL5-QUDI-366	IL5-QUDI-374	IL5-QUDI-382
F	IL7-QUDI-294	IL7-QUDI-302	IL7-QUDI-310	IL7-QUDI-318	IL7-QUDI-326	IL7-QUDI-334	IL7-QUDI-342	IL7-QUDI-350	IL7-QUDI-358	IL7-QUDI-366	IL7-QUDI-374	IL7-QUDI-382
	IL5-QUDI-295	IL5-QUDI-303	IL5-QUDI-311	IL5-QUDI-319	IL5-QUDI-327	IL5-QUDI-335	IL5-QUDI-343	IL5-QUDI-351	IL5-QUDI-359	IL5-QUDI-367	IL5-QUDI-375	IL5-QUDI-383
G	IL7-QUDI-295	IL7-QUDI-303	IL7-QUDI-311	IL7-QUDI-319	IL7-QUDI-327	IL7-QUDI-335	IL7-QUDI-343	IL7-QUDI-351	IL7-QUDI-359	IL7-QUDI-367	IL7-QUDI-375	IL7-QUDI-383
	IL5-QUDI-296	IL5-QUDI-304	IL5-QUDI-312	IL5-QUDI-320	IL5-QUDI-328	IL5-QUDI-336	IL5-QUDI-344	IL5-QUDI-352	IL5-QUDI-360	IL5-QUDI-368	IL5-QUDI-376	IL5-QUDI-384
H	IL7-QUDI-296	IL7-QUDI-304	IL7-QUDI-312	IL7-QUDI-320	IL7-QUDI-328	IL7-QUDI-336	IL7-QUDI-344	IL7-QUDI-352	IL7-QUDI-360	IL7-QUDI-368	IL7-QUDI-376	IL7-QUDI-384

Figure 4B. Layout of DNA Pro UDI Index Primer Plate in QIAseq Targeted DNA Pro UDI Sets C and D (96). Each well contains one pair of predispensed sample index primers plus universal primers for a single reaction in the Protocol: Universal PCR.

DNA Pro UDI Set E Plate (QUIDI-EA) in QIAseq Targeted DNA Pro UDI Set E (96)

	1	2	3	4	5	6	7	8	9	10	11	12
	IL5-QUDI-385	IL5-QUDI-393	IL5-QUDI-401	IL5-QUDI-409	IL5-QUDI-417	IL5-QUDI-425	IL5-QUDI-433	IL5-QUDI-441	IL5-QUDI-449	IL5-QUDI-457	IL5-QUDI-465	IL5-QUDI-473
A	IL7-QUDI-385	IL7-QUDI-393	IL7-QUDI-401	IL7-QUDI-409	IL7-QUDI-417	IL7-QUDI-425	IL7-QUDI-433	IL7-QUDI-441	IL7-QUDI-449	IL7-QUDI-457	IL7-QUDI-465	IL7-QUDI-473
	IL5-QUDI-386	IL5-QUDI-394	IL5-QUDI-402	IL5-QUDI-410	IL5-QUDI-418	IL5-QUDI-426	IL5-QUDI-434	IL5-QUDI-442	IL5-QUDI-450	IL5-QUDI-458	IL5-QUDI-466	IL5-QUDI-474
B	IL7-QUDI-386	IL7-QUDI-394	IL7-QUDI-402	IL7-QUDI-410	IL7-QUDI-418	IL7-QUDI-426	IL7-QUDI-434	IL7-QUDI-442	IL7-QUDI-450	IL7-QUDI-458	IL7-QUDI-466	IL7-QUDI-474
	IL5-QUDI-387	IL5-QUDI-395	IL5-QUDI-403	IL5-QUDI-411	IL5-QUDI-419	IL5-QUDI-427	IL5-QUDI-435	IL5-QUDI-443	IL5-QUDI-451	IL5-QUDI-459	IL5-QUDI-467	IL5-QUDI-475
C	IL7-QUDI-387	IL7-QUDI-395	IL7-QUDI-403	IL7-QUDI-411	IL7-QUDI-419	IL7-QUDI-427	IL7-QUDI-435	IL7-QUDI-443	IL7-QUDI-451	IL7-QUDI-459	IL7-QUDI-467	IL7-QUDI-475
	IL5-QUDI-388	IL5-QUDI-396	IL5-QUDI-404	IL5-QUDI-412	IL5-QUDI-420	IL5-QUDI-428	IL5-QUDI-436	IL5-QUDI-444	IL5-QUDI-452	IL5-QUDI-460	IL5-QUDI-468	IL5-QUDI-476
D	IL7-QUDI-388	IL7-QUDI-396	IL7-QUDI-404	IL7-QUDI-412	IL7-QUDI-420	IL7-QUDI-428	IL7-QUDI-436	IL7-QUDI-444	IL7-QUDI-452	IL7-QUDI-460	IL7-QUDI-468	IL7-QUDI-476
	IL5-QUDI-389	IL5-QUDI-397	IL5-QUDI-405	IL5-QUDI-413	IL5-QUDI-421	IL5-QUDI-429	IL5-QUDI-437	IL5-QUDI-445	IL5-QUDI-453	IL5-QUDI-461	IL5-QUDI-469	IL5-QUDI-477
E	IL7-QUDI-389	IL7-QUDI-397	IL7-QUDI-405	IL7-QUDI-413	IL7-QUDI-421	IL7-QUDI-429	IL7-QUDI-437	IL7-QUDI-445	IL7-QUDI-453	IL7-QUDI-461	IL7-QUDI-469	IL7-QUDI-477
	IL5-QUDI-390	IL5-QUDI-398	IL5-QUDI-406	IL5-QUDI-414	IL5-QUDI-422	IL5-QUDI-430	IL5-QUDI-438	IL5-QUDI-446	IL5-QUDI-454	IL5-QUDI-462	IL5-QUDI-470	IL5-QUDI-478
F	IL7-QUDI-390	IL7-QUDI-398	IL7-QUDI-406	IL7-QUDI-414	IL7-QUDI-422	IL7-QUDI-430	IL7-QUDI-438	IL7-QUDI-446	IL7-QUDI-454	IL7-QUDI-462	IL7-QUDI-470	IL7-QUDI-478
	IL5-QUDI-391	IL5-QUDI-399	IL5-QUDI-407	IL5-QUDI-415	IL5-QUDI-423	IL5-QUDI-431	IL5-QUDI-439	IL5-QUDI-447	IL5-QUDI-455	IL5-QUDI-463	IL5-QUDI-471	IL5-QUDI-479
G	IL7-QUDI-391	IL7-QUDI-399	IL7-QUDI-407	IL7-QUDI-415	IL7-QUDI-423	IL7-QUDI-431	IL7-QUDI-439	IL7-QUDI-447	IL7-QUDI-455	IL7-QUDI-463	IL7-QUDI-471	IL7-QUDI-479
	IL5-QUDI-392	IL5-QUDI-400	IL5-QUDI-408	IL5-QUDI-416	IL5-QUDI-424	IL5-QUDI-432	IL5-QUDI-440	IL5-QUDI-448	IL5-QUDI-456	IL5-QUDI-464	IL5-QUDI-472	IL5-QUDI-480
H	IL7-QUDI-392	IL7-QUDI-400	IL7-QUDI-408	IL7-QUDI-416	IL7-QUDI-424	IL7-QUDI-432	IL7-QUDI-440	IL7-QUDI-448	IL7-QUDI-456	IL7-QUDI-464	IL7-QUDI-472	IL7-QUDI-480

DNA Pro UDI Set F Plate (QUIDI-FA) in QIAseq Targeted DNA Pro UDI Set F (96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL5-QUDI-481 IL7-QUDI-481	IL5-QUDI-489 IL7-QUDI-489	IL5-QUDI-497 IL7-QUDI-497	IL5-QUDI-505 IL7-QUDI-505	IL5-QUDI-513 IL7-QUDI-513	IL5-QUDI-521 IL7-QUDI-521	IL5-QUDI-529 IL7-QUDI-529	IL5-QUDI-537 IL7-QUDI-537	IL5-QUDI-545 IL7-QUDI-545	IL5-QUDI-553 IL7-QUDI-553	IL5-QUDI-561 IL7-QUDI-561	IL5-QUDI-569 IL7-QUDI-569
B	IL5-QUDI-482 IL7-QUDI-482	IL5-QUDI-490 IL7-QUDI-490	IL5-QUDI-498 IL7-QUDI-498	IL5-QUDI-506 IL7-QUDI-506	IL5-QUDI-514 IL7-QUDI-514	IL5-QUDI-522 IL7-QUDI-522	IL5-QUDI-530 IL7-QUDI-530	IL5-QUDI-538 IL7-QUDI-538	IL5-QUDI-546 IL7-QUDI-546	IL5-QUDI-554 IL7-QUDI-554	IL5-QUDI-562 IL7-QUDI-562	IL5-QUDI-570 IL7-QUDI-570
C	IL5-QUDI-483 IL7-QUDI-483	IL5-QUDI-491 IL7-QUDI-491	IL5-QUDI-499 IL7-QUDI-499	IL5-QUDI-507 IL7-QUDI-507	IL5-QUDI-515 IL7-QUDI-515	IL5-QUDI-523 IL7-QUDI-523	IL5-QUDI-531 IL7-QUDI-531	IL5-QUDI-539 IL7-QUDI-539	IL5-QUDI-547 IL7-QUDI-547	IL5-QUDI-555 IL7-QUDI-555	IL5-QUDI-563 IL7-QUDI-563	IL5-QUDI-571 IL7-QUDI-571
D	IL5-QUDI-484 IL7-QUDI-484	IL5-QUDI-492 IL7-QUDI-492	IL5-QUDI-500 IL7-QUDI-500	IL5-QUDI-508 IL7-QUDI-508	IL5-QUDI-516 IL7-QUDI-516	IL5-QUDI-524 IL7-QUDI-524	IL5-QUDI-532 IL7-QUDI-532	IL5-QUDI-540 IL7-QUDI-540	IL5-QUDI-548 IL7-QUDI-548	IL5-QUDI-556 IL7-QUDI-556	IL5-QUDI-564 IL7-QUDI-564	IL5-QUDI-572 IL7-QUDI-572
E	IL5-QUDI-485 IL7-QUDI-485	IL5-QUDI-493 IL7-QUDI-493	IL5-QUDI-501 IL7-QUDI-501	IL5-QUDI-509 IL7-QUDI-509	IL5-QUDI-517 IL7-QUDI-517	IL5-QUDI-525 IL7-QUDI-525	IL5-QUDI-533 IL7-QUDI-533	IL5-QUDI-541 IL7-QUDI-541	IL5-QUDI-549 IL7-QUDI-549	IL5-QUDI-557 IL7-QUDI-557	IL5-QUDI-565 IL7-QUDI-565	IL5-QUDI-573 IL7-QUDI-573
F	IL5-QUDI-486 IL7-QUDI-486	IL5-QUDI-494 IL7-QUDI-494	IL5-QUDI-502 IL7-QUDI-502	IL5-QUDI-510 IL7-QUDI-510	IL5-QUDI-518 IL7-QUDI-518	IL5-QUDI-526 IL7-QUDI-526	IL5-QUDI-534 IL7-QUDI-534	IL5-QUDI-542 IL7-QUDI-542	IL5-QUDI-550 IL7-QUDI-550	IL5-QUDI-558 IL7-QUDI-558	IL5-QUDI-566 IL7-QUDI-566	IL5-QUDI-574 IL7-QUDI-574
G	IL5-QUDI-487 IL7-QUDI-487	IL5-QUDI-495 IL7-QUDI-495	IL5-QUDI-503 IL7-QUDI-503	IL5-QUDI-511 IL7-QUDI-511	IL5-QUDI-519 IL7-QUDI-519	IL5-QUDI-527 IL7-QUDI-527	IL5-QUDI-535 IL7-QUDI-535	IL5-QUDI-543 IL7-QUDI-543	IL5-QUDI-551 IL7-QUDI-551	IL5-QUDI-559 IL7-QUDI-559	IL5-QUDI-567 IL7-QUDI-567	IL5-QUDI-575 IL7-QUDI-575
H	IL5-QUDI-488 IL7-QUDI-488	IL5-QUDI-496 IL7-QUDI-496	IL5-QUDI-504 IL7-QUDI-504	IL5-QUDI-512 IL7-QUDI-512	IL5-QUDI-520 IL7-QUDI-520	IL5-QUDI-528 IL7-QUDI-528	IL5-QUDI-536 IL7-QUDI-536	IL5-QUDI-544 IL7-QUDI-544	IL5-QUDI-552 IL7-QUDI-552	IL5-QUDI-560 IL7-QUDI-560	IL5-QUDI-568 IL7-QUDI-568	IL5-QUDI-576 IL7-QUDI-576

Figure 4C. Layout of DNA Pro UDI Index Primer Plate in QIAseq Targeted DNA Pro UDI Sets E and F (96). Each well contains one pair of predispensed sample index primers plus universal primers for a single reaction in the Protocol: Universal PCR.

DNA Pro UDI Set G Plate (QUIDI-GA) in QIAseq Targeted DNA Pro UDI Set G (96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL5-QUDI-577 IL7-QUDI-577	IL5-QUDI-585 IL7-QUDI-585	IL5-QUDI-593 IL7-QUDI-593	IL5-QUDI-601 IL7-QUDI-601	IL5-QUDI-609 IL7-QUDI-609	IL5-QUDI-617 IL7-QUDI-617	IL5-QUDI-625 IL7-QUDI-625	IL5-QUDI-633 IL7-QUDI-633	IL5-QUDI-641 IL7-QUDI-641	IL5-QUDI-649 IL7-QUDI-649	IL5-QUDI-657 IL7-QUDI-657	IL5-QUDI-665 IL7-QUDI-665
B	IL5-QUDI-578 IL7-QUDI-578	IL5-QUDI-586 IL7-QUDI-586	IL5-QUDI-594 IL7-QUDI-594	IL5-QUDI-602 IL7-QUDI-602	IL5-QUDI-610 IL7-QUDI-610	IL5-QUDI-618 IL7-QUDI-618	IL5-QUDI-626 IL7-QUDI-626	IL5-QUDI-634 IL7-QUDI-634	IL5-QUDI-642 IL7-QUDI-642	IL5-QUDI-650 IL7-QUDI-650	IL5-QUDI-658 IL7-QUDI-658	IL5-QUDI-666 IL7-QUDI-666
C	IL5-QUDI-579 IL7-QUDI-579	IL5-QUDI-587 IL7-QUDI-587	IL5-QUDI-595 IL7-QUDI-595	IL5-QUDI-603 IL7-QUDI-603	IL5-QUDI-611 IL7-QUDI-611	IL5-QUDI-619 IL7-QUDI-619	IL5-QUDI-627 IL7-QUDI-627	IL5-QUDI-635 IL7-QUDI-635	IL5-QUDI-643 IL7-QUDI-643	IL5-QUDI-651 IL7-QUDI-651	IL5-QUDI-659 IL7-QUDI-659	IL5-QUDI-667 IL7-QUDI-667
D	IL5-QUDI-580 IL7-QUDI-580	IL5-QUDI-588 IL7-QUDI-588	IL5-QUDI-596 IL7-QUDI-596	IL5-QUDI-604 IL7-QUDI-604	IL5-QUDI-612 IL7-QUDI-612	IL5-QUDI-620 IL7-QUDI-620	IL5-QUDI-628 IL7-QUDI-628	IL5-QUDI-636 IL7-QUDI-636	IL5-QUDI-644 IL7-QUDI-644	IL5-QUDI-652 IL7-QUDI-652	IL5-QUDI-660 IL7-QUDI-660	IL5-QUDI-668 IL7-QUDI-668
E	IL5-QUDI-581 IL7-QUDI-581	IL5-QUDI-589 IL7-QUDI-589	IL5-QUDI-597 IL7-QUDI-597	IL5-QUDI-605 IL7-QUDI-605	IL5-QUDI-613 IL7-QUDI-613	IL5-QUDI-621 IL7-QUDI-621	IL5-QUDI-629 IL7-QUDI-629	IL5-QUDI-637 IL7-QUDI-637	IL5-QUDI-645 IL7-QUDI-645	IL5-QUDI-653 IL7-QUDI-653	IL5-QUDI-661 IL7-QUDI-661	IL5-QUDI-669 IL7-QUDI-669
F	IL5-QUDI-582 IL7-QUDI-582	IL5-QUDI-590 IL7-QUDI-590	IL5-QUDI-598 IL7-QUDI-598	IL5-QUDI-606 IL7-QUDI-606	IL5-QUDI-614 IL7-QUDI-614	IL5-QUDI-622 IL7-QUDI-622	IL5-QUDI-630 IL7-QUDI-630	IL5-QUDI-638 IL7-QUDI-638	IL5-QUDI-646 IL7-QUDI-646	IL5-QUDI-654 IL7-QUDI-654	IL5-QUDI-662 IL7-QUDI-662	IL5-QUDI-670 IL7-QUDI-670
G	IL5-QUDI-583 IL7-QUDI-583	IL5-QUDI-591 IL7-QUDI-591	IL5-QUDI-599 IL7-QUDI-599	IL5-QUDI-607 IL7-QUDI-607	IL5-QUDI-615 IL7-QUDI-615	IL5-QUDI-623 IL7-QUDI-623	IL5-QUDI-631 IL7-QUDI-631	IL5-QUDI-639 IL7-QUDI-639	IL5-QUDI-647 IL7-QUDI-647	IL5-QUDI-655 IL7-QUDI-655	IL5-QUDI-663 IL7-QUDI-663	IL5-QUDI-671 IL7-QUDI-671
H	IL5-QUDI-584 IL7-QUDI-584	IL5-QUDI-592 IL7-QUDI-592	IL5-QUDI-600 IL7-QUDI-600	IL5-QUDI-608 IL7-QUDI-608	IL5-QUDI-616 IL7-QUDI-616	IL5-QUDI-624 IL7-QUDI-624	IL5-QUDI-632 IL7-QUDI-632	IL5-QUDI-640 IL7-QUDI-640	IL5-QUDI-648 IL7-QUDI-648	IL5-QUDI-656 IL7-QUDI-656	IL5-QUDI-664 IL7-QUDI-664	IL5-QUDI-672 IL7-QUDI-672

DNA Pro UDI Set H Plate (QUIDI-HA) in QIAseq Targeted DNA Pro UDI Set H (96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL5-QUDI-673 IL7-QUDI-673	IL5-QUDI-681 IL7-QUDI-681	IL5-QUDI-689 IL7-QUDI-689	IL5-QUDI-697 IL7-QUDI-697	IL5-QUDI-705 IL7-QUDI-705	IL5-QUDI-713 IL7-QUDI-713	IL5-QUDI-721 IL7-QUDI-721	IL5-QUDI-729 IL7-QUDI-729	IL5-QUDI-737 IL7-QUDI-737	IL5-QUDI-745 IL7-QUDI-745	IL5-QUDI-753 IL7-QUDI-753	IL5-QUDI-761 IL7-QUDI-761
B	IL5-QUDI-674 IL7-QUDI-674	IL5-QUDI-682 IL7-QUDI-682	IL5-QUDI-690 IL7-QUDI-690	IL5-QUDI-698 IL7-QUDI-698	IL5-QUDI-706 IL7-QUDI-706	IL5-QUDI-714 IL7-QUDI-714	IL5-QUDI-722 IL7-QUDI-722	IL5-QUDI-730 IL7-QUDI-730	IL5-QUDI-738 IL7-QUDI-738	IL5-QUDI-746 IL7-QUDI-746	IL5-QUDI-754 IL7-QUDI-754	IL5-QUDI-762 IL7-QUDI-762
C	IL5-QUDI-675 IL7-QUDI-675	IL5-QUDI-683 IL7-QUDI-683	IL5-QUDI-691 IL7-QUDI-691	IL5-QUDI-699 IL7-QUDI-699	IL5-QUDI-707 IL7-QUDI-707	IL5-QUDI-715 IL7-QUDI-715	IL5-QUDI-723 IL7-QUDI-723	IL5-QUDI-731 IL7-QUDI-731	IL5-QUDI-739 IL7-QUDI-739	IL5-QUDI-747 IL7-QUDI-747	IL5-QUDI-755 IL7-QUDI-755	IL5-QUDI-763 IL7-QUDI-763
D	IL5-QUDI-676 IL7-QUDI-676	IL5-QUDI-684 IL7-QUDI-684	IL5-QUDI-692 IL7-QUDI-692	IL5-QUDI-700 IL7-QUDI-700	IL5-QUDI-708 IL7-QUDI-708	IL5-QUDI-716 IL7-QUDI-716	IL5-QUDI-724 IL7-QUDI-724	IL5-QUDI-732 IL7-QUDI-732	IL5-QUDI-740 IL7-QUDI-740	IL5-QUDI-748 IL7-QUDI-748	IL5-QUDI-756 IL7-QUDI-756	IL5-QUDI-764 IL7-QUDI-764
E	IL5-QUDI-677 IL7-QUDI-677	IL5-QUDI-685 IL7-QUDI-685	IL5-QUDI-693 IL7-QUDI-693	IL5-QUDI-701 IL7-QUDI-701	IL5-QUDI-709 IL7-QUDI-709	IL5-QUDI-717 IL7-QUDI-717	IL5-QUDI-725 IL7-QUDI-725	IL5-QUDI-733 IL7-QUDI-733	IL5-QUDI-741 IL7-QUDI-741	IL5-QUDI-749 IL7-QUDI-749	IL5-QUDI-757 IL7-QUDI-757	IL5-QUDI-765 IL7-QUDI-765
F	IL5-QUDI-678 IL7-QUDI-678	IL5-QUDI-686 IL7-QUDI-686	IL5-QUDI-694 IL7-QUDI-694	IL5-QUDI-702 IL7-QUDI-702	IL5-QUDI-710 IL7-QUDI-710	IL5-QUDI-718 IL7-QUDI-718	IL5-QUDI-726 IL7-QUDI-726	IL5-QUDI-734 IL7-QUDI-734	IL5-QUDI-742 IL7-QUDI-742	IL5-QUDI-750 IL7-QUDI-750	IL5-QUDI-758 IL7-QUDI-758	IL5-QUDI-766 IL7-QUDI-766
G	IL5-QUDI-679 IL7-QUDI-679	IL5-QUDI-687 IL7-QUDI-687	IL5-QUDI-695 IL7-QUDI-695	IL5-QUDI-703 IL7-QUDI-703	IL5-QUDI-711 IL7-QUDI-711	IL5-QUDI-719 IL7-QUDI-719	IL5-QUDI-727 IL7-QUDI-727	IL5-QUDI-735 IL7-QUDI-735	IL5-QUDI-743 IL7-QUDI-743	IL5-QUDI-751 IL7-QUDI-751	IL5-QUDI-759 IL7-QUDI-759	IL5-QUDI-767 IL7-QUDI-767
H	IL5-QUDI-680 IL7-QUDI-680	IL5-QUDI-688 IL7-QUDI-688	IL5-QUDI-696 IL7-QUDI-696	IL5-QUDI-704 IL7-QUDI-704	IL5-QUDI-712 IL7-QUDI-712	IL5-QUDI-720 IL7-QUDI-720	IL5-QUDI-728 IL7-QUDI-728	IL5-QUDI-736 IL7-QUDI-736	IL5-QUDI-744 IL7-QUDI-744	IL5-QUDI-752 IL7-QUDI-752	IL5-QUDI-760 IL7-QUDI-760	IL5-QUDI-768 IL7-QUDI-768

Figure 4D. Layout of DNA Pro UDI Index Primer Plate in QIAseq Targeted DNA Pro UDI Sets G and H (96). Each well contains one pair of predispensed sample index primers plus universal primers for a single reaction in the Protocol: Universal PCR.

4. Program a thermal cycler using the cycling conditions in Table 19, and refer to Table 20 for the cycle number to use which are dependent on the number of primers in the pool. Use the instrument's heated lid (>100°C), set up ramping rate at 2°C/S for all steps if the default ramping rate is more than 2°C/S.

Table 19. Incubation conditions for universal PCR

Step	Time	Temperature (°C)
Initial denaturation	2 min	98
Number of cycles (see Table 20)	15 s 30 s	98 62
1 cycle	3 min	72
Hold	∞	4

Table 20. Amplification cycles for universal PCR

Primers per pool	Cycle number (standard DNA)	Cycle number (FFPE DNA and cfDNA)
6–24	33	35
25–96	31	33
97–288	29	31
289–1056	28	30
1057–3072	27	29
3073–5999	26	28
6000–12,000	25	27
≥12,001	24	26

5. After the reaction is complete, place the reactions on ice and proceed to "Procedure: cleanup of universal PCR", next page. Alternatively, the samples can be stored at –20°C in a constant-temperature freezer for up to 3 days.

Procedure: cleanup of universal PCR

1. Keep the reaction on ice, add 80 µL QIAseq Beads to the completed universal PCR reaction; mix well by vortexing or pipetting up and down on ice at least 12 times with pipetting volume close to 180 µL.
2. Incubate for 5 min at room temperature.
3. Place the tubes/plate on magnetic rack for 5 min (tube) or 10 min (plate) to separate beads from supernatant. Once the solution has cleared, with the tubes/plate still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads as they contain the DNA of interest.
4. With the tubes/plate still on the magnetic stand, add 100 µL H2O to the bead, then add 80 µL QIAseq Bead Binding Buffer.
5. Take the tubes/plate off the magnetic stand; mix well by vortexing or pipetting up and down at least12 times with pipetting volume close to 180 µL.
6. Return the tubes/plate to the magnetic rack for 5 min (tube) or 10 min (plate). Once the solution has cleared, with the tubes/plate still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads as they contain the DNA of interest.
7. With the tubes/plate still on the magnetic stand, add 200 µL 80% ethanol to wash the beads. Carefully remove and discard the ethanol wash.
8. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol wash after this second wash. Remove the ethanol with a 200 µL pipet first, and then use a 10 µL pipet to remove any residual ethanol.
9. With the tubes/plate still on the magnetic stand, air dry at room temperature for 10 min.

   Note: Visually inspect that the pellet is completely dry. Over drying the beads will not affect DNA elution.

10. Remove the tubes/plate from the magnetic stand, and elute the DNA from the beads by adding 30 µL nuclease-free water. Mix well by pipetting or vortexing.
11. Return the tubes/plate to the magnetic rack until the solution has cleared.
12. Transfer 28 µL supernatant to clean tubes or plate.

    Proceed to “Recommendations: Library QC and Quantification”, page 45. Libraries can be normalized with QIAseq Universal Normalizer Kit according to Appentix E, page 63. Alternatively, the library can be stored at –30°C to –15°C in a constant-temperature freezer. Amplified libraries are stable for several months at –30°C to –15°C. Once quantification is performed, proceed to “Protocol: Sequencing Setup on Illumina MiSeq, NextSeq 500/550, NextSeq 1000/2000, MiniSeq, and NovaSeq”.

Recommendations: Library QC and Quantification

NGS library QC

After the library is constructed and purified, QC can be performed with QIAxcel or Agilent's Bioanalyzer or TapeStation to check for the correct size distribution of the library fragments and for the absence of primer dimers (approx <200 bp) and concentration. Majority library fragments prepared for Illumina instruments demonstrate a size distribution between 250–1000 bp (Figure 5 and Figure 6A). Library overamplification is normal (Figure 6B), and this should not affect the sequencing results. Overamplified libraries are usually single—stranded libraries with correct size but appear as "larger fragments" due to secondary structures. Amounts of DNA under the appropriate peaks can be used to quantify the libraries. However, due to the superior sensitivity of qPCR, we recommend quantifying the libraries using the QIAseq Library Quant System, especially when there are overamplified libraries (See "Preferred library quantification method", page 47). Alternaternatively, finished libraries can be normalized with the QIAseq Universal Normalizer Kit (Appendix E: Library Normalization with QIAseq Universal Normalizer Kit, page 63).

Recommended setting for checking QIAseq DNA Pro library on QIAxcel

QIAxcel Connect

- QIAxcel DNA High Sensitivity Kit (1200) (cat. no. 929012)
- Use method Default High Sensitivity with 1–10 µL library

QIAxcel Advanced or QIAxcel Connect

- QIAxcel DNA High Resolution Kit (cat. no. 929002)
- QX Alignment Marker: 15 bp/5 kb (cat. no. 929524)
- QX DNA Size Marker: 100bp – 2.5kb (cat. no. 929559)
- Use the Application Guide for Low-Concentration Libraries. To access guides and system files for Library QC, contact QIAGEN Technical Services.

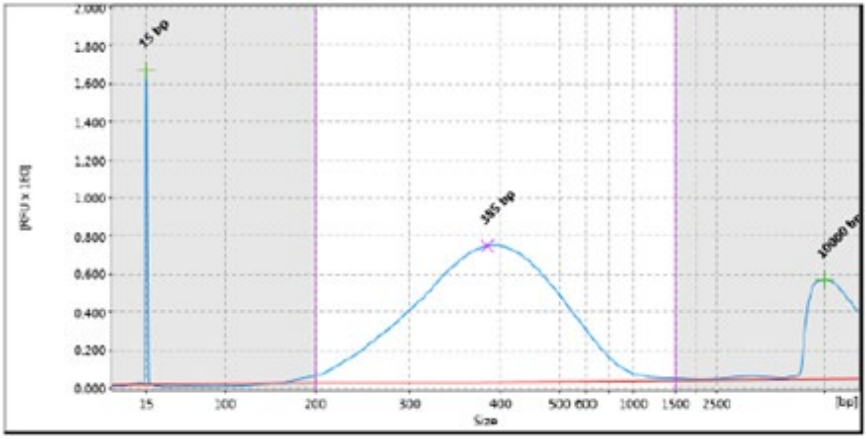
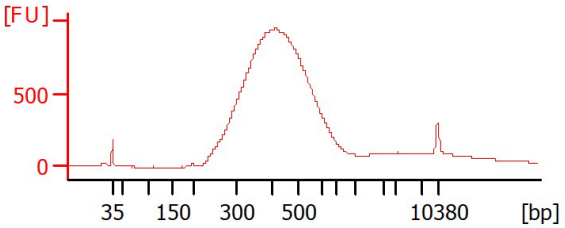


Figure 5. Sample QIAxcel image of QIAseq Targeted DNA Pro libraries for Illumina instruments. The library assessed using QIAxcel illustrates the size of the majority of the library fragments are between 250–1000 bp.

A: Library (without overamplification) prepared for Illumina instruments



B: Library (with overamplification) prepared for Illumina instruments

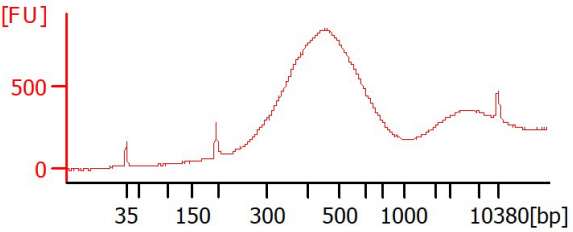


Figure 6. Sample Bioanalyzer images of QIAseq Targeted DNA Pro libraries for Illumina instruments. The size of the majority of the library fragments are between 250–1000 bp. **A**: Library without overamplification. **B**: Library with overamplification as indicated by the "larger fragment" peak.

Preferred library quantification method

The library yield measurements from the Bioanalyzer or TapeStation rely on fluorescence dyes that intercalate into DNA. These dyes cannot discriminate between molecules with or without adapter sequences, yet only complete QIAseq Targeted DNA Pro libraries with full adapter sequences will be sequenced. Due to the superior sensitivity of qPCR, we recommend quantifying the libraries using QIAseq Library Quant Assay Kit (cat. no. 333314), which contains laboratory-verified forward and reverse primers, together with a DNA standard. With this system, the correct dilution of the library can be determined for sequencing. Please refer to the relevant handbook (available at **www.qiagen.com**) for library quantification.

Protocol: Sequencing Setup on Illumina MiSeq, NextSeq 500/550, NextSeq 1000/2000, MiniSeq, and NovaSeq

Important points before starting

- Recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System or QIAseq Universal Normalizer Kit (see Preferred library quantification method, page 47).
- Paired-end sequencing should be used for the QIAseq Targeted DNA Pro on Illumina platform.
- To make sequencing preparation more convenient, download Illumina-compatible sample sheets for different sequencing instruments on **www.qiagen.com** from the Resources tab of the QIAseq Targeted DNA Pro.
- Paired-end sequencing of 149 bp should be used for QIAseq Targeted DNA Pro UDI libraries and dual 10 bp indices on Illumina platforms.
- For 2-channel sequencing chemistry platforms such as MiniSeq, NextSeq, and NovaSeq, 10% PhiX can be included in the run to improve sequencing quality. For complete instructions on how to denature sequencing libraries, and set up a sequencing run, please refer to the system-specific Illumina documents.

Sequencing preparations for MiSeq with QIAseq Targeted DNA Pro UDI Sets

1. When working with the QIAseq Targeted DNA Pro custom QIAseq 96-Unique Dual Index Sets, use Local Run Manager (LRM) v2 or LRM v3 on the instrument to upload a sample sheet (see the **Resources** tab of the QIAseq Targeted DNA Pro and download the appropriate template) and proceed with sequencing: Read 1 is 149 bp, Read 2 is 149 bp, and each Index Read is 10 bp.

2. **Sample Dilution and Pooling**: Dilute libraries to 2 or 4 nM for MiSeq. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.

   Note: Recommendations for library dilution concentrations are based on QIAseq Library Quant System or QIAseq Universal Normalizer Kit.

   Note: If combining libraries with the same number of primers, pool equal volumes of the individual libraries at 4 nM together. If the libraries have different primer numbers, then combine the libraries at volume ratios according to their number of primers. For example, Library A has 5000 primers at 4 nM, and Library B has 600 primers at 4 nM; combining 50 µL of Library A with 6 µL of Library B will result in similar coverage depth for both Libraries A and B in the same sequencing run.

3. **Library Preparation and Loading**: Prepare and load the library onto a MiSeq according to the MiSeq System Denature and Dilute Libraries Guide. The final library concentration is 10–12 pM on the MiSeq.

   Note: Recommendations for library loading concentrations are based on QIAseq Library Quant System or QIAseq Universal Normalizer Kit.

   Note: For loading with Qubit quantification, the recommend loading could be 4–7 pM depending on library concentration, over amplification et al, and adjust loading accordingly after the first run.

4. Upon completion of the sequencing run, proceed to Appendix C: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench, page 58.

Sequencing preparations for MiniSeq, NextSeq 500/550, and NextSeq 1000/2000 with QIAseq Targeted DNA Pro UDI Sets

1. When working with the QIAseq Targeted DNA Pro custom 96-Unique Dual Index Sets, use LRM v2 on the instrument to upload a sample sheet (see the **Resources** tab of the QIAseq Targeted DNA Pro and download the appropriate template) and proceed with sequencing: Read 1 is 149 bp, Read 2 is 149 bp, and each Index Read is 10 bp.

2. **Sample Dilution and Pooling**: Dilute libraries to 1 nM for MiniSeq, 0.5, 1, 2, or 4 nM for NextSeq 500/550, and 2 nM for NextSeq 1000/2000 onboard denature. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.

   Note: Recommendations for library dilution concentrations are based on QIAseq Library Quant System or QIAseq Universal Normalizer Kit.

   Note: If combining libraries with the same number of primers, pool equal volumes of the individual libraries at 4 nM together. If the libraries have different primer numbers, then combine the libraries at volume ratios according to their number of primers. For example, Library A has 5000 primers at 4 nM, and Library B has 600 primers at 4 nM; combining 50 µL Library A with 6 µL Library B will result in similar coverage depth for both Libraries A and B in the same sequencing run.

3. **Library Preparation and Loading**: Prepare and load the library onto a MiniSeq, NextSeq 500/550 or NextSeq 1000/2000 according to the MiniSeq or NextSeq System Denature and Dilute Libraries Guide. The final library concentration is 1.0–1.2 pM on the MiniSeq or NextSeq 500/550 and 650 pM for NextSeq 1000/2000 onboard denature loading.

   Note: Recommendations for library loading concentrations are based on QIAseq Library Quant System or QIAseq Universal Normalizer Kit.

   Note: for loading with Qubit quantification, the recommend loading could be 0.4–0.7 pM for MiniSeq and NextSeq 500/550 or 220-380 pM for NextSeq 1000/2000 onboard denature depending on library concentration, over amplification et al, and adjust loading accordingly after the first run.

4. Upon completion of the sequencing run, proceed to Appendix C: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench, page 58.

Sequencing preparations for NovaSeq with QIAseq Targeted DNA Pro UDI Index Sets

1. When working with the QIAseq Targeted DNA Pro custom QIAseq 96-Unique Dual Index Sets, upload a sample sheet (see the **Resources** tab of the QIAseq Targeted DNA Panel and download the appropriate template) and proceed with sequencing: Read 1 is 149 bp, Read 2 is 149 bp, and each Index Read is 10 bp.
2. **Sample Dilution and Pooling**: Dilute libraries to 10 nM for NovaSeq. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.

   Note: Recommendations for library dilution concentrations are based on QIAseq Library Quant System or QIAseq Universal Normalizer Kit.

   Note: If combining libraries with the same number of primers, pool equal volumes of the individual libraries at 10 nM together. If the libraries have different primer numbers, then combine the libraries at volume ratios according to their number of primers. For example, Library A has 5000 primers at 10 nM, and Library B has 600 primers at 10 nM; combining 50 µL Library A with 6 µL Library B will result in similar coverage depth for both Libraries A and B in the same sequencing run.
3. **Library Preparation and Loading**: Prepare and load the library onto a NovaSeq according to the NovaSeq 6000 Sequencing System Guide (part #1000000019358). The final pooled library concentration recommendation is between 1.0 and 1.5 nM yielding a final loading concentration of between 200 and 300 pM on the NovaSeq.

   Note: Recommendations for library loading concentrations are based on QIAseq Library Quant System or QIAseq Universal Normalizer Kit.

   Note: For loading with Qubit quantification, the recommend final pooled library concentration could be 0.4–0.7 nM depending on library concentration, over amplification et al, and adjust accordingly after the first run.

4. Upon completion of the sequencing run, proceed to Appendix C: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench, page 58.

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 yield	
a) Suboptimal reaction conditions due to low DNA quality	Make sure to use high-quality DNA to ensure optimal activity of library enzymes.
b) Inefficient targeted enrichment or universal PCR	Check to see if correct thermocycling condition is used during target enrichment or universal PCR.
Unexpected signal peaks	
a) Short peaks approx. 200 bp	These are primer–dimers from targeted enrichment PCR or universal PCR. The presence of primer dimers indicates either not enough DNA input or inefficient PCR reactions or issues during enzymatic cleanup reaction.
b) Larger library fragments after universal PCR	After the universal PCR, library fragments are larger than the intended peak and can be a PCR artifact due to overamplification of the DNA library. Overamplification of the library will not affect the QIAseq Targeted DNA Pro sequencing performance. Decreasing the number of universal PCR cycle numbers can reduce over-amplification.
Sequencing issues	
a) Too low or too high cluster density	Accurate library quantification is the key for optimal cluster density on any sequencing instrument. PCR-based quantification method is recommended. Other methods may lead to the incorrect quantification of the library especially when there is over amplification.
b) Very low clusters passing filter	Make sure that the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument.
Variant detection issues	
Known variants not detected	Variant detection sensitivity is directly related to the input DNA and read depth. Check Table 2 to see if the required input DNA and read depth are met for the specific variant detection application.

References

1. Xu, C., Gu, X., Padmanabhan R., Wu, Z., Peng, Q., DiCarlo, J., Wang, Y. (2019) smCounter2: an accurate low-frequency variant caller for targeted sequencing data with unique molecular identifiers. Bioinformatics 35(8).

Appendix A: Combining an Existing Panel with a Booster Panel

If additional primers need to be added into an existing panel, a Booster Panel with up to 100 primers can be ordered. To combine the existing panel with a Booster Panel, follow the volume ratio indicated in Table 21.

Table 21. Combining an Existing Panel (at 50 µL) with a Booster Panel

No. of primers in existing panel	Volume of existing panel to combine (µL)	Volume of booster panel to combine (µL)
1–2000	50	5
2001–4000	50	3.75
4001–12,000	50	2.5
12,001–20,000	50	1.25

Appendix B: FFPE DNA Quality and Quantity

Genomic DNA present in FFPE archives is usually damaged and fragmented to an uncertain extent. Commonly used DNA quantification methods, including spectrometers or fluorometers, do not differentiate between amplifiable and nonamplifiable DNA. Therefore, they cannot reliably measure the amplifiable amounts of DNA that are able to participate in the targeted enrichment step in the NGS workflow involving multiplex PCR, such as the QIAseq Targeted DNA Pro.

The QIAseq DNA QuantiMIZE System is a qPCR-based approach that determines the quantity and quality of the DNA amenable to PCR-based targeted enrichment prior to NGS. The system provides a cost-effective approach to qualify and quantify the DNA isolated from biological samples – mainly for FFPE samples. Please refer to the corresponding handbook for determining FFPE DNA quantity and quality with the QIAseq DNA QuantiMIZE System.

The QIAseq DNA QuantiMIZE System is recommended for determining FFPE DNA input for the QIAseq Targeted DNA Pro. If FFPE DNA is defined as high quality (quality control [QC] score ≤0.04) by QuantiMIZE, then up to 100 ng of DNA can be used. If the DNA is determined as low quality (QC score >0.04), then up to 250 ng of DNA can be used. The QC score of QuantiMIZE reflects the amount of amplifiable DNA present in the sample, therefore correlating with the number of UMIs that can be sequenced in the library (Figure 7, next page).

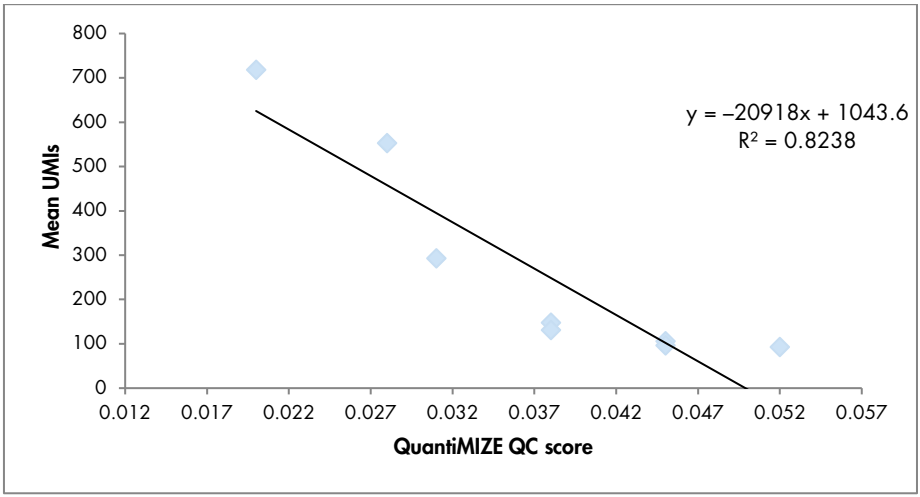


Figure 7. Correlation between QIAseq QuantiMIZE QC score and the number of UMIs.

Compared to the same amount of fresh DNA, only 10–50% of UMIs can be captured from FFPE DNA, depending on the quality. This is due to a lower amplifiable DNA amount present in the FFPE samples. Therefore, a higher input amount is recommended for FFPE DN-A samples to ensure that enough UMIs can be sequenced for variant detection.

However, if the quality of the FFPE DNA is not assessed by QIAseq QuantiMIZE kits, up to 100 ng can be used. If the FFPE DNA quality is high, an input of more than 100 ng will potentially overload the QIAseq Targeted DNA Pro system.

Appendix C: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench

After sequencing, the results can be analyzed using QIAseq targeted sequencing data analysis portal. Our data analysis pipeline will perform mapping to the reference genome, UMI counting, read trimming (removing primer sequences), and variant identification. Alternatively, data from the QIAseq Targeted DNA Pro can be analyzed using CLC Genomics Workbench, which allows you to optimize analysis parameters to your specific panel. The parameters can then be locked for routine use. Contact your account manager for further details.

1. Log in to the GeneGlobe® Data Analysis Center at **https://geneglobe.qiagen.com/us/product-groups/qiaseq-targeted-dna-panels**
2. Make selection as highlighted in Figure 8.

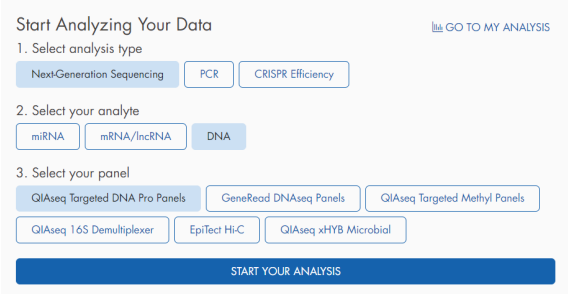


Figure 8.GeneGlobe Analysis pipeline selections for the QIAseq Targeted DNA Pro.

3. Click **START YOUR ANALYSIS**.

4. In the **Read Files** tab, select **BaseSpace** to upload files from **BaseSpace** or select **Uploaded** > **Upload New Files** to upload files from your local drive (Figure 9).

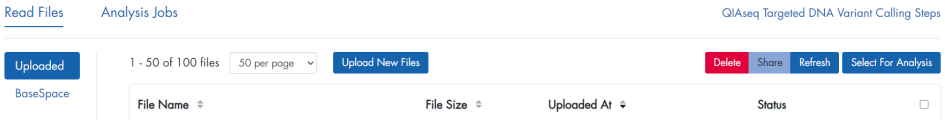


Figure 9. File Upload tab of the QIAseq Targeted DNA Pro Data Analysis Pipeline.

Note: All files that have been uploaded to GeneGlobe are listed under the **Read Files** tab. Using this tab, it is possible to delete files that are no longer needed and share files with collaborators.

5. Select the boxes next to the files that will be analyzed, and then click **Select For Analysis** (Figure 10).

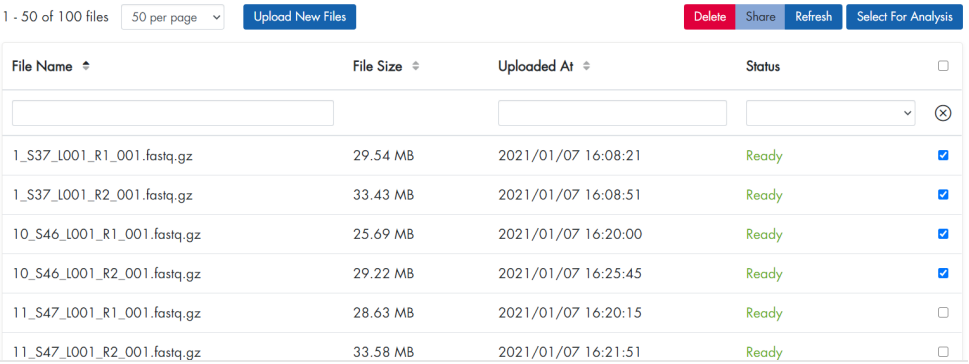


Figure 10. File selection for QIAseq Targeted DNA Pro data analysis pipeline.

Under the **Analysis Jobs** tab, configure the analysis per the drop-down menus as described below:

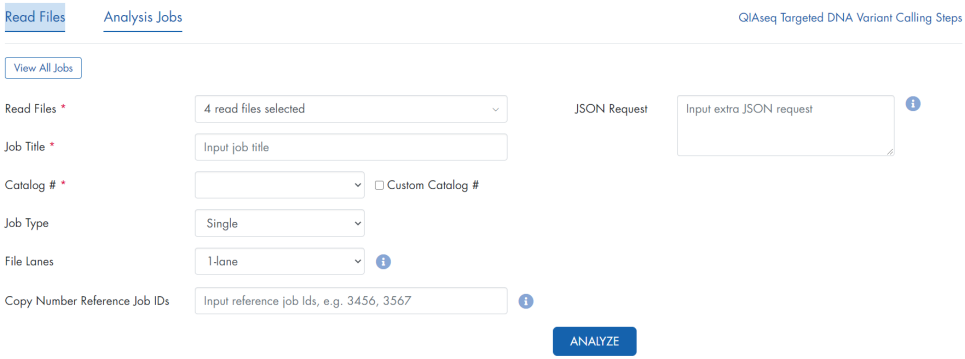


Figure 11. Analysis Jobs tab of the QIAseq Targeted DNA Pro data analysis pipeline.

- **Read Files**: Verify that the correct read files have been selected.
- **Job Title**: Enter a title for the analysis job.
- **Catalog #**: If using a catalog panel, select the number from the dropdown menu. If using a custom panel, enter the custom catalog number manually.
- **Job type**: Single or matched tumor/normal.
- **File lanes**:
  - For Illumina, choose **1-lane** if you set up your runs using MiSeq/HiSeq/NovaSeq/NextSeq concatenated.
  - Choose **4-lane** if you set up your runs using NextSeq individual lane files.
- **Copy Number Reference Job IDs**: For copy number analysis, normal sample(s) need(s) to be analyzed with the portal before case samples are set up. Enter the job ID corresponding to your control samples for copy number analysis.

6. Click **ANALYZE**. The analysis job status changes from **Queued**, to **In progress**, and then **Done successfully**.
7. Once the analysis is completed, output files can be downloaded by clicking **Download**.

   Note: Ultimately, detected variants can be interpreted with QCI Interpret.

Appendix D: Clean up TEPCR Reaction with QIAseq Bead

Keep QIAseq Beads on ice, don't warm up to room temperature. If mixing by pipetting, should pipette at close to maximum volume at least 12 times.

1. After TEPCR, transfer the samples to ice, mix each TEPCR reaction by pipetting, and transfer 20 µL from each reaction to a new PCR tube/plate on the ice.
2. On ice, add 80 µL H_2O to bring 20 µL reaction to 100 µL and mix, then add 100 µL QIAseq Beads on ice. Mix well by vortexing or pipetting up and down at least 12 times. Pipetting mix on ice and with pipetting volume about 190 µL.
3. Transfer tube/plate to room temperature, incubate for 5 min.
4. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates). After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.
5. With the beads still on the magnetic stand, add 200 µL 80% ethanol. Carefully remove and discard the wash.
6. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol after this second wash. Remove the ethanol with a 200 µL pipette first, and then use a 10 µL pipette to remove any residual ethanol.
7. With the beads still on the magnetic stand, air dry at room temperature for at least 10 min.

   Note: Visually inspect that the pellet is completely dry. Ethanol carryover to the next Universal PCR step will affect PCR efficiency. Over drying beads is fine and won't affect the elution.
8. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 28 µL nuclease-free water. Mix well by vortexing or pipetting.

9. Return the tube/plate to the magnetic rack until the solution has cleared.
10. Transfer 25 µL of the supernatant to clean tube/plate on ice.
11. Proceed with "Protocol: Universal PCR", page 36. Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer for up to 3 days.

Appendix E: Library Normalization with QIAseq Universal Normalizer Kit

Important points before starting

- Make sure your library yields are consistent with concentrations well above 15–20 nmol/L.
- Set up reactions on ice, mixing reactions by pipetting at close to maximum reaction volume on ice.
- Do not vortex any reagents or reactions.
- Prepare fresh 80% ethanol.
- 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.
- It is not required to equilibrate the QIAseq bead, Normalizer Reagent, and Normalizer Elution Buffer to room temperature before use.

Procedure

1. Set up a thermal cycler with a heated lid (>100°C) according to Table 22.

Table 22. Thermal cycling parameters

Step	Time	Temperature (°C)
Initial denaturation	2 min	98
2 cycles*	20 s	98
	30 s	60
	30 s	72
1 cycle	2 min	72
Hold	∞	4

* Perform 3-4 cycles, if your input library concentrations are generally low (<10 nmol/L) or only small volumes of input library (< 15 µL) are available.

2. On ice, prepare the library modification reaction mix according to Table 23. Mix the components in a PCR tube/strip or 96-well plate by pipetting at least 12 times with pipetting volume close to 50 µL.

Table 23. Reaction mix for Normalizer modification PCR

Component	Volume/reaction
HiFi PCR Master Mix, 2x	25 µL
Normalizer Primer Mix	1.5 µL
Finished QIAseq Targeted DNA Pro library	≤ 23.5 µL
RNase-free water	variable
Total	**50 µL**

3. Close PCR tube cap or seal plate with foil seal, transfer the PCR tube/strip or plate to the thermal cycler and start the program.
4. Once PCR is complete, transfer the whole reaction to a new PCR tube/plate and add 50 µL of homogenized QIAseq Beads to each reaction.
5. Mix by vortex or by thoroughly pipetting up and down at least 12 times at pipetting volume close to 100 µL. Then incubate the mixture for 5 min at room temperature.
6. Pellet the beads on a magnetic stand for 5 min or until the solution is clear.
7. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.
8. With the beads still on the magnetic stand, add 150 µL 80% ethanol. Carefully remove and discard the wash.
9. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol after this second wash. Remove the ethanol with a 200 µL pipette first, and then use a 10 µL pipette to remove any residual ethanol.
10. With the beads still on the magnetic stand, air dry at room temperature for at least 10 min.

Note: Visually inspect that the pellet is completely dry. Over drying beads is fine and won't affect the elution.

11. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 32.5 µL nuclease-free water. Mix well by vortexing or pipetting.
12. Return the tube/plate to the magnetic rack until the solution has cleared.
13. Transfer 30 µL of the supernatant to clean tube/plate on ice.
14. The collected supernatant contains the modified library and can be used for normalization. Proceed to QIAseq Library Normalization below. Alternatively, modified libraries can be stored for later use at –20°C.
15. Homogenize the bottle(s) of Normalizer Wash Buffer by vortexing. Then prepare an aliquot of wash buffer in 15 mL or 50 mL Falcon tubes and preheat to 55°C using a water bath or a heating block compatible with the receptacle. Prepare 400 µL of wash buffer × number of reactions (e.g., for 72 reactions, prepare 28.8 mL). Leave the wash buffer at 55°C until use.
16. Thoroughly mix the Normalizer Reagent by vortexing for 60 s. Make sure the bead pellet is completely dissolved and the solution is well homogenized.
17. 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.
18. Transfer 5 µL of homogenized Normalizer Reagent to a fresh PCR tube/plate using a 10 µL pipette or compatible repetitive pipette. Prepare 1 well for every library to be normalized.
19. Transfer 15 µL of the modified library to be normalized to the PCR tube/plate.
20. Seal the plate using a sealing foil or closed the tube cap and vortex to mix. Then, gently tap the tube/plate on the benchtop to collect most liquid.
21. Incubate for 10 min at room temperature to bind library fragments to the beads.
22. After the incubation is complete, pulse spin the tube/plate to collect all liquid at the bottom.
23. Add 180 µL pre-warmed Normalizer Wash Buffer to each tube/well.
24. Pellet the beads on a plate-format magnetic stand for 2 minutes or until the solution is clear.

Important: Make sure all beads have pelletized. If you are uncertain, leave the tube/plate on the magnet for 5 min.

25. Carefully discard the supernatant without disturbing the pellet. Leave the tube/plate on the magnet.
26. Add 200 µL pre-warmed Normalizer Wash Buffer to each well.
27. Move the tube/plate to switch positions on the magnet and force the beads to the opposite side of the tube/plate. Switch the tube/plate position 3 times to thoroughly wash the beads. Then wait until the solution is clear.

    Important: Make sure all beads have pelletized. If you are uncertain, leave the tube/plate on the magnet for 5 minutes.

28. Carefully discard the supernatant without disturbing the pellet. Then, using the same pipette, remove as much remaining liquid as possible. Proceed to the next step immediately.

    Note: Drying the beads is not required.

29. Remove the tube/plate from the magnet. Then, add 25 µL Normalizer Elution Buffer to each pellet.
30. Seal the plate using a sealing foil or close tube cap and mix well by vortexing. Make sure the pellet is completely dissolved.
31. Pulse spin the tube/plate at low force.

    Important: If a compact bead pellet has formed after pulse spin, vortex again to mix and pulse spin with smaller force.

32. Place the tube/plate in a thermal cycler and incubate for 5 min at 55°C with a heated lid.
33. Remove the plate from the thermal cycler and pulse spin to collect all liquid at the bottom, then pellet the beads on a magnetic stand for 2 minutes and wait until the solution is clear.
34. Carefully transfer 22 µL of the supernatant to a new tube/plate without aspirating beads.

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

35. Proceed to “Protocol: Sequencing Setup on Illumina MiSeq, NextSeq 500/550, NextSeq 1000/2000, MiniSeq, and NovaSeq”. Alternatively, the normalized libraries can be stored at –20°C for up to 3 months.

Ordering Information

Product	Contents	Cat. no.
QIAseq Targeted DNA Pro (12)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 12 samples; less than 200 genes	333651
QIAseq Targeted DNA Pro (96)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 96 samples; less than 200 genes	333655
QIAseq Targeted DNA Pro HC (12)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 12 samples; more than 200 genes	333661
QIAseq Targeted DNA Pro HC (96)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 96 samples; more than 200 genes	333665
QIAseq Targeted DNA Pro Custom (96)	All reagents (except indexes) for targeted DNA sequencing; custom panel for 96 samples	333675
QIAseq Targeted DNA Pro Booster (96)	Pool of primers used in combination with either catalogued or custom panels	333685
QIAseq Targeted DNA Pro Unique Dual Indices		
QIAseq Targeted DNA Pro UDI Set A (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set A (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	333455
QIAseq Targeted DNA Pro UDI Set B (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set B (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	333465

Product	Contents	Cat. no.
QIAseq Targeted DNA Pro UDI Set C (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set C (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	333475
QIAseq Targeted DNA Pro UDI Set D (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set D (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	333485
QIAseq Targeted DNA Pro UDI Set E (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set E (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	334015
QIAseq Targeted DNA Pro UDI Set F (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set F (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	334025
QIAseq Targeted DNA Pro UDI Set G (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set G (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	334035
QIAseq Targeted DNA Pro UDI Set H (96)	Box containing unique molecularly indexed adapters and primers, enough for a total of 96 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set H (of A, B, C, D, E, F, G, and H) required for multiplexing 768 samples in one run	334045

Product	Contents	Cat. no.
QIAseq Targeted DNA Pro UDI (12)	Box containing unique molecularly-indexed adapters and primers, enough for a total of 12 samples, for indexing up to 12 samples for targeted panel sequencing on Illumina platforms	333441
Related products		
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; assay format	333314
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
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
QIAamp Circulating Nucleic Acid Kit (50)	For 50 DNA preps: QIAamp Mini Columns, Tube Extenders (20 mL), QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors, and Collection Tubes (1.5 mL and 2 mL)	55114
QIAamp DNA FFPE Advanced UNG Kit (50)	For 50 preps: Uracil-N-glycosylase, QIAamp UCP MinElute columns, collection tubes, Deparaffinization Solution, Proteinase K, RNase A, RNase-free water and buffers	56704

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Document Revision History

Date	Changes
11/2021	Initial revision
12/2022	Corrected information in Tables, adjusted template, and style according to revised brand style guide

07/2024	• Updated Tables 3, 4, and 5
	  ○ Deleted Hiseq 2500, 3000, and 4000
	  ○ Added NovaSeq X
	• Updated Table 14 by adding <2000 and >=2000 primers temperature. Also removed the footnote "Use 67 °C for panels PHS-102Z, PHS-104Z, PHS-203Z, PHS-204Z and PHS-205Z".
	• Added QIAseq Targeted DNA Pro UDI Set E, F, G, and H (96) to title and footnote of Table 18.
	• Added QIAseq Targeted DNA Pro (96) primer plates: E, F, G, and H.
	• Added the following sections:
	  ○ Appendix D: Appendix D: Clean up TEPCR Reaction with QIAseq Bead
	  ○ Appendix E: Library Normalization with QIAseq Universal Normalizer Kit

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