November 2021

QIAseq® Targeted DNA Pro Handbook

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

Sample to Insight

QIAGEN

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	1700 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 Catalog no. No. of samples	(96) 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	Inquire	Inquire
PHS-201Z	Hereditary Breast and Ovarian Cancer Panel	Inquire	Inquire
PHS-202Z	Hereditary Colorectal Cancer Panel	Inquire	Inquire
PHS-203Z	Hematologic Malignancy Panel	Inquire	Inquire
PHS-204Z	Hereditary Prostate Cancer Panel	Inquire	Inquire
PHS-205Z	Hereditary Pancreatic Cancer Panel	Inquire	Inquire

* 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*	**Set A**	**Set B**	**Set C**	**Set D**
Catalog no.	**333455**	**333465**	**333475**	**333485**
No. of samples	**96**	**96**	**96**	**96**
DNA Pro UDI Set A Final Plate †	9 µl	N/A	N/A	N/A
DNA Pro UDI Set B Final Plate †	N/A	9 µl	N/A	N/A
DNA Pro UDI Set C Final Plate †	N/A	N/A	9 µl	N/A
DNA Pro UDI Set D Final Plate †	N/A	N/A	N/A	9 µl
AdP-IL5-Phased Adapter	180 µl	180 µl	180 µl	180 µl
SmP-IL5 TEPCR-F Primer	240 µl	240 µl	240 µl	240 µl

* 10 bp dual indices.

† Index Primer Plate (DNA Pro UDI Set A, B, C, and D Final Plate); 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.	**333441**
No. of samples	**12**
DNA Pro UDI 12 Index Final Plate †	9 µl
AdP-IL5-Phased Adapter	25 µl
SmP-IL5 TEPCR-F Primer	32 µl

* 10 bp dual indices.

† Index Primer Plate (DNA Pro UDI 12 Index Final Plate); 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 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 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 to 80 ng for fresh DNA or 100 to 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 been optimized also in combination with a specially formulated enrichment chemistry to achieve highly efficient enrichment on both regular and GC-rich regions at high multiplex levels. In addition, 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).

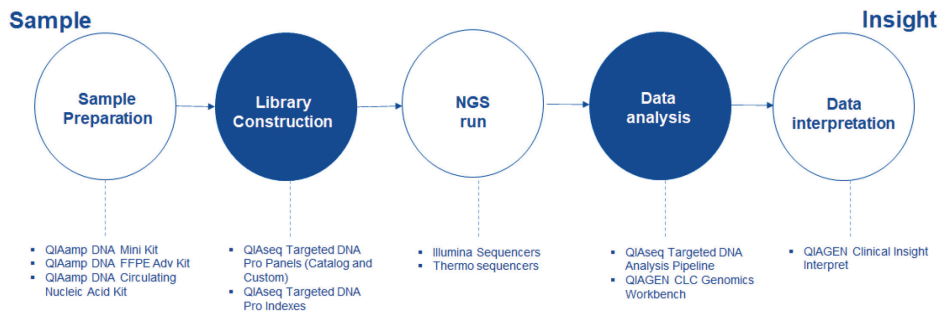


Figure 1. Overview of the Sample to Insight NGS workflow with the QIAseq Targeted DNA Pro. The complete Sample to Insight procedure begins with DNA extraction. Next is library construction and target enrichment with the QIAseq Targeted DNA Pro. Following NGS, data analysis is performed using the QIAseq 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 to 80 ng fresh DNA or 100 to 250 ng FFPE DNA (Figure 2). Lower input amounts are possible; however, this will lead to fewer sequenced UMI and reduced variant detection sensitivity.

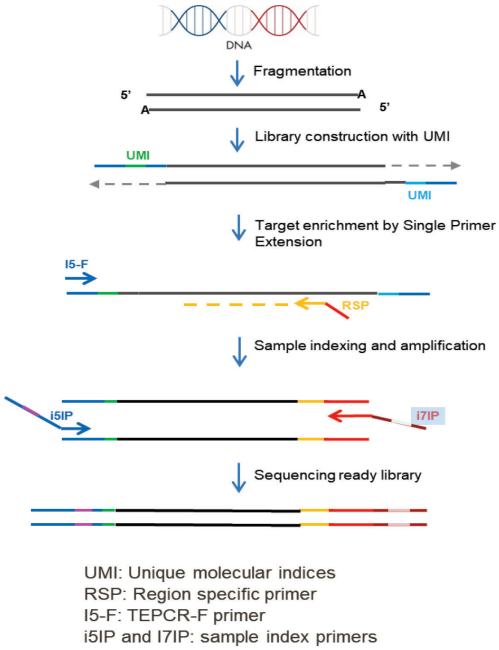


Figure 2. QIAseq Targeted DNA Pro workflow.

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 post-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. In addition, more consistent library construction can be achieved with the enzymatic cleanups since there are no more highly variable bead cleanups after the ligation and target enrichment PCR reactions. 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, as well as reducing the impact of low-level contamination during oligo synthesis and kit manufacturing, as well as carry over on the Illumina sequencing instrument itself. Hence, each sample will be assigned two unique indices to overcome the error introduced by image analysis, sequencing error, demultiplexing, and oligo synthesis contamination to reduce the reads mis-assignment 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. 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.

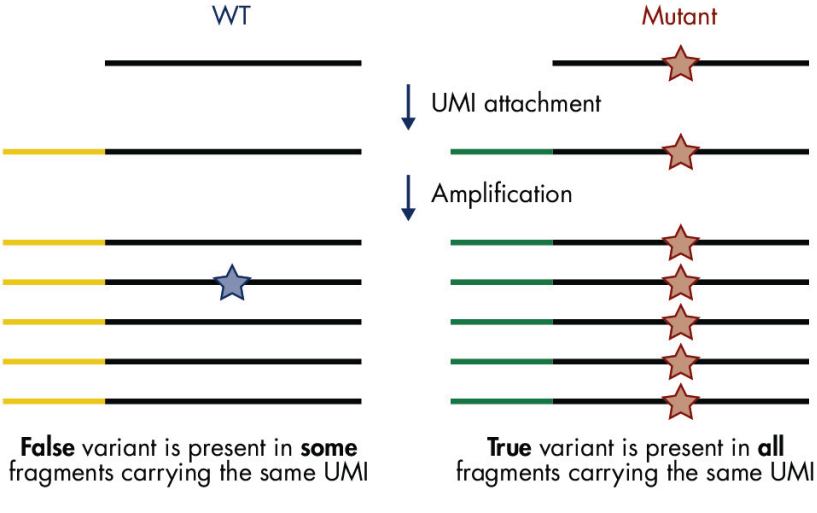


Figure 3. Principle of variant detection with UMIs. Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while false positives are mutations present in only one or a few reads within a UMI.

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, the QIAseq Targeted DNA Panel Analysis pipeline is available at **https://geneglobe.qiagen.com/us/analyze**. The pipeline automatically performs all steps necessary to generate a DNA sequence variant report from your raw 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
- 1.5 ml LoBind® tubes (Eppendorf®, cat. no. 022431021)
- PCR tubes (0.2 ml individual PCR tubes [VWR, cat. no. 20170-012], or tube strips [VWR, cat. no. 93001 118]) or 96-well PCR plates and caps
- Ice
- Microcentrifuge
- Thermal cycler
- Multichannel pipettes
- Single-channel pipettes
- QIAxcel® or Agilent® 2100 Bioanalyzer® (Agilent, cat. no. G2939BA) or Agilent TapeStation® (Agilent, cat. no. G2991AA)
- 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® 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. Do not omit the recommended RNase treatment step to remove RNA.

Note: If genomic DNA samples must be harvested from biological samples for which kits are not available, please contact 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 50) 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 consequence, 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 (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. This is enabled by filtering misassigned reads during the demultiplexing of individual samples, thus generating highly accurate output data.

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 in different sequencing platforms, based on panel size and read depth (Table 3, Table 4, and Table 5). Fine-tuning the read depth is possible after the first run.

Table 3. Number of multiplexed samples based on panel size with 500x mean 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
HiSeq 2500 rapid run	Dual flow cell v2	1200 M	2400	960	480	200
HiSeq 3000	8 Lanes per flow cell	5 B	10,000	4000	2000	833
HiSeq 4000	8 Lanes per flow cell	10 B	20,000	8000	4000	1666
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

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

Table 4. Number of multiplexed samples based on panel size with 2500x mean 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
HiSeq 2500 rapid run	Dual flow cell v2	1200 M	480	192	96	40
HiSeq 3000	8 Lanes per flow cell	5 B	2000	800	400	166
HiSeq 4000	8 Lanes per flow cell	10 B	4000	1600	800	333
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

* 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 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
HiSeq 2500 rapid run	Dual flow cell v2	1200 M	60	24	12	5
HiSeq 3000	8 Lanes per flow cell	5 B	250	100	50	20
HiSeq 4000	8 Lanes per flow cell	10 B	500	200	100	40
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

* 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.
- Do not vortex any reagents or reactions.

Procedure: FFPE DNA repair

Note: For FFPE DNA, set up a repair reaction first before doing "Fragmentation and End Prepare". For standard and cfDNA, go directly to "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 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 volume of master mix 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.

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 10–12 times 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 volume of master mix 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.

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 27.

Protocol: Adapter Ligation

Important points before starting

- The roughly 14 µl product from "Fragmentation and End Prepare", page 25, is the starting material for this protocol.
- Set up reactions 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 10–12 times 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 volume of master mix 10% greater than what is required for the total number of reactions.

Table 10. Reaction mix for adapter ligation

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

3. Program the thermal cycler according to Table 11.

   Important: Do not use the heated lid during the 20°C incubation step. Alternatively, can set up lid temperature 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 7–8 times and briefly centrifuge again.

2. Program the thermal cycler according to Table 12. Use the instrument's heated lid.

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 30.

   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 27, is the starting material for this protocol.
- Set up reactions 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. Keep QN Taq Polymerase on ice.
   1b. 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 7–8 times, and briefly centrifuge again.

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

Table 13. Reaction mix for Target Enrichment

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

1. 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).

Table 14. 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 2 min	98 65
1 cycle	3 min	72
Hold	∞	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

2. Place the target enrichment reaction in the thermal cycler and start the run.

3. After the reaction is complete, place the reactions on ice and proceed to "Procedure: TEPCR cleanup reaction" below. 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.

   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 7–8 times 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.

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 34.

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

Protocol: Universal PCR

Important points before starting

- The 25 µl product from "Protocol: Target Enrichment", page 30, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- **Important:** The QIAseq Targeted DNA Pro UDI (12) or QIAseq Targeted DNA Pro UDI Set A (96), Set B (96), Set C (96), or Set D (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 and no need to bring beads to room temperature before use.
- **Important:** 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; or 96-Index Plate on ice or at room temperature but immediately place on ice after being thawed. Keep QN Taq Polymerase on ice.
   1b. 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, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

3. For the QIAseq Targeted DNA Pro UDI plates, pierce the foil seal associated with each well that will be used, and transfer 5 µl (each well contains a forward primer and a reverse primer, each with a unique index) to the cleaned target-enriched DNA from "TEPCR cleanup reaction" sample tube/plate according to Table 18. Mix by pipetting up and down 7–8 times 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, and D (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, and D (96).

DNA Pro UDI 12 Index Plate 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 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 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 in QIAseq Targeted DNA Pro UDI Set C (96)

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

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

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

4. Program a thermal cycler using the cycling conditions in Table 19, and Table 20 indicates the cycle number to use dependent on the number of primers in the pool.

Table 19. Incubation conditions for universal PCR

Step	Time	Temperature (°C)
Initial denaturation	2 min	98
Number of cycles (see Table 20)	15 s	98
	30 s	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	32
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". 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. Add 80 µl QIAseq Beads to the completed universal PCR reaction; mix well by vortexing or pipetting up and down several times.

2. Incubate for 5 min at room temperature.

3. Place the tubes/plate on magnetic rack for 5 min 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 H_2O 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 several times.

6. Return the tubes/plate to the magnetic rack for 5 min. 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 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.

13. Proceed to “Recommendations: Library QC and Quantification”, page 40. Alternatively, the library can be stored at –30 to –15°C in a constant-temperature freezer. Amplified libraries are stable for several months at –30 to –15°C. Once quantification is performed, proceed to “Protocol: Sequencing Setup on Illumina MiSeq, NextSeq 500/550, MiniSeq, and NovaSeq”.

Recommendations: Library QC and Quantification

NGS library QC

After the library is constructed and purified, QC can be performed with QIAGEN's 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 and 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 42).

Recommended setting for checking QIAseq DNA Pro library on QIAxcel

- Refer to the Application Guide for Low-Concentration Libraries. To access guides and system files for Library QC, contact QIAGEN Technical Services.
- QIAxcel Cartridge: QIAxcel DNA High Resolution Kit (cat. no. 929002)
- QX Alignment Marker: 15 bp/10 kb (cat. no. 929523) or 15 bp/5 kb (cat. no. 929524)
- QX DNA Size Marker: 100bp – 2.5kb (cat. no. 929559)

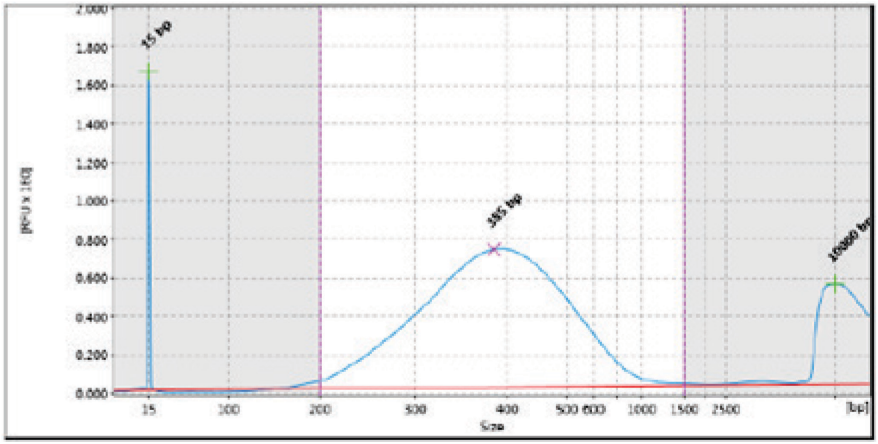
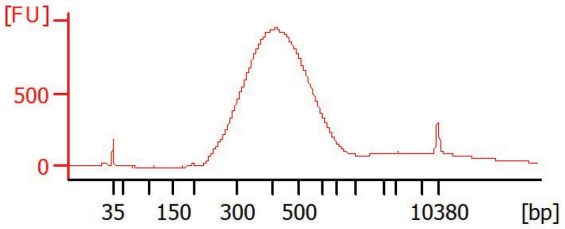


Figure 5. Sample QIAxcel image of QIAseq Targeted DNA Pro libraries for Illumina instruments. The library assessed using QIAxcel illustrates the size of the majority of the library fragments are between 250 and 1000 bp.

A: Library (without overamplification) prepared for Illumina instruments



B: Library (with overamplification) prepared for Illumina instruments

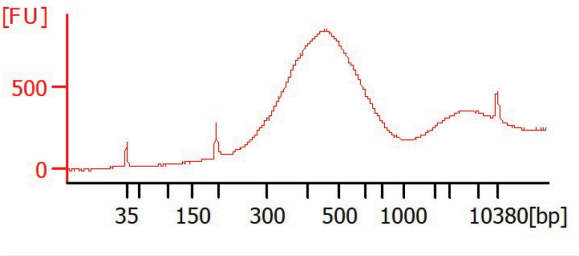


Figure 6. Sample Bioanalyzer images of QIAseq Targeted DNA Pro libraries for Illumina instruments. The size of the majority of the library fragments are between 250 and 1000 bp. A: Library without overamplification. B: Library with overamplification as indicated by the "larger fragment" peak.

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, QIAGEN's 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, MiniSeq, and NovaSeq

Important points before starting

- **Important:** Recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System (see Preferred library quantification method, page 42).
- **Important:** Paired-end sequencing **should** be used for the QIAseq Targeted DNA Pro on Illumina platform.
- **Important:** 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.
- **Important:** Paired-end sequencing of 149 bp should be used for QIAseq Targeted DNA Pro UDI libraries and dual 10 bp indices on Illumina platforms.
- **Important**: 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.

   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.

4. Upon completion of the sequencing run, proceed to Appendix C, page 52.

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

1. When working with the QIAseq Targeted DNA Pro custom QIAseq 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 and 0.5, 1, 2, or 4 nM for NextSeq. 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.

   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 or NextSeq 500/550 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.

   Note: Recommendations for library loading concentrations are based on QIAseq Library Quant System.

4. Upon completion of the sequencing run, proceed to Appendix C, page 52.

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.

   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.

4. Upon completion of the sequencing run, proceed to Appendix C, page 52.

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).

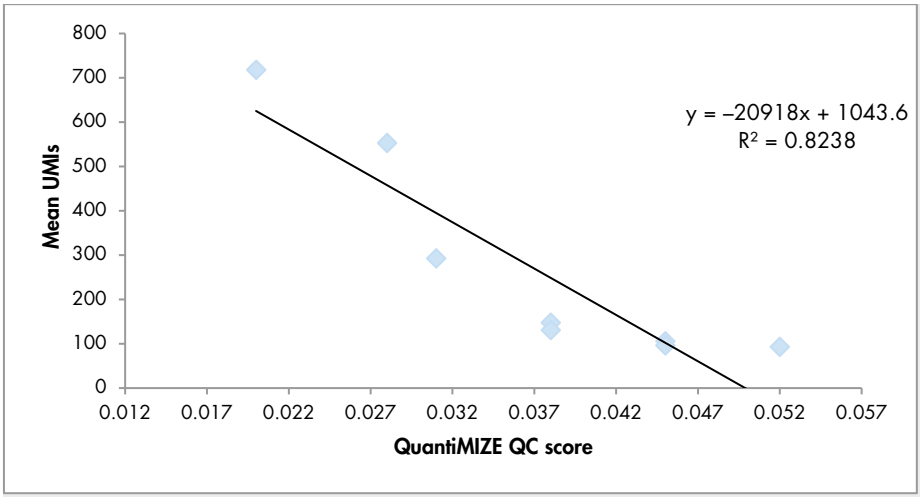


Figure 7. Correlation between QIAseq QuantiMIZE QC score and the number of UMIs.

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 DNA 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 QIAGEN's 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.

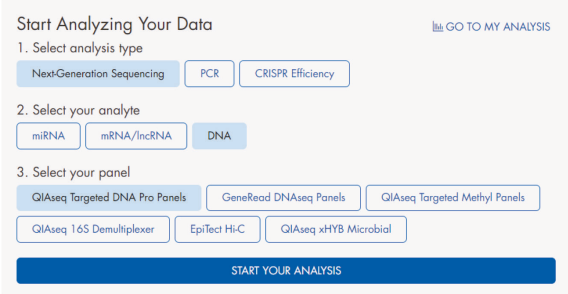


Figure 8. GeneGlobe Analysis pipeline selections for the QIAseq Targeted DNA Pro.

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).

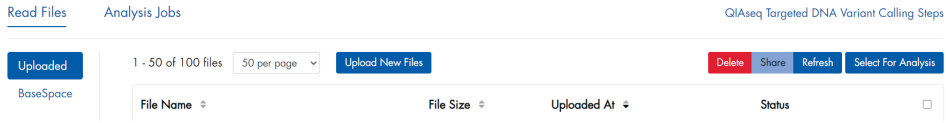


Figure 9. File Upload tab of the QIAseq Targeted DNA Pro Data Analysis Pipeline.

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).

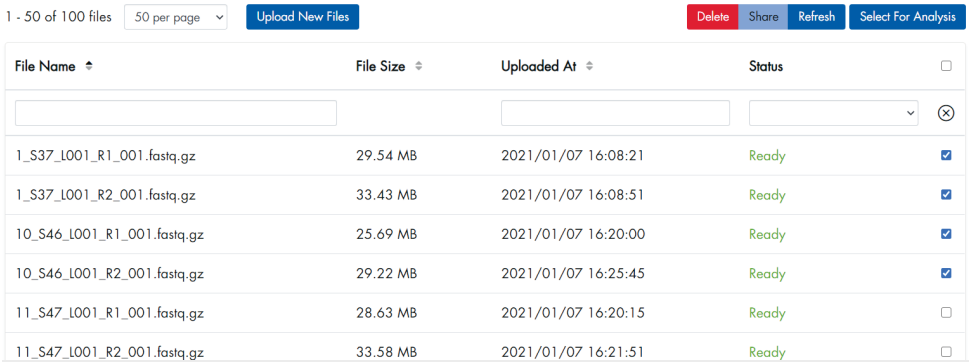


Figure 10. File selection for QIAseq Targeted DNA Pro data analysis pipeline.

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

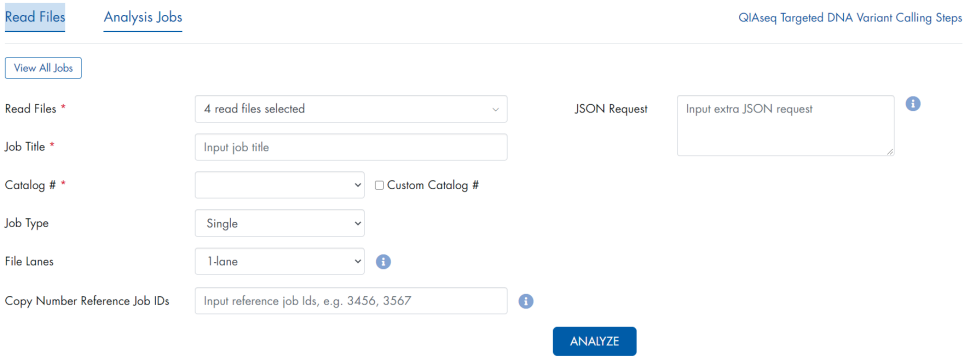


Figure 11. Analysis Jobs tab of the QIAseq Targeted DNA Pro data analysis pipeline.

- **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.

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 cataloged or custom panels	333685

Product	Contents	Cat. no.
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, and D) required for multiplexing 384 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, and D) required for multiplexing 384 samples in one run	333465
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, and D) required for multiplexing 384 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, and D) required for multiplexing 384 samples in one run	333485
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

Product	Contents	Cat. no.
Related products		
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; assay format	333314
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

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