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QIAseq® Targeted DNA Panel Handbook

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

Sample to Insight

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Kit Contents

QIAseq Targeted DNA Panel Catalog no. Number of samples	 333502 12	HC 333512 12	 333505 96	HC 333515 96	Extended 333545 96	Custom 333525 96
One pool of region-specific primers	75 µL	75 µL	600 µL	600 µL	600 µL	600 µL
Fragmentation Buffer, 10x	40 µL	40 µL	300 µL	300 µL	300 µL	300 µL
Fragmentation Enzyme Mix	75 µL	75 µL	600 µL	600 µL	600 µL	600 µL
FERA Solution	15 µL	15 µL	110 µL	110 µL	110 µL	110 µL
FG Solution	170 µL	170 µL	170 µL	170 µL	170 µL	170 µL
Ligation Buffer, 5x	160 µL	160 µL	1250 µL	1250 µL	1250 µL	1250 µL
DNA Ligase	75 µL	75 µL	600 µL	600 µL	600 µL	600 µL
Ligation Solution	125 µL	125 µL	970 µL	970 µL	970 µL	970 µL
Nuclease-free Water	1.5 ml	1.5 ml	10 ml	10 ml	10 ml	10 ml
TEPCR Buffer, 5x	60 µL	60 µL	500 µL	500 µL	500 µL	500 µL
UPCR Buffer, 5x	60 µL	60 µL	500 µL	500 µL	500 µL	500 µL
HotStarTaq® DNA Polymerase	30 µL	30 µL	240 µL	240 µL	240 µL	240 µL
One bottle containing QIAseq Beads	10 mL	10 mL	55 mL	55 mL	55 mL	55 mL

QIAseq Targeted DNA Booster Panel **Catalog no.** **Number of samples**	**(96)** **333535** **96**
One pool of region-specific primers	80 µL

Cat. no.	Product name	Total number of primers*	Panel size (bases)
DHS-001Z	Human Breast Cancer Panel	4831	370,942
DHS-002Z	Human Colorectal Cancer Panel	2929	215,328
DHS-003Z	Human Myeloid Neoplasms Panel	5887	436,672
DHS-005Z	Human Lung Cancer Panel	4149	318,059
DHS-101Z	Human Actionable Solid Tumor Panel	651	15,160
DHS-102Z	Human BRCA1 and BRCA2 Panel	223	16,405
DHS-103Z	Human BRCA1 and BRCA2 Plus Panel	348	25,590
DHS-104Z	Human Pharmacogenomics Panel	146	3313
DHS-105Z	Human Mitochondria Panel	222	16,570
DHS-110Z	Human HRR Panel	2303	56,485
DHS-3011Z	Human Inherited Disease Panel	11,579	838,627
DHS-3013Z	Human Cancer Predisposition Panel	5587	411,158
DHS-3501Z	Human Comprehensive Cancer Panel	11,311	836,670
DHS-6600Z	Tumor Mutational Burden Panel	19,121	1,335,689
DHS-7700Z	Human MSI Panel	92	5382
DHS-8800Z	Human TMB and MSI Panel	19,213	1,314,071

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

QIAseq Unique Dual Indices (UDI) V2 (96)

QIAseq Unique Dual Index Set V2* Catalog no. Number of samples	QIAseq UDI Set A V2 (96) 331745 96	QIAseq UDI Set B V2 (96) 331755 96	QIAseq UDI Set C V2 (96) 331765 96	QIAseq UDI Set D V2 (96) 331775 96
AUDI-96AX†	10 µL	N/A	N/A	N/A
AUDI-96BX†	N/A	10 µL	N/A	N/A
AUDI-96CX†	N/A	N/A	10 µL	N/A
AUDI-96DX†	N/A	N/A	N/A	10 µL
XUDI-96AX§	9 µL	N/A	N/A	N/A
XUDI-96BX§	N/A	9 µL	N/A	N/A
XUDI-96CX§	N/A	N/A	9 µL	N/A
XUDI-96DX§	N/A	N/A	N/A	9 µL
IL-Forward Primer (384)	310 µL	310 µL	310 µL	310 µL
QIAseq A Read1 Primer I (100 µM)	24 µL	24 µL	24 µL	24 µL

* 10 bp dual indices.

† Adapter Plate (AUDI-96AX, AUDI-96BX, AUDI-96CX, or AUDI-96DX); each plate contains 96 molecularly indexed adapters, with each well corresponding to one AUDI### sample index; each index is single-use.

§ Index Primer Plate (XUDI-96AX, XUDI-96BX, XUDI-96CX, or XUDI-96DX); each plate well contains one XUDI### index primer and IL-universal PCR primer pair for PCR amplification and sample indexing; each index is single-use.

QIAseq 96-Unique Dual Indices

QIAseq 96-Unique Dual Index Set* Catalog no. Number of samples	QIAseq 96-Unique Dual Index Set A 333725 384	QIAseq 96-Unique Dual Index Set B 333735 884
UDIN-96AA†	4 x 10 µL	N/A
UDIN-96BA†	N/A	4 x 10 µL
UDIS-96AK§	4	N/A
UDIS-96BK§	N/A	4
IL-Forward Primer (384)	310 µL	310 µL
QIAseq A Read1 Primer I (100 µM)	4 x 24 µL	4 x 24 µL
8-cap strips (24/bag)	4	4

* 10 bp dual indices.

† Adapter Plate (UDIN-96AA or UDIN-96BA); each plate contains 96 molecularly indexed adapters, with each well corresponding to one NQDIB### sample index; each index is single-use.

§ Dried Primer Plate (UDIS-96AK or UDIS-96BK); each array well contains one SQDIB### index primer and IL-universal PCR primer pair for PCR amplification and sample indexing; each kit can process up to 384 total samples.

QIAseq Combinatorial Dual Indices

QIAseq 12-Index I* (12 sample index for 48 samples on Illumina® platform) Catalog no. Number of samples	(48) 333714 48
IL-N7## Adapter contains 12 tubes of molecularly indexed adapters, with each tube corresponding to one sample index; each index can be used for up to 4 samples	20 µL
IL-S502 Index Primer	40 µL
IL-Forward Primer	40 µL
IL-Universal Primer	40 µL
QIAseq A Read1 Primer I (100 µM)	24 µL

* 8 bp dual indices.

QIAseq 96-Index I Set A, B, C, or D* Catalog no. No. of sample	QIAseq 96-Index I Set A 333727 384	QIAseq 96-Index I Set B 333737 384	QIAseq 96-Index I Set C 333747 384	QIAseq 96-Index I Set D 33757 384
IL-701NJ†	160 µL	N/A	160 µL	N/A
IL-716NJ†	N/A	160 µL	N/A	160 µL
IL-502SK§	4	4	N/A	N/A
IL-513SK§	N/A	N/A	4	4
IL-Forward Primer (384)	310 µL	310 µL	310 µL	310 µL
QIAseq A Read1 Primer I (100 µM)	4 x 24 µL	4 x 24 µL	4 x 24 µL	4 x 24 µL
12-cap strips	16	16	16	16

* 8 bp dual indices

† Adapter Plate (IL-7##NJ); each plate contains 12 molecularly indexed adapters, with each well corresponding to one sample index; each index can be used for up to 4 samples.

§ Dried Primer Plate with 4 index primer arrays (IL-5##K); each array well contains one IL-5## index primer and IL-universal PCR primer pair for PCR amplification and sample indexing; each kit can process up to 384 total samples.

QIAseq 8-Unique Dual Indices

QIAseq 8-Unique Dual Index Set* Catalog no. Number of samples	QIAseq 8-Unique Dual Index Set A 333715 48	QIAseq 8-Unique Dual Index Set B 333716 48
UDIN-8AA†	40 µL	N/A
UDIN-8BA†	N/A	40 µL
UDIS-8AK§	Dried	N/A
UDIS-8BK§	N/A	Dried
IL-Forward Primer (48)	40 µL	40 µL
QIAseq A Read1 Primer I (100 µM)	24 µL	24 µL
8-cap strips (24/bag)	4	4

* 8 bp dual indices.

† Adapter Plate (UDIN-8AA or UDIN-8BA); each plate contains 8 molecularly indexed adapters, with each well corresponding to one IL-N7## sample index; each index can be used for up to 6 samples.

§ Dried Primer Plate with 6 index primer arrays (UDIS-8AK or UDIS-8BK); each array well contains one IL-S5## index primer and IL-universal PCR primer pair for PCR amplification and sample indexing; each kit can process up to 48 total samples.

Shipping and Storage

QIAseq Targeted DNA Panels (except QIAseq Beads and Ligation Solution) 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 Ligation Solution are shipped on cold packs. The QIAseq Beads should be stored at 2–8°C. The Ligation Solution should be removed immediately upon receipt and stored at –30°C to –15°C in a constant-temperature freezer.

The QIAseq Index kits are shipped on dry ice and should be stored at –30°C to –15°C upon arrival. When stored correctly, the QIAseq Index kits are good until the expiration date printed on the kit label.

Intended Use

The QIAseq Targeted DNA Panels and QIAseq Index Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease. These products are for research use only.

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 Panel is tested against predetermined specifications, to ensure consistent product quality.

Introduction

The QIAseq Targeted DNA Panels enable Sample to Insight®, targeted next-generation sequencing (NGS) of DNA. This highly optimized solution facilitates ultrasensitive variant detection using integrated unique molecular indices (UMIs) from cells, tissue, and biofluids. The required amount of template for a single QIAseq targeted 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 Panels overcome these biases/artifacts by utilizing a highly optimized reaction chemistry whereby UMIs are integrated into a single gene-specific, primer-based targeted enrichment process. The QIAseq Targeted DNA Panels have 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. In addition, the panels are 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 Panels are a Sample to Insight solution for precision variant detection of targeted genomic regions using NGS (see Figure 1).

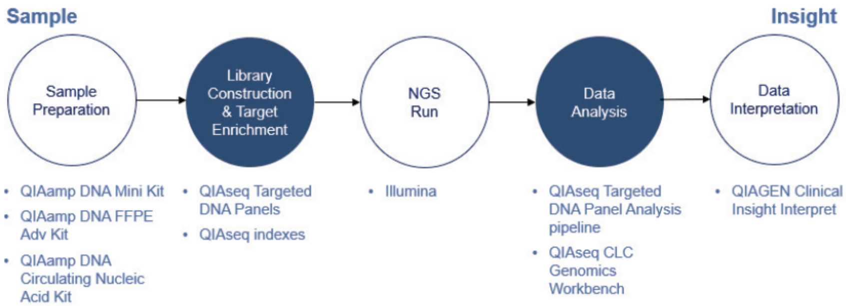


Figure 1. Overview of the Sample to Insight NGS workflow with the QIAseq Targeted DNA Panels. The complete Sample to Insight procedure starts with DNA extraction followed by library construction and target enrichment using QIAseq Targeted DNA Panels. After the NGS run, data analysis is performed using the QIAseq Targeted DNA Panel 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 Panels are provided as single tube primer mixes, with up to 20,000 primers per panel. The QIAseq Targeted DNA Panels are designed to enrich selected genes and regions using 10 to 80 ng fresh DNA or 100 to 250 ng FFPE DNA (see Figure 2). Lower input amounts are possible; however, this will lead to fewer sequenced UMI and reduced variant detection sensitivity.

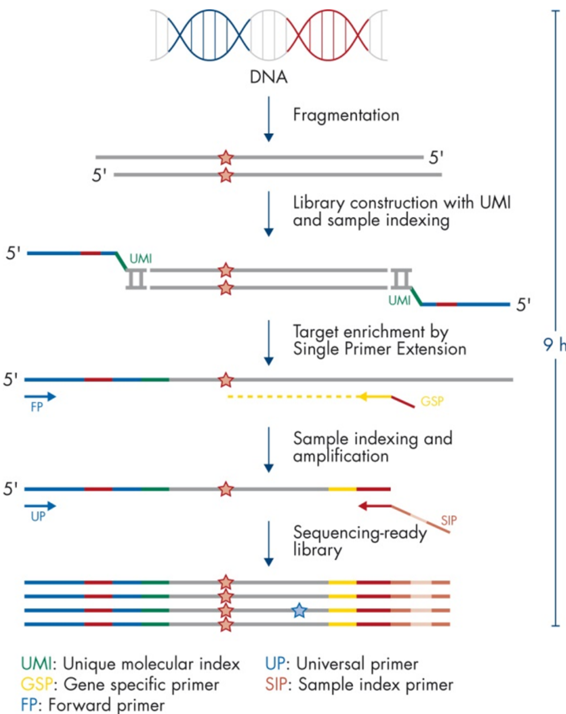


Figure 2. QIAseq Targeted DNA Panels workflow.

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 UMIs and sample index.

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. In addition, this ligated adapter also contains the first sample index.

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 additional sample indices.

NGS adapter and index technologies

The QIAseq Targeted DNA Panel Library Kits have the option of using either unique dual index (UDI) adapters or combinatorial dual index (CDI) adapters. The UDI adapters 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 mitigate errors from image analysis, sequencing error, demultiplexing, and oligo synthesis contamination, reducing reads mis-assignment to wrong samples.

Next-generation sequencing

The QIAseq Targeted DNA Panels are compatible with most medium- and high-throughput sequencers including Illumina NGS systems (MiniSeq®, MiSeq®, NextSeq® 500/550, HiSeq® 2500, HiSeq 3000/4000, and NovaSeq™ 6000). When using Illumina NGS systems, the QIAseq Targeted DNA libraries require a custom sequencing primer for Read 1 (QIAseq A Read 1 Primer I).

Principle of variant detection with UMIs

The principle of variant detection with UMIs is described in Figure 3. Indexed molecules may be amplified unevenly across the target regions due to intrinsic noise and sequence-dependent bias. 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.

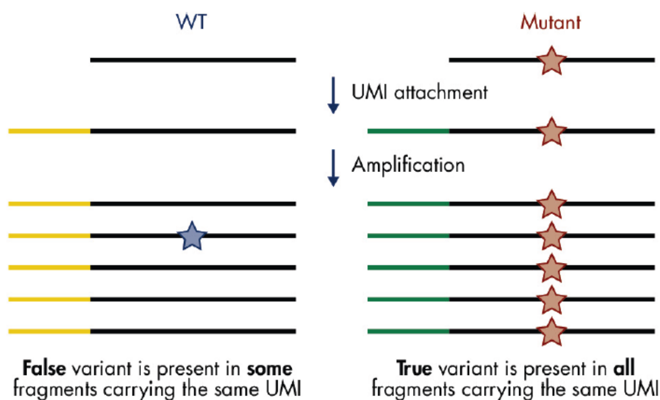


Figure 3. Principle of variant detection with UMIs. Each original molecule is tagged by a UMI. True variants are mutations present in the majority of reads within a UMI, while false positives are mutations present in only one or a few reads within a UMI.

Data analysis

The data for the QIAseq Targeted DNA Panels 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/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 the 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 Panels and the QIAseq Index Kit, the following are required:

- Ethanol, 80% (made fresh daily)[1]
- 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. 333404 or 333414)
- QIAxpert® or Thermo Fisher Scientific Qubit Fluorometer
- Quant-iT™ dsDNA Assay Kit (Thermo Fisher Scientific, cat. no. Q33120)

[1] 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 Kit	Animal/human tissues and cells	56604
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 or 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.

DNA quantification can also be performed using the high-sensitivity Quant-iT™ dsDNA Assay Kit (Thermo Fisher Scientific, cat. no. Q33120).

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 Panels. "Appendix B: FFPE DNA Quality and Quantity" 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 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 region of the QIAseq Targeted DNA Panel.

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 index sequences into the individual adapter molecules.

The QIAseq Targeted DNA Panel Library Kits include either a fully compatible UDI or CDI indexing solution. We recommend using the QIAseq 96-Unique Dual Index Sets. Each QIAseq Targeted DNA Panel Library Index Kit includes one of the following:

- QIAseq Unique Dual Index Set A V2 (96): AUDI-96AX paired with XUDI-96AX
- QIAseq Unique Dual Index Set B V2 (96): AUDI-96BX paired with XUDI-96BX
- QIAseq Unique Dual Index Set C V2 (96): AUDI-96CX paired with XUDI-96CX
- QIAseq Unique Dual Index Set D V2 (96): AUDI-96DX paired with XUDI-96DX
- QIAseq 96-Unique Dual Index Set A (384): UDIN-96AA paired with UDIS-96AK
- QIAseq 96-Unique Dual Index Set B (384): UDIN-96BA paired with UDIS-96BK
- QIAseq 12-Index I (48): IL-N701–IL-N707, IL-N710–IL-N712, IL-N714–IL-N715 all paired with IL-S502
- QIAseq 96-Index I Set A (384): IL-701NJ paired with IL-502SK
- QIAseq 96-Index I Set B (384): IL-716NJ paired with IL-502SK
- QIAseq 96-Index I Set C (384): IL-701NJ paired with IL-513SK
- QIAseq 96-Index I Set D (384): IL-716NJ paired with IL-513SK
- QIAseq 8-Unique Index Set A (48): UDIN-8AA paired with UDIS-8AK
- QIAseq 8-Unique Index Set B (48): UDIN-8BA paired with UDIS-8BK

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

contrast, CDI adapters use twelve i7 and eight i5 barcode motives that can be combined to form up to 96 CDIs.

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

To multiplex more than 96 libraries in a single sequencing run, combine kits with different sets, either the QIAseq 96-Unique Dual Index or the QIAseq 96-Index I (CDIs). For example, combining the unique dual QIAseq 96-Unique Dual Index V2 Set A (or B, or C, or D) (96) kits will allow the generation of 384 libraries with different unique dual sample indexes for 384-plex sequencing. For example, combining the combinatorial QIAseq 96-Index I Set A (or B, or C, or D) (96) kits will allow the generation of 384 libraries with different combinatorial sample indexes for 384-plex sequencing. For more information on the unique dual QIAseq 8-Unique Dual Index Set A (or B) (48) kits, please refer to "Appendix C: QIAseq 8-Unique Dual Index Sets".

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
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	16400	6560	3280	1366
NovaSeq 6000	S4 (4 lanes per flow cell)	20 B	40000	16000	8000	3332
NovaSeq X	1.5 B flow cell	3.2 B	6400	2560	1280	532
NovaSeq X	10 B flow cell	20 B	40,000	16,000	8000	3332
NovaSeq X	25 B flow cell	52 B	104,000	41,600	20,800	8663

* Based on 2 x 149 bp paired-end reads for 96-UDIs and 2 x 151 bp reads for CDIs and 8-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
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	1.5 B flow cell	3.2 B	1280	512	256	106
NovaSeq X	10 B flow cell	20 B	8000	3200	1600	666
NovaSeq X	25 B flow cell	52 B	20,800	8320	4160	1731

* Based on 2 x 149 bp paired-end reads for 96-UDIs and 2 x 151 bp paired-end reads for CDIs and 8-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
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	1.5 B flow cell	3.2 B	160	64	32	12
NovaSeq X	10 B flow cell	20 B	1000	400	200	80
NovaSeq X	25 B flow cell	52 B	2600	1040	520	208

* Based on 2 x 149 bp paired-end reads for 96-UDIs and 2 x 151 bp reads for CDIs and 8-UDIs.

N/A: Not applicable, no samples can be run.

Next-generation sequencing read-length recommendations

When using Illumina NGS systems, QIAseq Targeted DNA libraries require a custom sequencing primer for Read 1 (QIAseq A Read1 Primer I). QIAseq Targeted DNA Panel UDI libraries require 149 bp paired-end reads and dual 10-bp indices, while QIAseq Targeted DNA Panel CDI libraries require 151 bp paired-end reads and dual 8 bp indices. However, QIAseq Targeted DNA Panel 8-UDI libraries require 151 bp paired-end reads and dual 8 bp indices (see "Appendix C: QIAseq 8-Unique Dual Index Sets"for more details).

Protocol: Fragmentation, End-Repair, and A-addition

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:
  - Use 10–80 ng for standard DNA or cfDNA;
  - Use up to 250 ng of FFPE DNA if QIAseq QuantiMIZE kits have been used.
  - Use up to 100 ng of FFPE DNA if an alternative method was used. Lower input amounts are possible; however, this will lead to fewer sequenced UMIs and reduced variant detection sensitivity.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure

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, end-repair, and A-addition.
   a. Thaw Fragmentation Buffer, 10x; FERA Solution; and FG Solution if required at room temperature but place on ice after being thawed.
   b. Mix by flicking the tube, and centrifuge briefly.

Note: The Fragmentation Enzyme Mix should be removed from the freezer just before use and placed on ice. After use, immediately return the enzymes to the freezer.

3. On ice, prepare the fragmentation, end-repair, and A-addition 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 DNA input will improve variant detection sensitivity – particularly for FFPE DNA. See Appendix B: FFPE DNA Quality and Quantity" for more details.

Table 6. Reaction mix for fragmentation, end-repair, and A-addition

Component	Volume/reaction (standard, FFPE or pure cfDNA)	Volume/reaction (cfDNA contaminated with cellular DNA)
DNA*	Variable	Variable
Fragmentation Buffer, 10x	2.5 µL	2.5 µL
FERA Solution	0.75 µL	0.75 µL
FG Solution	–	1.25 µL
Nuclease-free Water	Variable	Variable
Total	**20 µL**	**20 µL**

* 10–80 ng for standard DNA or cfDNA. 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.

4. Add 5 µL Fragmentation Enzyme Mix to each reaction. Briefly centrifuge, mix by pipetting up and down at least 12 times (do not vortex) with pipetting volume close to 25 µL, and briefly centrifuge again.

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

5. Program the thermal cycler according to Table 7. Use the instrument’s heated lid.

Table 7. Cycling conditions for fragmentation, end-repair, and A-addition*

Step	Incubation temperature (°C)	Incubation time (standard DNA)	Incubation time (FFPE DNA)	Incubation time (cfDNA)
1	4	1 min	1 min	1 min
2	32*	24 min	14 min	14 min
3	72	30 min	30 min	30 min
4	4	Hold	Hold	Hold

* For Human Mitochondria Panel, use 8 min for both standard and FFPE DNA at 32°C incubation.

6. 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 pre-chilled and paused at 4°C.

7. Transfer the tubes/plate prepared in step 2 to the pre-chilled thermal cycler and resume the cycling program.
8. Upon completion, allow the thermal cycler to return to 4°C.
9. Place the samples on ice and immediately proceed to "Protocol: Adapter Ligation".

Protocol: Adapter Ligation

Important points before starting

- The 25 µL product from "Protocol: Fragmentation, End-Repair, and A-addition" is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- The QIAseq 96-Unique Dual Index Set V2 Adapter Plate (AUDI-96#X): A, B, C, or D used in the adapter ligation reaction must be paired with the matching QIAseq 96-Unique Dual Index Set V2 (XUDI-96#X) Primer Plate: A, B,C, or D used in the universal PCR amplification reaction.
  - The QIAseq 96-Unique Dual Index V2 (AUDI-96#X) adapters are sealed in a 96-well plate with pierceable aluminum heat sealing film.
  - It does not need to be removed; instead, puncture the film using standard 200 µL pipette tips to withdraw the appropriate adapter and adapter volume.
  - Thaw the adapter plate on ice or store at 4°C before usage. After it is fully thawed, centrifuge the plate at 1000 x *g* for 1 min.
- The QIAseq 96-Unique Dual Index Set Adapter Plate (UDIN-96#A): A or B used in the adapter ligation reaction must be paired with the matching QIAseq 96-Unique Dual Index Set (UDIS-96#K) Primer Plate: A or B used in the universal PCR amplification reaction.
  - The QIAseq 96-Unique Dual Index (UDIN-96#A) adapters are sealed in a 96-well plate with pierceable aluminum heat sealing film.
  - It does not need to be removed; instead, puncture the film using standard 200 µL pipette tips to withdraw the appropriate adapter and adapter volume.

    - Thaw the adapter plate on ice or store at 4°C before usage. After it is fully thawed, centrifuge the plate at 1000 x *g* for 1 min.
- The QIAseq 12-Index I Adapters (IL-N7##) are in individual tubes.
    - Thaw the tubes on ice or store at 4°C before usage. After the tubes are fully thawed, centrifuge the tubes.
- The QIAseq 96-Index I Set Adapter Plate (IL-7##NJ): A, B, C, or D used in the adapter ligation reaction must be paired with the matching QIAseq 96-Index I (IL-5##SK) Primer Plate: A, B, C, or D used in the universal PCR amplification reaction.
    - The QIAseq 96-Index I Set (IL-7##NJ) adapters are sealed in a 96-well plate that needs to have the seal removed.
    - Thaw the adapter plate on ice or store at 4°C before usage. After it is fully thawed, centrifuge the plate at 1000 x *g* for 1 min. Carefully withdraw the appropriate adapter and adapter volume.
- The QIAseq 8-Unique Dual Index Set Adapter Plate (UDIN-8#A): A or B used in the adapter ligation reaction must be paired with the matching QIAseq 8-Unique Dual Index Set (UDIS-8#K) Primer Plate: A or B used in the universal PCR amplification reaction.
    - The QIAseq 8-Unique Dual Index (UDIN-8#A) adapters are sealed in a 96-well plate with pierceable aluminum heat sealing film.
    - It does not need to be removed; instead, puncture the film using standard 200 µL pipette tips to withdraw the appropriate adapter and adapter volume.
    - Thaw the adapter plate on ice or store at 4°C before usage. After it is fully thawed centrifuge the plate at 1000 x *g* for 1 min.
- The QIAseq Beads are used for all reaction cleanups.
- 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

1. Prepare the reagents required for the DNA ligation.

   a. Thaw Ligation Buffer, 5x, and Ligation Solution at room temperature, but place on ice after being thawed.

   b. Mix by flicking the tube, and then centrifuge briefly.

   Note: DNA Ligase should be removed from the freezer just before use and placed on ice. After use, immediately return the enzyme to the freezer.

2. Prepare the adapter ligation mix according to Table 8. Briefly centrifuge, mix by pipetting up and down at least 12 times with pipette volume close to maximum mix volume, and briefly centrifuge again.

   Important: Only one single-indexed adapter should be used per ligation reaction. The QIAseq 96-Unique Dual Index set V2 or QIAseq 96-Unique Dual Index set adapter layout is described in Figure 4Figure 4, and Figure 6; use a multichannel pipette to pierce the foil prior to using a multichannel pipette to pipet the appropriate amount of adapters. Open one adapter tube at a time if using QIAseq 12-Index I adapters and avoid cross-contamination. For QIAseq 96-Index I adapters supplied in a plate (layout described in Figure 7), use a multichannel pipette to pipet the appropriate amount of adapters. Refer to "Appendix C: QIAseq 8-Unique Dual Index Sets" for the QIAseq 8-Unique Dual Index set adapter layout.

   Important: Pipet slowly to mix. The reaction mix is very viscous. Do not vortex.

   Note: If setting up more than one reaction, prepare a volume of master mix 20% greater than what is required for the total number of reactions.

Table 8. Reaction mix for adapter ligation

	Volume/reaction		
Component	**Standard DNA (µL)**	**FFPE DNA (µL)**	**cfDNA (µL)**
Fragmentation, end-repair, and A-addition reaction (already in tube)	25	25	25
Ligation Buffer, 5x	10	10	10
AUDI-96#X or UDIN-96#A or IL-N7## or IL-7##NJ or UDIN-8#A adapter*	2.8	2.8	0.5
DNA Ligase	5	5	5
Ligation Solution†	7.2	7.2	7.2
Nuclease-Free Water	–	–	2.3
Total	**50**	**50**	**50**

* This applies to AUDI-96#X for QIAseq 96-Unique Dual Index Set A, B, C, or D V2; or UDIN-96#A for QIAseq 96-Unique Dual Index Set A or B; or IL-N7## component applies to the adapters with up to a 12 sample index for QIAseq 12-Index I; or IL-7##NJ adapters with QIAseq 96-Index I A, B, C, or D set (CDIs); or UDIN-8#A for QIAseq 8-Unique Dual Index Set A or B.

† Ligation Solution is very viscous. It should be added into each reaction individually and not premixed with other components for a master mix. Do not coat the outside of the pipette tip with Ligation Solution or excess volume may be added.

QIAseq 96-Unique Dual Index Set A V2 adapter plate (96) (AUDI001-AUDI096)

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

QIAseq 96-Unique Dual Index Set B V2 adapter plate (96) (AUDI097-AUDI192)

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

Figure 4. Layout of the pierceable QIAseq 96-Unique Dual Index Set V2 Adapter Plates. QIAseq 96-Unique Dual Index Set A V2 (96) layout (AUDI001-AUDI096), Set B V2 (96) layout (AUDI097-AUDI192), Set C V2 (96) layout (AUDI193-AUDI288), and Set D V2 (96) layout (AUDI289-AUDI384).

QIAseq 96-Unique Dual Index Set C V2 adapter plate (96) (AUDI193-AUDI288)

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

QIAseq 96-Unique Dual Index Set D V2 adapter plate (96) (AUDI289-AUDI384)

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

Figure 5. (continued) Layout of the pierceable QIAseq 96-Unique Dual Index Set V2 Adapter Plates. QIAseq 96-Unique Dual Index Set A V2 (96) layout (AUDI001-AUDI096), Set B V2 (96) layout (AUDI097-AUDI192), Set C V2 (96) layout (AUDI193-AUDI288), and Set D V2 (96) layout (AUDI289-AUDI384).

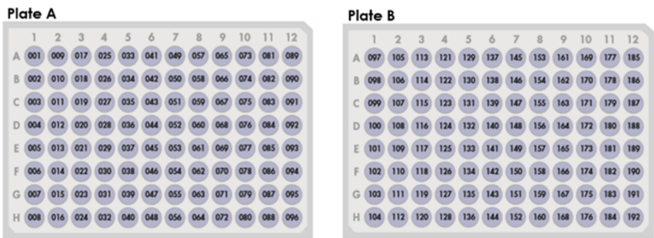


Figure 6. Layout of the pierceable QIAseq 96-Unique Dual Index Set Adapter Plates. QIAseq 96-Unique Dual Index Set A (96) layout (NQDIB001-NQDIB096) and QIAseq 96-Unique Dual Index Set B (96) layout (NQDIB097-NQDIB192).

IL-701NJ Adapter Plate in QIAseq 96-Index I Set A or C set

	1	2	3	4	5	6	7	8	9	10	11	12
A	N701	N702	N703	N704	N705	N706	N707	N710	N711	N712	N714	N715
B	N701	N702	N703	N704	N705	N706	N707	N710	N711	N712	N714	N715
C	N701	N702	N703	N704	N705	N706	N707	N710	N711	N712	N714	N715
D	N701	N702	N703	N704	N705	N706	N707	N710	N711	N712	N714	N715
E	–	–	–	–	–	–	–	–	–	–	–	–
F	–	–	–	–	–	–	–	–	–	–	–	–
G	–	–	–	–	–	–	–	–	–	–	–	–
H	–	–	–	–	–	–	–	–	–	–	–	–

IL-716NJ Adapter Plate in QIAseq 96-Index I Set B or D set

	1	2	3	4	5	6	7	8	9	10	11	12
A	N716	N718	N719	N720	N721	N722	N723	N724	N726	N727	N728	N729
B	N716	N718	N719	N720	N721	N722	N723	N724	N726	N727	N728	N729
C	N716	N718	N719	N720	N721	N722	N723	N724	N726	N727	N728	N729
D	N716	N718	N719	N720	N721	N722	N723	N724	N726	N727	N728	N729
E	–	–	–	–	–	–	–	–	–	–	–	–
F	–	–	–	–	–	–	–	–	–	–	–	–
G	–	–	–	–	–	–	–	–	–	–	–	–
H	–	–	–	–	–	–	–	–	–	–	–	–

Figure 7. Layout of sample adapters in QIAseq 96-Index I Set A, B, C, or D. Rows A through D of each plate have adapters. Rows E through H are empty. Each well in each row contains one sample adapter, and the amount of adapter in each well is enough for 8 samples.

3. Incubate the reactions in thermal cycler according to Table 9.

   Important: Do not use heated lid.

Table 9. Incubation conditions for DNA ligation

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

4. Once the run has finished,
   a. for standard/FFPE samples, add 50 µL Nuclease-Free Water to bring each sample to 100 µL;
   b. for cfDNA samples, add 30 µL Nuclease-Free Water to bring each sample to 80 µL.
5. For standard/FFPE samples, add 100 µL QIAseq Beads; For cfDNA samples, add 112 µL QIAseq Beads.

   Mix well by vortexing or pipetting up and down at least 12 times with pipetting volume close to 190 µL.
6. Incubate for 5 min at room temperature.
7. Place the tubes/plate on a magnetic rack for 10 min. Once the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads as they contain the DNA of interest.
8. With the beads still on the magnetic stand, add 200 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 10 min.

    Note: Visually inspect that the pellet is completely dry.

11. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 52 µL Nuclease-Free Water. Mix well by pipetting.
12. Return the tubes/plate to the magnetic rack until the solution has cleared.
13. Transfer 50 µL of the supernatant to clean tubes/plate.
14. For standard/FFPE samples, add 50 µL QIAseq Beads (for Human Mitochondria Panel use 35 µL). For cfDNA samples, add 70 µL QIAseq Beads. Mix well by vortexing or pipetting up and down at least 12 times.
15. Incubate for 5 min at room temperature.
16. 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.

    Important: Do not discard the beads as they contain the DNA of interest.

17. With the beads still on the magnetic stand, add 200 µL 80% ethanol. Carefully remove and discard the wash.
18. 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.

19. With the beads still on the magnetic stand, air dry at room temperature for 15 min.

Note: Visually inspect that the pellet is completely dry. Ethanol carryover to the target enrichment PCR step will affect enrichment PCR efficiency.

Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 12 µL Nuclease-Free Water. Mix well by pipetting.

20. Return the tube/plate to the magnetic rack until the solution has cleared.
21. Transfer 9.4 µL of the supernatant to clean tubes or plate.
22. Proceed with “Protocol: Target Enrichment”. Alternatively, the samples can be stored at −30 to −15°C in a constant-temperature freezer for up to 3 days.

Protocol: Target Enrichment

Important points before starting

- The 9.4 µL product from "Protocol: Adapter Ligation" is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- The QIAseq Beads are used for all reaction cleanups.
- 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

1. Prepare the reagents required for the target enrichment.
   a. Thaw TEPCR Buffer, 5x; QIAseq Targeted DNA Panel; and IL-Forward Primer at room temperature but place on ice after being thawed.
   b. Mix by flicking the tube, and then centrifuge briefly.

   Note: The HotStarTaq DNA Polymerase should be removed from the freezer just before use and placed on ice. After use, immediately return the enzyme to the freezer.

   Prepare the target enrichment mix according to Table 10. Briefly centrifuge, mix by pipetting up and down at least 12 times with a pipette volume close to 20 µL, and briefly centrifuge again.

Table 10. Reaction mix for target enrichment

Component	Volume/reaction (µL)
Sample (from "Protocol: Adapter Ligation")	9.4
TEPCR buffer, 5x	4
QIAseq Targeted DNA Panel	5
IL-Forward primer	0.8
HotStarTaq DNA Polymerase	0.8
Total	**20**

2. Program a thermal cycler using the cycling conditions in Table 11 (panel with <1500 primers/tube) or Table 12 (panel with ≥1500 primers/tube).

Table 11. Cycling conditions for target enrichment if number of primers <1500/tube

Step	Time	Temperature (°C)
Initial denaturation	13 min 2 min	95 98
8 cycles	15 s 10 min	98 68
1 cycle	5 min	72
Hold	∞	4

Table 12. Cycling conditions for target enrichment if number of primers ≥1500/tube

Step	Time (1500–12,000 primers/tube)	Time (>12,000 primers/tube)	Temperature (°C)
Initial denaturation	13 min 2 min	13 min 2 min	95 98
6 cycles	15 s 15 min	15 s 30 min	98 65
1 cycle	5 min	5 min	72
Hold	∞	∞	4

3. Place the target enrichment reaction in the thermal cycler and start the run.
4. Once the run has finished,
    a. for standard/FFPE samples, add 80 µL Nuclease-Free Water to bring each sample to 100 µL;
    b. For cfDNA samples, add 70 µL Nuclease-Free Water to bring each sample to 90 µL.
5. For standard/FFPE samples, add 100 µL QIAseq Beads (for Human Mitochondria Panel use 70 µL); For cfDNA samples, add 108 µL QIAseq Beads.

    Mix well by pipetting up and down at least 12 times with a pipeting volume close to 190 µL (170 µL for Human Mitochondria Panel).
6. Incubate for 5 min at room temperature.
7. 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.

    Important: Do not discard the beads as they contain the DNA of interest.
8. With the beads still on the magnetic stand, add 200 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 10 min.

    Note: Visually inspect that the pellet is completely dry. Ethanol carryover to the next universal PCR step will affect PCR efficiency.

11. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 14 µL Nuclease-Free Water. Mix well by pipetting.
12. Return the tube/plate to the magnetic rack until the solution has cleared.
13. Transfer 12 µL of the supernatant to clean tubes/plate.
14. Proceed with "Protocol: Universal PCR". Alternatively, the samples can be stored at −30 to −15°C in a constant-temperature freezer for up to 3 days.

Protocol: Universal PCR

Important points before starting

- The 12 µL product from "Protocol: Target Enrichment" is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- The QIAseq 96-Unique Dual Index Set V2 (XUDI-96#X) Index Primer Plate must be paired with the matching QIAseq 96-Unique Dual Index Set V2 (AUDI-96#X) Adapter Plate: A, B, C, or D used in the Adapter Ligation reaction.
    - The XUDI-96#X Index Primer Plate A, B, C, or D contain pre-dispensed index primers and a universal PCR primer that are sealed in a 96-well plate with pierceable aluminum heat sealing film.
    - It does not need to be removed; instead, puncture the film using standard 200 µL pipette tips to withdraw the appropriate index primer and index primer volume.
    - Thaw the index primer plate on ice or store at 4°C before usage. After it is fully thawed, centrifuge the plate at 1000 x *g* for 1 min.
- The QIAseq 96-Unique Dual Index Set (UDIS-96#K) Index Primer Plate must be paired with the matching QIAseq 96-Unique Dual Index Set (UDIN-96#A) Adapter Plate: A or B used in the Adapter Ligation reaction.
    - The UDIS-96#K Index Primer Plate A or B contain pre-dispensed dried index primers and a universal PCR primer and are sealed in an aluminum pouch.
    - Add components directly in the UDIS-96#K Index Primer plates for the universal PCR reaction. See Figure 11 for the layout of the index primers in the plate.

- The QIAseq 12-Index I Adapter (IL-S502) is in an individual tube and must be paired with the IL-N7## tubes.
- Thaw the tubes on ice or store at 4°C before usage. After the tubes are fully thawed centrifuge the tubes.
- The QIAseq 96-Index I Set A, B, C, or D (IL-5##SK) Plate must be paired with the matching QIAseq 96-Index I Set (IL-7##NJ) Adapter Plate: A, B, C, or D used in the Adapter Ligation reaction.
    - The QIAseq 96-Index I Set (IL-5##SK) plates contain pre-dispensed, dried index primers and the universal PCR primer and are sealed in an aluminum pouch.
-
    - Add the universal PCR reaction components directly in the appropriate IL-5##SK Index Primer plates. See Figure 11 for the layout of the index primers in the plate.
- The QIAseq 8-Unique Dual Index Set (UDIS-8#K) Index Primer Plate must be paired with the matching QIAseq 8-Unique Dual Index Set (UDIN-8#A) Adapter Plate: A or B used in the Adapter Ligation reaction.
    - The UDIS-8#K Index Primer Plate A or B contain pre-dispensed dried index primers and a universal PCR primer that are sealed in an aluminum pouch.
    - Add components directly in the UDIS-8#K Index Primer plates for the universal PCR reaction. See "Appendix C: QIAseq 8-Unique Dual Index Sets" for more details.
- The QIAseq Beads are used for all reaction cleanups.
- 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

1. Prepare the reagents required for the universal PCR.
   a. Thaw the index primer plate XUDI-96#X and UPCR Buffer, 5x, on ice. Bring the appropriate IL-S502 tube, IL-5##SK plate, UDIS-96#K plate, or UDIS-8#K plate to room temperature.
   b. Mix by flicking the tube, and then centrifuge briefly.

   Note: The HotStarTaq DNA Polymerase should be removed from the freezer just before use and placed on ice. After use, immediately return the enzyme to the freezer.
2. Prepare the universal PCR according to Table 13a, Table 13b, or Table 14, depending on which index set is being used. Briefly centrifuge, mix by pipetting up and down at least 12 times and briefly centrifuge again.

Table 13a. Reaction components for universal PCR if using QIAseq 96-Unique Dual Index Set A, B, C or D V2*

Component	Volume/reaction (µL)
Sample (from "Protocol: Target Enrichment")	12
UPCR Buffer, 5x	4
HotStarTaq DNA Polymerase	1
XUDI-96#X Index Primer	3
Total	**20**

* Applies to QIAseq 96-Unique Dual Index Set V2 XUDI-96#X Index Primer Plate in A, B, C, or D set. The final library dual sample index is determined by the unique AUDI-96#X Adapter Plate and the QIAseq XUDI-96#X Index Primer Plate. Total sample index level can be up to 384-plex if using QIAseq 96-Unique Dual Index Set A, B, C, and D V2 together.

Table 13b. Reaction components for universal PCR if using QIAseq 96-Unique Dual Index Set A or B*; or QIAseq 96-Index I Set A, B, C, or D†; or QIAseq 8-Unique Dual Index Set A or B§

Component	Volume/reaction (µL)
Sample (from "Protocol: Target Enrichment")	12
UPCR Buffer, 5x	4
HotStarTaq DNA Polymerase	1
Nuclease-free Water	3
Total	**20**

* Applies to QIAseq UDIS-96#K Index Primer Plate in A or B set. The final library dual sample index is determined by the unique UDIN-96#A Adapter Plate and the QIAseq UDIS-96#K Index Primer Plate. Total sample index level can be up to 192-plex if using QIAseq 96-Unique Dual Index Set A and B together.

† Applies to QIAseq IL-5##SK Index Primer Plate in A, B, C, or D set. The final library dual sample index is determined by the combination of the IL-7##NJ Adapter Plate and the QIAseq IL-5##SK Index Primer Plate. Total sample index level can be up to 384-plex if using QIAseq 96-Index I Set A, B, C, and D together.

§ Applies to QIAseq UDIS-8#K Index Primer Plate in A or B set. The final library dual sample index is determined by the unique UDIN-8#A Adapter Plate and the QIAseq UDIS-8#K Index Primer Plate. Total sample index level can be up to 16-plex if using QIAseq 8-Unique Dual Index Set A and B together.

Table 14. Reaction mix for universal PCR if using QIAseq 12-Index I (48)

Component	Volume/reaction (µL)
Sample (from "Protocol: Target Enrichmentt")	12
UPCR Buffer, 5x	4
IL-Universal Primer	0.8
IL-S502 Index Primer	0.8
HotStarTaq DNA Polymerase	1
Nuclease-free Water	1.4
Total	**20**

QIAseq 96-Unique Dual Index Set A V2 index primer plate (96) (XUDI001-XUDI096)

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

QIAseq 96-Unique Dual Index Set B V2 index primer plate (96) (XUDI097-XUDI192)

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

Figure 8. Layout of the QIAseq 96-Unique Dual Index Set V2 Index Primer Plates. QIAseq 96-Unique Dual Index Set A V2 (96) layout (XUDI001–XUDI096), Set B V2 (96) layout (XUDI097–XUDI192), Set C V2 (96) layout (XUDI193–XUDI288), and Set D V2 (96) layout (XUDI289–XUDI384).

QIAseq 96-Unique Dual Index Set C V2 index primer plate (96) (XUDI193-XUDI288)

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

QIAseq 96-Unique Dual Index Set D V2 index primer plate (96) (XUDI289-XUDI384)

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

Figure 9. (continued) Layout of the QIAseq 96-Unique Dual Index Set V2 Index Primer Plates. QIAseq 96-Unique Dual Index Set A V2 (96) layout (XUDI001–XUDI096), Set B V2 (96) layout (XUDI097–XUDI192), Set C V2 (96) layout (XUDI193–XUDI288), and Set D V2 (96) layout (XUDI289–XUDI384).

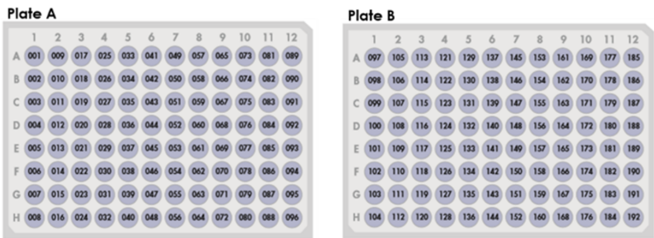


Figure 10. Layout of the QIAseq 96-Unique Dual Index Set Index Primer Plates. QIAseq 96-Unique Dual Index Set A (96) layout (SQDIB001–SQDIB096) and QIAseq 96-Unique Dual Index Set B (96) layout (SQDIB097–SQDIB192).

IL-502SK Index Primer Plate in QIAseq 96-Index I Set A or B set

	1	2	3	4	5	6	7	8	9	10	11	12
A	S502	S502	S502	S502	S502	S502	S502	S502	S502	S502	S502	S502
B	S503	S503	S503	S503	S503	S503	S503	S503	S503	S503	S503	S503
C	S505	S505	S505	S505	S505	S505	S505	S505	S505	S505	S505	S505
D	S506	S506	S506	S506	S506	S506	S506	S506	S506	S506	S506	S506
E	S507	S507	S507	S507	S507	S507	S507	S507	S507	S507	S507	S507
F	S508	S508	S508	S508	S508	S508	S508	S508	S508	S508	S508	S508
G	S510	S510	S510	S510	S510	S510	S510	S510	S510	S510	S510	S510
H	S511	S511	S511	S511	S511	S511	S511	S511	S511	S511	S511	S511

IL-513SK Index Primer Plate in QIAseq 96-Index I Set C or D set

	1	2	3	4	5	6	7	8	9	10	11	12
A	S513	S513	S513	S513	S513	S513	S513	S513	S513	S513	S513	S513
B	S515	S515	S515	S515	S515	S515	S515	S515	S515	S515	S515	S515
C	S516	S516	S516	S516	S516	S516	S516	S516	S516	S516	S516	S516
D	S517	S517	S517	S517	S517	S517	S517	S517	S517	S517	S517	S517
E	S518	S518	S518	S518	S518	S518	S518	S518	S518	S518	S518	S518
F	S520	S520	S520	S520	S520	S520	S520	S520	S520	S520	S520	S520
G	S521	S521	S521	S521	S521	S521	S521	S521	S521	S521	S521	S521
H	S522	S522	S522	S522	S522	S522	S522	S522	S522	S522	S522	S522

Figure 11. Layout of IL-5##SK Index Primer Plate in QIAseq 96-Index I Set A, B, C, or D. Each well contains one pre-dispensed dried sample index primer and a universal primer pair for a single reaction. In universal PCR step 1, IL-7##NJ Adapter Plate in A, B, C, or D set used in ligation must be paired with the appropriate IL-5##SK Index Primer Plate in A, B, C, or D set, respectively.

3. Program a thermal cycler using the cycling conditions in Table 15 (cycling program) and Table 16 (cycle number).

Table 15. Cycling conditions for universal PCR

Step	Time	Temperature (°C)
Initial denaturation	13 min 2 min	95 98
Number of cycles (see Table 16 below)	15 s 2 min	98 60
1 cycle	5 min	72
Hold	∞	4

Table 16. Amplification cycles for universal PCR

Primers per pool	Cycle number	
	Standard DNA	cfDNA and FFPE DNA
6–24	26	28
25–96	24	26
97–288	22	24
289–1056	21	23
1057–1499	20	22
1500–3072	21	23
3073–4999	20	22
5000–12,000	19	21
≥12,001	18	20

4. After the reaction is complete, place the reactions on ice and,
   a. for standard/FFPE samples, add 80 µL Nuclease-Free Water to bring each sample to 100 µL;
   b. for cfDNA samples, add 70 µL Nuclease-Free Water to bring each sample to 90 µL.
5. For standard/FFPE samples, add 100 µL QIAseq Beads (for Human Mitochondria Panel use 70 µL); For cfDNA samples, add 108 µL QIAseq Beads.

   Mix well by vortexing or pipetting up and down at least 12 times with a pipetting volume close to 190 µL (170 µL for Human Mitochondria Panel).
6. Incubate for 5 min at room temperature.
7. Place the tubes/plate on magnetic rack for 5 min (for tubes) or 10 min (for plates) to separate beads from supernatant. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads as they contain the DNA of interest.
8. With the beads still on the magnetic stand, add 200 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 10 min.

    Note: Visually inspect that the pellet is completely dry.
11. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 30 µL Nuclease-Free Water. Mix well by pipetting.
12. Return the tubes/plate to the magnetic rack until the solution has cleared.

13. Transfer 28 µL supernatant to clean tubes or plate.

    Proceed to "Recommendations: Library QC and Quantification". Alternatively, the library can be stored in a –30 to –15°C in a constant 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, NextSeq 1000/2000, MiniSeq, and NovaSeq".

Recommendations: Library QC and Quantification

NGS Library QC

After the library has been constructed and purified, QC can be performed with the QIAxcel, 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 with the High Sensitivity DNA Kit. Libraries prepared for Illumina instruments demonstrate a size distribution between 300–1000 bp (see Figure 12 and Figure 13a). Library overamplification is normal (see Figure 13b), 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 over-amplified libraries (See "Preferred library quantification method").

Recommended setting for checking QIAseq DNA Panel 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.

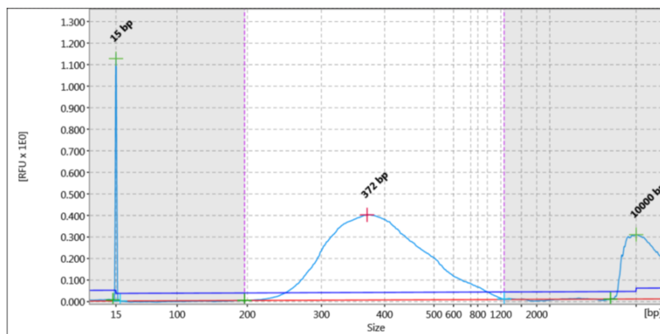
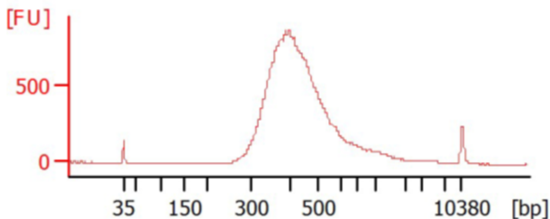


Figure 12. Sample QIAxcel image of QIAseq Targeted DNA Panel libraries for Illumina instruments. The library assessed using QIAxcel illustrates the size of the majority of the library fragments are between 300–1000 bp.

A: Library (without overamplification) prepared for Illumina instruments



B: Library (with overamplification) prepared for Illumina instruments

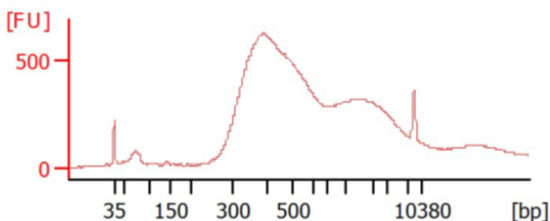


Figure 13. Sample Bioanalyzer images of QIAseq Targeted DNA Panel libraries for Illumina instruments. The size of the majority of the library fragments are between 300–1000 bp. **A**: Library without overamplification. **B**: Library with overamplification as indicated by the "larger fragment" peak.

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 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, 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 (see "Preferred library quantification method").
- QIAseq A Read1 Primer I (Custom Read 1 Sequencing Primer) must be used when performing sequencing on Illumina platform.
- QIAseq A Read1 Primer I (the Custom Read 1 Sequencing Primer) goes into the following specific reagent cartridge positions:
    - MiniSeq Position #15
    - MiSeq Position #18
    - NextSeq 500/550 Position #7
    - NextSeq 1000/2000 Custom well 1 or 2
    - NovaSeq 6000 Position #5
    - NovaSeq X Position CP1
- During sequencing run set up, make sure using custom sequencing read 1 primer is selected.
- HT1 buffer used for custom sequencing primer dilution needs to be ordered separately for NextSeq 1000/2000 sequencing kit, Illumina (cat. no. 20015892).

- Paired-end sequencing should be used for the QIAseq Targeted DNA Panel on Illumina platform.
- To make sequencing preparation more convenient, download Illumina-compatible sample sheets for different sequencing instruments on the Resources tab at **www.qiagen.com/QIAseqTargetedDNAPanel**
- Paired-end sequencing of 149 bp should be used for QIAseq Targeted DNA 96-Unique Index Set libraries and dual 10 bp indices on Illumina platforms.
- Paired-end sequencing of 151 bp should be used for QIAseq Targeted DNA 12-Index I, 96-Index I, and 8-Unique Index Set libraries and dual 8 bp indices on Illumina platforms.
- For complete instructions on how to denature sequencing libraries, prepare custom sequencing primers, and set up a sequencing run, please refer to the system-specific Illumina documents.
- Instrument-specific imagery is included to aid in sequencing preparations.

Sequencing preparations for MiSeq with QIAseq 96-Unique Dual Index Sets

1. When working with the QIAseq Targeted DNA Panel custom QIAseq 96-Unique Dual Index Sets V2 or QIAseq 96-Unique Dual Index Sets, use Local Run Manager (LRM) v2 or later on the instrument to upload a sample sheet (see the Product Resources tab at **www.qiagen.com/QIAseqTargetedDNAPanel** 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. Custom sequencing primer for Read 1 preparation and loading: Use 597 µL HT1 (Hybridization Buffer) to dilute 3 µL QIAseq A Read 1 Custom Primer I (provided) to obtain a final concentration of 0.5 µM. Load 600 µL of the diluted QIAseq A Read 1 Primer I to position 18 of the MiSeq reagent cartridge (Figure 10). For more details, please refer to the Illumina protocol: **miseq_using_custom_primers_15041638_b.pdf** for the MiSeq.
5. During sequencing run set up, make sure using custom read 1 sequencing primer is selected.

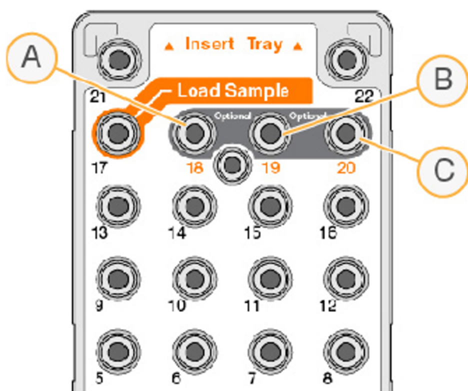


Figure 14. MiSeq reagent cartridge. **A**: Position 18 for Read 1 Custom Primer; **B and C**: not relevant.

6. Upon completion of the sequencing run, proceed to "Appendix D: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench".

Sequencing preparations for MiSeq with QIAseq 12-Index I, QIAseq 96 Index I Sets, and QIAseq 8-Unique Index Sets

1. When working with the QIAseq Targeted DNA Panel custom QIAseq 12-Index I, QIAseq 96-Index I Sets, and QIAseq 8-Unique Index Sets, use LRM V2 or later on the instrument to upload a sample sheet (see the Product Resources tab at **www.qiagen.com/QIAseqTargetedDNAPanel** and download the appropriate template) and proceed with sequencing: Read 1 is 151 bp, Read 2 is 151 bp, and each Index Read is 8 bp.

   or

   Set up the sample sheet with Custom Sequencing Read 1 primer using Illumina Experiment Manager v1.2, or later (Figure 15). Sample index of QIAseq Targeted DNA Panel is

compatible with Illumina Nextera XT v2 adapter sample index system. Set the parameters as follows:

- Category: Select **Other**
- Select Application: Select **FASTQ Only**
- Sample Prep Kit: Select **Nextera XT v2**
- Index Reads: Select **2**
- Read Type: Select **Paired End** Read
- Cycles for both Read 1 and 2: Select **151**
- Check **Custom Primer for Read 1**
- Check **Use Adapter Trimming**

Figure 15. Sample sheet using Illumina Experiment Manager.

2. Follow steps 2–5 in "Sequencing preparations for MiSeq with QIAseq 96-Unique Dual Index Sets".

Sequencing preparations for NextSeq 500/550 and NextSeq 1000/2000 with QIAseq 96-Unique Index Sets

1. When working with the QIAseq Targeted DNA Panel custom QIAseq 96-Unique Dual Index Sets V2 or QIAseq 96-Unique Dual Index Sets, use LRM V2 or later on the instrument to upload a sample sheet (see the Product Resources tab at **www.qiagen.com/QIAseqTargetedDNAPanel** 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 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.

   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 NextSeq 500/550, or NextSeq 1000/2000 according to the NextSeq System Denature and Dilute Libraries Guide. The final library concentration is 1.0–1.2 pM on the NextSeq 500/550 and 650 pM for NextSeq 1000/2000 onboard denature loading.

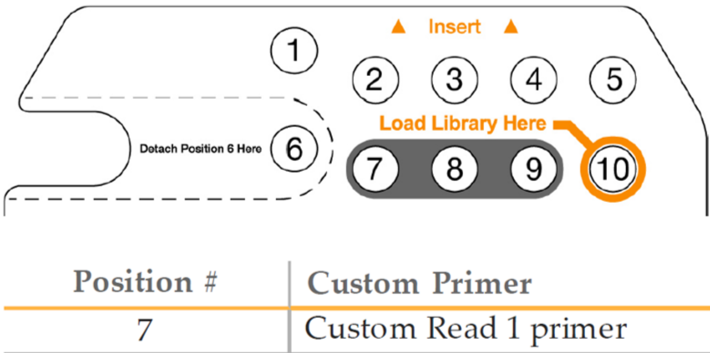
   Note: Recommendations for library loading concentrations are based on QIAseq Library Quant System.

Note: For P3/P4 flow cell with XLEAP-SBS chemistry kit, load 488 pM that's about 25% less compared to standard SBS according to Illumina recommendation.

4. Custom sequencing primer for Read 1 preparation and loading on NextSeq 500/550: Use 1994 µL HT1 (Hybridization Buffer) to dilute 6 µL QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 2 mL of the diluted QIAseq A Read 1 Primer I to position 7 of the NextSeq 500/550 reagent cartridge (see Figure 16).
5. Custom sequencing primer for Read 1 preparation and loading on NextSeq 1000/2000: Use 598.2 µL HT1 (Hybridization Buffer) to dilute 1.8 µL QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 600 µL of the diluted QIAseq A Read 1 Primer I to custom well 1 or custom well 2 of the NextSeq1000/2000 reagent cartridge (see Figure 17)

Note: For allother steps, refer to run setup workflow as described in the Illumina NextSeq 500/550 or NextSeq 1000/2000 System Guide.

Note: HT1 buffer needs to be ordered seperately for NextSeq 1000/2000 sequencing, Illumina (cat. no. 20015892).



Position #	Custom Primer
7	Custom Read 1 primer

Figure 16. NextSeq 500/550 reagent cartridge.

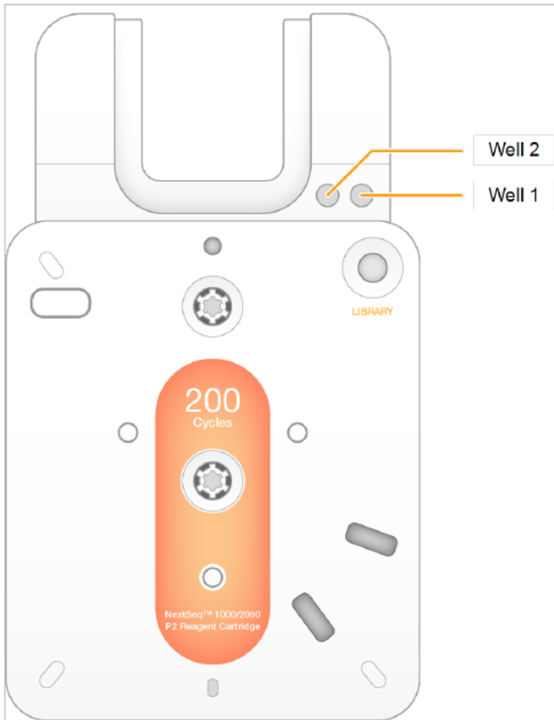


Figure 17. NextSeq 1000/2000 reagent cartridge.

6. During sequencing run set up, make sure Custom Read 1 sequencing primer is selected.
7. Upon completion of the sequencing run, proceed to "Appendix D: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench"

Sequencing preparations for NextSeq 500/550 and NextSeq 1000/2000 with QIAseq 12-Index I, QIAseq 96-Index I Sets, and QIAseq 8-Unique Index Sets

1. When working with the QIAseq Targeted DNA Panel custom QIAseq 12-Index I, QIAseq 96-Index I Sets, and QIAseq 8-Unique Index Sets, use LRM V2 or later on the instrument to upload a sample sheet (see the Product Resources tab at **www.qiagen.com/QIAseqTargetedDNAPanel** and download the appropriate template) and proceed with sequencing: Read 1 is 151 bp, Read 2 is 151 bp, and each Index Read is 8 bp.
2. Follow steps 2–6 in "Sequencing preparations for NextSeq 500/550 and NextSeq 1000/2000 with QIAseq 96-Unique Index Sets".

Sequencing preparations for MiniSeq with QIAseq 96-Unique Index Sets

1. When working with the QIAseq Targeted DNA Panel custom QIAseq 96-Unique Dual Index Sets V2 or QIAseq 96-Unique Dual Index Sets, use LRM V2 or later on the instrument to upload a sample sheet (see the Product 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 0.5, 1, 2, or 4 nM for MiniSeq. 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 the 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 according to the MiniSeq System Denature and Dilute Libraries Guide. The final library concentration is 1.0–1.2 pM on the MiniSeq.

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

4. Custom sequencing primer for Read 1 preparation and loading: Use 997 µL HT1 (Hybridization Buffer) to dilute 3 µL QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 1 mL of the diluted QIAseq A Read 1 Primer I to position 15 of the MiniSeq reagent cartridge (Figure 12).

   Note: All other steps refer to run setup workflow as described in the MiniSeq System Guide (part #1000000002695).

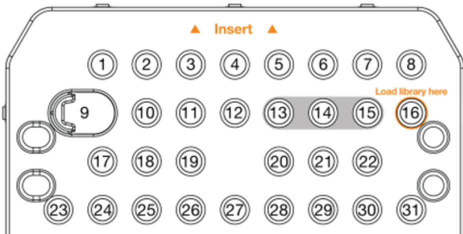


Figure 18. MiniSeq reagent cartridge.

5. During sequencing run set up, make sure using custom read 1 sequencing primer is selected.

6. Upon completion of the sequencing run, proceed to "Appendix D: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench".

Sequencing preparations for MiniSeq with QIAseq 12-Index I, QIAseq 96-Index I Sets, and QIAseq 8-Unique Index Sets

1. When working with the QIAseq Targeted DNA Panel custom QIAseq 12-Index I, QIAseq 96-Index I Sets, and QIAseq 8-Unique Index Sets, use LRM V2 or later on the instrument to upload a sample sheet (see the Product Resources tab at **www.qiagen.com/QIAseqTargetedDNAPanel** and download the appropriate template) and proceed with sequencing: Read 1 is 151 bp, Read 2 is 151 bp, and each Index Read is 8 bp.
2. Follow steps 2–5 in "Sequencing preparations for MiSeq with QIAseq 96-Unique Dual Index Sets".

Sequencing preparations for NovaSeq with QIAseq 96-Unique Index Sets

1. When working with the QIAseq Targeted DNA Panel custom QIAseq 96-Unique Dual Index Sets V2 or QIAseq 96-Unique Dual Index Sets, upload a sample sheet (see the Product Resources tab at **www.qiagen.com/QIAseqTargetedDNAPanel** 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 4 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 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 NovaSeq according to the NovaSeq 6000 or NovaSeq X Sequencing System Guide. The final pooled library concentration recommendation is between 2–4 nM yielding a final loading concentration of between 140–200 pM on the NovaSeq.

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

4. Custom sequencing primer for Read 1 preparation and loading on NovaSeq 6000:
   a. S4 Mode: Use 3489.5 µL HT1 (Hybridization Buffer) to dilute 10.5 µL QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 3.5 mL of the diluted QIAseq A Read 1 Primer I to position 5 of the NovaSeq 6000 reagent cartridge (Figure 19).
   b. SP/S1/S2 Mode: Use 1994 µL HT1 (Hybridization Buffer) to dilute 6 µL QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 2 mL of the diluted QIAseq A Read 1 Primer I to position 5 of the NovaSeq 6000 reagent cartridge (Figure 19).
5. Custom sequencing primer for Read 1 preparation and loading on NovaSeq X:
   a. 10 B and 25 B flow cell: Use 4985 µL NovaSeq X Series Custom-Primer buffer to dilute 15 µL QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 5 mL of the diluted QIAseq A Read 1 Primer I to position CP1 of the NovaSeq X reagent cartridge (Figure 20).
   b. 1.5 B flow cell: Use 2991 µL NovaSeq X Series Custom-Primer buffer to dilute 9 µL QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 3 mL of the diluted QIAseq A Read 1 Primer I to position CP1 of the NovaSeq X reagent cartridge (Figure 20).

Note: For all other steps, refer to run setup workflow as described in the NovaSeq 6000 and NovaSeq X Sequencing System Guide.

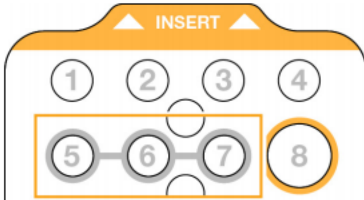


Figure 19. NovaSeq 6000 reagent cartridge.

Figure 20. NovaSeq X reagent cartridge.

6. During sequencing run set up, make sure using custom read 1 sequencing primer is selected.
7. Upon completion of the sequencing run, proceed to "Appendix D: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench".

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	QIAseq Beads need to be completely dried before elution. Ethanol carryover to targeted enrichment and universal PCR will affect PCR reaction efficiency.
Unexpected signal peaks	
a) Short peaks approx. 140 and 180 bp	These are primer–dimers from targeted enrichment PCR (approx. 140 bp) or universal PCR (approx.180 bp). The presence of primer dimers indicates either not enough DNA input or inefficient PCR reactions or handling issues with bead purifications.
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 over-amplification of the DNA library. Overamplification of the library will not affect the QIAseq Targeted DNA Panels sequencing performance. Decreasing the number of universal PCR cycle numbers can reduce over-amplification.

	Comments and suggestions
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 the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument. In addition, the QIAseq A Read 1 Primer I (100 µM) Custom Read 1 Sequencing primer must be used when sequencing on any Illumina platform.
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, UMI numbers, and read depth are met for the specific variant detection application.

References

1. Xu, C., Nezami Ranjbar, M.R., Wu, Z., DiCarlo, J., Wang, Y. (2017) Detecting very low allele fraction variants using targeted DNA sequencing and a novel molecular barcode-aware variant caller. BMC Genomics. 18, 5.

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 17.

Table 17. 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 Panels.

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 Panels. 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 (see Figure 21).

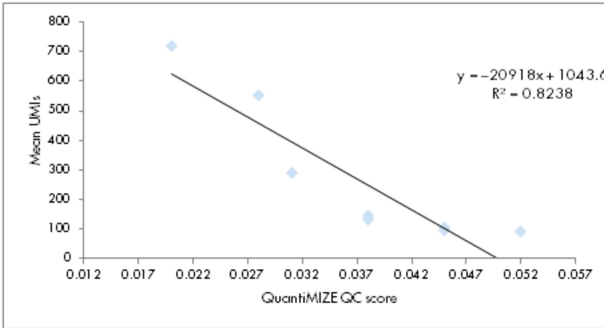


Figure 21. Correlation between QIAseq QuantiMIZE QC score and the number of UMIs.

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 system.

Appendix C: QIAseq 8-Unique Dual Index Sets

The barcode sequences used in the QIAseq 8-Unique Dual Index Sets correspond to Illumina adapter barcodes from Nextera XT v2. The layout used in the QIAseq 8-Unique Dual Index Sets are described in Figure 22 and Figure 23.

UDIN-8AA Adapter Plate in QIAseq 8-Unique Dual Index Set A (48)

	1	2	3	4	5	6	7	8	9	10	11	12
A	N701											
B	N702											
C	N703											
D	N704											
E	N706											
F	N707											
G	N712											
H	N714											

UDIN-8BA Adapter Plate in QIAseq 8-Unique Dual Index Set B (48)

	1	2	3	4	5	6	7	8	9	10	11	12
A	N716											
B	N720											
C	N721											
D	N722											
E	N726											
F	N727											
G	N728											
H	N729											

Figure 22. Layout of sample adapters in QIAseq 8-Unique Dual Index Set A or B. Only column 1 of each plate have adapters. Columns 2 through 12 are empty. Each well in column 1 contains one sample adapter, and the amount of adapter in each well is enough for 6 samples.

UDIS-8AK Index Primer Plate in QIAseq 8-Unique Dual Index Set A (48)

	1	2	3	4	5	6	7	8	9	10	11	12
A	S502	S502	S502	S502	S502	S502						
B	S503	S503	S503	S503	S503	S503						
C	S505	S505	S505	S505	S505	S505						
D	S506	S506	S506	S506	S506	S506						
E	S507	S507	S507	S507	S507	S507						
F	S508	S508	S508	S508	S508	S508						
G	S510	S510	S510	S510	S510	S510						
H	S511	S511	S511	S511	S511	S511						

UDIS-8BK Index Primer Plate in QIAseq 8-Unique Dual Index Set B (48)

	1	2	3	4	5	6	7	8	9	10	11	12
A	S513	S513	S513	S513	S513	S513						
B	S515	S515	S515	S515	S515	S515						
C	S516	S516	S516	S516	S516	S516						
D	S517	S517	S517	S517	S517	S517						
E	S518	S518	S518	S518	S518	S518						
F	S520	S520	S520	S520	S520	S520						
G	S521	S521	S521	S521	S521	S521						
H	S522	S522	S522	S522	S522	S522						

Figure 23. Layout of UDIS-8#K Index Primer Plate in QIAseq 8-Unique Dual Index Set A or B. Each well contains one pre-dispensed dried sample index primer and a universal primer pair for a single reaction. In the universal PCR step 1, the UDIN-8#A Adapter Plate in A or B set used in the ligation reaction, must be paired with the appropriate UDIS-8#K Index Primer Plate in A or B set, respectively. For the universal PCR step, mix the components directly into the single-use pierceable plate.

Appendix D: 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 Panels 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 24.

Figure 24. GeneGlobe Analysis pipeline selections for the QIAseq Targeted DNA Panels.

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 25 on the facing page).

Figure 25. File Upload tab of the QIAseq Targeted DNA Panel 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 26).

1 - 50 of 100 files | 50 per page | Upload New Files | Delete | Share | Refresh | Select For Analysis

File Name	File Size	Uploaded At	Status	
1_S37_L001_R1_001.fastq.gz	29.54 MB	2021/01/07 16:08:21	Ready	☑
1_S37_L001_R2_001.fastq.gz	33.43 MB	2021/01/07 16:08:51	Ready	☑
10_S46_L001_R1_001.fastq.gz	25.69 MB	2021/01/07 16:20:00	Ready	☑
10_S46_L001_R2_001.fastq.gz	29.22 MB	2021/01/07 16:25:45	Ready	☑
11_S47_L001_R1_001.fastq.gz	28.63 MB	2021/01/07 16:20:15	Ready	☐
11_S47_L001_R2_001.fastq.gz	33.58 MB	2021/01/07 16:21:51	Ready	☐

Figure 26. File selection for QIAseq Targeted DNA Panel data analysis pipeline.

Under the Analysis Jobs tab, configure the analysis per the drop-down menus as described in Figure 27 below:

Read Files | Analysis Jobs | QIAseq Targeted DNA Variant Calling Steps

View All Jobs

Read Files * : 4 read files selected
Job Title * : Input job title
Catalog # * : ☐ Custom Catalog #
Job Type : Single
File Lanes : 1-lane
Copy Number Reference Job IDs : Input reference job Ids, e.g. 3456, 3567
JSON Request : Input extra JSON request

ANALYZE

Figure 27. Analysis Jobs tab of the QIAseq Targeted DNA Panel 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/NextSeq concatenated. Choose 4-lane if you set up your runs using NextSeq individual lane files. Choose 1-lane for Ion Torrent™ 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 Panel (12)	ALL reagents (except indexes) for targeted DNA sequencing; fixed panel for 12 samples; less than 200 genes	333502
QIAseq Targeted DNA Panel (96)	ALL reagents (except indexes) for targeted DNA sequencing; fixed panel for 96 samples; less than 200 genes	333505
QIAseq Targeted DNA HC Panel (12)	ALL reagents (except indexes) for targeted DNA sequencing; fixed panel for 12 samples; more than 200 genes	333512
QIAseq Targeted DNA HC Panel (96)	ALL reagents (except indexes) for targeted DNA sequencing; fixed panel for 96 samples; more than 200 genes	333515
QIAseq Targeted DNA Custom Panel (96)	ALL reagents (except indexes) for targeted DNA sequencing; Custom panel for 96 samples	333525
QIAseq Targeted DNA Extended Panel (96)	ALL reagents (except indexes) for targeted DNA sequencing; Extended panel for 96 samples	333545
QIAseq Targeted DNA Booster Panel (96)	Pool of primers used in combination with either cataloged or custom panels	333535[1]
QIAseq Unique Dual Indices V2		
QIAseq 96-Unique Dual Index Set A V2 (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	331745
QIAseq 96-Unique Dual Index Set B V2 (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	331755
QIAseq 96-Unique Dual Index Set C V2 (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	331765

[1] Visit **www.qiagen.com/GeneGlobe** to search for and order these products.

Product	Contents	Cat. no.
QIAseq 96-Unique Dual Index Set D V2 (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	331775
QIAseq Unique Dual Indices		
QIAseq 96-Unique Dual Index Set A (384)	Box containing unique molecularly indexed adapters and primers, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set A (of A and B) required for multiplexing 192 samples in one run	333725
QIAseq 96-Unique Dual Index Set B (384)	Box containing unique molecularly indexed adapters and primers, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; Set B (of A and B) required for multiplexing 192 samples in one run	333735
QIAseq 8-Unique Dual Index Set A (48)	Box containing unique molecularly-indexed adapters and primers, enough for a total of 48 samples, for indexing up to 8 samples for targeted panel sequencing on Illumina platforms; Set A (of A and B) required for multiplexing 16 samples in one run	333715
QIAseq 8-Unique Dual Index Set B (48)	Box containing unique molecularly-indexed adapters and primers, enough for a total of 48 samples, for indexing up to 8 samples for targeted panel sequencing on Illumina platforms; Set B (of A and B) required for multiplexing 16 samples in one run	333716
QIAseq Combinatorial Dual Indices		
QIAseq 12-Index I (48)	Box containing molecularly indexed adapters and primers, enough for a total of 48 samples, for indexing up to 12 samples for targeted panel sequencing on Illumina platforms	333714
QIAseq 96-Index I Set A (384)	Box containing molecularly indexed adapters and primers, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; 1 of 4 sets required for multiplexing 384 samples	333727
QIAseq 96-Index I Set B (384)	Box containing molecularly indexed adapters and primers, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; 2 of 4 sets required for multiplexing 384 samples	333737

Product	Contents	Cat. no.
QIAseq 96-Index I Set C (384)	Box containing molecularly indexed adapters and primers, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; 3 of 4 sets required for multiplexing 384 samples	333747
QIAseq 96-Index I Set D (384)	Box containing molecularly indexed adapters and primers, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; 4 of 4 sets required for multiplexing 384 samples	333757
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	Description of changes
02/2020	QIAseq bead component size changed from 7 mL to 10 ml.
03/2021	Introduction of UDIs. Updated the Kit Contents section. Updated Figure 1. Updated Table 1. Qubit added as recommendation for DNA quantification. GeneRead Library Quant Array removed from Preferred library quantification method. Protocol: Sequencing Setup on Illumina MiSeq and, NextSeq 500/550, MiniSeq and NovaSeq updated to include sequencing preparations for MiniSeq and Novaseq. QIAseq Targeted DNA Panel Analysis pipeline portal updated in Appendix D.
08/2021	Updated the list of product names in Kit Contents section. Changed the ranges of the required amount of template for a single QIAseq Targeted sequencing reaction. Updated the sentence about quantification based on mass calculations. Included a phrase about placing buffers and solutions on ice after being thawed. Updated the number of minutes as to how long the tubes/plate be placed on a magnetic rack. Updated Figure 6 title and Tables 11, 12, and 16. Updated the “Important points before starting” section under “Protocol: Universal PCR”. Updated “Recommendations: Library QC and Quantification” section. Changed the dimmer size in the Troubleshooting Guide. Updated the “Ordering Information” section.
06/2025	Addition of QIAseq 96-Unique Dual Index Sets (A,B,C, and D) V2.

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