June 2021

QIAseq® Targeted DNA Panel Handbook

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

Sample to Insight

QIAGEN

Contents

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

QIAseq 12-Index L **(12 sample index for 48 samples on Ion Torrent® platform)** **Catalog no.** **Number of samples**	**(48)** **333764** **48**
LT-BC# 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	25 µl
LT-P1 Primer	40 µl
LT-Forward Primer	40 µl
LT-Universal Primer	40 µl

QIAseq 96-Index L **(96 sample index for 384 samples on Ion Torrent® platform)** **Catalog no.** **Number of samples**	**(384)** **333777** **384**
LT-BC1-96 Adapter Plate; each plate contains 96 molecularly indexed adapters, each well corresponding to one sample index; kit can process up to 384 total samples	20 µl per well
LT-P1 Primer	310 µl
LT-Forward Primer	310 µl
LT-Universal Primer	310 µl
12-cap strips	16

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-003Y	Human Myeloid Neoplasms E Panel	2511	184,219
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-3015Z	Human Inherited Disease Sub-Panel	5619	413,655
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.

Shipping and Storage

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

The QIAseq Index Kits are shipped on dry ice and should be stored at –30 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.

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 all 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 not platform-specific and are compatible with most medium- and high-throughput sequencers, including Illumina and Ion Torrent systems.

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 (Figure 1).

Figure 1. Overview of the Sample to Insight NGS workflow with QIAseq Targeted DNA Panels. The complete Sample to Insight procedure begins with DNA extraction. Next is library construction and target enrichment with the QIAseq Targeted DNA Panels. Following NGS, 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 QIAGEN Clinical Insight Interpret.

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 (Figure 2). Lower input amounts are possible; however, this will lead to fewer sequenced UMI and reduced variant detection sensitivity.

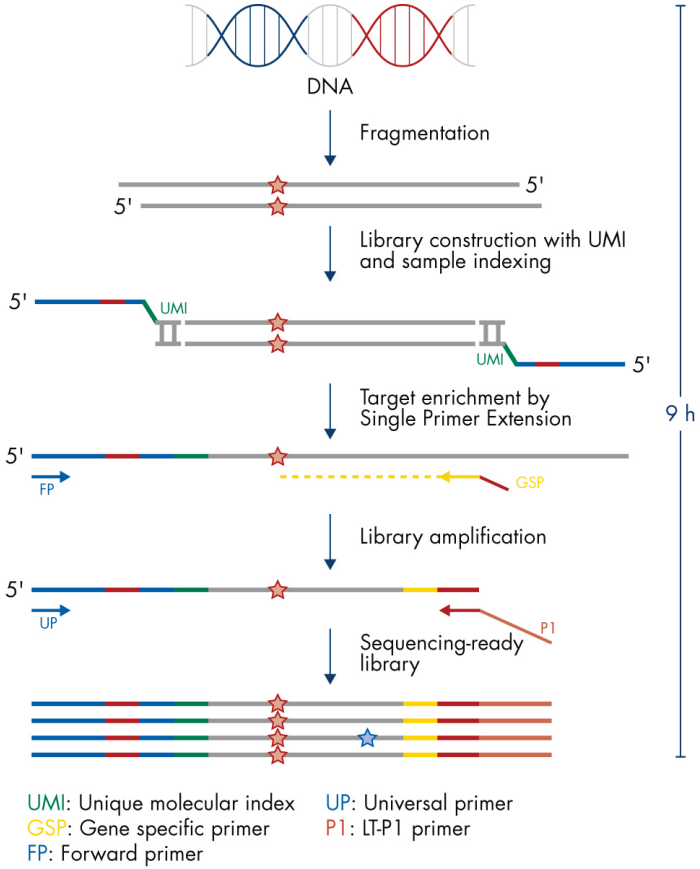


Figure 2. QIAseq Targeted DNA Panels workflow.

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 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.

Next-generation sequencing

The QIAseq Targeted DNA Panels are compatible with most medium- and high-throughput sequencers including Thermo Fisher Scientific Ion Torrent systems (Ion Personal Genome Machine® (Ion PGM®), Ion Proton®, and Ion S5®). When using Ion Torrent systems, 200 bp (or longer) single reads are required, except for the Human Mitochondria Panel where 300 bp (or longer) single reads are needed. For best result, 400 bp reads are recommended for all panels.

Principle of variant detection with UMIs

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

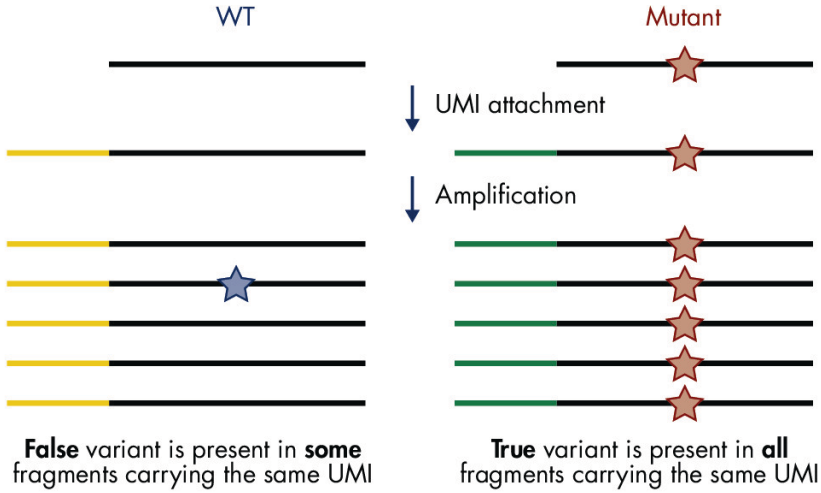


Figure 3. Principle of variant detection with UMIs. Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while false positives are mutations present in only one or a few reads within a UMI. Description of the variant calling algorithm can be found and downloaded from Xu et al (1).

Data analysis

Data for 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/us/analyze**. The pipeline automatically performs all steps necessary to generate a DNA sequence variant report from your raw NGS data. An explanation of the principles of UMI-directed variant detection and the features of the primary sequence analysis output can be found at Xu et al (1).

All detected variants can be further interpreted using QCI Interpret for QIAseq.

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)*
- Nuclease-free pipette tips and tubes
- LoBind® tubes, 1.5 ml (Eppendorf®, cat. no. 022431021)
- PCR tubes, 0.2 ml; 96-well PCR plates; or PCR strips 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)

* 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 quality

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 Tissue Kit	Animal/human tissues and cells	56404
QIAamp DNA FFPE Adv Kit	Animal/human tissues and cells	56604
QIAamp DNA FFPE UNG Kit	Animal/human tissues and cells	56704
QIAamp Circulating Nucleic Acid Kit	Animal and human plasma and serum	55114

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

DNA quantification

The concentration and purity should 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 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™) can be very inaccurate. Appendix B (page 57) provides detailed information for FFPE DNA quality assessment and input amount. For FFPE DNA we recommend Qubit or qPCR for quantification.

DNA integrity

DNA integrity can be checked using the QIAxcel or Agilent Bioanalyzer. 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 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

* 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.

Sample multiplexing level is determined by the size of the panel, required depth of coverage, and sequencing platform read capacity. For the Ion Torrent platforms, up to 96 sample indexes are available per run. 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	1000 primers	2500 primers	5000 primers	12,000 primers
Ion PGM	318 Chip v2	5 M†	7	2	1	N/A
Ion GeneStudio S5	Ion 530 Chip	15 M†	21	8	4	1
Ion GeneStudio S5	Ion 540 Chip	60 M†	84	33	16	7
Ion GeneStudio S5	Ion 550 Chip	100–130 M†	168	66	32	14
Ion Proton	Ion P1 Chip	80 M†	112	44	22	9
Genexus	Ion Torrent GX5 Chip	12 M† per lane (has four lanes; 48 M† in total)	67	26	13	5

* Based on 200 bp read on Ion Torrent platforms.

† Single reads only (specifically for Ion Torrent).

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

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

Instrument	Version	Capacity	1000 primers	2500 primers	5000 primers	12,000 primers
Ion Torrent PGM	318 Chip v2	5 M†	1	N/A	N/A	N/A
Ion GeneStudio S5	Ion 530 Chip	15 M†	4	1	N/A	N/A
Ion GeneStudio S5	Ion 540 Chip	60 M†	16	6	3	1
Ion GeneStudio S5	Ion 550 Chip	100 M†	32	12	6	2
Ion Proton	Ion P1 Chip	80 M†	22	8	4	1
Genexus	Ion Torrent GX5 Chip	12 M† per lane (has four lanes; 48 M† in total)	13	5	2	1

* Based on 200 bp read on Ion Torrent platforms.

† Single reads only (specifically for Ion Torrent).

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	1000 primers	2500 primers	5000 primers	12,000 primers
Ion Torrent PGM	318 Chip v2	5 M†	N/A	N/A	N/A	N/A
Ion GeneStudio S5	Ion 530 Chip	15 M†	N/A	N/A	N/A	N/A
Ion GeneStudio S5	Ion 540 Chip	60 M†	2	N/A	N/A	N/A
Ion GeneStudio S5	Ion 550 Chip	100–130 M†	4	N/A	N/A	N/A
Ion Proton	Ion P1 Chip	80 M†	2	1	N/A	N/A
Genexus	Ion Torrent GX5 Chip	12 M† per lane (has four lanes; 48 M† in total)	1	N/A	N/A	N/A

* Based on 200 bp read on Ion Torrent platforms.

† Single reads only (specifically for Ion Torrent).

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

NGS read-length recommendations

When using Ion Torrent systems, 200 bp (or longer) single reads are required, except for the Human Mitochondria Panel where 300 bp (or longer) single reads are needed. For best result, 400 bp reads are recommended for all panels.

Protocol: QIAseq Targeted DNA Panel for Ion Torrent Instruments

Important points before starting

- This protocol covers all procedures required for the preparation of libraries for Ion Torrent sequencers from "standard DNA" (i.e., cells or tissues), FFPE DNA, and cfDNA.
- Before setting up the reaction, it is critical to accurately determine the amount of the input DNA (10 to 80 ng for standard DNA or cfDNA; up to 250 ng of FFPE DNA can be used, if the QIAseq QuantiMIZE kits have been used. If an alternative method was used to determine the concentration of FFPE DNA, then up to 100 ng DNA can be used). Lower input amounts are possible; however, this will lead to fewer sequenced UMIs and reduced variant detection sensitivity.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- The QIAseq Beads are used for all reaction cleanups.
- Important: Prepare fresh 80% ethanol daily.
- Reaction and cleanup procedures can be performed in either PCR tubes or 96-well plate.
- Upon completion of the library preparation, the QIAseq Library Quant System can be used for library quantification.

Protocol: Fragmentation, end-repair, and A-addition

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.

   2a. Thaw Fragmentation Buffer, 10x; FERA Solution; and FG Solution if required at room temperature and then place on ice.

2b. 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 (page 57) 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**

* For standard DNA or cfDNA, 10–80 ng. Use up to 250 ng of FFPE DNA if the 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 7–8 times (do not vortex), and briefly centrifuge again.

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

5. Program a 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	Incubation time (standard DNA)	Incubation time (FFPE DNA)	Incubation time (cfDNA)
1	4°C	1 min	1 min	1 min
2	32°C*	24 min	14 min	14 min
3	72°C	30 min	30 min	30 min
4	4°C	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", page 25.

Protocol: Adapter Ligation

Important points before starting

- The 25 µl product from "Protocol: Fragmentation, end-repair, and A-addition", page 22, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- **Important**: The QIAseq LT-BC# Adapters in QIAseq 12-Index L with up to 12 sample indices.
    - **Important**: Thaw the QIAseq 12-Index L tubes on ice or store at 4°C before usage. After the tubes are fully thawed, centrifuge the tubes.
- **Important**: The QIAseq LT-BC# Adapters in QIAseq 96-index L with up to 96 sample indices.
    - The QIAseq LT-BC# Adapters in QIAseq 96-index L are sealed in a 96-well plate that needs to have the indented flat 12-cap strips removed. See Figure 4 for the layout of the index primers in the plate.
    - **Important**: 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 Beads are used for all reaction cleanups.
- **Important**: Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

Procedure: Adapter ligation

1. Prepare the reagents required for the DNA ligation.

   1a. Thaw Ligation Buffer, 5x, and Ligation Solution at room temperature and then place on ice.

   1b. 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 10–12 times, and briefly centrifuge again.

   Important: Only one single-indexed adapter should be used per ligation reaction. Open one adapter tube at a time if using 12-index adapters and avoid cross-contamination. For 96-index adapters supplied in a plate (layout described in Figure 4), use a multichannel pipet to pipet the appropriate amount of adapters.

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

Table 8. Reaction mix for adapter ligation

Component	Volume/reaction, standard DNA	Volume/reaction, FFPE DNA	Volume/reaction, cfDNA
Fragmentation, end-repair, and A-addition reaction (already in tube)	25 µl	25 µl	25 µl
Ligation Buffer, 5x	10 µl	10 µl	10 µl
LT-BC# adapter*	2.8 µl	2.8 µl	0.5 µl
DNA Ligase	5 µl	5 µl	5 µl
Ligation solution†	7.2 µl	7.2 µl	7.2 µl
Nuclease-free Water	0	0	2.3 µl
Total	**50 µl**	**50 µl**	**50 µl**

* This component applies to QIAseq LT-BC# adapters in QIAseq 12-index L and QIAseq 96-index L with up to 12 or 96 sample indices, respectively.

† 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.

LT-BC1-96 Adapter Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BC1	BC2	BC3	BC4	BC5	BC6	BC7	BC8	BC9	BC10	BC11	BC12
B	BC13	BC14	BC15	BC16	BC17	BC18	BC19	BC20	BC21	BC22	BC23	BC24
C	BC25	BC26	BC27	BC28	BC29	BC30	BC31	BC32	BC33	BC34	BC35	BC36
D	BC37	BC38	BC39	BC40	BC41	BC42	BC43	BC44	BC45	BC46	BC47	BC48
E	BC49	BC50	BC51	BC52	BC53	BC54	BC55	BC56	BC57	BC58	BC59	BC60
F	BC61	BC62	BC63	BC64	BC65	BC66	BC67	BC68	BC69	BC70	BC71	BC72
G	BC73	BC74	BC75	BC76	BC77	BC78	BC79	BC80	BC81	BC82	BC83	BC84
H	BC85	BC86	BC87	BC88	BC89	BC90	BC91	BC92	BC93	BC94	BC95	BC96

Figure 4. Layout of sample index adapters in QIAseq 96-Index L. Each well contains one sample adapter. The amount of adapter in each well is sufficient for 4 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	Incubation time
1	4°C	1 min
2	20°C	15 min
3	4°C	Hold

4. After the reaction is complete, place the reactions on ice and proceed with "Cleanup of adapter-ligated DNA".

Cleanup of adapter-ligated DNA

5. Once the run has finished, for standard/FFPE samples, add 50 µl nuclease-free water to bring each sample to 100 µl. For cfDNA samples, add 30 µl nuclease-free water to bring each sample to 80 µl.

6. For standard/FFPE samples, add 100 µl QIAseq Beads. For cfDNA samples, add 112 µl QIAseq Beads. Mix well by pipetting up and down several times.

7. Incubate for 5 min at room temperature.

8. 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.

9. With the beads still on the magnetic stand, add 200 µl 80% ethanol. Carefully remove and discard the wash.

10. 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 pipet to remove any residual ethanol.

11. 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.

12. 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.

13. Return the tubes/plate to the magnetic rack until the solution has cleared.

14. Transfer 50 µl of the supernatant to clean tubes/plate.

15. For standard/FFPE samples, add 65 µl QIAseq Beads (for Human Mitochondria Panel use 50 µl). For cfDNA samples, add 70 µl QIAseq Beads. Mix well by pipetting up and down several times.

16. Incubate for 5 min at room temperature.

17. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates). 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.

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

19. 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.

20. 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.

21. Return the tube/plate to the magnetic rack until the solution has cleared.

22. Transfer 9.4 µl of the supernatant to clean tubes or plate.

23. 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", page 25, 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.
- **Important**: Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

Procedure: Target enrichment

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

   Note: 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 target enrichment mix according to Table 10. Briefly centrifuge, mix by pipetting up and down 7–8 times and briefly centrifuge again.

Table 10. Reaction mix for target enrichment

Component	Volume/reaction
Adapter-ligated DNA from "Cleanup of adapter-ligated DNA"	9.4 µl
TEPCR buffer, 5x	4 µl
QIAseq Targeted DNA Panel	5 µl
LT-Forward Primer	0.8 µl
HotStarTaq DNA Polymerase	0.8 µl
Total	**20 µl**

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

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

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

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
Initial denaturation	13 min 2 min	13 min 2 min	95°C 98°C
6 cycles	15 s 15 min	15 s 30 min	98°C 65°C
1 cycle	5 min	5 min	72°C
Hold	∞	∞	4°C

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

5. After the reaction is complete, place the reactions on ice and proceed with "Cleanup of target enrichment". Alternatively, the samples can be stored at –20°C in a constant-temperature freezer for up to 3 days.

Cleanup of target enrichment

1. Once the run has finished, for all sample types, add 60 µl nuclease-free water to bring each sample to 80 µl.

2. For standard/FFPE samples, add 104 µl QIAseq Beads (for Human Mitochondria Panel use 80 µl). For cfDNA samples, add 112 µl QIAseq Beads. Mix well by pipetting up and down several times.

3. Incubate for 5 min at room temperature.

4. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates). After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

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

5. With the beads still on the magnetic stand, add 200 µl 80% ethanol. Carefully remove and discard the wash.

6. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol after this second wash. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.

7. With the beads still on the magnetic stand, air dry at room temperature for 10 min.

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

8. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 16 µl nuclease-free water. Mix well by pipetting.

9. Return the tube/plate to the magnetic rack until the solution has cleared.

10. Transfer 13.4 µl of the supernatant to clean tubes/plate.

11. Proceed with "Protocol: Universal PCR", page 34. 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 13.4 µl product from "Protocol: Target Enrichment", page 30, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- The QIAseq Beads are used for all reaction cleanups.
- **Important**: Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

Procedure: universal PCR

1. Prepare the reagents required for the universal PCR.

   1a. Thaw UPCR Buffer, 5x, at room temperature and bring the LT-Universal Primer and LT-P1 Primer to room temperature and then place on ice.

   1b. 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 13. Briefly centrifuge, mix by pipetting up and down 7–8 times and briefly centrifuge again.

Table 13. Reaction mix for universal PCR

Component	Volume/reaction
Target enriched DNA from "Cleanup of target enrichment"	13.4 µl
UPCR buffer, 5x	4 µl
LT-Universal Primer	0.8 µl
LT-P1 Primer	0.8 µl
HotStarTaq DNA Polymerase	1 µl
Total	**20 µl**

3. Program a thermal cycler using the cycling conditions in Table 14 (cycling program) and Table 15 (number of cycles).

Table 14. Cycling conditions for universal PCR

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

Table 15. Amplification cycles for universal PCR

Primers per pool	Number of cycles, standard DNA	Number of cycles, 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, for all sample types, add 60 µl nuclease-free water to bring each sample to 80 µl.

5. For standard/FFPE samples, add 104 µl QIAseq Beads (for Human Mitochondria Panel use 80 µl). For cfDNA samples, add 112 µl QIAseq Beads. Mix well by pipetting up and down several times.

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. 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 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.

14. Proceed to "Recommendations: Library QC and Quantification", page 38. Alternatively, the library can be stored in a –30 to –15°C in a constant-temperature freezer. Amplified libraries are stable for several months at –30 to –15°C. Once quantification is performed proceed to "Protocol: Sequencing Setup for Ion Torrent Instruments", page 40.

Recommendations: Library QC and Quantification

NGS library QC

After the library is constructed and purified, the QIAxcel or Bioanalyzer can be used to check the fragment size and concentration with the High Sensitivity DNA Kit. Libraries prepared for Ion Torrent instruments demonstrate a size distribution between 200 and 1000 bp (Figure 5, page 38). Amounts of DNA under the appropriate peaks can be used to quantify the libraries. However, due to the superior sensitivity of qPCR, we recommend quantifying the libraries using the QIAseq Library Quant System, especially when there are overamplified libraries (See "Preferred library quantification method", page 39).

Library prepared for Ion Torrent instruments

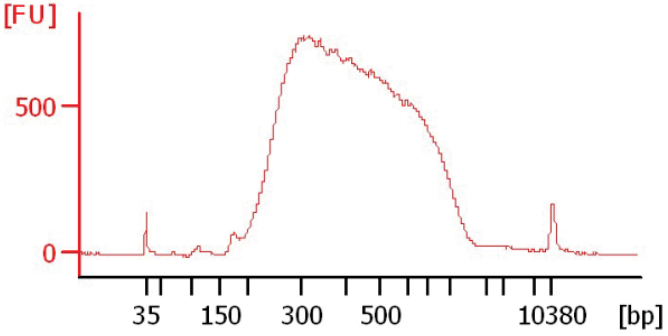


Figure 5. Sample Bioanalyzer image of QIAseq Targeted DNA Panel libraries for Ion Torrent instruments. The size of the majority of the library fragments are between 200 and 1000 bp.

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 for Ion Torrent Instruments

Important: Recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System (See Appendix C, page 59).

After the library is constructed, follow Appendix C (page 59) to determine the library dilution factor (which dilutes libraries to 4 pM for PGM or 50 pM for Ion Chef™,/S5), and dilute each individual library according to this factor.

Libraries with a different sample index can be combined in equimolar amounts if similar sequencing depth is needed for each library. If combining libraries with the same number of primers, pool equal volumes of the individual libraries at 4 pM together. If combining libraries with different primer numbers, mix the libraries at a volume ratio according to their number of primers. For example, Library A has 5000 primers at 4 pM, and Library B has 600 primers at 4 pM; 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.

After combining libraries with different indexes, proceed to template preparation and sequencing according to the manufacturer instructions. The sample index of QIAseq Targeted DNA Panels for the Ion Torrent is compatible with the Ion Xpress adapter sample index system. When using Ion Torrent systems, 200 bp (or longer) single reads are required, except for the Human Mitochondria Panel where 300 bp (or longer) single reads are needed. For best result, 400 bp reads are recommended for all panels.

Upon completion of the sequencing run, proceed with "Protocol: Downloading Individual Unaligned BAM File with a Multiplex Sample on Ion PGM Sequencer", page 52.

QIAseq Targeted DNA Panel Ion Chef and S5 set up

Important points before starting

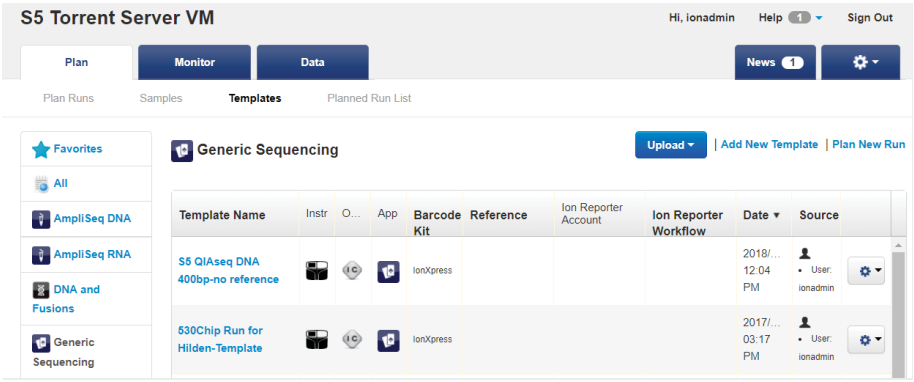
- Following recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System:
  Ion Chef: 50 pM
  Ion OneTouch™: 4 pM
- Do 400 bp read length for optimal result. Our data analysis tool requires complete read length from UMIs to gene specific primers. Use 200 bp read length can result in up to 30% reads dropping due to incomplete read length.
- Set up sequencing without reference genome to get unaligned .bam file for our data analysis. In addition, due to the special read structure of QIAseq Targeted DNA Panel Library, read starting with 12 bp UMI and 11 bp common sequences, selecting reference genome could result in many short reads dropped or identified as low-quality reads.
- However, in case the sequencing is set up with reference genome, the data can be reanalyzed without reference genome.
- Do not run plugin or Ion reporter if possible. Due to the library special structure, it could pose potential problem for correct reads processing.

Create a Planned Run

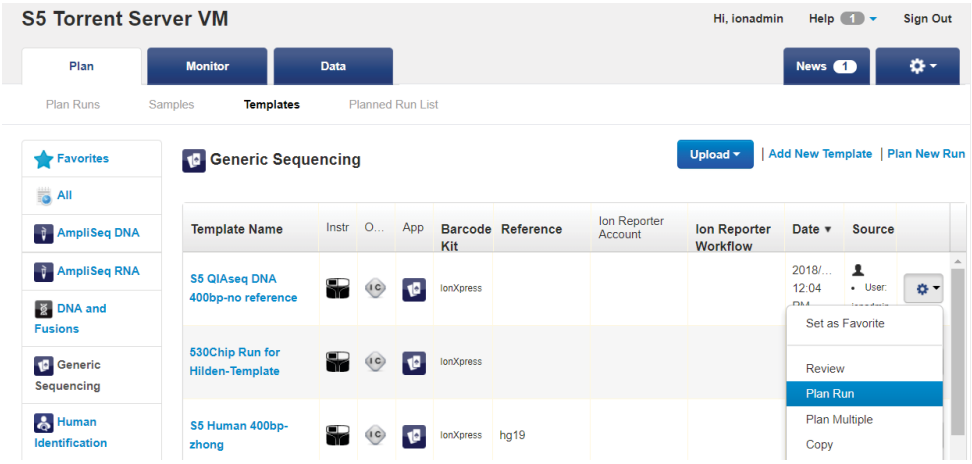
1. Log in to the Torrent server via the Torrent Browser.

2. Select the **Plan** tab, click **Templates**, select the application that you want to run, then click either:

   Plan New Run on the right side to plan a new run using the generic template for the selected application.

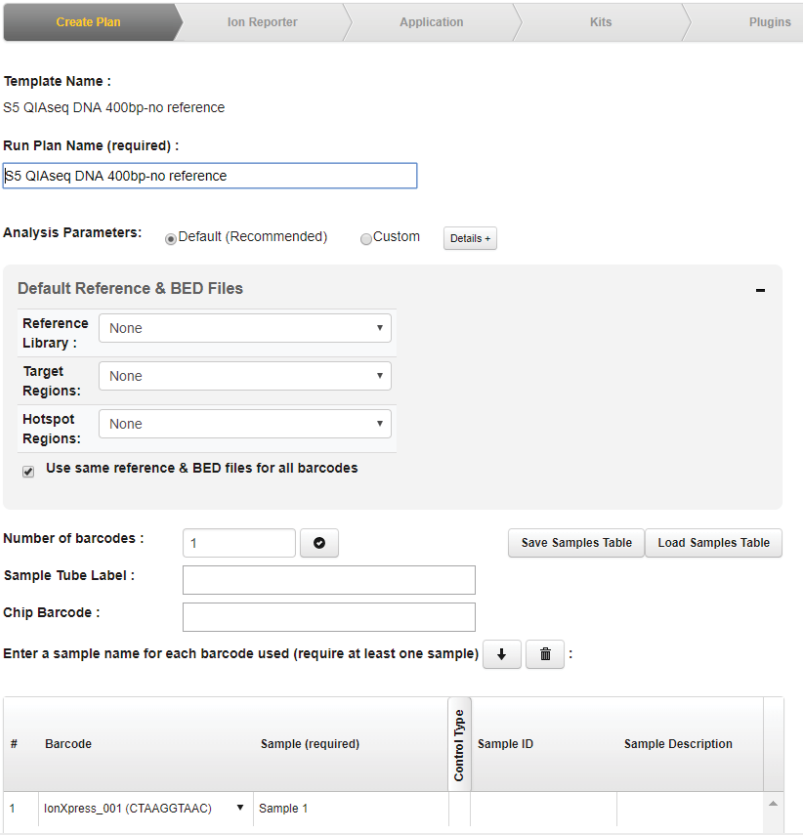


   Plan Run in the dropdown menu under the **Settings** tab to the right of the existing template you select from the template list.

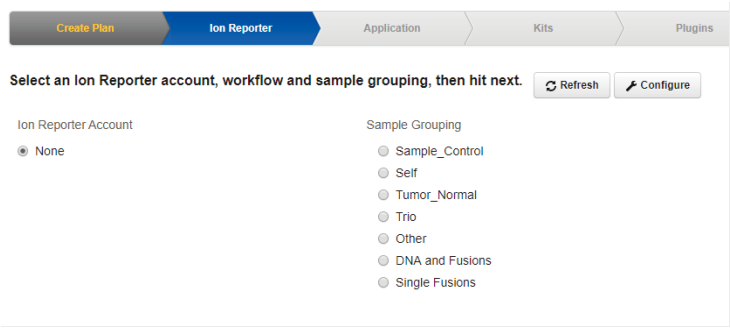


3. In the **Planned Run** wizard, under **Create Plan** tab, make appropriate selections as below.

   Important: Do not select reference genome if possible.



4. Under the **Ion Reporter** tab, select **None**.



5. Under **Application** tab, select **DNA** for **Application** and **Other** for **Target Technique**.

6. Under **Kits** tab, make the following selection:

Instrument: Ion S5 System

Chip Type: Select the appropriate chip type from dropdown list

Library Kit Type: Ion Xpress Plus Fragment Library Kit

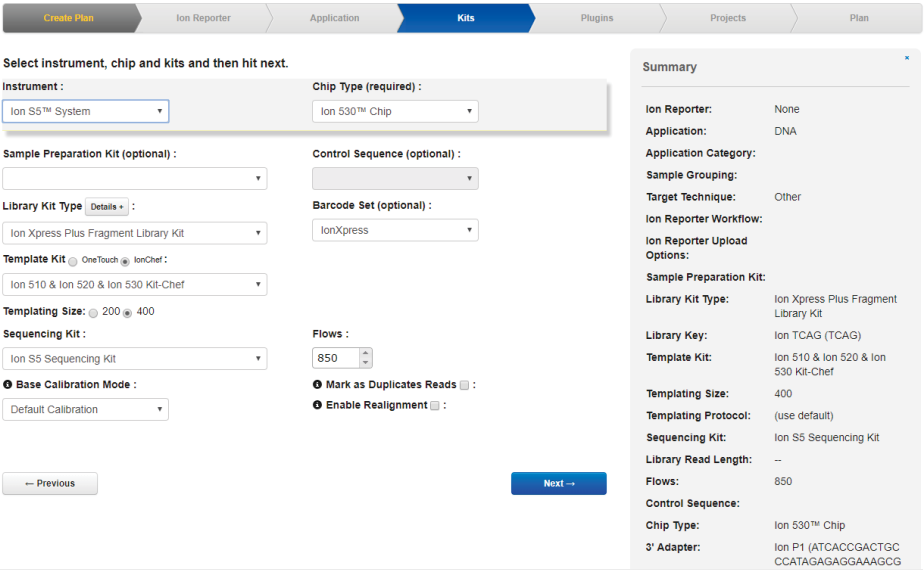
Barcode Set: IonXpress

Template Kit: IonChef

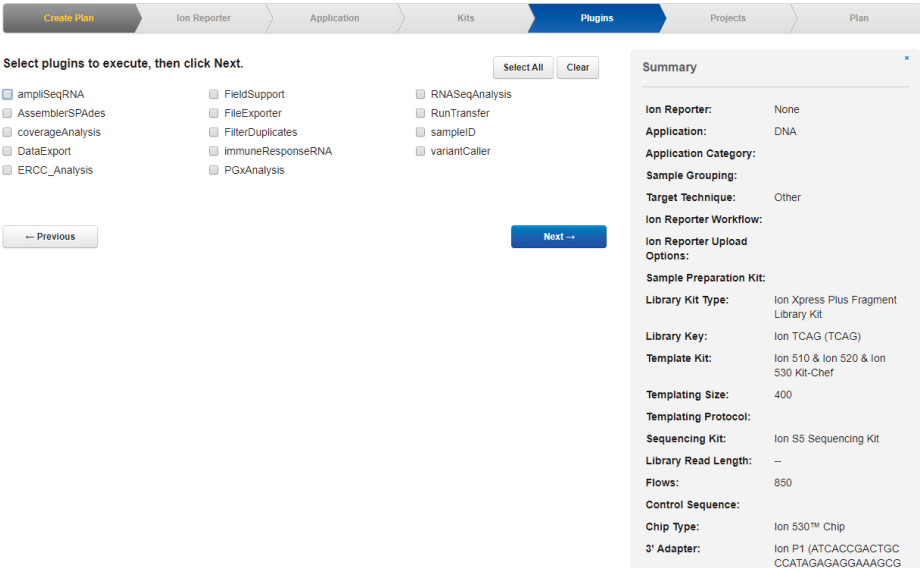
Templating Size: 400

Sequencing Kit: Ion S5 Sequencing Kit

Flows: 850



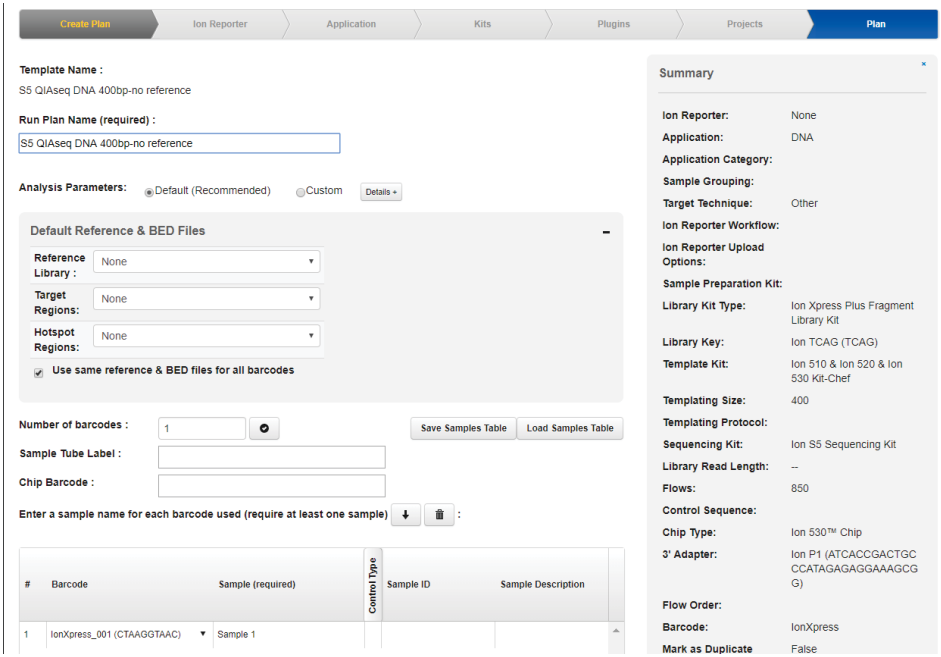
7. Under **Plugins** tab, leave everything unselected.



8. Select or create appropriate project under **Projects** tab.

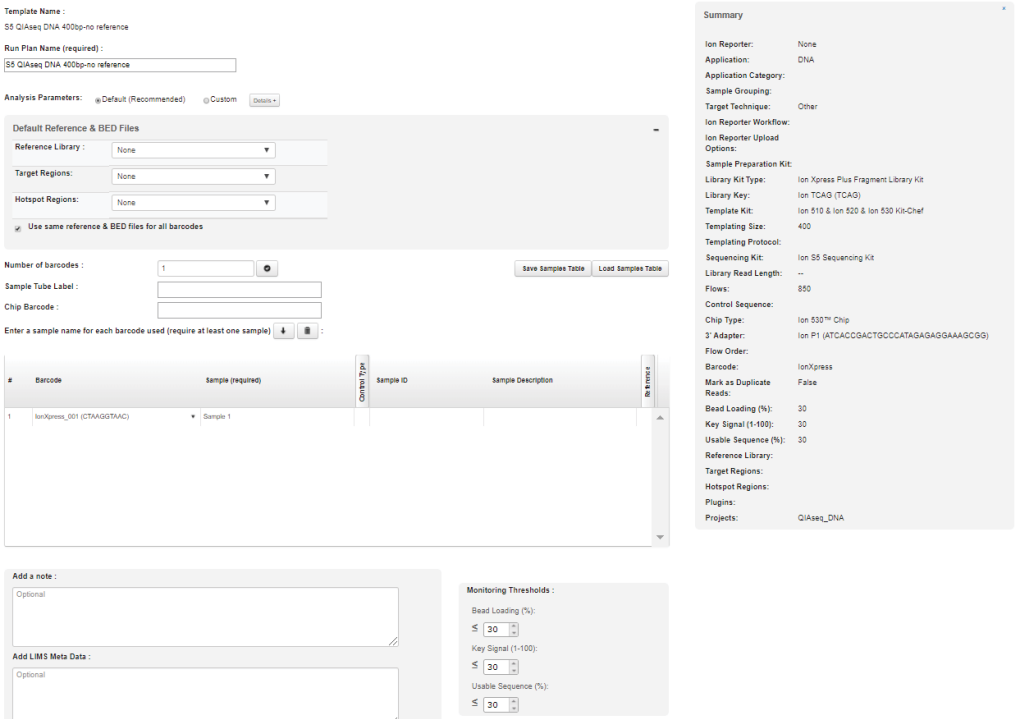
9. Under the **Plan** tab:

   Run Plan Name (required): Enter a Planned Run name
   Analysis Parameters: Default (Recommended)
   Default Reference and BED Files: none
   Sample Tube Label: Enter or scan the barcodes of the Ion Chef Library Sample Tubes
   Chip Barcode: Scan the barcodes of the chip



10. When you have completed your selections, click "**Plan Run**" at the bottom right of the **Plan** tab screen to save the run. The run is listed on the Planned Runs page under the name that you specified and is automatically used by the Ion Chef System when the associated sample is loaded.

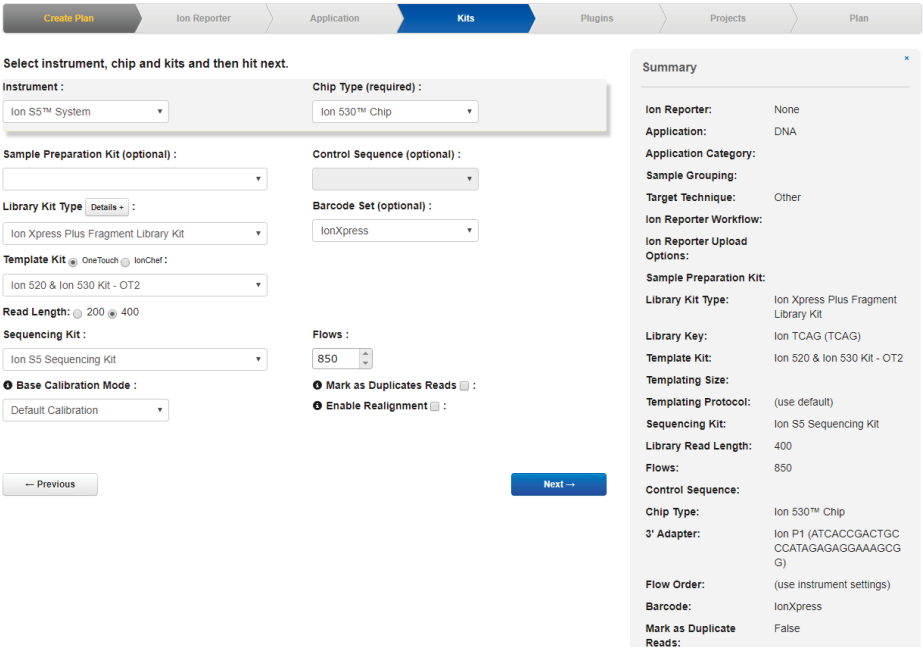
11. Here is the overview of all settings:



12. Run the Ion Chef System according to manufacturer's instruction.

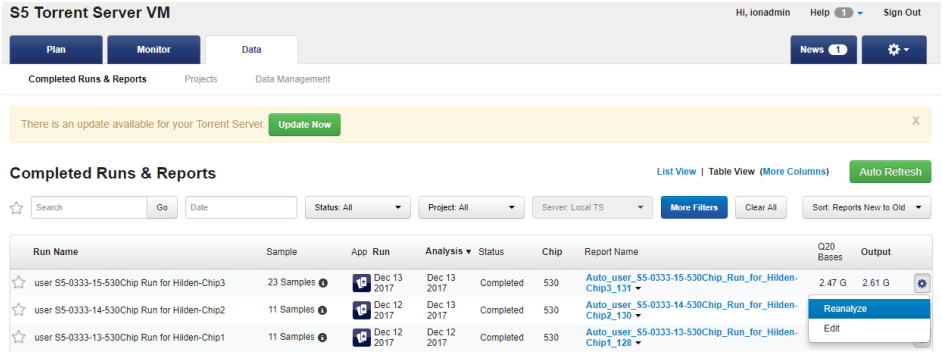
13. When the run is complete, unload the Ion Chef instrument and sequence the chips immediately on S5 according to manufacturer's instruction.

14. If template is prepared by OneTouch instead of Ion Chef, under the **Kits** tab, select **OneTouch** for **Template kit**. Other sequencing parameters set up on S5 should be the same as above described with Ion Chef.



Reanalyze Data with No Reference Genome

1. If the run was set up with a reference genome, the data can be reanalyzed with no reference genome. Under the **Data** tab, find your run, click the setting icon at the right side, and select **Reanalyze**.

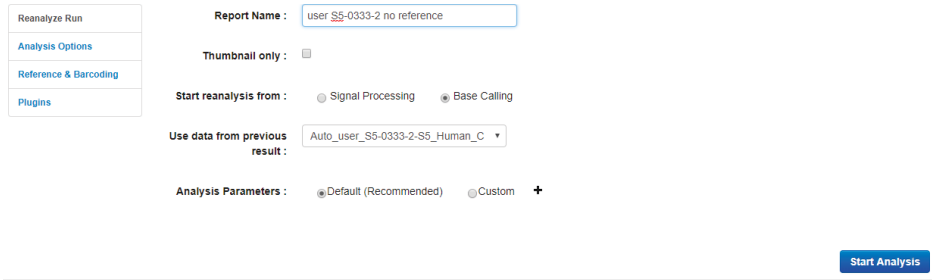


2. Click the **Reanalyze Run** tab at the left, select the following:

   Report Name: give the reanalyzing data a difference name than the original one

   Start reanalysis from: Base Calling

   Use data from previous result: should be already automatically selected with the run you want to reanalyze



3. Click the **Analysis Options** tab at the left, select the following:

Library Key: TCAG

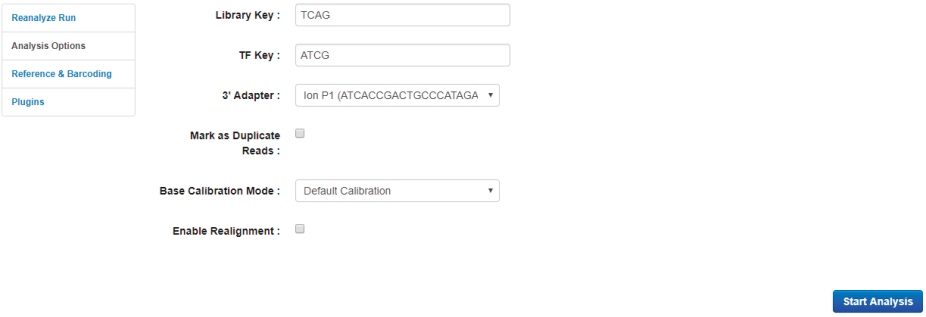
TF Key: ATCG

3′ Adapter: Ion P1

Mark as Duplicate Reads: not selected

Base Calibration Mode: Default Calibration

Enable Realignment: not selected

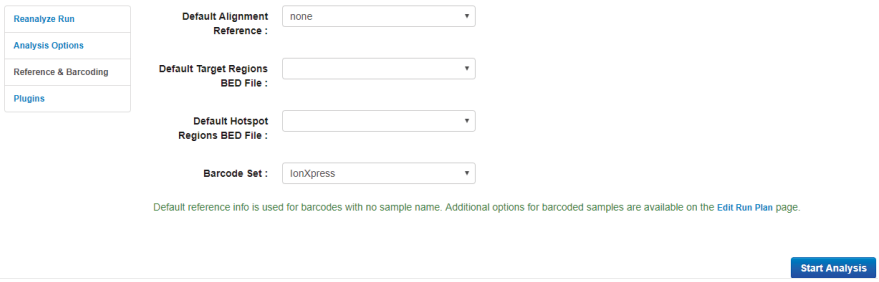


4. Click the **Reference & Barcoding** tab at the left, select the following:

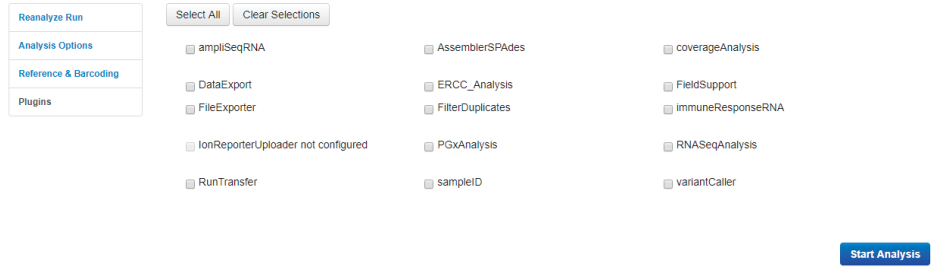
Default Alignment Reference: none

Default Target Regions and Default Hotspot Regions BED File: not selected

Barcode Set: IonXpress

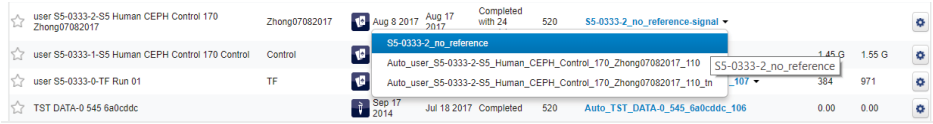


5. Click the **Plugins** tab at the left; do not select any plugins.



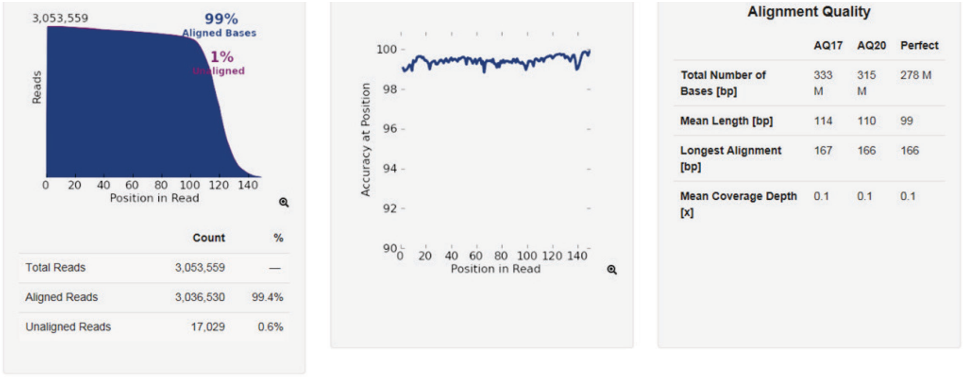
6. Click **Start Analysis**, the data will be reanalyzed with no reference genome sequence.

7. After reanalyzing, go back to the **Data** tab and find the run that was selected for analyzing. The report that was reanalyzed with no reference genome will be found under the report name drop-down list.



Protocol: Downloading Individual Unaligned BAM File with a Multiplex Sample on Ion PGM Sequencer

1. Upon completion of the sequencing run, navigate to the report page on the Torrent Browser. Locate the **Output Files** section near the end of the report (Figure 6).



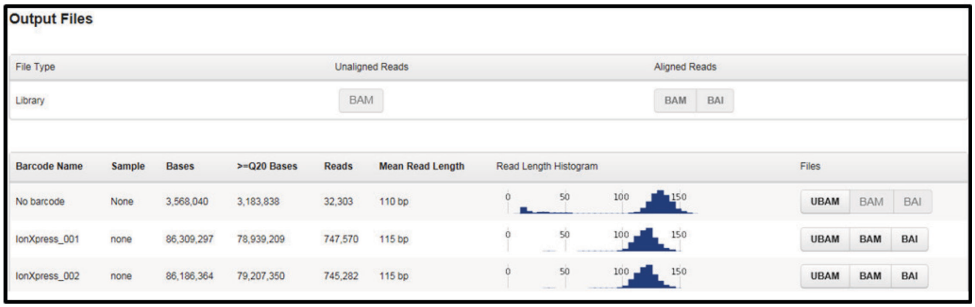


Figure 6. Torrent Browser report page and output files.

2. Click the **UBAM** button in the row corresponding to the individually indexed samples and column labeled **Files** in the table (Figure 7). These are the unaligned reads in BAM format, with the index separated for each sample. Save the .ubam file to a local disk. The file is usually several hundred megabytes to several gigabytes, depending on the size of the sequencing chip being used.

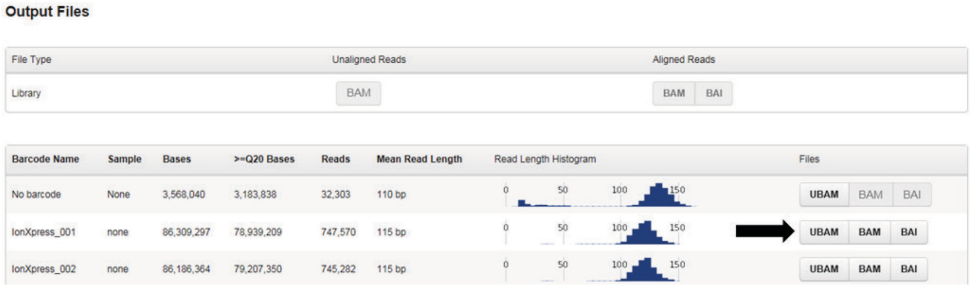


Figure 7. Unaligned reads in BAM format.

3. Locate the file that was just downloaded to the local disk. The individual .UBAM files will be needed to perform analysis using the QIAseq Targeted Sequencing Data Analysis Portal or the QIAGEN CLC Genomics Workbench.

4. Proceed to "Appendix C: Data Analysis Using QIAGEN's QIAseq Targeted Sequencing Data Analysis Portal or CLC Genomics Workbench", page 59.

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 TEPCR or UPCR	The QIAseq Beads need to be completely dried before elution; overdrying beads won't affect elution in this workflow. Ethanol carryover to TEPCR and UPCR will affect PCR reaction efficiency.
Unexpected signal peaks	
a) Short peaks around 75 and 180 bp	These are primer–dimers from TEPCR (approx. 75 bp) or UPCR (approx. 180 bp). The presence of primer dimers indicates either not enough DNA input or inefficient PCR reactions.
b) Larger library fragments after UPCR	After the UPCR, library fragments are larger than the intended peak and can be a PCR artifact due to overamplification of the DNA library. Overamplification of the library won't affect the QIAseq Targeted DNA Panels sequencing performance. Decreasing the number of UPCR cycle numbers can reduce overamplification.
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 overamplification.
Variant detection issues	
Known variants not detected	Variant detection sensitivity is directly related to the input DNA and read depth. Check Table 3 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 16.

Table 16. Combining an existing panel (at 50 µl) with a booster panel

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

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 100 ng of DNA can be used. If the DNA is determined as low quality (QC score >0.04), then 250 ng of DNA can be used. The QC score of QuantiMIZE reflects the amount of amplifiable DNA present in the sample, therefore correlating with the number of UMIs that can be sequenced in the library (Figure 8, next page).

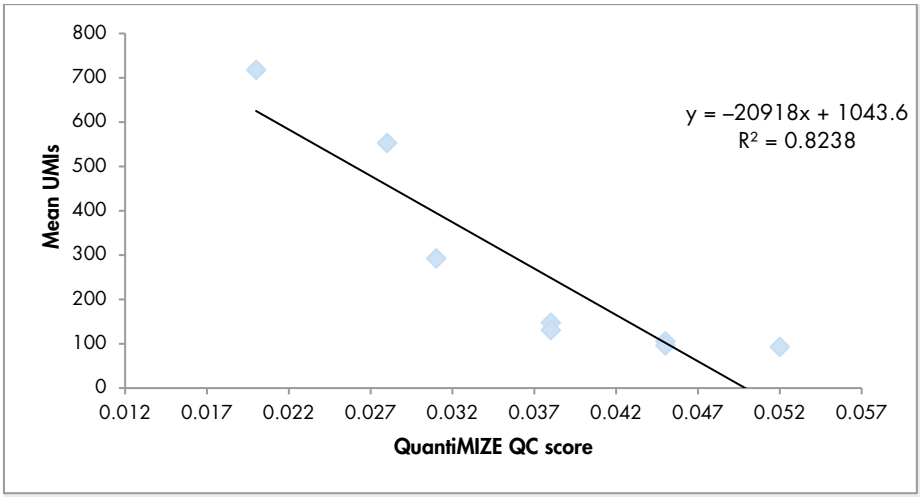


Figure 8. Correlation between QIAseq QuantiMIZE QC score and the number of UMIs.

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: 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 9.

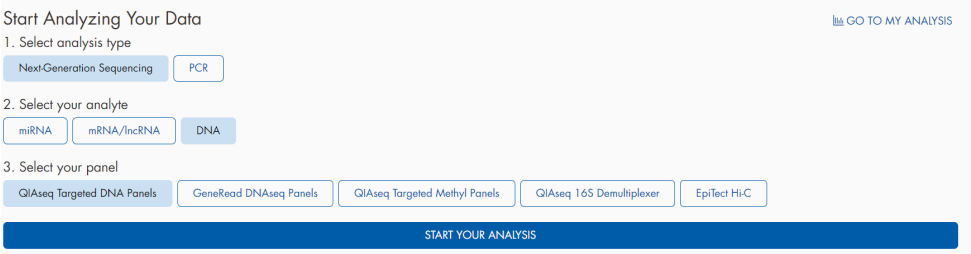


Figure 9. GeneGlobe Analysis pipeline selections for the QIAseq Targeted DNA Panels.

3. Click **START YOUR ANALYSIS**.

4. In the **Read Files** tab, select **Uploaded** > **Upload New Files** to upload files from your local drive (Figure 10).

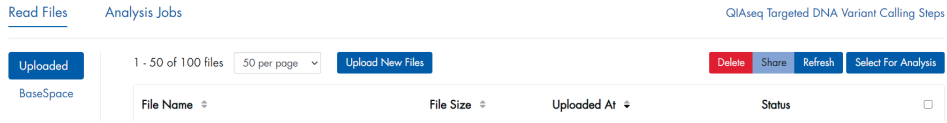


Figure 10. File Upload tab of the QIAseq Targeted DNA Panel Data Analysis Pipeline.

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 11).

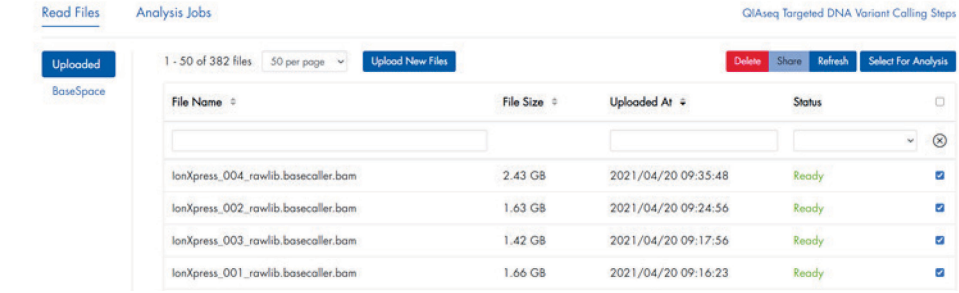


Figure 11. File selection for the QIAseq Targeted DNA Panel data analysis pipeline

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

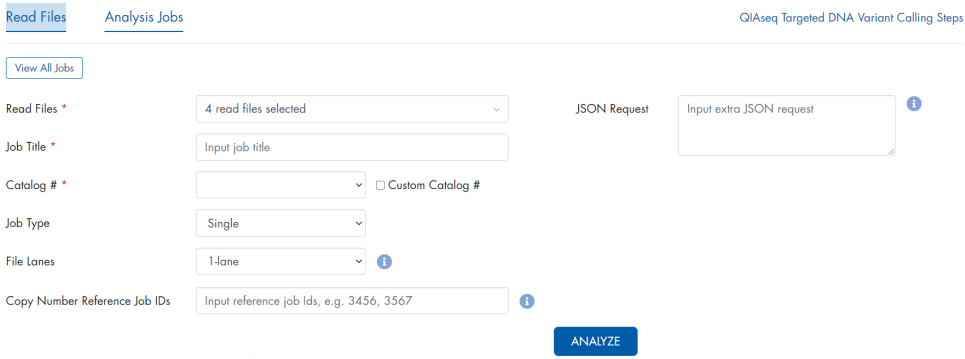


Figure 12. Analysis Jobs tab of the QIAseq Targeted DNA Panel data analysis pipeline

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 drop-down menu. If using a custom panel, enter the custom catalog number manually.

Job type: Single or matched tumor/normal.

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.

7. Click **ANALYZE**. The analysis job status changes from **Queued** to **In progress**, and then **Done** successfully.

8. 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
QIAseq 12-Index L (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 Ion Torrent platforms	333764

Product	Contents	Cat. no.
QIAseq 96-Index L (384)	Box containing molecularly-indexed adapters and primers in arrays, enough for a total of 384 samples – for indexing up to 96 samples for targeted panel sequencing on Ion Torrent platforms	333777
Related products		
QIAseq Library Quant Array Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; array format	333304
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; assay format	333314
QIAseq DNA QuantiMIZE Array Kit	qPCR arrays for optimizing amount of input DNA and PCR cycling conditions for targeted enrichment of FFPE DNA	333404
QIAseq DNA QuantiMIZE Assay Kit	qPCR assays for optimizing amount of input DNA and PCR cycling conditions for targeted enrichment of FFPE DNA	333414
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 Tissue Kit	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404
QIAamp DNA FFPE Advanced Kit	For 50 preps: QIAamp UCP MinElute Columns, collection tubes, Deparaffinization Solution, Proteinase K, RNase A, RNase-free water and buffers	56604

Product	Contents	Cat. no.
QIAamp DNA FFPE UNG Kit	For 50 preps: Uracil-N-glycosylase, QIAamp UCP MinElute columns, collection tubes, Deparaffinization Solution, Proteinase K, RNase A, RNase-free water and buffers	56704

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
06/2021	Initial release

Notes

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