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QIAseq[®] Targeted DNA Pro Handbook

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

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Kit Contents

QIAseq Targeted DNA Pro Catalog no. No. of samples	333651 12	HC 333661 12	333655 96	HC 333665 96	Custom 333675 96
One pool of region-specific primers	90 µL	90 µL	700 µL	700 µL	700 µL
FFPE repair reagent	15 µL	15 µL	80 µL	80 µL	80 µL
FFPE repair enzyme	17 µL	17 µL	120 µL	120 µL	120 µL
FX Buffer, 10x	23 µL	23 µL	170 µL	170 µL	170 µL
5X WGS FX Mix	42 µL	42 µL	330 µL	330 µL	330 µL
FG Solution	170 µL	170 µL	170 µL	170 µL	170 µL
UPH Ligation Buffer, 2.5x	288 µL	288 µL	1 152 µL	1 152 µL	1 152 µL
DNA Ligase	75 µL	75 µL	330 µL	330 µL	330 µL
Ligation Cleanup Reagent	30 µL	30 µL	240 µL	240 µL	240 µL
TEPCR Buffer, 5x	3 x 60 µL	3 x 60 µL	3 x 500 µL	3 x 500 µL	3 x 500 µL
TEPCR modifier	30 µL	30 µL	230 µL	230 µL	230 µL
TEPCR Cleanup Reagent	80 µL	80 µL	600 µL	600 µL	600 µL
UPCR Buffer, 5x	500 µL	500 µL	4 x 500 µL	4 x 500 µL	4 x 500 µL
QN Taq Polymerase	2 x 60 µL	2 x 60 µL	2 x 450 µL	2 x 450 µL	2 x 450 µL
Nuclease-free water	1.5 mL	1.5 mL	10 mL	10 mL	10 mL
One bottle containing QIAseq Bead Binding Buffer	10.2 mL	10.2 mL	10.2 mL	10.2 mL	10.2 mL
One bottle containing QIAseq Beads	10 mL	10 mL	10 mL	10 mL	10 mL
QIAseq Targeted DNA Pro Booster (96) Catalog no. No. of samples				333685 96	
One pool of region-specific primers				80 µL	

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

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

QIAseq Targeted DNA Pro Index L (24)

QIAseq Targeted DNA Pro Index L (24)

Catalog no.

333492

No. of samples

24 (µL)

QILT-12A Index Adapter Plate*	5
LT-Forward Primer	40
LT-P1 primer	40
LT-Universal Primer	310
SmP-LTP1 UPCR-R Primer	120

* Index Adapter Plate; each plate contains 24 wells of molecularly indexed adapters, with each well corresponding to one sample index; each index is single use.

QIAseq Targeted DNA Pro Index L (12)

QIAseq Targeted DNA Pro Index L (12)

Catalog no.

333491

No. of samples

12 (µL)

QILT-24A Index Adapter Plate*	5
LT-Forward Primer	40
LT-P1 primer	40
LT-Universal Primer	310
SmP-LTP1 UPCR-R Primer	60

* Index Adapter Plate; each plate contains 12 wells of molecularly indexed adapters, with each well corresponding to one sample index; each index is single use.

Shipping and Storage

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

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

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

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

Intended Use

The QIAseq Targeted DNA Pro Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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

Safety Information

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq Targeted DNA Pro is tested against predetermined specifications, to ensure consistent product quality.

Introduction

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

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

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

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

The QIAseq Targeted DNA Pro Kit has also been optimized in combination with a specially formulated enrichment chemistry to achieve highly efficient enrichment on both regular and GC-rich regions at high multiplex levels. Additionally, the 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 Pro Kit is a Sample to Insight solution for precision variant detection of targeted genomic regions using NGS (Figure 1).

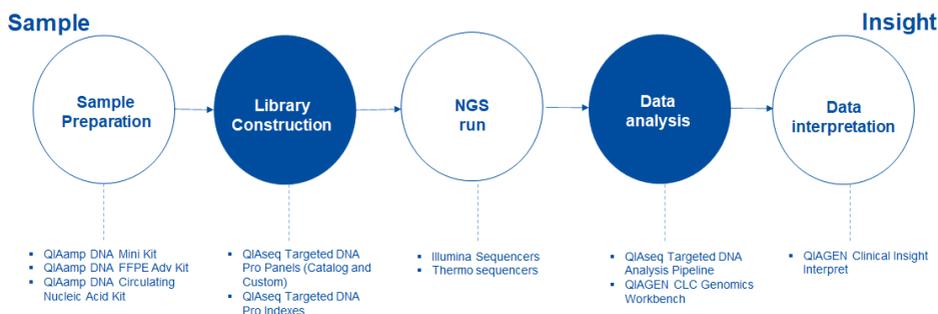
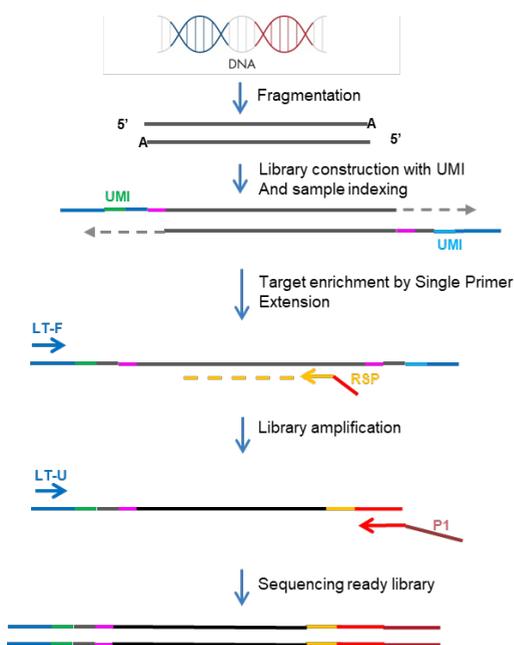


Figure 1. Overview of the Sample to Insight NGS workflow with the QIAseq Targeted DNA Pro Kit. The complete Sample to Insight procedure begins with DNA extraction. Next is library construction and target enrichment with the QIAseq Targeted DNA Pro Kit. Following NGS, data analysis is performed using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

Principle and procedure

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



UMI: Unique molecular indices
RSP: Region specific primer
LT-F: TEPCR-F primer
LT-U and P1: Universal PCR primers

Figure 2. QIAseq Targeted DNA Pro workflow.

Fragmentation

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

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

UMI assignment

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

Target enrichment and final library construction

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

Enzymatic cleanup after ligation and target enrichment PCR

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

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

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. 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. However, target region coverage can be better achieved by counting the number of UMIs rather than counting the number of total reads for each region. Sequence reads having different UMIs represent different original molecules, while sequence reads having the same UMIs are the result of PCR duplication from one original molecule. Errors from PCR amplification and from the sequencing process may also be present in final reads that lead to false positive variants in sequencing results. These artifactual variants can be greatly reduced by calling variants across all reads within a unique UMI instead of picking up variants at the original read level.

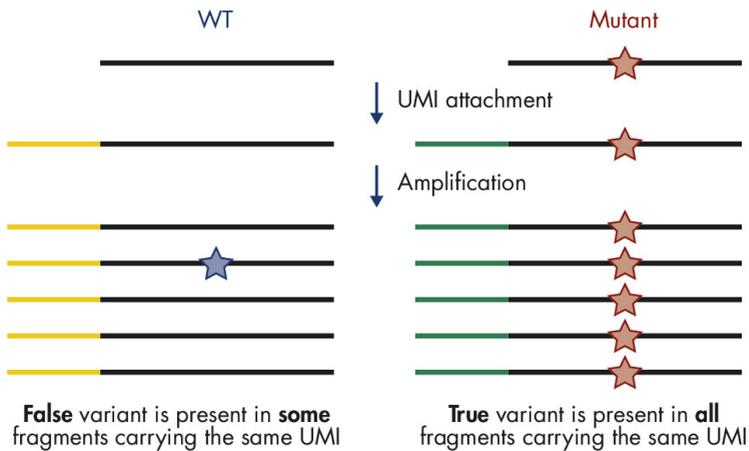


Figure 3. Principle of variant detection with UMIs. Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while false positives are mutations present in only one or a few reads within a UMI.

Data analysis

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

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

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

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

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

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

Important Notes

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

DNA isolation and quality check

The most important prerequisite for DNA sequence analysis is consistent, high-quality DNA from every experimental sample. Therefore, sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, or other contaminants may either degrade the DNA or decrease the efficiency of, if not block completely, the enzymatic activity necessary for optimal targeted enrichment. Sample purity can be checked with the QIAxpert.

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

Important: Do not use DEPC-treated water.

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

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

Table 1. Recommended kits for purification of genomic DNA

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

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

DNA quantification

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

DNA integrity

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

Specific recommendations for FFPE DNA

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

targeted enrichment NGS workflow, such as the QIAseq Targeted DNA Pro Kit. Appendix B (page 59) provides detailed information for FFPE DNA quality assessment and input amount.

DNA input amount and sequencing depth

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

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

Variant frequency (%)	Input (ng)	Reads/UMI	Mean read
5	10	4	7200
1	40	4	25,600

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

Variant detection

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

Sequencing capacity and sample multiplex level

Sample multiplexing is one of the most important NGS tools for increasing throughput and reducing costs. It works by combining multiple samples to be processed together in a single sequencing run; as a result, sequencing reads need to be demultiplexed by reassigning each single read to its original source library. This is facilitated by the integration of sample index sequences into the individual library molecules.

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

- QIAseq Targeted DNA Pro Index L (24): DNA Pro 24 index L adapter plate
- QIAseq Targeted DNA Pro Index L (12): DNA Pro 12 index L adapter plate

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

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

Instrument	Version	Capacity	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 raw read 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 raw read 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. For best result, 400 bp reads are recommended for all panels.

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

Important points before starting

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

Procedure: FFPE DNA repair

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

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

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

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

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

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

Table 6. Reaction mix for FFPE DNA repair

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

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

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

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

Table 7. Incubation conditions for FFPE DNA Repair reaction

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

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

Procedure: fragmentation and end prepare

1. Thaw nucleic acid samples on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and then return to ice.
2. Prepare the reagents required for fragmentation and end prepare.
 - 2a. Thaw 10x FX Buffer and FG Solution on ice or, if required, at room temperature but immediately place on ice after being thawed. Keep 5X WGS FX Mix on ice.
 - 2b. Mix all reagents by flicking the tube, and centrifuge briefly.
3. On ice, prepare the fragmentation and end prepare mix according to Table 8. Briefly centrifuge, mix by pipetting up and down 10–12 times and briefly centrifuge again.

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

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

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

Table 8. Reaction mix for fragmentation and end prepare

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

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

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

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

6. Transfer the tubes/plate prepared in step 3 to the prechilled thermal cycler and resume the cycling program.

Table 9. Incubation conditions for fragmentation and end prepare

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

7. Upon completion, allow the thermal cycler to return to 4°C.
8. Place the samples on ice and immediately proceed to “Protocol: Adapter Ligation”, page 25.

Protocol: Adapter Ligation

Important points before starting

- The roughly 14 μL product from “Procedure: fragmentation and end prepare”, page 23, is the starting material for this protocol.
- Set up reactions on ice, mixing reactions by pipetting on ice.
- Do not vortex any reagents or reactions.
- Use the QIAseq Pro LT-## Adapters in QIAseq Pro Index L (12) and (24) with up to 12 and 24 sample indices, respectively.
 - The adapter plate is sealed with pierceable aluminum heat sealing film.
 - Puncture the film using standard 200 μL pipet tips to transfer the appropriate amount of adapter to tube/plate for the ligation reaction.
 - Thaw the QIAseq Targeted DNA Pro Index L (12) or (24) adapter plate on ice or store at 4°C before usage. After it is fully thawed, centrifuge the plate at 1000 $\times g$ for 1 min.

Procedure: adapter ligation

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

Important: Only one single-indexed adapter should be used per ligation reaction. Layout of the QIAseq Targeted DNA Pro Index L (12) or (24) adapter plate is described in Figure 4. Use a multichannel pipet to pipet the appropriate amount of adapters

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

QIIL-12A Index Adapter Plate in QIAseq Targeted DNA Pro Index L (12)

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

QIIL-24A Index Adapter Plate in QIAseq Targeted DNA Pro Index L (24)

	1	2	3	4	5	6	7	8	9	10	11	12
A	LT-1	LT-9	LT-17									
B	LT-2	LT-10	LT-18									
C	LT-3	LT-11	LT-19									
D	LT-4	LT-12	LT-20									
E	LT-5	LT-13	LT-21									
F	LT-6	LT-14	LT-22									
G	LT-7	LT-15	LT-23									
H	LT-8	LT-16	LT-24									

Figure 4. Layout of sample index adapters in QIAseq Targeted DNA Pro Index L (12) and (24). Each well contains one sample adapter. The amount of adapter in each well is for a single reaction.

Table 10. Reaction mix for adapter ligation

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

3. Program the thermal cycler according to Table 11.

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

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

Table 11. Incubation conditions for DNA ligation

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

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

Procedure: ligation cleanup reaction

1. After ligation, transfer the sample to ice and add 2 μ L of Ligation Cleanup Reagent to each sample. Briefly centrifuge, mix by pipetting up and down 7–8 times and briefly centrifuge again.
2. Program the thermal cycler according to Table 12. Use the instrument's heated lid.
3. Before adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.
4. Transfer the tubes/plate prepared in step 1 to the prechilled thermal cycler and resume the cycling program.

Table 12. Incubation conditions for ligation cleanup reaction

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

5. Upon completion, allow the thermal cycler to return to 4°C.
6. Place the samples on ice and immediately proceed to "Protocol: Target Enrichment", page 29.

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

Important: Keep samples on ice all the time; do not leave them at room temperature.

Protocol: Target Enrichment

Important points before starting

- The 30 μ L product from “Protocol: Adapter Ligation”, page 25, is the starting material for this protocol.
- Set up reactions on ice, mixing reactions by pipetting on ice.
- Do not vortex any reagents or reactions.

Procedure: target enrichment

1. Prepare the reagents required for the Target Enrichment PCR (TEPCR).
 - 1a. Thaw TEPCR Buffer, 5x; QIAseq Targeted DNA Pro Panel; SmP-IL5 TEPCR-F Primer; and TEPCR modifier on ice (or at room temperature, but immediately place on ice after being thawed).
 - 1b. Keep QN Taq Polymerase on ice.
 - 1c. Mix all reagents by flicking the tube, and then centrifuge briefly
2. Prepare the target enrichment mix according to Table 13. Briefly centrifuge, mix by pipetting up and down 7–8 times, and briefly centrifuge again.

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

Table 13. Reaction mix for Target Enrichment

Component	Volume/reaction (µL)
Cleaned Adapter-ligated DNA from "Ligation Cleanup Reaction" (already in tube)	30
TEPCR Buffer, 5x	10
QIAseq Targeted DNA Pro Panel	6.3
LT-Forward Primer	0.5
TEPCR modifier	1.95
QN Taq Polymerase	2.4
Total	51.15

3. Program a thermal cycler using the cycling conditions in Table 14 (panel with <12,000 primers/tube) or Table 15 (panel with ≥12,000 primers/tube).

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

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

* Use 67 °C for panels PHS-102Z, PHS-104Z, PHS-203Z, PHS-204Z and PHS-205Z.

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

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

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

- After the reaction is complete, place the reactions on ice and proceed to “Procedure: TEPCR cleanup reaction” below. Alternatively, the samples can be stored at -20°C in a constant-temperature freezer for up to 3 days.

Procedure: TEPCR cleanup reaction

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

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

- Prepare the TEPCR cleanup mix according to Table 16. Briefly centrifuge, mix by pipetting up and down 7–8 times and briefly centrifuge again.

Table 16. Reaction mix for TEPCR cleanup

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

- Program the thermal cycler according to Table 17. Use the instrument’s heated lid.
- Before adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4°C , pause the program.

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

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

Table 17. Incubation conditions for TEPCR cleanup reaction

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

6. Upon completion, allow the thermal cycler to return to 4°C.
7. Place the samples on ice and immediately proceed to “Protocol: Universal PCR”, page 33.

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

Important: Keep samples on ice.

Protocol: Universal PCR

Important points before starting

- The 25 μ L product from “Protocol: Target Enrichment”, page 29, is the starting material for this protocol.
- Set up reactions on ice, mixing reactions by pipetting on ice.
- Do not vortex any reagents or reactions.
- The QIAseq Beads and QIAseq Bead Binding Buffer are used for universal PCR reaction cleanup, and there is no need to bring beads and buffer to room temperature before use.
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

Procedure: universal PCR

1. Prepare the reagents required for the universal PCR.
 - 1a. Thaw UPCR Buffer, 5x; LT-Universal Primer; LT-P1 Primer; and SmP-LTP1 UPCR-R Primer on ice (or at room temperature, but immediately place on ice after being thawed).
 - 1b. Keep QN Taq Polymerase on ice.
 - 1c. Mix by flicking the tube, and then centrifuge briefly
2. Prepare the universal PCR in cleaned target-enriched DNA tube/plate from TEPCR cleanup reaction according to Table 18.

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

3. Mix by pipetting up and down 7–8 times and briefly centrifuge again.

Table 18. Reaction components for universal PCR

Component	Volume/reaction (µL)
Cleaned target-enriched DNA from "TEPCR Cleanup Reaction"	25
UPCR Buffer, 5x	20
LT-Universal Primer	4
LT-P1 Primer	1
SmP-LTP1 UPCR-R Primer	4
QN Taq Polymerase	4.8
Nuclease-free water	41.2
Total	100

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

Table 19. Incubation conditions for universal PCR

Step	Time	Temperature (°C)
Initial denaturation	2 min	98
Number of cycles (see Table 16)	15 s	98
	30 s	62
1 cycle	3 min	72
Hold	∞	4

Table 20. Amplification cycles for universal PCR

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

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

Procedure: cleanup of universal PCR

1. Add 80 μL QIAseq Beads to the completed universal PCR reaction; mix well by vortexing or pipetting up and down 10–12 times.
2. Incubate for 5 min at room temperature.
3. 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 tubes/plate still on the magnetic stand, carefully remove and discard the supernatant.
Important: Do not discard the beads as they contain the DNA of interest.
4. With the tubes/plate still on the magnetic stand, add 100 μL H₂O to the bead, then add 80 μL QIAseq Bead Binding Buffer.
5. Take the tubes/plate off the magnetic stand; mix well by vortexing or pipetting up and down 10–12 times.

6. Return the tubes/plate to the magnetic rack for 5 min. Once the solution has cleared, with the tubes/plate still on the magnetic stand, carefully remove and discard the supernatant.

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

7. With the tubes/plate still on the magnetic stand, add 200 μL 80% ethanol to wash the beads. Carefully remove and discard the ethanol wash.

8. Repeat the ethanol wash.

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

9. With the tubes/plate still on the magnetic stand, air dry at room temperature for 10 min.

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

10. Remove the tubes/plate from the magnetic stand, and elute the DNA from the beads by adding 30 μL nuclease-free water. Mix well by pipetting or vortexing.

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

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

13. Proceed to “Recommendations: Library QC and Quantification”, page 37. Alternatively, the library can be stored at -30 to -15°C in a constant-temperature freezer. Amplified libraries are stable for several months at -30 to -15°C . Once quantification is performed, proceed to “Protocol: Sequencing Setup”.

Recommendations: Library QC and Quantification

NGS library QC

After the library is constructed and purified, QC can be performed with QIAGEN's QIAxcel or Agilent's Bioanalyzer or TapeStation to check for the correct size distribution of the library fragments and for the absence of primer dimers (approx. <200 bp) and concentration. Majority library fragments prepared for Ion instruments demonstrate a size distribution between 250 and 1000 bp (Figure 5 and Figure 6). 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).

Recommended setting for checking QIAseq DNA Pro library on QIAxcel

QIAxcel Connect

- QIAxcel DNA High Sensitivity Kit (1200) (cat. no. 929012)
- Use method Default High Sensitivity with 1–10 μ L 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: 100 bp – 2.5 kb (cat. no. 929559).
- Use the Application Guide for Low-Concentration Libraries. To access guides and system files for Library QC, contact QIAGEN Technical Services.

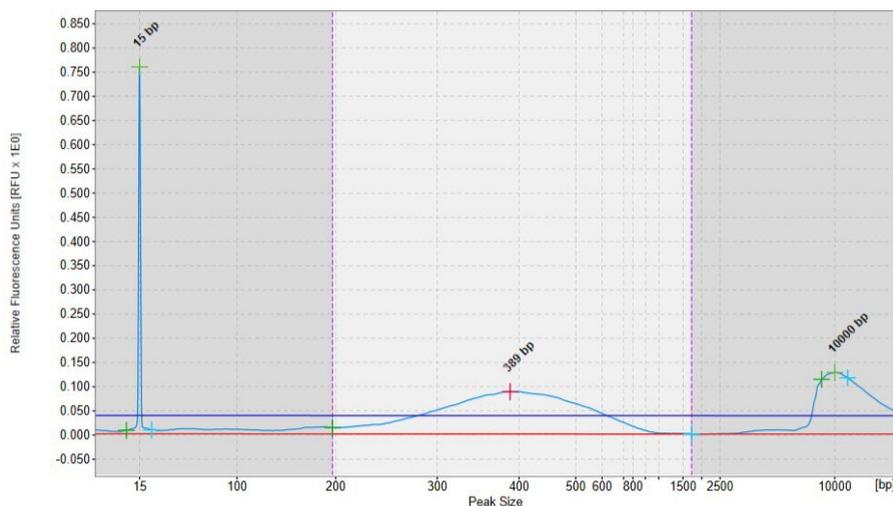


Figure 5. Sample QIAxcel image of QIAseq Targeted DNA Pro libraries for Ion instruments. The library assessed using QIAxcel illustrates the size of the majority of the library fragments are between 250 and 1000 bp.

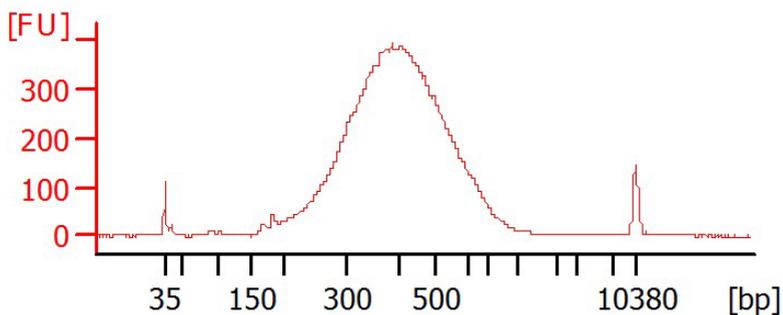


Figure 6. Sample Bioanalyzer images of QIAseq Targeted DNA Pro libraries for Ion instruments. The size of the majority of the library fragments are between 250 and 1000 bp.

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 Pro libraries with full adapter sequences will be sequenced. Due to the superior sensitivity of qPCR, we recommend quantifying the libraries using QIAGEN's QIAseq Library Quant Assay Kit (cat. no. 333314), which contains laboratory-verified forward and reverse primers, together with a DNA standard. With this system, the correct dilution of the library can be determined for sequencing. Please refer to the relevant handbook (available at www.qiagen.com) for library quantification.

Protocol: Sequencing Setup Ion Torrent Instruments

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, page 39).
- After the library is constructed, follow “Preferred library quantification method” (page 39) 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 μ L of Library A with 6 μ L 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 the QIAseq Targeted DNA Pro Kit for the Ion Torrent is compatible with the Ion Xpress adapter sample index system. When using Ion Torrent systems, at least 200 bp (or longer) single reads are required. 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 54.

Protocol: QIAseq Targeted DNA Pro Ion Chef and S5 Setup

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

Note: For loading with Qubit quantification, recommend to load 1.5-2.5 fold less depending on library concentration, over amplification et al, and adjust loading accordingly after the first run.

- 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 Pro 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 (Figure 7a), click **Templates** (Figure 7b), **select** the application that you want to run, then click either:
 - 2a. **Plan New Run** (Figure 7c) on the right side to plan a new run using the generic template for the selected application;

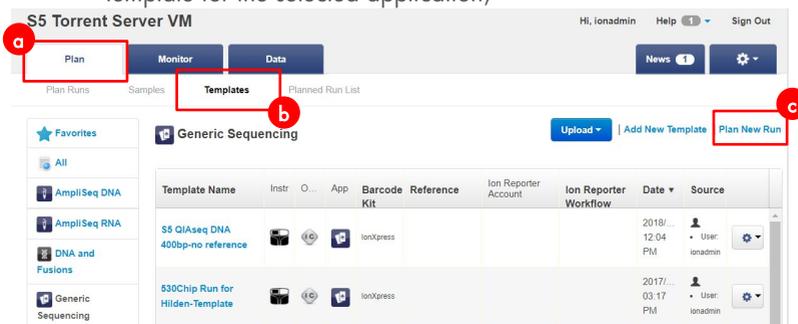


Figure 7. Planning a New Run in Torrent Server. (7a) Plan Tab. (7b) Templates tab. (7c) Plan New Run link.

- 2b. **Plan Run** (Figure 8a) in the dropdown menu under the **Settings** tab (Figure 8b) to the right of the existing template you select from the template list.

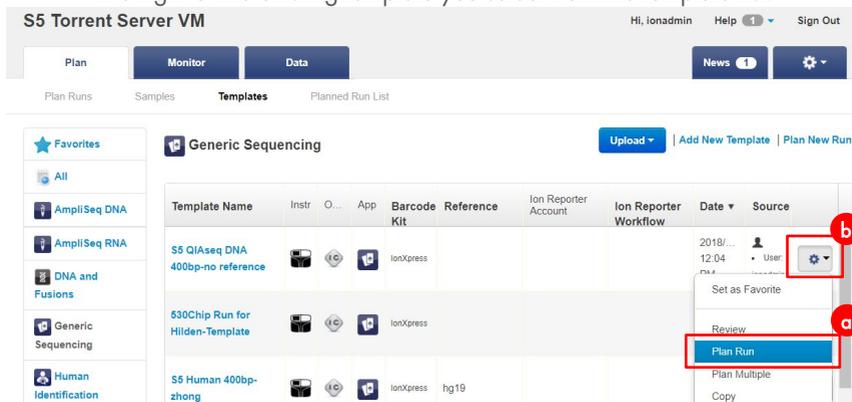


Figure 8. Running an Existing Template in Torrent Server. (8a) Plan Run dropdown menu. (8b) Setting tab of an existing template.

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

Important: Do not select reference genome if possible.

Create Plan Ion Reporter Application Kits Plugins

Template Name :
S5 QIaseq DNA 400bp-no reference

Run Plan Name (required) :
S5 QIaseq DNA 400bp-no reference

Analysis Parameters: Default (Recommended) Custom [Details +](#)

Default Reference & BED Files

Reference Library : None

Target Regions: None

Hotspot Regions: None

Use same reference & BED files for all barcodes

Number of barcodes : 1 [Save Samples Table](#) [Load Samples Table](#)

Sample Tube Label :

Chip Barcode :

Enter a sample name for each barcode used (require at least one sample) ↓ 🗑️ :

#	Barcode	Sample (required)	Control Type	Sample ID	Sample Description
1	IonXpress_001 (CTAAGGTAAC)	Sample 1			

Figure 9. Planned Run Wizard tab. First step in running an existing template, with dropdown menus for available selections.

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

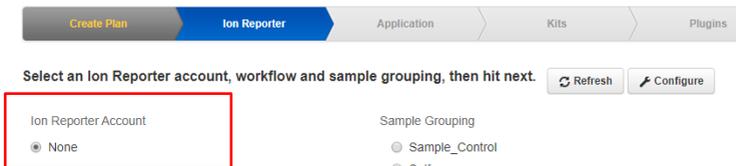


Figure 10. Ion Reporter Tab with the option "None" selected for Ion Reporter Account.

5. Under **Application** tab, select **DNA** (Figure 11 a) for Application and **Other** (Figure 11b) for Target Technique.

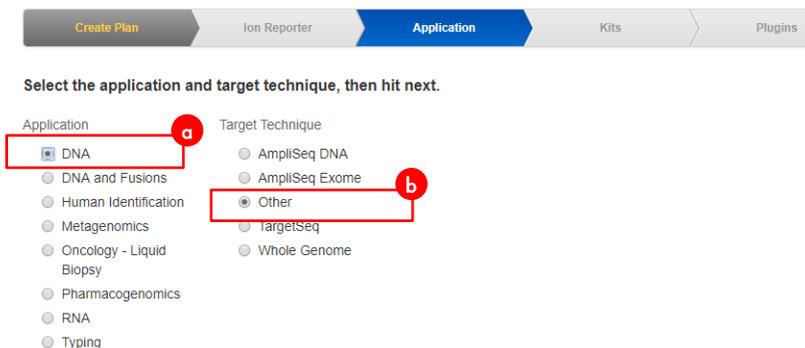


Figure 11. Application Tab. (11a) "DNA" option selected under Application. (11b) "Other" option selected under Target Technique.

6. Under **Kits** tab (Figure 12), make the following selection:

- **Instrument:** Ion S5 System (Figure 12a)
- **Chip Type:** Select the appropriate chip type from dropdown list (Figure 12b)
- **Library Kit Type:** Ion Xpress Plus Fragment Library Kit (Figure 12c)
- **Barcode Set:** IonXpress (Figure 12d)
- **Template Kit:** IonChef (Figure 12e)
- **Templating Size:** 400 (Figure 12f)
- **Sequencing Kit:** Ion S5 Sequencing Kit (Figure 12g)
- **Flows:** 850 (Figure 12h)

Select instrument, chip and kits and then hit next.

Instrument : Ion S5™ System (a)

Chip Type (required) : Ion 530™ Chip (b)

Sample Preparation Kit (optional) : Ion Xpress Plus Fragment Library Kit (c)

Control Sequence (optional) : IonXpress (d)

Library Kit Type [Details +] : Ion Xpress Plus Fragment Library Kit (c)

Barcode Set (optional) : IonXpress (d)

Template Kit [OneTouch] [IonChef] : Ion 510 & Ion 520 & Ion 530 Kit-Chef (e)

Templating Size : 200 [400] (f)

Sequencing Kit : Ion S5 Sequencing Kit (g)

Flows : 850 (h)

Base Calibration Mode : Default Calibration

Mark as Duplicates Reads [x] :

Enable Realignment [x] :

← Previous Next →

Summary

Ion Reporter:	None
Application:	DNA
Application Category:	
Sample Grouping:	
Target Technique:	Other
Ion Reporter Workflow:	IonXpress
Ion Reporter Upload Options:	
Sample Preparation Kit:	
Library Kit Type:	Ion Xpress Plus Fragment Library Kit
Library Key:	Ion TCAG (TCAG)
Template Kit:	Ion 510 & Ion 520 & Ion 530 Kit-Chef
Templating Size:	400
Templating Protocol:	(use default)
Sequencing Kit:	Ion S5 Sequencing Kit
Library Read Length:	--
Flows:	850
Control Sequence:	
Chip Type:	Ion 530™ Chip
Adapter:	Ion P1 (ATCACCGACTGC CCATAGAGAGGAAAGCG)

Figure 12. Kits tab with 8 selections. (12a) Ion S5 System under Instrument selection.; (12b) Chip Type dropdown list; (12c) Ion Xpress Plus Fragment Library Kit under Library Kit Type selection; (12d) IonXpress under Barcode Set selection; (12e) IonChef under Template Kit selection; (12f) 400 option under Templating Size selection; (12g) Ion S5 Sequencing Kit under Sequencing Kit selection; (12h) 850 option under Flows selection.

7. Under **Plugins** tab (Figure 13), leave everything unselected.

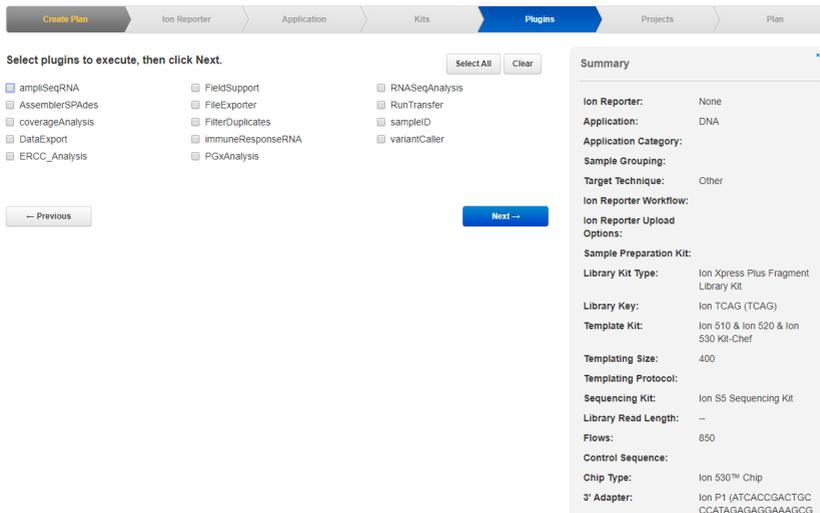


Figure 13. Plugins Tab with every option unselected.

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

9. Under the **Plan** tab (Figure 14):

- **Run Plan Name (required):** Enter Run Plan Name (Figure 14a)
- **Analysis Parameters:** Default (Recommended) (Figure 14b)
- **Default Reference and BED Files:** none (Figure 14c)
- **Number of barcodes:** Enter or scan the barcodes of the Ion Chef Library (Figure 14d)
- **Sample Tube Label:** Enter label of the Sample Tube (Figure 14e)
- **Chip Barcode:** Scan the barcodes of the chip (Figure 14f)

Figure 14. Plan Tab with 6 selections. (14a) Text area to input Run Plan name; (14b) Recommended Default settings for Analysis Parameters; (14c) No options selected for Default Reference and BED Files section; (14d) Text box to input or show the scanned barcode of the Ion Chef Library in Number of barcodes section; (14e) Text box to input the label for the Sample Tube; (14f) Text box to input or show scanned Chip Barcode.

- 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.

Here is the overview of all settings (Figure 15):

Template Name :
S5 QIAsSeq DNA 400bp-no reference

Run Plan Name (required):
S5 QIAsSeq DNA 400bp-no reference

Analysis Parameters: @Default (Recommended) @Custom

Default Reference & BED Files

Reference Library: None

Target Regions: None

Hotspot Regions: None

Use same reference & BED files for all barcodes

Number of barcodes: 1

Sample Tube Label:

Chip Barcode:

Enter a sample name for each barcode used (require at least one sample)

#	Barcode	Sample (required)	Sample ID	Sample Description	Barcode
1	IonXpress_001 (CTAAGGTAAC)	Sample 1			

Add a note:
Optional

Add LIMS Meta Data:
Optional

Monitoring Thresholds:

Bead Loading (%): ≤ 30

Key Signal (1-100): ≤ 30

Usable Sequence (%): ≤ 30

Summary

Ion Reporter: None

Application: DNA

Application Category:

Sample Grouping:

Target Technique: Other

Ion Reporter Workflow:

Ion Reporter Upload Options:

Sample Preparation Kit:

Library Kit Type: Ion Xpress Plus Fragment Library Kit

Library Key: Ion TCAG (TCAG)

Template Kit: Ion S10 & Ion S20 & Ion S30 Kit-Chef

Templating Size: 400

Templating Protocol:

Sequencing Kit: Ion S5 Sequencing Kit

Library Read Length: ...

Flows: 800

Control Sequence:

Chip Type: Ion 530™ Chip

3' Adapter: Ion P1 (ATCACCGACTGCCCATAGAGGAAAGCGG)

Flow Order:

Barcode: IonXpress

Mark as Duplicate: False

Reads:

Bead Loading (%): 30

Key Signal (1-100): 30

Usable Sequence (%): 30

Reference Library:

Target Regions:

Hotspot Regions:

Plugins:

Projects: QIAsSeq_DNA

Figure 15. Overview of completed settings for the planned Run.

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

Note: If template is prepared by OneTouch instead of Ion Chef, under the Kits tab, select **OneTouch** for Template kit (Figure 16). Other sequencing parameters set up on S5 should be the same as above described with Ion Chef.

Create Plan > Ion Reporter > Application > **Kits** > Plugins > Projects > Plan

Select instrument, chip and kits and then hit next.

Instrument :

Sample Preparation Kit (optional) :

Library Kit Type Details + :

Template Kit OneTouch IonChef :

Read Length:

Sequencing Kit :

Base Calibration Mode :

Chip Type (required) :

Control Sequence (optional) :

Barcode Set (optional) :

Flows :

Mark as Duplicates Reads

Enable Realignment

← Previous
Next →

Summary

Ion Reporter: None

Application: DNA

Application Category:

Sample Grouping:

Target Technique: Other

Ion Reporter Workflow:

Ion Reporter Upload Options:

Sample Preparation Kit:

Library Kit Type: Ion Xpress Plus Fragment Library Kit

Library Key: Ion TCAG (TCAG)

Template Kit: Ion 520 & Ion 530 Kit - OT2

Templating Size:

Templating Protocol: (use default)

Sequencing Kit: Ion S5 Sequencing Kit

Library Read Length: 400

Flows: 850

Control Sequence:

Chip Type: Ion 530™ Chip

3' Adapter: Ion P1 (ATCACCGACTGC CCATAGAGAGGAAAGCG G)

Flow Order: (use instrument settings)

Barcode: IonXpress

Mark as Duplicate Reads: False

Figure 16. Kits Tab if the template is prepared by OneTouch. The sequencing parameters are set same with the parameters of Ion Chef.

Protocol: 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 (Figure 17a), click the setting icon at the right side (Figure 17b), and select **Reanalyze** (Figure 17c).

S5 Torrent Server VM

Hi, Ionadmin Help Sign Out

Plan Monitor Data

Completed Runs & Reports Projects Data Management

There is an update available for your Torrent Server [Update Now](#)

Completed Runs & Reports [List View](#) | [Table View \(More Columns\)](#) [Auto Refresh](#)

Search Go Date Status: All Project: All Server: Local TS More Filters Clear All Sort: Reports New to Old

Run Name	Sample	App Run	Analysis	Status	Chip	Report Name	Q20 Bases	Output
user S5-0333-15-530Chip Run for Hidden-Chip3	23 Samples	Dec 13 2017	Dec 13 2017	Completed	530	Auto_user_S5-0333-15-530Chip_Run_for_Hidden-Chip3_151	2.47 G	2.61 G
user S5-0333-14-530Chip Run for Hidden-Chip2	11 Samples	Dec 12 2017	Dec 13 2017	Completed	530	Auto_user_S5-0333-14-530Chip_Run_for_Hidden-Chip2_130		
user S5-0333-13-530Chip Run for Hidden-Chip1	11 Samples	Dec 12 2017	Dec 12 2017	Completed	530	Auto_user_S5-0333-13-530Chip_Run_for_Hidden-Chip1_128		

Figure 17. Data Tab when reanalyzing with no reference genome. (17a) Column for Runs; (17b) Setting icon of a specific run; (17c) Reanalyze option.

2. Click the **Reanalyze Run** tab at the left; select the following:
 - **Report Name:** give the reanalyzing data a different name than the original one (Figure 18a)
 - **Start reanalysis from:** Base Calling (Figure 18b).
 - **Use data from previous result:** should be automatically selected with the run you want to reanalyze (Figure 18c).



Figure 18. Tab for Reanalyzing Run. (18a) Text box to input Report Name which is supposed to be different from the original name; (18b) Selected Base Calling option to start reanalysis from; (18c) Automatically selected data from the previous result.

3. Click the **Analysis Options** tab at the left (Figure 19); select the following:

- **Library Key:** TCAG (Figure 19a)
- **TF Key:** ATCG (Figure 19b)
- **3' Adapter:** Ion P1 (Figure 19c)
- **Mark as Duplicate Reads:** not selected (Figure 19d)
- **Base Calibration Mode:** Default Calibration (Figure 19e)
- **Enable Realignment:** not selected (Figure 19f)

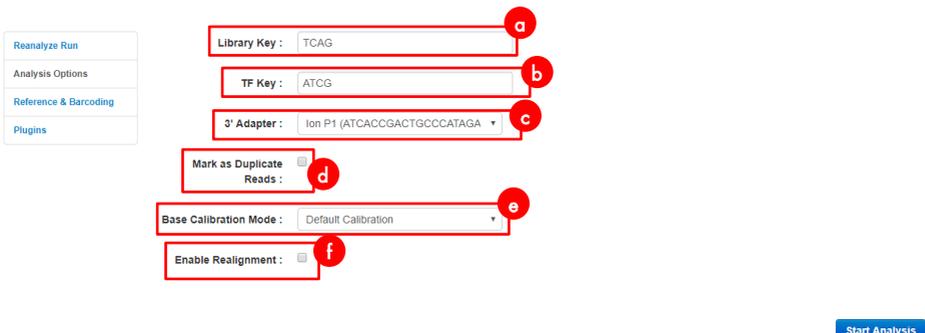


Figure 19. Analysis Options tab for reanalyzing run. (19a) Library Key "TCAG"; (19b) TF Key "ATCG"; (19c) Ion P1 option selected for 3' Adapter section; (19d) Unchecked box for Mark as Duplicate Reads section; (19e) Default Calibration option selected for Base Calibration Mode; (19f) Unchecked box for Enable Realignment section.

- Click the **Reference & Barcoding** tab at the left (Figure 20); select the following:
 - Default Alignment Reference:** none (Figure 20a)
 - Default Target Regions and Default Hotspot Regions BED File:** not selected (Figure 20b)
 - Barcode Set:** IonXpress (Figure 20c)

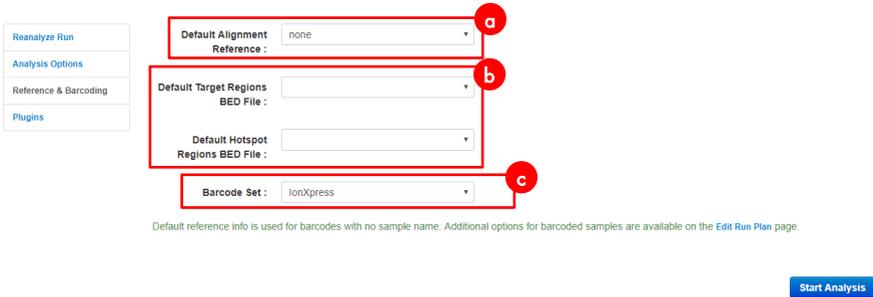


Figure 20. Reference & Barcoding tab for reanalyzing run. (20a) “None” option selected for Default Alignment Reference section; (20b) None selected for Default Target Regions section, and none selected for Default Hotspot Regions BED File; (20c) IonXpress option selected for Barcode Set section.

- Click the **Plugins** tab at the left (Figure 21); do not select any plugins.

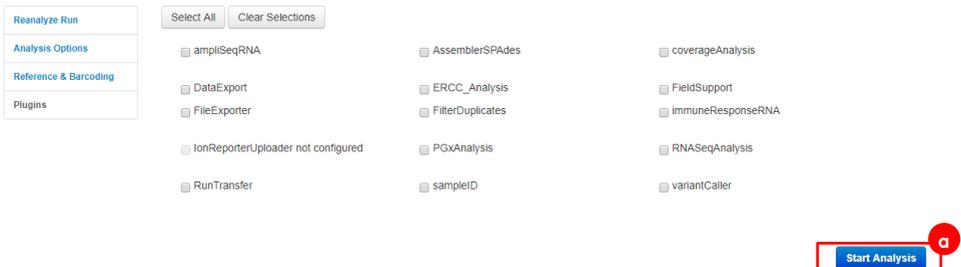


Figure 21. Plugins tab for reanalyzing run. No plugins are selected. (21a) Start Analysis button that will reanalyze the run without the reference genome sequence.

- Click **Start Analysis** (Figure 21a); the data will be reanalyzed with no reference genome sequence.

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 23).

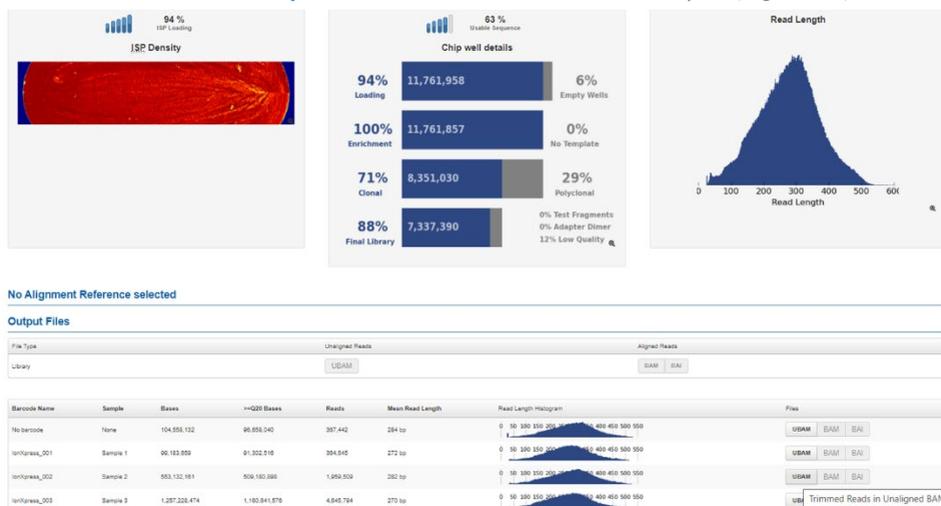


Figure 23. Torrent Browser report page and output files.

2. Click the **UBAM** button in the row corresponding to the individually indexed samples and column labeled **Files** in the table (Figure 24). 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.

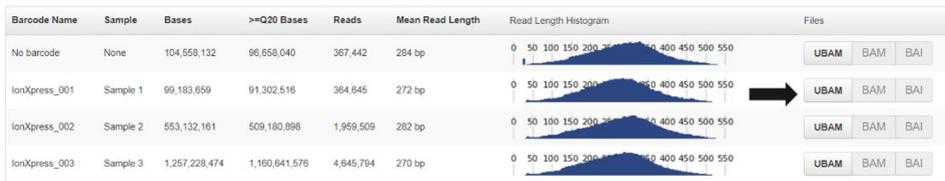


Figure 24. Unaligned reads in BAM format.

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 61.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Comments and suggestions

Low library yield

- | | |
|--|--|
| a) Suboptimal reaction conditions due to low DNA quality | Make sure to use high-quality DNA to ensure optimal activity of library enzymes. |
| b) Inefficient targeted enrichment or universal PCR | Check to see if correct thermocycling condition is used during target enrichment or universal PCR. |

Unexpected signal peaks

- | | |
|---|--|
| a) Short peaks approx. 200 bp | These are primer-dimers from TEPCR or universal PCR. The presence of primer-dimers indicates either not enough DNA input or inefficient PCR reactions or issues during enzymatic cleanup reaction. |
| b) Larger library fragments after universal PCR | After the universal PCR, library fragments are larger than the intended peak and can be a PCR artifact due to overamplification of the DNA library. Overamplification of the library will not affect the QIAseq Targeted DNA Pro sequencing performance. Decreasing the number of universal PCR cycle numbers can reduce over-amplification. |

Sequencing issues

- | | |
|------------------------|--|
| a) Too low ISP loading | Accurate library quantification is the key for optimal chip loading on any Ion 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) High polyclonal | Make sure that the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument. |

Variant detection issues

- | | |
|-----------------------------|--|
| Known variants not detected | Variant detection sensitivity is directly related to the input DNA and read depth. Check Table 2 to see if the required input DNA and read depth are met for the specific variant detection application. |
|-----------------------------|--|

References

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

Appendix A: Combining an Existing Panel with a Booster Panel

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

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

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

Appendix B: FFPE DNA Quality and Quantity

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

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

The QIAseq DNA QuantiMIZE System is recommended for determining FFPE DNA input for the QIAseq Targeted DNA Pro Kit. If FFPE DNA is defined as high quality (quality control [QC] score ≤ 0.04) by QuantiMIZE, then up to 100 ng of DNA can be used. If the DNA is determined as low quality (QC score > 0.04), then up to 250 ng of DNA can be used. The QC score of QuantiMIZE reflects the amount of amplifiable DNA present in the sample, therefore correlating with the number of UMIs that can be sequenced in the library (Figure 25, next page).

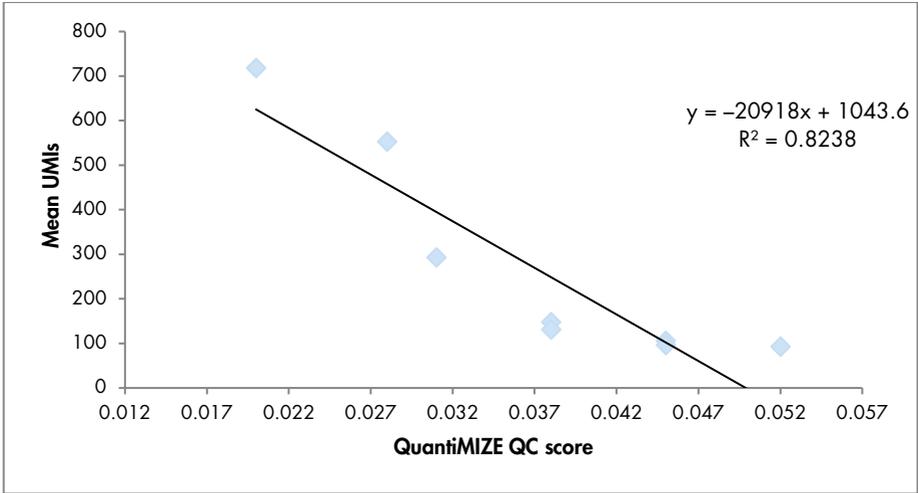


Figure 25. Correlation between QIAseq QuantiMIZE QC score and the number of UMIs.

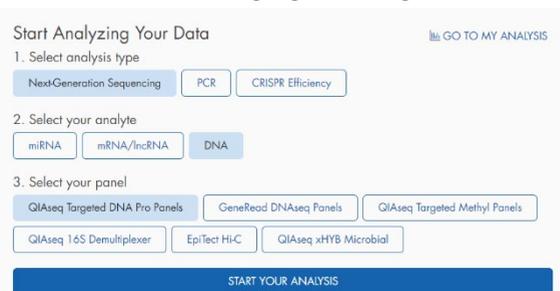
Compared to the same amount of fresh DNA, only 10–50% of UMIs can be captured from FFPE DNA, depending on the quality. This is due to a lower amplifiable DNA amount present in the FFPE samples. Therefore, a higher input amount is recommended for FFPE DNA samples to ensure that enough UMIs can be sequenced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

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

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

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

1. Log in to the GeneGlobe® Data Analysis Center at <https://geneglobe.qiagen.com/us/product-groups/qiaseq-targeted-dna-panels>
2. Make selection as highlighted in Figure 26.



The screenshot shows a web interface titled "Start Analyzing Your Data" with a "GO TO MY ANALYSIS" link. It is divided into three numbered steps:

- 1. Select analysis type:** Three buttons are shown: "NextGeneration Sequencing" (highlighted in blue), "PCR", and "CRISPR Efficiency".
- 2. Select your analyte:** Three buttons are shown: "miRNA", "miRNA/lncRNA", and "DNA" (highlighted in blue).
- 3. Select your panel:** A grid of buttons is shown. The first row contains "QIAseq Targeted DNA Pro Panels" (highlighted in blue), "GeneRead DNAseq Panels", and "QIAseq Targeted Methyl Panels". The second row contains "QIAseq 16S Demultiplexer", "EpiTect Hi-C", and "QIAseq xHYB Microbial".

At the bottom of the form is a large blue button labeled "START YOUR ANALYSIS".

Figure 26. GeneGlobe Analysis pipeline selections for the QIAseq Targeted DNA Pro. NextGeneration Sequencing is selected for analysis type; DNA is selected for analyte; and QIAseq Targeted DNA Pro Panels is selected as panel.

3. Click **START YOUR ANALYSIS**.
4. In the **Read Files** tab, select **Uploaded** > **Upload New Files** to upload files from your local drive (Figure 27).

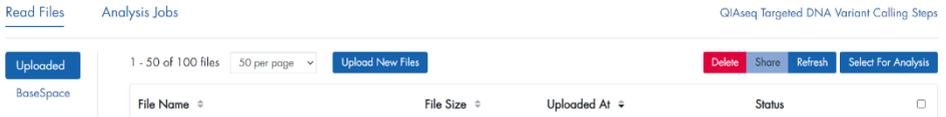


Figure 27. File Upload tab of the QIAseq Targeted DNA Pro Data Analysis Pipeline.

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

5. Select the boxes next to the files that will be analyzed, and then click **Select For Analysis** (Figure 28).

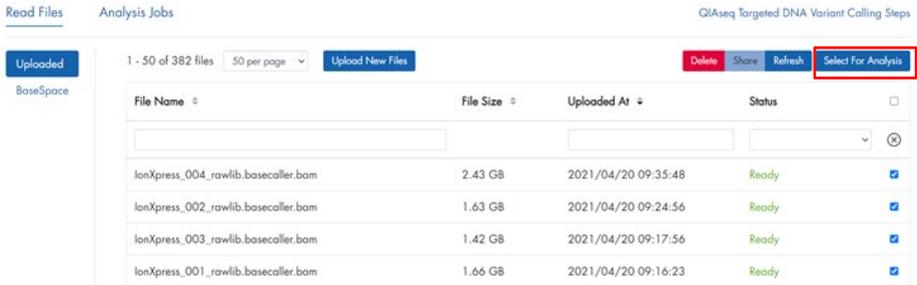


Figure 28. File selection for QIAseq Targeted DNA Pro data analysis pipeline.

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

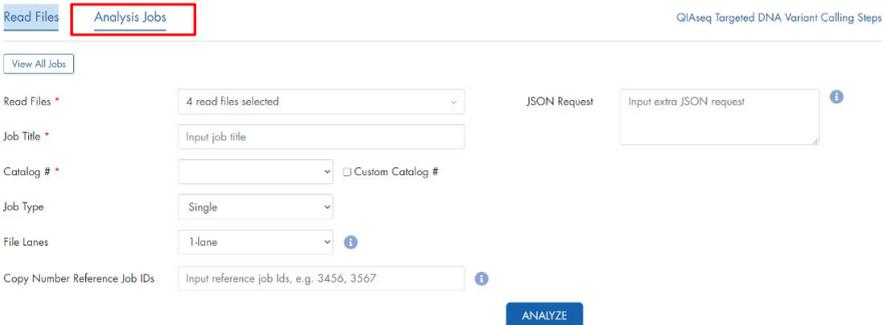


Figure 29. Analysis Jobs tab of the QIAseq Targeted DNA Pro data analysis pipeline.

- **Read Files:** Verify that the correct read files have been selected.
 - **Job Title:** Enter a title for the analysis job.
 - **Catalog #:** If using a catalog panel, select the number from the dropdown menu. If using a custom panel, enter the custom catalog number manually.
 - **Job type:** Single or matched tumor/normal.
 - **File lanes:** 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 Pro (12)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 12 samples; less than 200 genes	333651
QIAseq Targeted DNA Pro (96)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 96 samples; less than 200 genes	333655
QIAseq Targeted DNA Pro HC (12)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 12 samples; more than 200 genes	333661
QIAseq Targeted DNA Pro HC (96)	All reagents (except indexes) for targeted DNA sequencing; fixed panel for 96 samples; more than 200 genes	333665
QIAseq Targeted DNA Pro Custom (96)	All reagents (except indexes) for targeted DNA sequencing; custom panel for 96 samples	333675
QIAseq Targeted DNA Pro Booster (96)	Pool of primers used in combination with either cataloged or custom panels	333685
QIAseq Targeted DNA Pro Index L (12)	Box containing molecularly-indexed adapters and primers, enough for a total of 12 samples – for indexing up to 12 samples for targeted panel sequencing on Ion Torrent platforms	333491
QIAseq Targeted DNA Pro Index L (24)	Box containing molecularly-indexed adapters and primers, enough for a total of 24 samples – for indexing up to 24 samples for targeted panel sequencing on Ion Torrent platforms	333492
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

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
12/2021	Initial revision
12/2022	Updated content. Adjusted format according to new template and brand style guide. Added Figure labels and descriptions.

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