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# QIAseq<sup>®</sup> Multimodal Panel HT Handbook

Consolidated targeted next-generation sequencing of DNA and RNA

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# Kit Contents

QIAseq Multimodal Panel HT Catalog no. Number of samples	(12) 334932 12	HC (12) 334942 12	(96) 334935 96	HC (96) 334945 96	Custom (96) 334955 96
Multimodal DHS Panel (DNA)	120 µL	120 µL	960 µL	960 µL	960 µL
Multimodal VHS Panel (RNA)	96 µL	96 µL	768 µL	768 µL	768 µL
Fragmentation Buffer, 10x	40 µL	40 µL	192 µL	192 µL	192 µL
Fragmentation Enzyme Mix	90 µL	90 µL	384 µL	384 µL	384 µL
FERA Solution	15 µL	15 µL	60 µL	60 µL	60 µL
Side Reaction Reducer	48 µL	48 µL	192 µL	192 µL	192 µL
FG Solution	170 µL	170 µL	170 µL	170 µL	170 µL
DNA Ligase	75 µL	75 µL	600 µL	600 µL	600 µL
UPH Ligation Buffer, 2.5x	288 µL	288 µL	1152 µL x 2	1152 µL x 2	1152 µL x 2
DNA Ligation Adapter	42 µL	42 µL	325 µL	325 µL	325 µL
ATP Solution	36 µL	36 µL	290 µL	290 µL	290 µL
PAP Enzyme	12 µL	12 µL	96 µL	96 µL	96 µL
PAP Dilution Buffer, 10x	24 µL	24 µL	192 µL	192 µL	192 µL
T4 Polynucleotide Kinase	12 µL	12 µL	125 µL	125 µL	125 µL
EZ Reverse Transcriptase	15 µL	15 µL	150 µL	150 µL	150 µL
RNase Inhibitor	12 µL	12 µL	96 µL	96 µL	96 µL
Multimodal RT Primer	12 µL	12 µL	96 µL	96 µL	96 µL
Multimodal RT Buffer, 5x	60 µL	60 µL	480 µL	480 µL	480 µL
Multimodal RT Enhancer	12 µL	12 µL	48 µL	48 µL	48 µL
TEPCR Buffer, 5x	60 µL x 2	60 µL x 2	500 µL x 2	500 µL x 2	500 µL x 2

<b>QIAseq Multimodal Panel HT</b>	<b>(12)</b>	<b>HC (12)</b>	<b>(96)</b>	<b>HC (96)</b>	<b>Custom (96)</b>
<b>Catalog no.</b>	<b>334932</b>	<b>334942</b>	<b>334935</b>	<b>334945</b>	<b>334955</b>
<b>Number of samples</b>	<b>12</b>	<b>12</b>	<b>96</b>	<b>96</b>	<b>96</b>
QN Taq Polymerase	60 µL	60 µL	480 µL	480 µL	480 µL
Nuclease-free Water	1 tube	1 tube	10 mL	10 mL	10 mL
QIAseq Beads	10 mL	10 mL	38.4 mL x 2	38.4 mL x 2	38.4 mL x 2
QIAseq Bead Binding Buffer	10.2 mL	10.2 mL	10.2 mL x 2	10.2 mL x 2	10.2 mL x 2

### QIAseq Multimodal Panel HT

<b>Catalog no.</b>	<b>Product name</b>	<b>Total number of primers</b>	
		<b>DNA Panel</b>	<b>RNA Panel</b>
QHS-005Z	Human Lung Cancer Panel	4149	487
QHS-006Z	Human Sarcoma Panel	11243	665
QHS-009Z	Human Leukemia Panel	6244	1116
QHS-5000Z	Human Pan Cancer Panel	19995	2571

## Indexing for Separated Enrichment Workflow (SW) for Targeted Enrichment

**Note:** Follow “Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment”.

<b>QIAseq Multimodal Index I SW (12)</b> (DNA and RNA indexing for 12 samples using the Separated Enrichment Workflow for Targeted Enrichment) Catalog no. Number of samples	(24)   333982 12
<b>Multimodal N7 Plate SW (12): MTIN-125WK</b>	1
Each plate allows N7 indexing of 12 samples: 12 for DNA and 12 for RNA, with each well in the plate being single use. There are dried N7 index primers for DNA and RNA libraries in separate wells of the same plate. The plates can be cut into columns to enable indexing of the desired number of samples.	
<b>Multimodal S5 Plate (12): MTIS-12K</b>	1
Each plate allows S5 indexing of 12 samples: 12 for DNA and 12 for RNA, with each well in the plate being single use. The dried S5 indexes for DNA libraries are pre-mixed with universal DNA primer. The dried S5 indexes for RNA libraries are pre-mixed with universal RNA primer. The plates can be cut into columns to enable indexing of the desired number of samples.	
UPCR Buffer, 5x	60 µL x 3
DNA qPCR AMP Set	12 µL
RNA qPCR AMP Set	12 µL
QIAseq A Read 1 Primer I (100 µM)	24 µL
Multimodal Read 2 Primer (100 µM)	24 µL
Optical Thin-wall 8-cap Strips	24 strips

<b>QIAseq Multimodal HT SW 96 UDI*</b> <b>(DNA and RNA indexing for 96 samples using the Separated Enrichment Workflow for Targeted Enrichment)</b> <b>Catalog no.</b>	<b>(96)</b>  <b>333986</b> <b>MTSW-96A</b> <b>MTSW-96X</b> <b>MTSW-96K</b> <b>96</b>
<b>Number of samples</b>	

<b>Multimodal N7 Plate SW (96) for DNA: MTIN-96DNA/K/X</b> Each plate allows N7 indexing of 96 DNA samples. Each well in the plate is single use and contains dried N7 index primers for DNA libraries.	1
<b>Multimodal N7 Plate SW (96) for RNA: MTIN-96RNA/K/X</b> Each plate allows N7 indexing of 96 RNA samples. Each well in the plate is single use and contains dried N7 index primers for RNA libraries.	1
<b>Multimodal S5 Plate (96) for DNA: MTIS-96DNA/K/X</b> Each plate allows S5 indexing of 96 DNA samples: Each well in the plate is single use and contains dried S5 indexes for DNA libraries pre-mixed with universal DNA primer.	1
<b>Multimodal S5 Plate (96) for RNA: MTIS-96RNA/K/X</b> Each plate allows S5 indexing of 96 RNA samples: Each well in the plate is single use and contains dried S5 indexes for RNA libraries pre-mixed with universal RNA primer.	1
UPCR Buffer, 5x	500 µL x 3
DNA qPCR AMP Set	96 µL
RNA qPCR AMP Set	96 µL
QIAseq A Read 1 Primer I (100 µM)	4 x 24 µL
Multimodal Read 2 Primer (100 µM)	4 x 24 µL
Optical Thin-wall 8-cap Strips	48 strips

\* QIAseq Multimodal HT SW 96 UDI is a variant configuration product under the umbrella of cat. no. 333986, meaning that it is available in a variety of plastics. MTSW-96A is a hard-shell, full-skirted clear plate; MTSW-96X is a hard-shell, full-skirted white plate; and MTSW-96K is a non-skirted cuttable (in columns) plate.

Indexing for Combined Enrichment Workflow (CW) for Targeted Enrichment

**Note:** Follow “Appendix B: Combined Enrichment Workflow for Targeted Enrichment”.

QIAseq Multimodal Index I (12) (DNA and RNA indexing for 12 samples using the Combined Enrichment Workflow for Targeted Enrichment) Catalog no. Number of samples	(24)  333962 12
Multimodal N7 Plate (12): MTIN-12K	1
Each plate allows N7 indexing of 12 samples: 12 for DNA and 12 for RNA. Each well in the plate is single use. There are dried N7 index primers for DNA and RNA, mixed in the same well. The plates can be cut into columns to enable indexing of the desired number of samples.	
Multimodal S5 Plate (12): MTIS-12K	1
Each plate allows S5 indexing of 12 samples: 12 for DNA and 12 for RNA, with each well in the plate being single use. The dried S5 indexes for DNA libraries are pre-mixed with universal DNA primer. The dried S5 indexes for RNA libraries are pre-mixed with universal RNA primer. The plates can be cut into columns to enable indexing of the desired number of samples.	
UPCR Buffer, 5x	60 µL x 3
DNA qPCR AMP Set	12 µL
RNA qPCR AMP Set	12 µL
QIAseq A Read 1 Primer I (100 µM)	24 µL
Multimodal Read 2 Primer (100 µM)	24 µL
Optical Thin-wall 8-cap Strips	24 strips



<b>QIAseq Multimodal HT CW 96 UDI*</b> <b>(DNA and RNA indexing for 96 samples using the Combined Enrichment Workflow for Targeted Enrichment)</b> <b>Catalog no.</b>	<b>(96)</b>  <b>333979</b> <b>MTCW-96A</b> <b>MTCW-96X</b> <b>MTCW-96K</b> <b>96</b>
<b>Number of samples</b>	
<b>Multimodal N7 Plate (96): MTIN-96ABA/K/X</b> Each plate allows N7 indexing of 96 samples. Each well in the plate is single use, containing dried N7 index primers specific for DNA libraries mixed with N7 index primers specific for RNA libraries.	1
<b>Multimodal S5 Plate (96) for DNA: MTIS-96DNA/K/X</b> Each plate allows S5 indexing of 96 DNA samples: Each well in the plate is single use and contains dried S5 indexes for DNA libraries pre-mixed with universal DNA primer.	1
<b>Multimodal S5 Plate (96) for RNA: MTIS-96RNA/K/X</b> Each plate allows S5 indexing of 96 RNA samples: Each well in the plate is single use and contains dried S5 indexes for RNA libraries pre-mixed with universal RNA primer.	1
UPCR Buffer, 5x	500 µL x 3
DNA qPCR AMP Set	96 µL
RNA qPCR AMP Set	96 µL
QIAseq A Read 1 Primer I (100 µM)	4 x 24 µL
Multimodal Read 2 Primer (100 µM)	4 x 24 µL
Optical Thin-wall 8-cap Strips	48 strips

\* QIAseq Multimodal HT CW 96 UDI is a variant configuration product under the umbrella of cat. no. 333979, meaning that it is available in a variety of plastics. MTCW-96A is a hard-shell, full-skirted clear plate; MTCW-96X is a hard-shell, full-skirted white plate; and MTCW-96K is non-skirted cuttable (in columns) plate.

# Shipping and Storage

QIAseq Multimodal Panels HT (except QIAseq Beads, QIAseq Bead Binding Buffer, and Nuclease-free Water) are shipped on dry ice and should be stored immediately upon receipt at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

QIAseq Beads, QIAseq Bead Binding Buffer, and Nuclease-free Water are shipped in a separate box on cold packs. Upon receipt, QIAseq Beads, QIAseq Bead Binding Buffer, and Nuclease-free Water should be stored at  $2-8^{\circ}\text{C}$ .

QIAseq Multimodal Index Kits are shipped on dry ice and should be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  upon arrival.

Under these conditions, the components are stable until the expiry date indicated on the label.

## Intended Use

QIAseq Multimodal Panels HT and QIAseq Multimodal 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](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

# Quality Control

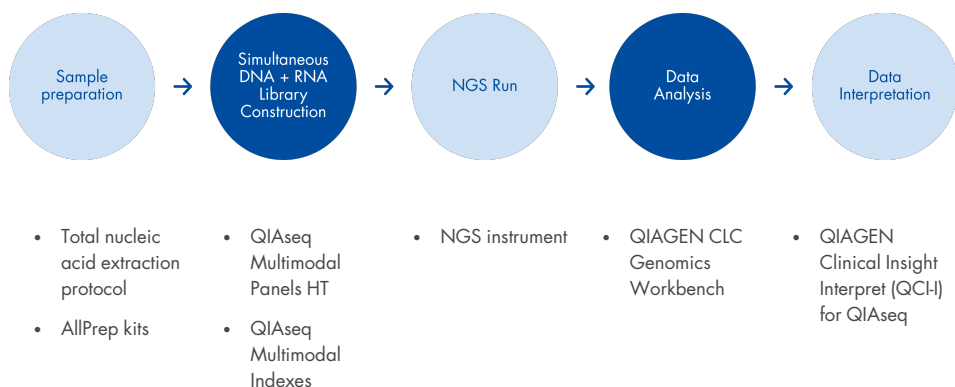
In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAseq Multimodal Panels HT is tested against predetermined specifications to ensure consistent product quality.

# Introduction

Recent advancements in NGS have enabled the analysis of single nucleotide variants (SNVs), InDels (insertions–deletions), and copy number variants (CNVs) from DNA, and the analysis of fusions and gene expression levels from RNA. Existing solutions, however, only allow users to perform such DNA and RNA analyses using two separate workflows: one for DNA and one for RNA. Additionally, these solutions require separate inputs of DNA and RNA, making the sequencing of low-yielding samples very difficult.

To overcome the limitations of existing solutions, the QIAseq Multimodal Panels HT have been developed. QIAseq Multimodal Panels HT enable Sample to Insight®, simultaneous targeted next-generation sequencing (NGS) of DNA and RNA using total nucleic acids in a single-tube workflow. Resulting DNA and RNA libraries can be sequenced together for cost effectiveness. This highly optimized solution facilitates ultrasensitive DNA variant detection as well as fusions and gene expression detection from RNA using integrated unique molecular indexes (UMIs) from cells, tissue, and biofluids. The starting material for QIAseq Multimodal can be total nucleic acid or separately isolated DNA and RNA.

The QIAseq Multimodal Panels HT use a targeted approach by enriching specific genomic or transcriptomic regions, which allows for increased sequencing depth and sample throughput while minimizing cost. More importantly, QIAseq Multimodal Panels HT enable simultaneous enrichment for both DNA and RNA regions of interest using total nucleic acids as input, thereby saving precious biological material, maximizing library prep efficiency, and reducing handling errors. Using a robust chemistry with integrated UMIs, QIAseq Multimodal Panels HT enable sensitive detection of DNA and RNA analytes of interest. Furthermore, data analysis tools have been developed to perform all steps necessary to generate both a DNA sequence variant report as well as an RNA fusion and gene expression report from NGS data. Collectively, QIAseq Multimodal Panels are a Sample to Insight solution for consolidated targeted DNA and RNA analysis using NGS (Figure 1).



**Figure 1. Overview of the Sample to Insight NGS workflow with QIAseq Multimodal Panels HT.** The complete Sample to Insight procedure begins with total nucleic acid or AllPrep® (separate DNA and RNA) extractions. Next is library construction and target enrichment with QIAseq Multimodal Panels HT. Following NGS, data analysis is performed using the QIAseq Multimodal Panel Analysis Software pipeline in Genomics Workbench. Ultimately, detected variants can be interpreted with QIAGEN Clinical Insight Interpret (QCI®-I) for QIAseq.

## Principle and procedure

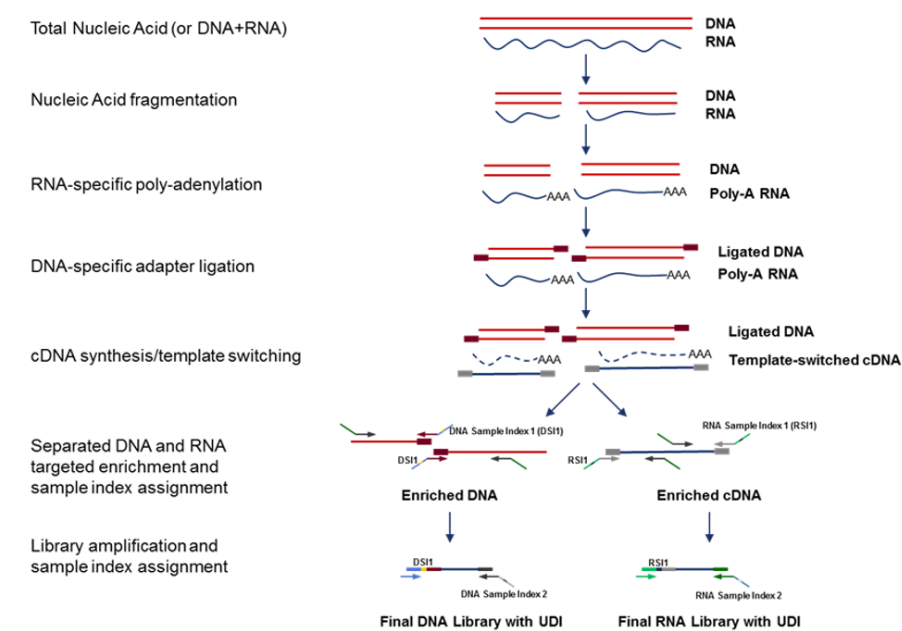
QIAseq Multimodal Panels HT enable the simultaneous enrichment and library prep of DNA+RNA, with up to 25,000 primers per panel (20,000 DNA + 5000 RNA). For DNA, the recommended input range is 10–40 ng for fresh samples or 40–250 ng for FFPE samples. For RNA, the optimal amount of starting material depends on the relative abundance of the transcripts of interest. Lower-abundance transcripts require more RNA; high-abundance transcripts require less RNA. The recommended amount input range is 10–250 ng for fresh samples or 100–250 ng for FFPE samples (up to 500 ng for “severely” fragmented FFPE sample, with “severely” being defined as samples that have less than 40% of fragments >200 nt by smear analysis on the Bioanalyzer®). When working with total nucleic acid samples, input amounts should be based on DNA, because RNA is usually in vast excess to DNA. Lower input amounts are possible; however, this will lead to fewer sequenced UMIs and reduced variant detection sensitivity. The following reactions occur in a streamlined, single-tube workflow (Figure 2 on the next page).

## Nucleic acid fragmentation

RNA molecules are heat fragmented and DNA molecules are enzymatically fragmented, end repaired, and A-tailed within a single controlled multienzyme reaction.

## RNA polyadenylation

Specific to RNA, synthetic polyadenylation is performed to create a binding site for subsequent reverse transcription.



**Figure 2. QIAseq Multimodal Panels HT workflow, using the Separated Enrichment Workflow (SW) for Targeted Enrichment.** Alternatively, the Combined Enrichment Workflow (CW) for Targeted Enrichment can be performed using “Appendix B: Combined Enrichment Workflow for Targeted Enrichment”.

## DNA ligation

Specific to DNA, UMI-containing adapters are ligated at the 3' ends of the molecules. The UMI is a 12-base fully random sequence, which statistically provides  $4^{12}$  possible sequences per adapter and ensures that each molecule receives a UMI sequence. In addition, this adapter contains a binding site for subsequent target enrichment.

## RNA reverse transcription and template switching

Specific to RNA, reverse transcription and template switching are performed. For reverse transcription, the anchored oligo-dT primer contains a 10-base fully random UMI sequence, and the template switching oligonucleotide also contains a 10-base fully random UMI sequence. This allows each RNA molecule to be tagged with a unique UMI, regardless of which strand it was derived from. Lastly, the reverse transcription and template switching oligonucleotides each contain the same binding site for subsequent target enrichment.

## Target enrichment

Two protocols are provided for target enrichment. The recommended, default workflow is "Separated Enrichment Workflow (SW) for Targeted Enrichment":

- Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment
- Appendix B: Combined Enrichment Workflow for Targeted Enrichment

**Important:** Table 1 outlines important points to consider when choosing between the two protocols.

Table 1. Target enrichment options

Choose ...	For ...	Remarks
<p>“Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment ”</p> <p><b>Note:</b> Samples are split before targeted enrichment, so the input DNA recommendations would be doubled, compared to Combined Enrichment Workflow (CW) for Targeted Enrichment.</p>	Maximal panel specificity	With this protocol, when considering sequencing read budgets, the DNA panel and the RNA panel can be considered separately. For example, if you are working with a DNA panel of 10,000 primers and an RNA panel of 1000 primers, the read budget ultimately needed for the DNA library must be based on 10,000 primers and the read budget ultimately needed for the RNA library must be based on 1000 primers.
	Flexibility for Custom Multimodal Primer Panel design	Separated targeted DNA and RNA enrichment prevents the need to account for potential interactions (i.e., dimerization potential) between DNA and RNA primer pool
	Custom Multimodal Primer Panels with specific primer numbers	When the number of DNA+RNA primers is $\geq 12,000$
	QIAseq Multimodal Panel QHS-5000Z	QIAseq Multimodal Pan Cancer Panel (QHS-5000Z) is designed exclusively for the “Separated Targeted DNA and RNA Enrichment in Separate Tubes” workflow
“Appendix B: Combined Enrichment Workflow for Targeted Enrichment”	Maximal detection sensitivity of DNA and RNA variants, but at the expense of sequencing read allocation, since there is no splitting of samples	Points to note with the combined protocol are that RNA primers will amplify DNA, and DNA primers have the possibility of amplifying RNA. As a result, when considering sequencing read budgets, the panel size needs to be accounted for as “DNA+RNA” primers. For example, if you are working with a DNA panel of 10,000 primers and an RNA panel of 1000 primers, the read budget ultimately needed for both the DNA and the RNA library must be based on 11,000 primers.

For both DNA and RNA, target enrichment is performed post-UMI assignment to ensure that molecules containing UMIs are sufficiently enriched in the sequenced library. For enrichment, ligated DNA molecules and reverse-transcribed/template-switched cDNA molecules are subject to several cycles of targeted PCR using the QIAseq enrichment technology. This reaction includes highly optimized chemistry to amplify traditionally difficult regions using pools of DNA and RNA region-specific primers. Universal primers complementary to the DNA adapter-binding sequence and reverse-transcription/template-switching oligonucleotides



ensure specificity for DNA and RNA molecules and assign the N7 index, which is one of the 2 sample unique dual indexes (UDIs). The N7 indexes are listed on [www.qiagen.com/QIAseqMultimodalPanelsHT](http://www.qiagen.com/QIAseqMultimodalPanelsHT)

## Library amplification

A Universal PCR is ultimately carried out separately on DNA and RNA libraries to both optimally amplify each library as well as add the second UDI. Collectively, DNA and RNA libraries for a given sample have their own unique dual indexes. The S5 indexes are listed on [www.qiagen.com/QIAseqMultimodalPanelsHT](http://www.qiagen.com/QIAseqMultimodalPanelsHT)

**Important:** The sample recommendations for the UDIs should be maintained between the N7 and S5 indexes.

## Next-generation sequencing

QIAseq Multimodal Panels HT are compatible with Illumina NGS platforms including iSeq 100, MiniSeq®, MiSeq®, NextSeq® 500/550, NextSeq 1000/2000, and NovaSeq® 6000/X Plus. QIAseq Multimodal Panels HT are compatible with Element Biosciences Aviti NGS platform. When using Illumina or Element Biosciences NGS systems, QIAseq Multimodal libraries require a custom sequencing primer for Read 1 (QIAseq A Read 1 Primer I), custom sequencing primer for Read 2 (Multimodal Read 2 Primer), and 149 bp paired-end reads.

QIAseq Multimodal Panels HT are compatible with other sequencing platforms after a conversion step.

## Data analysis

Data from QIAseq Multimodal Panels HT can be analyzed using the QIAGEN CLC Genomics Workbench and Biomedical Genomics Analysis plugin, allowing you to optimize analysis parameters for your specific panels. You can easily identify the relevant analysis for your panel using the QIAseq Panel Analysis Assistant. The parameters can then be locked for routine use. All detected variants can be further interpreted using QCI-Interpret.

Please note that the QIAseq Multimodal Panels HT are designed against genome build GRCh37/hg19 for the DNA Panel and GRCh38/hg38 for the RNA Panel. BED files are provided in the respective genome build.

The CLC analysis pipeline requires that both BED files are compatible with genome build GRCh38/hg38. After importing the DNA Panel BED file into CLC using GRCh37/hg19, it needs to be lifted over from GRCh37/hg19 to GRCh38/hg38 using the CLC lift over tool. Instructions can be found here:

- [resources.qiagenbioinformatics.com/manuals/biomedicalgenomicsanalysis/current/User\\_Manual.pdf](https://resources.qiagenbioinformatics.com/manuals/biomedicalgenomicsanalysis/current/User_Manual.pdf)
- [resources.qiagenbioinformatics.com/manuals/biomedicalgenomicsanalysis/current/index.php?manual=Convert\\_Annotation\\_Track\\_Coordinates.html](https://resources.qiagenbioinformatics.com/manuals/biomedicalgenomicsanalysis/current/index.php?manual=Convert_Annotation_Track_Coordinates.html)

# Important Notes

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

## Total nucleic acid isolation

Supplementary protocols for the simultaneous isolation of total nucleic acid (DNA+RNA) from cells and tissue, blood, or FFPE samples are available at [www.qiagen.com/TotalNucleicAcid](http://www.qiagen.com/TotalNucleicAcid)

**Note:** For quantification of DNA and RNA from total nucleic acid samples, we recommend the high-sensitivity Quant-iT™ dsDNA Assay Kit (Thermo Fisher Scientific, cat. no. Q33120) for DNA and the Quant-iT RNA Assay Kit (Thermo Fisher Scientific, cat. no. Q33140) for RNA. When working with total nucleic acid samples, input amounts should be based on DNA because RNA is usually in vast excess to DNA.

## Simultaneous purification of DNA and RNA into separate eluates

The QIAGEN kits listed in Important Notes are recommended for the preparation of DNA and RNA samples from cells, tissues, and FFPE tissues. For whole blood, we recommend the PAXgene® Blood DNA Kit (cat. no. 761133) and the PAXgene 96 Blood RNA Kit (cat. no. 762331) or QIAasympy PAXgene Blood RNA Kit (cat. no. 762635).

**Note:** If samples must be harvested from biological samples for which kits are not available, please contact QIAGEN Technical Services ([support.qiagen.com](mailto:support.qiagen.com)) for suggestions.

**Table 2. Recommended AllPrep kits for simultaneous purification of DNA and RNA into separate eluates**

Kit	Starting material	Cat. no.
AllPrep DNA/RNA Mini Kit	Cells and tissue	80204
AllPrep DNA/RNA FFPE Kit	FFPE samples	80234

Specific recommendations for FFPE samples (total nucleic acid or DNA)

If FFPE samples are used for QIAseq Multimodal Panels HT, the QIAseq DNA QuantiMIZE kit (cat. no. 333414) are strongly recommended for determining the quality of each FFPE sample. “Appendix A: FFPE Sample Quality and Quantity” on page 70 provides detailed information for FFPE DNA quality assessment and input amount.

# 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 Multimodal Panels HT and the QIAseq Multimodal Index Kit, the following are required:

- EvaGreen® Dye, 20x, in water (Biotium, cat. no. 31000-T or 31000)
- 80% ethanol (made fresh daily)\*
- Nuclease-free pipette tips and tubes
- 1.5 mL DNA LoBind® Tubes (Eppendorf, cat. no. 022431021)
- PCR tubes (0.2 mL individual tubes [VWR, cat. no. 20170-012] or tube strips [VWR, cat. no. 93001118]) or plates
- Ice
- Microcentrifuge
- Thermal cycler
- Magnet for bead cleanups:
  - Tubes: MagneSphere® Technology Magnetic Separation Stand (Promega, cat. no. Z5342)
  - Plates: DynaMag™-96 Side Magnet (Thermo Fisher Scientific Inc., cat. no. 12331D)
- 2100 Bioanalyzer (Agilent®, cat. no. G2939BA)

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

- Agilent High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626)
- qPCR Instrument
- The following controls can be used to validate the performance of the QIAseq Multimodal Panel HT:
  - SeraSeq® Lung & Brain CNV Mix, +6Copies (0710-0415)
  - Seraseq MyeloidMutation DNA Mix (0710-0408)
  - Seraseq Tri-LvlTumor Mut DNA Mixv2 HC (0710-0089)
  - Seraseq gDNA TMBMix Score 20 WT (0710-1324)
  - Seraseq gDNA TMBMix Score 20 tumor (0710-1324)
  - Seraseq gDNA TMBMix Score 7 WT (0710-1326)
  - Seraseq gDNA TMBMix Score 7 tumor (0710-1326)
  - Seraseq Myeloid Fusion RNA(710-0407)
  - Seraseq FFPE Tumor Fusion RNA v4 Reference Material (710-0496)
  - Seraseq Fusion RNA Mix v4 (0710-0497)
  - Seraseq FFPE NTRK Fusion RNA Reference Material (0710-1031)
  - NA24385 (from Coriell Institute, control for copy number detection)

# Protocol: Nucleic Acid Fragmentation, Standard Samples

## Important points before starting

- This protocol describes fragmentation of nucleic acids from “standard samples” (e.g., cells or tissue). For fragmentation of FFPE samples, please refer to “Protocol: Nucleic Acid Fragmentation, FFPE Samples” on page 27.
- This protocol is designed to work with either total nucleic acid eluates (those containing DNA+RNA) or separate DNA and RNA eluates.
- When performing “Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment”, the recommended amount of DNA is 20–80 ng.
- When performing “Appendix B: Combined Enrichment Workflow for Targeted Enrichment”, the recommended amount of DNA is 10–40 ng.
- The recommended amount of RNA is 10–250 ng total RNA. When working with total nucleic acid samples, we recommend basing the input on the amount of quantified DNA.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

## Procedure

1. Thaw nucleic acid samples on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and then return to ice.

2. Prepare the reagents required for the fragmentation.
  - a. Thaw Fragmentation Buffer, 10x, and FERA Solution at room temperature (15–25°C).
  - b. Mix by flicking the tube, and then centrifuge briefly.

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

3. On ice, prepare the fragmentation mix according to Table 3a (standard samples) or Table 3b (cfDNA samples). Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 3a. Reaction mix for nucleic acid fragmentation**

Component	Volume/reaction
DNA (see input recommendation in the “Protocol: Nucleic Acid Fragmentation, Standard Samples” section)*	Variable A
RNA (see input recommendation in the “Protocol: Nucleic Acid Fragmentation, Standard Samples” section)*	Variable B
Fragmentation Buffer, 10x	2 µL
FERA Solution	0.6 µL
Fragmentation Enzyme Mix	4 µL
Nuclease-free Water	13.4 µL – variable A (DNA) – variable B (RNA)
<b>Total</b>	<b>20 µL</b>

\* Instead of adding DNA and RNA separately, total nucleic acid containing both DNA and RNA can be added. If adding total nucleic acid, base the input amount on DNA.



**Table 3b. Reaction mix for nucleic acid fragmentation of cfDNA samples**

Component	Volume/reaction (pure cfDNA)	Volume/reaction (cfDNA contaminated with cellular DNA)
DNA (see input recommendation in the “Protocol: Nucleic Acid Fragmentation, Standard Samples” section)*	Variable A	Variable A
RNA (see input recommendation in the “Protocol: Nucleic Acid Fragmentation, Standard Samples” section)*	Variable B	Variable B
Fragmentation Buffer, 10x	2 µL	2 µL
FERA Solution	0.6 µL	0.6 µL
FG Solution	–	1.25
Fragmentation Enzyme Mix	4 µL	4 µL
Nuclease-free Water	Variable	Variable
<b>Total</b>	<b>20 µL</b>	<b>20 µL</b>

\* Instead of adding DNA and RNA separately, total nucleic acid containing both DNA and RNA can be added. If adding total nucleic acid, base the input amount on DNA.

4. Program the thermal cycler according to Table 4. Use the instrument’s heated lid.

**Table 4. Incubation conditions for nucleic acid fragmentation**

Step	Temperature (°C)	Time for standard sample (min)	Time for cfDNA (min)
1	4	1	1
2	32	24	14
3	72	30	30
4	4	Hold	Hold

5. Prior to 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 program.
7. Upon completion, allow the thermal cycler to return to 4°C.
8. Place the samples on ice and immediately proceed to “Protocol: RNA Polyadenylation” on page 30.

# Protocol: Nucleic Acid Fragmentation, FFPE Samples

## Important points before starting

- This protocol describes fragmentation of nucleic acids from FFPE samples. For fragmentation of “standard samples” (e.g., cells or tissue), please refer to “Protocol: Nucleic Acid Fragmentation, Standard Samples”.
- This protocol is designed to work with either total nucleic acid eluates (those containing DNA+RNA) or separate DNA and RNA eluates.
- The recommended amount of FFPE DNA is up to 250 ng DNA if QIAseq QuantiMIZE kits have been used (see “Appendix A: FFPE Sample Quality and Quantity” on page 70). If an alternative method was used to determine the concentration of FFPE DNA, then up to 100 ng DNA can be used. For better results, we recommend that first-time users start with 250 ng FFPE RNA (up to 500 ng for “severely” fragmented FFPE sample, with “severely” being defined as samples that have less than 40% of fragments >200 nt by smear analysis on the Bioanalyzer). When working with total nucleic acid samples, we recommend basing the input on the amount of quantified DNA.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

## Procedure

1. Thaw nucleic acid samples on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and then return to ice.

2. Prepare the reagents required for the fragmentation.
- a. Thaw Fragmentation Buffer, 10x, and FERA Solution at room temperature.
  - b. Mix by flicking the tube, and centrifuge briefly.

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

3. On ice, prepare the fragmentation mix according to Table 5. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 5. Reaction mix for nucleic acid fragmentation**

Component	Volume/reaction
DNA (see input recommendation in the “Protocol: Nucleic Acid Fragmentation, FFPE Samples” section)*	Variable A
RNA (see input recommendation in the “Protocol: Nucleic Acid Fragmentation, FFPE Samples” section)*	Variable B
Fragmentation Buffer, 10x	2 µL
FERA Solution	0.6 µL
Side Reaction Reducer	1.6 µL
Nuclease-free Water	11.8 µL – variable A (DNA) – variable B (RNA)
<b>Total</b>	<b>16 µL</b>

\* Instead of adding DNA and RNA separately, total nucleic acid containing both DNA and RNA can be added. If adding total nucleic acid, base the input amount on DNA.

4. Incubate for 15 min at 37°C and then place on ice.

5. Add 4  $\mu$ L of Fragmentation Enzyme Mix to each reaction. Briefly centrifuge, mix by pipetting up and down 10–12 times (do not vortex), and then briefly centrifuge again.

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

6. Program the thermal cycler according to Table 6. Use the instrument’s heated lid.

**Table 6. Incubation conditions for nucleic acid fragmentation**

Step	Temperature ( $^{\circ}$ C)	Time (min)
1	4	1
2	32	14
3	72	30
4	4	Hold

7. Prior to adding the tubes/plate to a thermal cycler, start the program. When the thermal cycler reaches 4 $^{\circ}$ C, pause the program.

**Important:** The thermal cycler must be prechilled and paused at 4 $^{\circ}$ C.

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

9. Upon completion, allow the thermal cycler to return to 4 $^{\circ}$ C.

10. Place the samples on ice and immediately proceed to “Protocol: RNA Polyadenylation” on the next page.

# Protocol: RNA Polyadenylation

## Important points before starting

- Set up reactions on ice.
- Do not vortex any reagents or reactions.

## Procedure

1. Prepare the reagents required for the polyadenylation.
  - a. Thaw PAP Dilution Buffer, 10x, and ATP Solution on ice.
  - b. Mix by flicking the tube, and then centrifuge briefly.

**Note:** T4 Polynucleotide Kinase and PAP Enzyme should be removed from the freezer and placed on ice just before use. After use, immediately return the enzymes to the freezer.
2. 1 Prepare 1x PAP Dilution Buffer by diluting 2  $\mu\text{L}$  of the 10x PAP Dilution Buffer with 18  $\mu\text{L}$  Nuclease-free Water.
3. Use the 1x PAP Dilution Buffer to dilute an aliquot of the PAP Enzyme from 5 to 2 U/ $\mu\text{L}$ . For FFPE samples: titer to 1 U/ $\mu\text{L}$  (or 0.5 U/ $\mu\text{L}$ ). Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.
4. Prepare the RNA polyadenylation mix according to Table 7. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 7. Reaction mix for RNA polyadenylation**

Component	Volume/reaction (μL)
Fragmentation reaction (already in tube)	20
ATP Solution	1.25
T4 Polynucleotide Kinase	1
Diluted PAP Enzyme (2 U/μL)*	1
Nuclease-free Water	1.75
<b>Total</b>	<b>25</b>

\* Ensure PAP Enzyme has been diluted from its stock 5 U/μL concentration to 2 U/μL using 1x PAP Dilution Buffer (1 U/μL FFPE samples).

5. Incubate the reactions in a thermal cycler according to Table 8. Use the instrument's heated lid.

**Table 8. Incubation conditions for RNA polyadenylation**

Step	Temperature (°C)	Time (min)
1	4	1
2	30	10
3	4	Hold

6. Upon completion, place the reactions on ice and proceed to "Protocol: DNA Ligation", next section.

# Protocol: DNA Ligation

## Important points before starting

- The product from “Protocol: RNA Polyadenylation” is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

## Procedure

1. Prepare the reagents required for the DNA ligation.
  - a. Thaw DNA Ligation Adapter and UPH Ligation Buffer, 2.5x, at room temperature.
  - b. Mix by flicking the tube, and then centrifuge briefly.

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



2. Prepare the DNA ligation mix according to Table 9. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 9. Reaction mix for DNA ligation**

Component	Volume/reaction (µL)
RNA polyadenylation reaction (already in tube)	25
UPH Ligation Buffer, 2.5x	24
DNA Ligation Adapter	3.36
DNA Ligase	6
Nuclease-free Water	1.64
<b>Total</b>	<b>60</b>

3. Incubate the reactions in a thermal cycler according to Table 10.

**Important:** Do not use the heated lid.

**Table 10. Incubation conditions for DNA ligation**

Step	Temperature (°C)	Time (min)
1	4	1
2	20	15
3	4	Hold

4. Add 40 µL of Nuclease-free Water to bring each sample to 100 µL.
5. Add 130 µLQIAseq Beads, and then mix by vortexing.
6. Incubate for 5 min at room temperature.

7. Place the tubes/plate wells on a magnetic rack for 2 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 because they contain the DNA of interest.

**Note:** For plates, the following may improve performance. After 8 min, remove 130  $\mu\text{L}$  supernatant. Leave it on the magnetic stand for 2 min and remove 90  $\mu\text{L}$  supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10  $\mu\text{L}$  pipette to remove the remaining supernatant.

8. Add 80  $\mu\text{L}$  of Nuclease-free Water to resuspend the beads and then 128  $\mu\text{L}$  of QIAseq NGS Bead Binding Buffer. Mix by vortexing and incubate for 5 min at room temperature.
9. Place the tubes/plate on a magnetic rack for 2 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 because they contain the DNA of interest.

**Note:** For plates, the following may improve performance. After 8 min, remove 108  $\mu\text{L}$  supernatant. Leave it on the magnetic stand for 2 min and remove 90  $\mu\text{L}$  supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10  $\mu\text{L}$  pipette to remove the remaining supernatant.

10. With the beads still on the magnetic stand, add 200  $\mu\text{L}$  of 80% ethanol. Rotate the tube 2–3 times or move the plate side-to-side between the two column positions of the magnet to wash the beads. Carefully remove and discard the wash.
11. Repeat the ethanol wash.

**Important:** Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200  $\mu\text{L}$  pipette tip first, spin down briefly, and then use a 10  $\mu\text{L}$  pipette tip to remove any residual ethanol.

12. With the beads still on the magnetic stand, air-dry at room temperature for 5–10 min.  
**Note:** Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.
13. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 19  $\mu$ L Nuclease-free Water. Mix well by pipetting.
14. Return the tubes/plate wells to the magnetic rack until the solution has cleared.
15. Transfer 16.62  $\mu$ L of the supernatant to clean tubes/plate.
16. Proceed to “Protocol: Reverse Transcription”, on the next page. Alternatively, the samples can be stored at  $-80^{\circ}\text{C}$  in a constant-temperature freezer.

# Protocol: Reverse Transcription

## Important points before starting

- The 16.62  $\mu\text{L}$  product from “Protocol: DNA Ligation” on page 32 is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

## Procedure

1. Prepare the reagents required for the reverse transcription.
  - a. Thaw the Multimodal RT Primer, Multimodal RT Buffer (5x), and Multimodal RT Enhancer at room temperature.
  - b. Mix by flicking the tube, and then centrifuge briefly.

**Note:** The RNase Inhibitor and EZ Reverse Transcriptase should be removed from the freezer and placed on ice just before use. After use, immediately return the enzymes to the freezer.

2. Prepare the reverse transcription mix according to Table 11. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 11. Reaction mix for reverse transcription**

Component	Volume/reaction (μL)
Sample (from "Protocol: DNA Ligation")	16.62
Multimodal RT Primer	1
Multimodal RT Buffer, 5x	5
Multimodal RT Enhancer	0.5
RNase Inhibitor	0.63
EZ Reverse Transcriptase	1.25
<b>Total</b>	<b>25</b>

3. Incubate the reactions in a thermal cycler according to Table 12. Use the instrument's heated lid.

**Table 12. Incubation conditions for reverse transcription**

Step	Temperature (°C)	Time (min)
1	4	1
2	25	10
3	42	45
4	70	15
5	4	Hold

4. Add 75 μL of Nuclease-free Water to bring each sample to 100 μL.

5. Add 130  $\mu\text{L}$  QIAseq Beads and mix by vortexing or by pipetting up and down several times.
6. Incubate for 5 min at room temperature.
7. Place the tubes/plate on a magnetic rack for 2 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 because they contain the DNA of interest.

**Note:** For plates, the following may improve performance. After 8 min, remove 130  $\mu\text{L}$  supernatant. Leave it on the magnetic stand for 2 min and remove 90  $\mu\text{L}$  supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10  $\mu\text{L}$  pipette to remove the remaining supernatant.

8. With the beads still on the magnetic stand, add 200  $\mu\text{L}$  of 80% ethanol. Rotate the tube 2–3 times or move the plate side-to-side between the 2 column positions of the magnet to wash the beads. 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  $\mu\text{L}$  pipette tip first, spin down briefly, and then use a 10  $\mu\text{L}$  pipette tip to remove any residual ethanol.

10. With the beads still on the magnetic stand, air-dry at room temperature for 5–10 min.

**Note:** Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required. Ethanol carryover to the next universal PCR step will affect PCR efficiency.

11. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 22.4  $\mu$ L Nuclease-free Water.

**Important:** If performing the Combined Enrichment Workflow (CW) for Targeted Enrichment (“Appendix B: Combined Enrichment Workflow for Targeted Enrichment” on page 72), elute by adding 15  $\mu$ L Nuclease-free Water.

12. Return the tube/plate to the magnetic rack until the solution has cleared.
13. Transfer 10.2  $\mu$ L of the eluate to each of two clean tubes/plate wells per sample.

**Important:** If performing the Combined Enrichment Workflow (CW) for Targeted Enrichment (“Appendix B: Combined Enrichment Workflow for Targeted Enrichment” on page 72), transfer 12.4  $\mu$ L of the eluate to clean tubes/plate wells and proceed to the Appendix B protocol.

14. Proceed to “Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment” on the next page. Alternatively, the samples can be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

# Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment

## Important points before starting

- Two 10.2 µL aliquots of the product from “Protocol: Reverse Transcription” are the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- The final Library dual sample index is determined by the combination of the QIAseq Multimodal N7 Plate and the QIAseq Multimodal S5 Plate. QIAseq Beads are used for all reaction cleanups.

**Note:** The required combinations of indexes are described in the sequencing sample setup sheets: Sample Sheet Multimodal UDI Set 96 ([www.qiagen.com/PROM-20735](http://www.qiagen.com/PROM-20735)).

- 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.
- To use this protocol, one of the following QIAseq Multimodal N7 index plates is required:
  - QIAseq Multimodal Index I SW (12) (cat. no. 333982): MTIN-12SWK (shown in Table 14)
  - QIAseq Multimodal HT SW 96 UDI (cat. no. 333986): MTIN-96DNA/K/X and MTIN-96RNA/K/X (shown in Table 15a and Table 15b)



- MTIN-12SWK is a 12-reaction format enabling the indexing of 12 DNA and 12 RNA libraries. There are dried N7 index primers for DNA and RNA libraries, in separate wells of the same plate. The plates can be cut in columns to enable indexing of the desired number of samples.
- MTIN-96DNA/K/X and MTIN-96RNA/K/X enable the N7 indexing of 96 DNA samples and 96 RNA samples, respectively, using separate plates.

## Procedure

1. Prepare the reagents required for target enrichment.
  - a. Thaw TEPCR Buffer, 5x; Multimodal DHS Panel (DNA); and Multimodal VHS Panel (RNA); and bring the QIAseq Multimodal N7 index plate to room temperature.
  - b. Mix by flicking the tube, and then centrifuge briefly.

**Note:** QN Taq Polymerase should be removed from the freezer and placed on ice just before use. After use, immediately return the enzyme to the freezer.

2. Prepare the target enrichment mix according to Table 13. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 13. Reaction mix for separated target enrichment**

Component	Separate DNA (μL)	Separate RNA (μL)
Sample (from "Protocol: Reverse Transcription")	10.2	10.2
TEPCR Buffer, 5x	4	4
Multimodal DHS Panel (DNA)	5	0
Multimodal VHS Panel (RNA)	0	4
QN Taq Polymerase	0.8	0.8
Nuclease-free Water	0	1
<b>Total</b>	<b>20</b>	<b>20</b>

3. Add the 20 μL target enrichment reaction mix into a well of a QIAseq Multimodal N7 index plate (Table 14, Table 15a, and Table 15b).

**Important:** Put any unused plate(s) back in the foil back and keep in  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for long-term storage.

**Important:** Index primers for DNA and RNA should be used in pairs. For example, in MTIN-12SWK (Table 14), use well A1 for DNA and well A7 for RNA for sample 1, use well B1 for DNA and well B7 for RNA for sample 2, etc. For MTIN-96DNA/K/X and MTIN-96RNA/K/X (Table 15a and Table 15b), well A1 for DNA (MTIN-96DNA/K/X) should be paired with well A1 for RNA (MTIN-96RNA/K/X), well A2 for DNA should be paired with well A2 for RNA, etc.

Table 14. Layout of QIAseq Multimodal N7 index plate MTIN-12SWK

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	DNAp-M001 S1	DNAp-M009 S9	–	–	–	–	RNAp-M049 S1	RNAp-M057 S9	–	–	–	–
<b>B</b>	DNAp-M002 S2	DNAp-M010 S10	–	–	–	–	RNAp-M050 S2	RNAp-M058 S10	–	–	–	–
<b>C</b>	DNAp-M003 S3	DNAp-M011 S11	–	–	–	–	RNAp-M051 S3	RNAp-M059 S11	–	–	–	–
<b>D</b>	DNAp-M004 S4	DNAp-M012 S12	–	–	–	–	RNAp-M052 S4	RNAp-M060 S12	–	–	–	–
<b>E</b>	DNAp-M005 S5	–	–	–	–	–	RNAp-M053 S5	–	–	–	–	–
<b>F</b>	DNAp-M006 S6	–	–	–	–	–	RNAp-M054 S6	–	–	–	–	–
<b>G</b>	DNAp-M007 S7	–	–	–	–	–	RNAp-M055 S7	–	–	–	–	–
<b>H</b>	DNAp-M008 S8	–	–	–	–	–	RNAp-M056 S8	–	–	–	–	–

Table 15a. Layout of QIAseq Multimodal N7 index plates MTIN-96DNA/K/X

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	DNAp-M001 S1	DNAp-M009 S9	DNAp-M017 S17	DNAp-M025 S25	DNAp-M033 S33	DNAp-M041 S41	DNAp-M097 S49	DNAp-M105 S57	DNAp-M113 S65	DNAp-M121 S73	DNAp-M129 S81	DNAp-M137 S89
<b>B</b>	DNAp-M002 S2	DNAp-M010 S10	DNAp-M018 S18	DNAp-M026 S26	DNAp-M034 S34	DNAp-M042 S42	DNAp-M098 S50	DNAp-M106 S58	DNAp-M114 S66	DNAp-M122 S74	DNAp-M130 S82	DNAp-M138 S90
<b>C</b>	DNAp-M003 S3	DNAp-M011 S11	DNAp-M019 S19	DNAp-M027 S27	DNAp-M035 S35	DNAp-M043 S43	DNAp-M099 S51	DNAp-M107 S59	DNAp-M115 S67	DNAp-M123 S75	DNAp-M131 S83	DNAp-M139 S91
<b>D</b>	DNAp-M004 S4	DNAp-M012 S12	DNAp-M020 S20	DNAp-M028 S28	DNAp-M036 S36	DNAp-M044 S44	DNAp-M100 S52	DNAp-M108 S60	DNAp-M116 S68	DNAp-M124 S76	DNAp-M132 S84	DNAp-M140 S92
<b>E</b>	DNAp-M005 S5	DNAp-M013 S13	DNAp-M021 S21	DNAp-M029 S29	DNAp-M037 S37	DNAp-M045 S45	DNAp-M101 S53	DNAp-M109 S61	DNAp-M117 S69	DNAp-M125 S77	DNAp-M133 S85	DNAp-M141 S93
<b>F</b>	DNAp-M006 S6	DNAp-M014 S14	DNAp-M022 S22	DNAp-M030 S30	DNAp-M038 S38	DNAp-M046 S46	DNAp-M102 S54	DNAp-M110 S62	DNAp-M118 S70	DNAp-M126 S78	DNAp-M134 S86	DNAp-M142 S94
<b>G</b>	DNAp-M007 S7	DNAp-M015 S15	DNAp-M023 S23	DNAp-M031 S31	DNAp-M039 S39	DNAp-M047 S47	DNAp-M103 S55	DNAp-M111 S63	DNAp-M119 S71	DNAp-M127 S79	DNAp-M135 S87	DNAp-M143 S95
<b>H</b>	DNAp-M008 S8	DNAp-M016 S16	DNAp-M024 S24	DNAp-M032 S32	DNAp-M040 S40	DNAp-M048 S48	DNAp-M104 S56	DNAp-M112 S64	DNAp-M120 S72	DNAp-M128 S80	DNAp-M136 S88	DNAp-M144 S96

**Table 15b. Layout of QIAseq Multimodal N7 index plates MTIN-96RNA/K/X**

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	RNAp-M049	RNAp-M057	RNAp-M065	RNAp-M073	RNAp-M081	RNAp-M089	RNAp-M145	RNAp-M153	RNAp-M161	RNAp-M169	RNAp-M177	RNAp-M185
	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
<b>B</b>	RNAp-M050	RNAp-M058	RNAp-M066	RNAp-M074	RNAp-M082	RNAp-M090	RNAp-M146	RNAp-M154	RNAp-M162	RNAp-M170	RNAp-M178	RNAp-M186
	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
<b>C</b>	RNAp-M051	RNAp-M059	RNAp-M067	RNAp-M075	RNAp-M083	RNAp-M091	RNAp-M147	RNAp-M155	RNAp-M163	RNAp-M171	RNAp-M179	RNAp-M187
	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
<b>D</b>	RNAp-M052	RNAp-M060	RNAp-M068	RNAp-M076	RNAp-M084	RNAp-M092	RNAp-M148	RNAp-M156	RNAp-M164	RNAp-M172	RNAp-M180	RNAp-M188
	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
<b>E</b>	RNAp-M053	RNAp-M061	RNAp-M069	RNAp-M077	RNAp-M085	RNAp-M093	RNAp-M149	RNAp-M157	RNAp-M165	RNAp-M173	RNAp-M181	RNAp-M189
	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
<b>F</b>	RNAp-M054	RNAp-M062	RNAp-M070	RNAp-M078	RNAp-M086	RNAp-M094	RNAp-M150	RNAp-M158	RNAp-M166	RNAp-M174	RNAp-M182	RNAp-M190
	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
<b>G</b>	RNAp-M055	RNAp-M063	RNAp-M071	RNAp-M077	RNAp-M087	RNAp-M095	RNAp-M151	RNAp-M159	RNAp-M167	RNAp-M175	RNAp-M183	RNAp-M191
	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
<b>H</b>	RNAp-M056	RNAp-M064	RNAp-M072	RNAp-M080	RNAp-M088	RNAp-M096	RNAp-M152	RNAp-M160	RNAp-M168	RNAp-M176	RNAp-M184	RNAp-M192
	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

- Briefly centrifuge, mix by pipetting up and down 8 times, and then briefly centrifuge again.

**Note:** If only one column is used, cut that column from the cuttable plate and proceed to the next step.

- Program a thermal cycler according to Table 16c or Table 16d based on the number of DNA or RNA primers (not the combined DNA + RNA primer total).

**Important:** Set up the ramp rate of the thermal cycler to  $\leq 2^{\circ}\text{C/s}$  to ensure best performance.

**Table 16c. Cycling conditions for target enrichment if the number of primers is <3000/tube**

Step	Time	Temperature ( $^{\circ}\text{C}$ )
Initial denaturation	2 min	98
8 cycles	15 s	98
	2 min	68
Hold	$\infty$	4

**Table 16d. Cycling conditions for target enrichment if the number of primers is  $\geq 3000$ /tube**

Step	Time (3000–12,000 primers/tube)	Time (>12,000 primers/tube)	Temperature (°C)
Initial denaturation	2 min	2 min	98
6 cycles	15 s	15 s	98
	4 min	8 min	65
Hold	$\infty$	$\infty$	4

6. Place the target enrichment reaction in the thermal cycler and start the run.
7. Once the run has finished, add 80  $\mu\text{L}$  of Nuclease-free Water to bring each sample to 100  $\mu\text{L}$ .
8. Follow the steps below for standard, cfDNA, and FFPE samples:
  - a. **For standard and cfDNA samples:** Add 80  $\mu\text{L}$  QIAseq Beads for standard or 100  $\mu\text{L}$  QIAseq Beads for cfDNA sample and mix by vortexing or by pipetting up and down several times.
  - b. **For FFPE samples:**
    - Add 50  $\mu\text{L}$  QIAseq Beads to the 100  $\mu\text{L}$  reactions. Mix by vortexing.
    - Incubate for 5 min at room temperature.
    - Place the reaction on a magnet rack for 2 min (for tubes) or 10 min (for plates).
    - Carefully transfer the supernatant to a new tube:  
**Important:** Keep the supernatant because it contains the target.
    - Add another 40  $\mu\text{L}$  QIAseq Beads to the supernatant. Mix by vortexing.
9. Incubate for 5 min at room temperature.
10. Place the tubes/plate on a magnetic rack for 2 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 because they contain the DNA of interest.

**Note:** For plates, the following may improve performance. After 8 min, remove 100  $\mu$ L supernatant. Leave it on the magnetic stand for 2 min and remove the remaining supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10  $\mu$ L pipette to remove the residues.

11. With the beads still on the magnetic stand, add 200  $\mu$ L of 80% ethanol. Rotate the tube 2–3 times or move the plate side-to-side between the 2 column positions of the magnet to wash the beads. Carefully remove and discard the wash.

12. Repeat the ethanol wash.

**Important:** Completely remove all traces of the ethanol after this second wash. Remove the ethanol with a 200  $\mu$ L pipette first, spin down briefly, and then use a 10  $\mu$ L pipette to remove any residual ethanol.

13. With the beads still on the magnetic stand, air-dry at room temperature for 5–10 min.

**Note:** Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required. Ethanol carryover to the next universal PCR step will affect PCR efficiency.

14. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 14  $\mu$ L Nuclease-free Water. Mix well by pipetting.
15. Return the tube/plate to the magnetic rack until the solution has cleared.
16. Transfer 12  $\mu$ L of the supernatant to clean tubes/plate.
17. Proceed to “Protocol: qPCR Determination of Universal PCR Cycles”. Alternatively, the samples can be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

# Protocol: qPCR Determination of Universal PCR Cycles

## Important points before starting

- Use 2  $\mu$ L of the product from “Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment”, or “Appendix B: Combined Enrichment Workflow for Targeted Enrichment”, as the starting material for each of the reaction mixes.
- EvaGreen Dye, 20x in water, is required for this procedure and must be purchased from Biotium (cat. nos. 31000-T and 31000).
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

## Procedure

1. Prepare the reagents required for the qPCR.
  - a. Thaw UPCR Buffer, 5x; DNA qPCR AMP Set; and RNA qPCR AMP. Set at room temperature.
  - b. Mix by flicking the tube, and then centrifuge briefly.

**Note:** QN Taq Polymerase should be removed from the freezer and placed on ice just before use. After use, immediately return the enzyme to the freezer.
2. Prepare the qPCR reactions according to Table 17 for DNA Library or Table 18 for RNA Library. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 17. Reaction mix for qPCR of DNA Library**

Component	Volume/reaction (µL)
DNA Sample [from "Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment"]	2
or	
Sample [from "Appendix B: Combined Enrichment Workflow for Targeted Enrichment"]	
UPCR Buffer, 5x	2
Nuclease-free Water	4.1
DNA qPCR AMP Set	1
QN Taq Polymerase	0.4
EvaGreen Dye, 20x in water*	0.5
<b>Total</b>	<b>10</b>

\* Must be purchased from Biotium (cat. nos. 31000-T and 31000).



**Table 18. Reaction mix for qPCR of RNA Library**

Component	Volume/reaction (µL)
RNA Sample [from "Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment"]	2
or	
Sample [from "Appendix B: Combined Enrichment Workflow for Targeted Enrichment"]	
UPCR Buffer, 5x	2
Nuclease-free Water	4.1
RNA qPCR AMP Set	1
QN Taq Polymerase	0.4
EvaGreen Dye, 20x in water*	0.5
<b>Total</b>	<b>10</b>

\* Must be purchased from Biotium (cat. nos. 31000-T and 31000).

3. Program a qPCR instrument using the cycling conditions for standard samples (Table 19a) or FFPE samples (Table 19b).

**Note:** No melting curve is required.

**Table 19a. Reaction mix for qPCR of Library – Standard samples**

Step	Time	Temperature (°C)
Hold	2 min	98
<b>2-step cycling</b>		
Denaturation	15 s	98
Annealing/Extension*	30 s	62
<b>Cycle number</b>	<b>30 cycles</b>	
Hold	∞	4

\* Perform fluorescence data collection.

**Table 19b. Reaction mix for qPCR of Library – FFPE samples**

Step	Time	Temperature (°C)
Hold	2 min	98
<b>2-step cycling</b>		
Denaturation	15 s	98
Annealing/Extension*	2 min	62
<b>Cycle number</b>	<b>30 cycles</b>	
Hold	∞	4

\* Perform fluorescence data collection.

4. Following the reaction, determine the  $C_T$  values. Based on the  $C_T$  values, the number of universal PCR cycles is defined as  $C_{T(qPCR)} + 3$ , for both the DNA and RNA libraries.

For example, if the DNA qPCR is  $C_T = 19$ , then perform 22 cycles for DNA universal PCR. If the RNA qPCR is  $C_T = 15$ , then perform 18 cycles for RNA universal PCR.

For FFPE samples, use  $C_{T(qPCR)} + 5$  for better library yield.

Alternative method: When the run has finished, observe the amplification plot in "Log View" and define the baseline using "auto baseline". Using the "Log View" of the amplification plot, determine the cycle in which the amplification curve reaches its Plateau Phase, and use 2 cycles fewer. For example, if the plateau phase is reached when the  $C_T$  is 18, then 16 is the required number of universal PCR amplification cycles (For FFPE samples, use 18 cycles for better Library yield).

5. Once the amplification cycles for universal PCR have been determined, proceed to "Protocol: Universal PCR ", next section.

# Protocol: Universal PCR

## Important points before starting

- Use the 9  $\mu$ L product from “Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment”, or “Appendix B: Combined Enrichment Workflow for Targeted Enrichment”, as the starting material for each of the reaction mixes.
- The number of cycles required for amplification are determined in “Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment”.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- QIAseq Multimodal S5 index plates enable indexing of 12 or 96 samples via the following plates.
  - QIAseq Multimodal Index I SW (12) (cat. no. 333982): MTIS-12K (shown in Table 22)
  - QIAseq Multimodal HT SW 96 UDI (cat. no. 333986): MTIS-96DNA/K/X and MTIS-96RNA/K/X (shown in Table 23a and Table 23b)
  - QIAseq Multimodal Index I (12) (cat. no. 333962): MTIS-12K (shown in Table 22)
  - QIAseq Multimodal HT CW 96 UDI (cat. no. 333979): MTIS-96DNA/K/X and MTIS-96RNA/K/X (shown in Table 23a and Table 23b)

**Note:** MTIS-12K is the same plate found in cat. nos. 333982 and 333962. MTIS-96DNA/K/X and MTIS-96RNA/K/X are the same plates found in cat. nos. 333986 and 333979.

- MTIS-12K enables the S5 indexing of 12 DNA and 12 RNA libraries. The dried S5 indexes for DNA libraries are pre-mixed with universal DNA primer. The dried S5 indexes for RNA libraries are pre-mixed with universal RNA primer. The plates can be cut in columns to enable indexing of the desired number of samples.
- MTIN-96DNA/K/X and MTIN-96RNA/K/X enable the S5 indexing of 96 DNA samples and 96 RNA samples, respectively, using separate plates. The dried S5 indexes for DNA libraries are pre-mixed with universal DNA primer. The dried S5 indexes for RNA libraries are pre-mixed with universal RNA primer.
- The required combinations of indexes are described in the sequencing sample setup sheets (Sample Sheet Multimodal UDI Set 96, accessible from [www.qiagen.com/PROM-20735](http://www.qiagen.com/PROM-20735)).
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.

## Procedure

1. Prepare the reagents required for universal PCR.
  - a. Thaw UPCR Buffer, 5x, and bring the QIAseq Multimodal S5 Plate to room temperature.
  - b. Mix by flicking the tube, and then centrifuge briefly.

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

2. Prepare the universal PCR according to Table 20 for DNA Library or Table 21 for RNA Library. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

**Table 20. Reaction mix for universal PCR of DNA Library**

Component	Volume/reaction (μL)
DNA Sample [from "Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment"]	9
or	
Sample [from "Appendix B: Combined Enrichment Workflow for Targeted Enrichment"]	
UPCR Buffer, 5x	5
Nuclease-free Water	10
QN Taq Polymerase	1
<b>Total</b>	<b>25</b>

Table 21. Reaction mix for universal PCR of RNA Library

Component	Volume/reaction (µL)
RNA Sample [from "Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment"]	9
or	
Sample [from "Appendix B: Combined Enrichment Workflow (CW) for Targeted Enrichment"]	
UPCR Buffer, 5x	5
Nuclease-free Water	10
QN Taq Polymerase	1
Total	25

3. To the QIAseq Multimodal S5 index plate (Table 22 or Table 23a and Table 23b) add the 25 µL reaction mix for universal PCR of DNA Library to the DNA wells or plate and add the 25 µL reaction mix for universal PCR of RNA Library to the RNA wells or plate.

**Important:** S5 index primers for DNA and RNA should be used in pairs, both within themselves and with the N7 index primers. For example, in MTIS-12K (Table 22), use well A1 for DNA and well A7 for RNA for sample 1; use well B1 for DNA and well B7 for RNA for sample 2; etc. For MTIS-96DNA/K/X and MTIS-96RNA/K/X (Table 23a and Table 23b), well A1 for DNA (MTIS-96DNA/K/X) should be paired with well A1 for RNA (MTIS-96RNA/K/X), well A2 for DNA should be paired with well A2 for RNA, etc.

Table 22. Layout of QIAseq Multimodal S5 index plate MTIS-12K

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	SGDIB001	SGDIB009					SGDIB049	SGDIB057				
	<b>S1</b> uDNA Pr	<b>S9</b> uDNA Pr					<b>S1</b> uRNA Pr	<b>S9</b> uRNA Pr				
<b>B</b>	SGDIB002	SGDIB010					SGDIB050	SGDIB058				
	<b>S2</b> uDNA Pr	<b>S10</b> uDNA Pr					<b>S2</b> uRNA Pr	<b>S10</b> uRNA Pr				
<b>C</b>	SGDIB003	SGDIB011					SGDIB051	SGDIB059				
	<b>S3</b> uDNA Pr	<b>S11</b> uDNA Pr					<b>S3</b> uRNA Pr	<b>S11</b> uRNA Pr				
<b>D</b>	SGDIB004	SGDIB012					SGDIB052	SGDIB060				
	<b>S4</b> uDNA Pr	<b>S12</b> uDNA Pr					<b>S4</b> uRNA Pr	<b>S12</b> uRNA Pr				
<b>E</b>	SGDIB005						SGDIB053					
	<b>S5</b> uDNA Pr						<b>S5</b> uRNA Pr					
<b>F</b>	SGDIB006						SGDIB054					
	<b>S6</b> uDNA Pr						<b>S6</b> uRNA Pr					
<b>G</b>	SGDIB007						SGDIB055					
	<b>S7</b> uDNA Pr						<b>S7</b> uRNA Pr					
<b>H</b>	SGDIB008						SGDIB056					
	<b>S8</b> uDNA Pr						<b>S8</b> uRNA Pr					

Table 23a. Layout of QIAseq Multimodal S5 index plates MTIS-96DNA/K/X

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	SGDIB001	SGDIB009	SGDIB017	SGDIB025	SGDIB033	SGDIB041	SGDIB097	SGDIB105	SGDIB113	SGDIB121	SGDIB129	SGDIB137
	<b>S1</b> uDNA Pr	<b>S9</b> uDNA Pr	<b>S17</b> uDNA Pr	<b>S25</b> uDNA Pr	<b>S33</b> uDNA Pr	<b>S41</b> uDNA Pr	<b>S49</b> uDNA Pr	<b>S57</b> uDNA Pr	<b>S65</b> uDNA Pr	<b>S73</b> uDNA Pr	<b>S81</b> uDNA Pr	<b>S89</b> uDNA Pr
<b>B</b>	SGDIB002	SGDIB010	SGDIB018	SGDIB026	SGDIB034	SGDIB042	SGDIB098	SGDIB106	SGDIB114	SGDIB122	SGDIB130	SGDIB138
	<b>S2</b> uDNA Pr	<b>S10</b> uDNA Pr	<b>S18</b> uDNA Pr	<b>S26</b> uDNA Pr	<b>S34</b> uDNA Pr	<b>S42</b> uDNA Pr	<b>S50</b> uDNA Pr	<b>S58</b> uDNA Pr	<b>S66</b> uDNA Pr	<b>S74</b> uDNA Pr	<b>S82</b> uDNA Pr	<b>S90</b> uDNA Pr
<b>C</b>	SGDIB003	SGDIB011	SGDIB019	SGDIB027	SGDIB035	SGDIB043	SGDIB099	SGDIB107	SGDIB115	SGDIB123	SGDIB131	SGDIB764
	<b>S3</b> uDNA Pr	<b>S11</b> uDNA Pr	<b>S19</b> uDNA Pr	<b>S27</b> uDNA Pr	<b>S35</b> uDNA Pr	<b>S43</b> uDNA Pr	<b>S51</b> uDNA Pr	<b>S59</b> uDNA Pr	<b>S67</b> uDNA Pr	<b>S75</b> uDNA Pr	<b>S83</b> uDNA Pr	<b>S91</b> uDNA Pr
<b>D</b>	SGDIB004	SGDIB012	SGDIB020	SGDIB028	SGDIB036	SGDIB044	SGDIB100	SGDIB108	SGDIB116	SGDIB124	SGDIB132	SGDIB140
	<b>S4</b> uDNA Pr	<b>S12</b> uDNA Pr	<b>S20</b> uDNA Pr	<b>S28</b> uDNA Pr	<b>S36</b> uDNA Pr	<b>S44</b> uDNA Pr	<b>S52</b> uDNA Pr	<b>S60</b> uDNA Pr	<b>S68</b> uDNA Pr	<b>S76</b> uDNA Pr	<b>S84</b> uDNA Pr	<b>S92</b> uDNA Pr
<b>E</b>	SGDIB005	SGDIB013	SGDIB021	SGDIB029	SGDIB037	SGDIB045	SGDIB101	SGDIB109	SGDIB117	SGDIB125	SGDIB133	SGDIB141
	<b>S5</b> uDNA Pr	<b>S13</b> uDNA Pr	<b>S21</b> uDNA Pr	<b>S29</b> uDNA Pr	<b>S37</b> uDNA Pr	<b>S45</b> uDNA Pr	<b>S53</b> uDNA Pr	<b>S61</b> uDNA Pr	<b>S69</b> uDNA Pr	<b>S77</b> uDNA Pr	<b>S85</b> uDNA Pr	<b>S93</b> uDNA Pr
<b>F</b>	SGDIB006	SGDIB014	SGDIB761	SGDIB030	SGDIB038	SGDIB046	SGDIB102	SGDIB110	SGDIB118	SGDIB126	SGDIB134	SGDIB142
	<b>S6</b> uDNA Pr	<b>S14</b> uDNA Pr	<b>S22</b> uDNA Pr	<b>S30</b> uDNA Pr	<b>S38</b> uDNA Pr	<b>S46</b> uDNA Pr	<b>S54</b> uDNA Pr	<b>S62</b> uDNA Pr	<b>S70</b> uDNA Pr	<b>S78</b> uDNA Pr	<b>S86</b> uDNA Pr	<b>S94</b> uDNA Pr
<b>G</b>	SGDIB007	SGDIB015	SGDIB023	SGDIB031	SGDIB763	SGDIB047	SGDIB103	SGDIB111	SGDIB119	SGDIB127	SGDIB135	SGDIB143
	<b>S7</b> uDNA Pr	<b>S15</b> uDNA Pr	<b>S23</b> uDNA Pr	<b>S31</b> uDNA Pr	<b>S39</b> uDNA Pr	<b>S47</b> uDNA Pr	<b>S55</b> uDNA Pr	<b>S63</b> uDNA Pr	<b>S71</b> uDNA Pr	<b>S79</b> uDNA Pr	<b>S87</b> uDNA Pr	<b>S95</b> uDNA Pr
<b>H</b>	SGDIB008	SGDIB016	SGDIB024	SGDIB032	SGDIB040	SGDIB048	SGDIB104	SGDIB112	SGDIB120	SGDIB128	SGDIB136	SGDIB144
	<b>S8</b> uDNA Pr	<b>S16</b> uDNA Pr	<b>S24</b> uDNA Pr	<b>S32</b> uDNA Pr	<b>S40</b> uDNA Pr	<b>S48</b> uDNA Pr	<b>S56</b> uDNA Pr	<b>S64</b> uDNA Pr	<b>S72</b> uDNA Pr	<b>S80</b> uDNA Pr	<b>S88</b> uDNA Pr	<b>S96</b> uDNA Pr



Table 23b. Layout of QIAseq Multimodal S5 index plates MTIS-96RNA/K/X

	1	2	3	4	5	6	7	8	9	10	11	12
	SQDIB049	SQDIB057	SQDIB065	SQDIB073	SQDIB081	SQDIB089	SQDIB015	SQDIB153	SQDIB161	SQDIB169	SQDIB177	SQDIB185
A S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB050	SQDIB058	SQDIB066	SQDIB074	SQDIB082	SQDIB090	SQDIB146	SQDIB154	SQDIB162	SQDIB170	SQDIB178	SQDIB186
B S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB051	SQDIB059	SQDIB067	SQDIB075	SQDIB083	SQDIB091	SQDIB147	SQDIB155	SQDIB163	SQDIB171	SQDIB179	SQDIB187
C S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB052	SQDIB060	SQDIB068	SQDIB076	SQDIB084	SQDIB092	SQDIB148	SQDIB156	SQDIB164	SQDIB172	SQDIB180	SQDIB188
D S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB053	SQDIB061	SQDIB069	SQDIB077	SQDIB085	SQDIB093	SQDIB149	SQDIB157	SQDIB165	SQDIB173	SQDIB181	SQDIB189
E S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB054	SQDIB062	SQDIB070	SQDIB078	SQDIB086	SQDIB094	SQDIB150	SQDIB158	SQDIB166	SQDIB174	SQDIB182	SQDIB190
F S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB055	SQDIB063	SQDIB071	SQDIB079	SQDIB087	SQDIB095	SQDIB151	SQDIB159	SQDIB167	SQDIB175	SQDIB183	SQDIB191
G S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB056	SQDIB064	SQDIB072	SQDIB080	SQDIB088	SQDIB096	SQDIB152	SQDIB160	SQDIB168	SQDIB176	SQDIB184	SQDIB192
H S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96	
	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr

4. Program a thermal cycler using the cycling conditions for standard samples (Table 24c) or FFPE samples (Table 24d).

**Important:** Set up the ramp rate of the thermal cycler at  $\leq 2^{\circ}\text{C}/\text{s}$ .

**Table 24c. Cycling conditions for universal PCR – standard samples**

Step	Time	Temperature ( $^{\circ}\text{C}$ )
Hold	2 min	98
<b>2-step cycling</b>		
Denaturation	15 s	98
Annealing/Extension	30 s	62
Cycle number	Based on Protocol: qPCR Determination of Universal PCR Cycles” on page 47	
Hold	$\infty$	4

**Table 24d. Cycling conditions for universal PCR – FFPE samples**

Step	Time	Temperature ( $^{\circ}\text{C}$ )
Hold	2 min	98
<b>2-step cycling</b>		
Denaturation	15 s	98
Annealing/Extension	2 min	62
Cycle number	Based on “Protocol: qPCR Determination of Universal PCR Cycles” on page 47	
Hold	$\infty$	4

5. After the reaction is complete, add 75  $\mu\text{L}$  of Nuclease-free Water to bring each sample to 100  $\mu\text{L}$ .

6. Add 80  $\mu$ L QIAseq Beads for standard/FFPE sample or 100  $\mu$ L QIAseq Beads for cfDNA sample, and then mix by vortexing or pipetting up and down several times.
7. Incubate for 5 min at room temperature.
8. Place the tubes/plate on a magnetic rack for 2 min (for tubes) or 10 min (for plates) to separate the beads from the supernatant.

After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

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

**Note:** For plates, the following may improve performance. After 8 min, remove 100  $\mu$ L supernatant. Leave it on the magnetic stand for 2 min and remove 80  $\mu$ L supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10  $\mu$ L pipette to remove the remaining supernatant.

9. With the beads still on the magnetic stand, add 200  $\mu$ L of 80% ethanol. Rotate the tube 2–3 times or move the plate side-to-side between the 2 column positions of the magnet to wash the beads. 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  $\mu$ L pipette tip first, spin down briefly, and then use a 10  $\mu$ L pipette tip to remove any residual ethanol.

11. With the beads still on the magnetic stand, air-dry at room temperature for 5–10 min.

**Note:** Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.

12. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 20  $\mu$ L Nuclease-free Water. Mix well by pipetting.
13. Return the tube/plate to the magnetic rack until the solution has cleared.

14. Transfer 18  $\mu\text{L}$  of the supernatant to clean tubes/plate.
15. Proceed to “Recommendations: Library QC and Quantification”, next section. Alternatively, the samples can be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

# Recommendations: Library QC and Quantification

## NGS library QC

QC can be performed with the QIAxcel® Connect, Agilent Bioanalyzer, or TapeStation®. Check for the correct size distribution of library fragments (~400–500 bp median size) and for the absence of adapters or adapter-dimers (~<200 bp) (Figure 3).

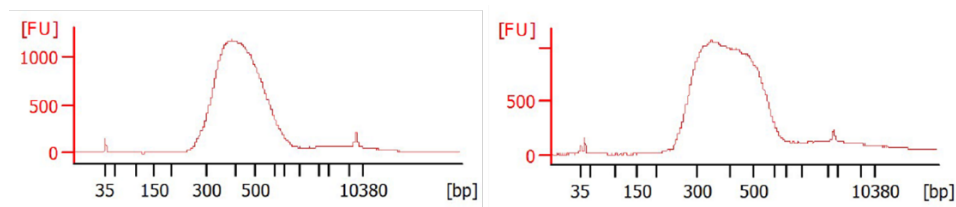


Figure 3. QIAseq Multimodal Targeted DNA (left) and Targeted RNA (right) libraries.

## Preferred library quantification method

The library yield measurements from the Bioanalyzer or TapeStation rely on fluorescence dyes that intercalate into DNA or RNA. These dyes cannot discriminate between molecules with or without adapter sequences, yet only complete QIAseq Multimodal libraries with full adapter sequences will be sequenced. As a result, a real-time PCR-based method is highly recommended for accurate quantification of the prepared QIAseq Multimodal Panels library.

# Protocol: Sequencing Setup on Illumina MiSeq and NextSeq550

## Important points before starting

- Recommendations for Library dilution concentrations and Library loading concentrations are based on QIAseq Library Quant System.
- QIAseq A Read 1 Primer I (Custom Read 1 Sequencing Primer) and Multimodal Read 2 Primer (Custom Read 2 Sequencing Primer) must be used when performing sequencing on Illumina platform.
- QIAseq A Read 1 Primer I (Custom Read 1 Sequencing Primer) goes into the following specific reagent cartridge positions:
  - MiSeq position #18
  - NextSeq position #7
- Multimodal Read 2 Primer (the Custom Read 2 Sequencing Primer) goes into the following specific reagent cartridge positions:
  - MiSeq position #20
  - NextSeq position #8
- Paired-end sequencing should be used for QIAseq Multimodal libraries on Illumina platforms.
  - Read 1: 149 bp
  - Read 2: 149 bp
  - Custom Index 1: 10 bp
  - Custom Index 2: 10 bp

- For complete instructions on how to denature sequencing libraries, prepare custom index primers, and set up a sequencing run, please refer to the system-specific Illumina documents.
- Instrument-specific imagery is included to aid in sequencing preparations.

For sequencing on other Illumina Instruments, please refer to our Template files available on the **Resources** section of the product page ([www.qiagen.com/QIAseqMultimodalPanelsHT](http://www.qiagen.com/QIAseqMultimodalPanelsHT)).

## Sequencing preparations for MiSeq

1. Download the appropriate template from the **Resources** tab of the QIAseq Multimodal Panel (Sample Sheet Multimodal 96 UDI is accessible from [qiagen.com/PROM-20735](http://qiagen.com/PROM-20735), which is also used for Indexes 1–12).
2. On the template:
  - a. Modify Investigator Name, Date, Sample ID, and Sample Name.
 

**Important:** We recommend adding -DNA in the Sample name of a DNA Library and -RNA for an RNA Library to allow automatic parsing of the DNA and RNA libraries during data analysis. If the libraries are not labeled, they must be manually parsed into either the DNA or RNA box.
  - b. Delete any unused index pairs and save the sample sheet for uploading.
  - c. Read 1 is 149 bp, Read 2 is 149 bp, and each Index Read is 10 bp.
3. **Sample dilution and pooling:** Dilute libraries to 4 nM for MiSeq. Combine 4 nM DNA libraries in equal amount in volume to make a DNA Library. Combine 4 nM RNA libraries in equal amount in volume to make a RNA Library.

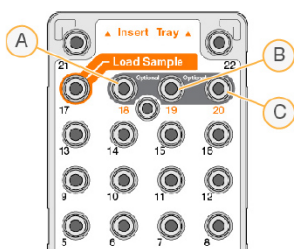
Use Table 32a and Table 32b or Reads Allocation Template ([www.qiagen.com/PROM-16466](http://www.qiagen.com/PROM-16466)) to get the reads needed for each Library and obtain numbers of Library to be loaded on MiSeq. Based on the ratio of reads needed for DNA or RNA Library, make a

Library pool by combining DNA Library and RNA Library at the same ratio in volume. For example, if 10 M reads for DNA Library and 2 M for RNA Library are needed, combine 10  $\mu\text{L}$  of each 4 nM DNA Library and 2  $\mu\text{L}$  of each 4 nM RNA Library to make the Library pool.

4. **Library preparation and loading:** Prepare and load library on a MiSeq according to the *MiSeq System Denature and Dilute libraries Guide*. The final Library concentration is 10–12 pM on MiSeq.

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

5. **Custom sequencing primer for Read 1 preparation and loading:** Use 597  $\mu\text{L}$  HT1 (hybridization buffer) from the Illumina Sequencing Kit to dilute 3  $\mu\text{L}$  of QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.5  $\mu\text{M}$ . Load 600  $\mu\text{L}$  of the diluted QIAseq A Read 1 Primer I to position 18 of the MiSeq reagent cartridge (Figure 4). For more details, please refer to Illumina's *MiSeq System: Custom Primers Guide*.
6. **Custom sequencing primer for Read 2 preparation and loading:** Use 597  $\mu\text{L}$  HT1 (hybridization buffer) from the Illumina Sequencing Kit to dilute 3  $\mu\text{L}$  of Multimodal Read 2 Primer (provided) to obtain a final concentration of 0.5  $\mu\text{M}$ . Load 600  $\mu\text{L}$  of the diluted QIAseq Read 2 Primer to position 20 of the MiSeq reagent cartridge (Figure 4). For more details, please refer to Illumina's *MiSeq System: Custom Primers Guide*.



**Figure 4. MiSeq reagent cartridge.** A: position 18 for Read 1 Custom Primer; C: position 20 for Read 2 Custom Primer.



## Sequencing preparations for NextSeq550

1. Sample dilution and pooling: Dilute each Library to 0.5, 1, 2, or 4 nM for NextSeq.
2. Use Table 32a and Table 32b or Reads Allocation Template ([www.qiagen.com/PROM-16466](http://www.qiagen.com/PROM-16466)) to get the reads needed for each Library and obtain numbers of Library to be loaded on NextSeq. Based on ratio of reads needed for DNA or RNA Library, make a Library pool by combining DNA Library and RNA Library at the same ratio in volume. For example, if 10 M reads for DNA Library and 2 M for RNA Library are needed, combine 10  $\mu$ L of each diluted DNA Library and 2  $\mu$ L of each diluted RNA Library to make the Library pool.

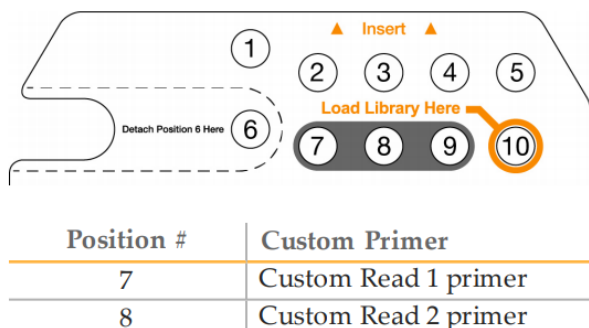
**Library preparation and loading:** Prepare and load Library onto a NextSeq according to the NextSeq System Denature and Dilute libraries Guide. The final Library concentration is 1.2–1.5 pM on NextSeq.

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

3. **Custom sequencing primer for Read 1 preparation and loading:** Use 1994  $\mu$ L HT1 (hybridization buffer) from the Illumina Sequencing Kit to dilute 6  $\mu$ L of QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3  $\mu$ M. Load 2 mL of the diluted QIAseq A Read 1 Primer I to position 7 of the NextSeq reagent cartridge (Figure 5).

**Note:** All other steps refer to run setup workflow as described in the NextSeq 500 System Guide (part no. 15046563) or NextSeq 550 System Guide (part no. 15069765-02).

4. **Custom sequencing primer for Read 2 preparation and loading:** Use 1994  $\mu$ L HT1 (hybridization buffer) from the Illumina Sequencing Kit to dilute 6  $\mu$ L of Multimodal Read 2 Primer to obtain a final concentration of 0.3  $\mu$ M. Load 2 mL of the diluted Multimodal Read 2 Primer to position 8 of the NextSeq reagent cartridge (Figure 5).



**Figure 5. NextSeq reagent cartridge.**

- When working with the QIAseq Multimodal custom UDIs, use Local Run Manager (LRM) V2 on the instrument to upload sample sheet (see “Important points before starting” section of Appendix B on page 72 for downloading the appropriate template and modifying the template) and proceed with sequencing: Read 1 is 149 bp, Read 2 is 149 bp, and each Index Read is 10 bp.

# 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](http://www.qiagen.com/FAQ/FAQList.aspx) (for contact information, visit [www.qiagen.com](http://www.qiagen.com)).

Comments and suggestions	
Low library yield	
a) Suboptimal reaction conditions due to low sample quality	Make sure to use high-quality samples to ensure optimal activity of the library enzymes.
b) Inefficient targeted enrichment or universal PCR	QIAseq beads need to be completely dried before elution. Ethanol carryover to targeted enrichment and universal PCR will affect PCR reaction efficiency.
Unexpected signal peaks	
a) Short peaks <200 bp	These are primer–dimers from targeted enrichment or universal PCR (<200 bp). The presence of primer–dimers indicates either not enough DNA/RNA input or inefficient PCR reactions or handling issues with bead purifications.
b) Larger DNA 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 won't affect the sequencing performance. Decreasing the number of universal PCR 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 incorrect quantification of the library, especially when there is overamplification.
b) Very low clusters passing filter	Make sure the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument. In addition, the QIAseq A Read 1 Primer I (100 µM) Custom Read 1 Sequencing Primer and Custom Multimodal Read 2 Primer (100 µM) <b>must</b> be used when sequencing on any Illumina platform.

## Variant detection issues

Comments and suggestions

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Known variants not detected	Variant detection sensitivity is directly related to the input DNA and read depth. Check Table 32 (page 81), Table 33 (page 84), and Table 34 (page 88) to determine if the required input DNA, UMI numbers, and read depth are met for the specific variant detection application.
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# Reference

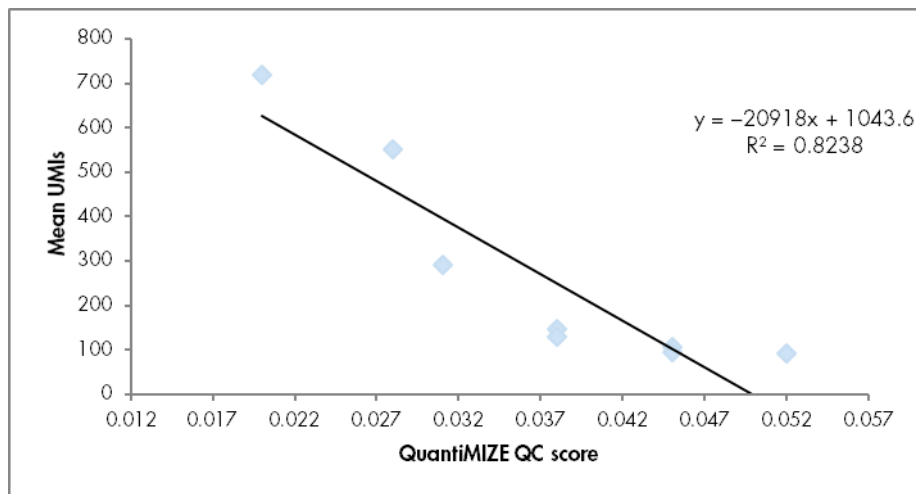
1. Xu, C., Nezami Ranjbar, M.R., Wu, Z., DiCarlo, J., Wang, Y. (2017). Detecting very low allele fraction variants using targeted DNA sequencing and a novel molecular barcode-aware variant caller. BMC Genomics. 18, 5.

## Appendix A: FFPE Sample Quality and Quantity

High-sensitivity Quant-iT™ dsDNA Assay Kit (Thermo Fisher Scientific, cat. no. Q33120) is recommended for quantification of DNA from FFPE total nucleic acid samples. This kit or other methods like Nanodrop® or QIAxpert® can be used for quantification of FFPE DNA (in separate elutes with RNA).

Genomic DNA present in FFPE archives is usually damaged and fragmented to an uncertain extent. 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.

FFPE DNA input can be determined by the following: If FFPE DNA is defined as high quality (quality control [QC] score  $\leq 0.04$ ) 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 6).



**Figure 6. 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 Multimodal Panels system.

# Appendix B: Combined Enrichment Workflow for Targeted Enrichment

## Important points before starting

- The 12.4 µL product from “Protocol: Reverse Transcription” is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- The final library dual sample index is determined by the combination of the QIAseq Multimodal N7 Plate and the QIAseq Multimodal S5 Plate. QIAseq Beads are used for all reaction cleanups.
- To use this protocol, one of the following is required:
  - QIAseq Multimodal Index I (12) (cat. no. 333962): MTIN-12K (shown in Table 26)
  - QIAseq Multimodal HT CW 96 UDI (cat. no. 333979): MTIN-96ABA/K/X (shown in Table 27)

MTIN-12K is a 12-reaction format enabling the indexing of 12 DNA and 12 RNA libraries. There are dried N7 index primers for both DNA and RNA, mixed in the same well. The plates can be cut in columns to enable indexing of the desired number of samples.

MTIN-96ABA/K/X is a 96-reaction format enabling the indexing of 96 DNA and 96 RNA libraries. There are dried N7 index primers for both DNA and RNA, mixed in the same well.

- **Important:** The required combinations of indexes are described in the sequencing sample setup sheets:
  - Sample Sheet Multimodal UDI Set 96: [www.qiagen.com/PROM-20735](http://www.qiagen.com/PROM-20735)



- **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

1. Prepare the reagents required for target enrichment.
  - a. Thaw TEPCR Buffer, 5x; Multimodal DHS Panel (DNA); and Multimodal VHS Panel (RNA); and bring QIAseq Multimodal N7 index plate to room temperature.
  - b. Mix by flicking the tube, and then centrifuge briefly.

**Note:** QIAseq Multimodal N7 Plate only needs to be centrifuged, not mixed.

**Note:** QN Taq 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 25. Briefly centrifuge, mix by pipetting up and down 10–12 times, and then briefly centrifuge again.

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

Table 25. Reaction mix for combined target enrichment

Component	Volume/reaction (µL)
Sample (from "Protocol: Reverse Transcription")	12.4
TEPCR Buffer, 5x	8
Multimodal DHS Panel (DNA)	10
Multimodal VHS Panel (RNA)	8
QN Taq Polymerase	1.6
Total	40

3. Add the 40  $\mu$ L target enrichment reaction mix into a well of a QIAseq Multimodal N7 index plate (Table 26 or Table 27).

**Important:** Put any unused plate(s) back in the foil back and keep in  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for long-term storage.

**Table 26. Layout of QIAseq Multimodal N7 index plate MTIN-12K**

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	DNAp-M001	DNAp-M009										
	<b>S1</b>	<b>S9</b>	–	–	–	–	–	–	–	–	–	–
	RNAp-M049	RNAp-M057										
<b>B</b>	DNAp-M002	DNAp-M010										
	<b>S2</b>	<b>S10</b>	–	–	–	–	–	–	–	–	–	–
	RNAp-M050	RNAp-M058										
<b>C</b>	DNAp-M003	DNAp-M011										
	<b>S3</b>	<b>S11</b>	–	–	–	–	–	–	–	–	–	–
	RNAp-M051	RNAp-M059										
<b>D</b>	DNAp-M004	DNAp-M012										
	<b>S4</b>	<b>S12</b>	–	–	–	–	–	–	–	–	–	–
	RNAp-M052	RNAp-M060										
<b>E</b>	DNAp-M005											
	<b>S5</b>	–	–	–	–	–	–	–	–	–	–	–
	RNAp-M053											
<b>F</b>	DNAp-M006											
	<b>S6</b>	–	–	–	–	–	–	–	–	–	–	–
	RNAp-M054											
<b>G</b>	DNAp-M007											
	<b>S7</b>	–	–	–	–	–	–	–	–	–	–	–
	RNAp-M055											
<b>H</b>	DNAp-M008											
	<b>S8</b>	–	–	–	–	–	–	–	–	–	–	–
	RNAp-M056											

Table 27. Layout of QIAseq Multimodal N7 index plate MTIN-96ABA/K/X

	1	2	3	4	5	6	7	8	9	10	11	12
DNAp-M001	DNAp-M009	DNAp-M017	DNAp-M025	DNAp-M033	DNAp-M041	DNAp-M097	DNAp-M105	DNAp-M113	DNAp-M121	DNAp-M129	DNAp-M137	
<b>A 51</b>	<b>S9</b>	<b>S17</b>	<b>S25</b>	<b>S33</b>	<b>S41</b>	<b>S49</b>	<b>S57</b>	<b>S65</b>	<b>S73</b>	<b>S81</b>	<b>S89</b>	
RNAp-M049	RNAp-M057	RNAp-M065	RNAp-M073	RNAp-M081	RNAp-M089	RNAp-M145	RNAp-M153	RNAp-M161	RNAp-M169	RNAp-M177	RNAp-M185	
DNAp-M002	DNAp-M010	DNAp-M018	DNAp-M026	DNAp-M034	DNAp-M042	DNAp-M098	DNAp-M106	DNAp-M114	DNAp-M122	DNAp-M130	DNAp-M138	
<b>B 52</b>	<b>S10</b>	<b>S18</b>	<b>S26</b>	<b>S34</b>	<b>S42</b>	<b>S50</b>	<b>S58</b>	<b>S66</b>	<b>S74</b>	<b>S82</b>	<b>S90</b>	
RNAp-M050	RNAp-M058	RNAp-M066	RNAp-M074	RNAp-M082	RNAp-M090	RNAp-M146	RNAp-M154	RNAp-M162	RNAp-M170	RNAp-M178	RNAp-M186	
DNAp-M003	DNAp-M011	DNAp-M019	DNAp-M027	DNAp-M035	DNAp-M043	DNAp-M099	DNAp-M107	DNAp-M115	DNAp-M123	DNAp-M131	DNAp-M139	
<b>C 53</b>	<b>S11</b>	<b>S19</b>	<b>S27</b>	<b>S35</b>	<b>S43</b>	<b>S51</b>	<b>S59</b>	<b>S67</b>	<b>S75</b>	<b>S83</b>	<b>S91</b>	
RNAp-M051	RNAp-M059	RNAp-M067	RNAp-M075	RNAp-M083	RNAp-M091	RNAp-M147	RNAp-M155	RNAp-M163	RNAp-M171	RNAp-M179	RNAp-M187	
DNAp-M004	DNAp-M012	DNAp-M020	DNAp-M028	DNAp-M036	DNAp-M044	DNAp-M100	DNAp-M108	DNAp-M116	DNAp-M124	DNAp-M132	DNAp-M140	
<b>D 54</b>	<b>S12</b>	<b>S20</b>	<b>S28</b>	<b>S36</b>	<b>S44</b>	<b>S52</b>	<b>S60</b>	<b>S68</b>	<b>S76</b>	<b>S84</b>	<b>S92</b>	
RNAp-M052	RNAp-M060	RNAp-M068	RNAp-M076	RNAp-M084	RNAp-M092	RNAp-M1148	RNAp-M156	RNAp-M164	RNAp-M172	RNAp-M180	RNAp-M188	
DNAp-M005	DNAp-M013	DNAp-M021	DNAp-M029	DNAp-M037	DNAp-M045	DNAp-M101	DNAp-M109	DNAp-M117	DNAp-M125	DNAp-M133	DNAp-M141	
<b>E 55</b>	<b>S13</b>	<b>S21</b>	<b>S29</b>	<b>S37</b>	<b>S45</b>	<b>S53</b>	<b>S61</b>	<b>S69</b>	<b>S77</b>	<b>S85</b>	<b>S93</b>	
RNAp-M053	RNAp-M061	RNAp-M069	RNAp-M077	RNAp-M085	RNAp-M093	RNAp-M149	RNAp-M157	RNAp-M165	RNAp-M173	RNAp-M181	RNAp-M189	
DNAp-M006	DNAp-M014	RNAp-M022	DNAp-M030	DNAp-M038	DNAp-M046	DNAp-M102	DNAp-M110	DNAp-M118	DNAp-M126	DNAp-M134	DNAp-M142	
<b>F 56</b>	<b>S14</b>	<b>S22</b>	<b>S30</b>	<b>S38</b>	<b>S46</b>	<b>S54</b>	<b>S62</b>	<b>S70</b>	<b>S78</b>	<b>S86</b>	<b>S94</b>	
RNAp-M054	RNAp-M062	RNAp-M070	RNAp-M078	RNAp-M086	RNAp-M094	RNAp-M150	RNAp-M158	RNAp-M166	RNAp-M174	RNAp-M182	RNAp-M190	
DNAp-M007	DNAp-M015	DNAp-M023	DNAp-M031	DNAp-M039	DNAp-M047	DNAp-M103	DNAp-M111	DNAp-M119	DNAp-M127	DNAp-M135	DNAp-M143	
<b>G 57</b>	<b>S15</b>	<b>S23</b>	<b>S31</b>	<b>S39</b>	<b>S47</b>	<b>S55</b>	<b>S63</b>	<b>S71</b>	<b>S79</b>	<b>S87</b>	<b>S95</b>	
RNAp-M055	RNAp-M063	RNAp-M071	RNAp-M079	RNAp-M087	RNAp-M095	RNAp-M151	RNAp-M159	RNAp-M167	RNAp-M175	RNAp-M183	RNAp-M191	
DNAp-M008	DNAp-M016	DNAp-M024	DNAp-M032	DNAp-M040	DNAp-M048	DNAp-M104	DNAp-M112	DNAp-M120	DNAp-M128	DNAp-M136	DNAp-M144	
<b>H 58</b>	<b>S16</b>	<b>S24</b>	<b>S32</b>	<b>S40</b>	<b>S48</b>	<b>S56</b>	<b>S64</b>	<b>S72</b>	<b>S80</b>	<b>S88</b>	<b>S96</b>	
RNAp-M056	RNAp-M064	RNAp-M072	RNAp-M080	RNAp-M088	RNAp-M096	RNAp-M152	RNAp-M160	RNAp-M168	RNAp-M176	RNAp-M184	RNAp-M192	

4. Briefly centrifuge, mix by pipetting up and down 8 times, and then briefly centrifuge again.

**Note:** If only a column is used, cut that column from the cuttable plate and proceed to the next step.

5. Program a thermal cycler using the cycling conditions in Table 28a (DNA+RNA primers <3000) or Table 28b (DNA+RNA primers ≥3000).

Table 28a. Cycling conditions for target enrichment if DNA+RNA primers<3000

Step	Time	Temperature (°C)
Initial denaturation	2 min	98
8 cycles	15 s	98
	10 min	68
Hold	∞	4

**Table 28b. Cycling conditions for target enrichment if number of primers  $\geq 3000$ /tube**

Step	Time	Temperature (°C)
Initial denaturation	2 min	98
8 cycles	15 s	98
	4 min	65
Hold	$\infty$	4

6. Place the target enrichment reaction in the thermal cycler and start the run.
7. Once the run has finished, add 60  $\mu\text{L}$  of Nuclease-free Water to bring each sample to 100  $\mu\text{L}$ .
8. Add 80  $\mu\text{L}$  QIAseq Beads for standard/FFPE sample or 100  $\mu\text{L}$  QIAseq Beads for cfDNA and mix by vortexing or by pipetting up and down several times.
9. Incubate for 5 min at room temperature.
10. Place the tubes/plate on a magnetic rack for 2 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 because they contain the DNA of interest.

**Important:** When working with FFPE or cfDNA/RNA sample, add 100  $\mu\text{L}$  of Nuclease-free Water to resuspend beads, and then, add 100  $\mu\text{L}$  of QIAseq Bead Binding Buffer. Mix by vortexing or pipetting up and down. Repeat steps 10 and 11.

**Note:** For plates, the following may improve performance. After 8 min, remove 100  $\mu\text{L}$  supernatant. Leave it on the magnetic stand for 2 min and remove 90  $\mu\text{L}$  supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10  $\mu\text{L}$  pipette to remove the remaining supernatant.

11. With the beads still on the magnetic stand, add 200  $\mu$ L of 80% ethanol. Rotate the tube 2–3 times or move the plate side-to-side between the 2 column positions of the magnet to wash the beads. Carefully remove and discard the wash.
12. Repeat the ethanol wash.

**Important:** Completely remove all traces of the ethanol after this second wash. Remove the ethanol with a 200  $\mu$ L pipette tip first, spin down briefly, and then use a 10  $\mu$ L pipette tip to remove any residual ethanol.
13. With the beads still on the magnetic stand, air-dry at room temperature for 5–10 min or longer.

**Note:** Visually inspect the pellet to confirm that it is completely dry. Ethanol carryover to the next universal PCR step will affect PCR efficiency.
14. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 25  $\mu$ L Nuclease-free Water. Mix well by pipetting.
15. Return the tube/plate to the magnetic rack until the solution has cleared.
16. Transfer 24  $\mu$ L of the supernatant to clean tubes/plate. This will be used in the next 2 protocols.
17. Proceed to “Protocol: qPCR Determination of Universal PCR Cycles” on page 47. Alternatively, the samples can be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

# Appendix C: 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 29 or Table 30.

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

<b>QIAseq Targeted RNA Booster Panel</b>	<b>(96)</b>
<b>Catalog no.</b>	<b>334635</b>
<b>Number of samples</b>	<b>96</b>
One pool of region-specific primers	80 µL

Table 29. Combining an existing DNA panel (at 50 µL) with a booster panel

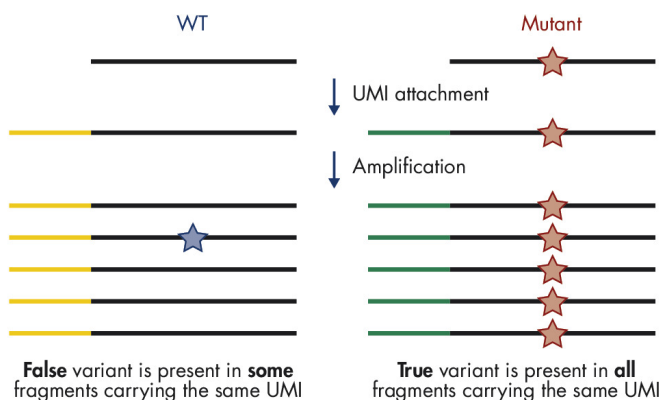
No. of primers in existing panel	Volume of existing panel to combine (µL)	Volume of booster panel to combine (µL)
1–2000	50	5
2001–4000	50	3.75
4001–12,000	50	2.5
12,001–20,000	50	1.25

Table 30. Combining an existing RNA panel (at 40 µL) with a booster panel

No. of primers in existing panel	Volume of existing panel to combine (µL)	Volume of booster panel to combine (µL)
1–2000	40	5
2001–4000	40	3.75
4001–5000	40	2.5

## Appendix D: Principle of Variant Detection with UMIs

The principle of variant detection with UMIs is described in Figure 7. 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 artefactual 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 7. 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 [doi.org/10.1186/s12864-016-3425-4](https://doi.org/10.1186/s12864-016-3425-4) (Xu et al., 2017).

# Appendix E: Nucleic Acid Input Amount and Sequencing Depth

The number of UMIs captured from the original DNA sample correlates with the DNA input amount and sequencing read depth. Adequate sequencing of captured UMIs requires relatively deep sequencing coverage. Table 31 provides guidance on variant detection with fresh DNA amounts at different depths of coverage. Additionally, 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 per UMI) to generate a sufficient amount of UMIs.

**Table 31. Suggested fresh DNA input amount and read depth coverage for variant detection\***

Variant frequency	Input (ng)	Read pairs/UMI	Mean read depth (coverage)
5%	20 <sup>†</sup>	4	7200x
5%	40 <sup>†</sup>	2	3640x
1%	80 <sup>†</sup>	4	25,600x

\* Variant detection is based on 90% sensitivity on the entire region of the QIAseq Multimodal DHS (DNA) Panel.

<sup>†</sup> If performing the procedure in “Appendix B: Combined Enrichment Workflow for Targeted Enrichment”, use half of the input amounts listed.

As RNA expression levels for each transcript varies broadly between different samples, there is no definitive calculation for a required number of reads. Based on previous testing with RNA libraries, allocation of 5000 reads per primer is a reasonable starting point. Table 32a (Separated Targeted DNA and RNA Enrichment) and Table 32b (Combined Targeted DNA+RNA Enrichment) provide recommendations for the number of reads that should be allocated for the prepared QIAseq Multimodal DNA and RNA libraries. As a note, the QIAseq Multimodal Pan Cancer Panel (QHS-5000Z) is designed exclusively for the Separated Targeted DNA and RNA Enrichment workflow.



Table 32a. Read allocation for cataloged QIAseq Multimodal DNA and RNA libraries (Separated Targeted DNA and RNA Enrichment)

Per DNA library	Input	Primer no.	QHS-005Z	QHS-006Z	QHS-009Z	QHS-5000Z*
		DNA RNA	4149 487	6244 1116	11243 665	19995 2571
		Coverage (X)	Reads allocated to each DNA library (M) <sup>†</sup>			
5% VAF	20 ng <sup>‡</sup>	7200	30	45	81	144
5% VAF	40 ng <sup>‡</sup>	3640	15	23	41	73
1% VAF	80 ng <sup>‡</sup>	25,600	106	160	288	512
Reads allocated to each RNA library (M)*						
Per RNA library		5000	2	6	3	13

\* QIAseq Multimodal Pan Cancer Panel (UHS-5000Z) is designed exclusively for the “Separated Targeted DNA and RNA Enrichment” workflow.

<sup>†</sup> Reads allocated to each library (M) = coverage x primer number/10<sup>6</sup>

<sup>‡</sup> Since samples are split before targeted enrichment, the recommended DNA input is doubled, compared to combined targeted enrichment workflow.

Table 32b. Read allocation for cataloged QIAseq Multimodal DNA and RNA libraries (Combined Targeted DNA+RNA Enrichment)

		Primer no. DNA RNA	QHS-005Z 4149 487	QHS-006Z 11243 665	QHS-009Z 6244 1116
Per DNA library	Input	Coverage (X)	Reads allocated to each DNA library (M) <sup>†</sup>		
5% VAF	10 ng	7200	33	86	53
5% VAF	20 ng	3640	17	43	27
1% VAF	40 ng	25,600	119	305	188
Reads allocated to each RNA library (M) <sup>*</sup>					
Per RNA library		5000	23	60	37

<sup>\*</sup> Reads allocated to each library (M) = coverage x primer number/10<sup>6</sup>

# Appendix F: Sample Multiplexing

## Recommendations for Illumina Sequencing Platforms

Sample multiplexing level is determined by the size of the panel, required depth of coverage, and sequencing platform total output. For the Illumina platforms, sample indexes are available to multiplex up to 96 samples, one targeted DNA, and one targeted RNA library per sample, 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 calculated for QIAseq Multimodal DNA and RNA libraries prepared using the Separated Targeted DNA and RNA Enrichment workflow (Table 33c to Table 33f) or the Combined Targeted DNA+RNA Enrichment workflow (Table 34g to Table 34j). Fine-tuning the read depth is possible after the first run. See read allocation and sample multiplexing template for the custom panel (accessible at [www.qiagen.com/PROM-16466](http://www.qiagen.com/PROM-16466)).

Table 33c. Number of multiplexed samples (one DNA and one RNA library for each sample) based on DNA or RNA panel size (Separated targeted DNA and RNA Enrichment workflow) with 500x mean coverage for DNA library and 5000 reads/primer for the RNA library\*

		Primer no.	QHS-005Z	QHS-006Z	QHS-009Z	QHS-5000Z
		DNA	4149	11243	6244	19995
		RNA	487	665	1116	2571
Instrument	Version	Capacity (paired-end reads)				
MiniSeq	Mid output	16M	3	1	1	N/A
MiniSeq	High output	50M	11	5	5	2
MiSeq	v2	30M	6	3	3	1
NextSeq 500/550	Mid output	260M	57	29	29	11
NextSeq 500/550	High output	800M	177	89	91	35
NextSeq 1000/2000	P1	200M	44	22	22	8
NextSeq 1000/2000	P2	800M	177	89	91	35
NextSeq 1000/2000	P3	2400M	531	267	273	105
NovaSeq 6000	SP (per flow cell)	1.6B	354	178	183	70

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

\* Based on 2 x 149 bp paired-end reads.

Table 33d. Number of multiplexed samples (one DNA and one RNA library for each sample) based on DNA or RNA panel size (Separated targeted DNA and RNA Enrichment workflow) with 3640x mean coverage for DNA library and 5000 reads/primer for the RNA library\*

			Primer no.	QHS-005Z	QHS-006Z	QHS-009Z	QHS-5000Z
			DNA	4149	11243	6244	19995
Instrument	Version	Capacity (paired-end reads)	RNA	487	665	1116	2571
MiniSeq	Mid output	16M	N/A	N/A	N/A	N/A	N/A
MiniSeq	High output	50M	2	1	1	N/A	N/A
MiSeq	v2	30M	1	N/A	1	N/A	N/A
NextSeq 500/550	Mid output	260M	14	5	9	3	
NextSeq 500/550	High output	800M	45	18	28	9	
NextSeq 1000/2000	P1	200M	11	4	7	2	
NextSeq 1000/2000	P2	800M	45	18	28	9	
NextSeq 1000/2000	P3	2400M	135	54	84	27	
NovaSeq 6000	SP (per flow cell)	1.6B	91	36	56	18	

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

\* Based on 2 x 149 bp paired-end reads.

**Table 33e. Number of multiplexed samples (one DNA and one RNA library for each sample) based on DNA or RNA panel size (Separated targeted DNA and RNA Enrichment workflow) with 7200x mean coverage for DNA library and 5000 reads/primer for the RNA library\***

			Primer no.	QHS-005Z	QHS-006Z	QHS-009Z	QHS-5000Z
			DNA	4149	11243	6244	19995
Instrument	Version	Capacity (paired-end reads)	RNA	487	665	1116	2571
MiniSeq	Mid output	16M	N/A	N/A	N/A	N/A	N/A
MiniSeq	High output	50M	1	N/A	N/A	N/A	N/A
MiSeq	v2	30M	N/A	N/A	N/A	N/A	N/A
NextSeq 500/550	Mid output	260M	8	3	5	1	
NextSeq 500/550	High output	800M	24	9	15	5	
NextSeq 1000/2000	P1	200M	6	2	3	1	
NextSeq 1000/2000	P2	800M	24	9	15	5	
NextSeq 1000/2000	P3	2400M	72	27	45	15	
NovaSeq 6000	SP (per flow cell)	1.6B	49	18	31	10	

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

\* Based on 2 x 149 bp paired-end reads.

Table 33f. Number of multiplexed samples (one DNA and one RNA library for each sample) based on DNA or RNA panel size (Separated targeted DNA and RNA Enrichment workflow) with 25,600x mean coverage for DNA library and 5000 reads/primer for the RNA library\*

		Primer no. DNA RNA	QHS-005Z 4149 487	QHS-006Z 11243 665	QHS-009Z 6244 1116	QHS-5000Z 19995 2571
Instrument	Version	Capacity (paired-end reads)				
MiniSeq	Mid output	16M	N/A	N/A	N/A	N/A
MiniSeq	High output	50M	N/A	N/A	N/A	N/A
MiSeq	v2	30M	N/A	N/A	N/A	N/A
NextSeq 500/550	Mid output	260M	2	N/A	1	N/A
NextSeq 500/550	High output	800M	7	2	4	1
NextSeq 1000/2000	P1	200M	1	N/A	1	N/A
NextSeq 1000/2000	P2	800M	7	2	4	1
NextSeq 1000/2000	P3	2400M	21	6	12	3
HiSeq 4000	8 lanes per flow cell	10B	92	34	60	19
NovaSeq 6000	SP (per flow cell)	1.6B	14	5	9	3

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

\* Based on 2 x 149 bp paired-end reads.

Table 34g. Number of multiplexed samples (one DNA and one RNA library for each sample) based on a DNA+RNA panel size (Combined targeted DNA+RNA Enrichment workflow) with 500x mean coverage for the DNA library and 5000 reads/primer for the RNA library\*

		Primer no. DNA RNA	QHS-005Z 4149 487	QHS-006Z 11243 665	QHS-009Z 6244 1116
Per DNA library	Input	Coverage (X)	Reads allocated to each DNA library (M) <sup>†</sup>		
MiniSeq	Mid output	16M	N/A	N/A	N/A
MiniSeq	High output	50M	1	N/A	1
MiSeq	v2	30M	1	N/A	N/A
NextSeq 500/550	Mid output	260M	10	3	6
NextSeq 500/550	High output	800M	31	12	19
NextSeq 1000/2000	P1	200M	7	3	4
NextSeq 1000/2000	P2	800M	31	12	19
NextSeq 1000/2000	P3	2400M	93	36	57
HiSeq 4000	8 lanes per flow cell	10B	392	152	247
NovaSeq 6000	SP (per flow cell)	1.6B	62	24	39

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

\* Based on 2 x 149 bp paired-end reads.



**Table 34h. Number of multiplexed samples (one DNA and one RNA library for each sample) based on a DNA+RNA panel size (Combined targeted DNA+RNA Enrichment workflow) with 3640x mean coverage for the DNA library and 5000 reads/primer for the RNA library\***

		Primer no. DNA RNA	QHS-005Z 4149 487	QHS-006Z 11243 665	QHS-009Z 6244 1116
Per DNA library	Input	Coverage (X)	Reads allocated to each DNA library (M) <sup>†</sup>		
MiniSeq	Mid output	16M	N/A	N/A	N/A
MiniSeq	High output	50M	1	N/A	N/A
MiSeq	v2	30M	N/A	N/A	N/A
NextSeq 500/550	Mid output	260M	6	2	4
NextSeq 500/550	High output	800M	19	7	12
NextSeq 1000/2000	P1	200M	4	1	3
NextSeq 1000/2000	P2	800M	19	7	12
NextSeq 1000/2000	P3	2400M	57	21	36
HiSeq 4000	8 lanes per flow cell	10B	249	97	157
NovaSeq 6000	SP (per flow cell)	1.6B	39	15	25

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

\* Based on 2 x 149 bp paired-end reads.

Table 34i. Number of multiplexed samples (one DNA and one RNA library for each sample) based on a DNA+RNA panel size (Combined targeted DNA+RNA Enrichment workflow) with 7200x mean coverage for the DNA library and 5000 reads/primer for the RNA library\*

		Primer no. DNA RNA	QHS-005Z 4149 487	QHS-006Z 11243 665	QHS-009Z 6244 1116
Per DNA library	Input	Coverage (X)	Reads allocated to each DNA library (M) <sup>†</sup>		
MiniSeq	Mid output	16M	N/A	N/A	N/A
MiniSeq	High output	50M	N/A	N/A	N/A
MiSeq	v2	30M	N/A	N/A	N/A
NextSeq 500/550	Mid output	260M	4	1	2
NextSeq 500/550	High output	800M	14	5	8
NextSeq 1000/2000	P1	200M	3	1	2
NextSeq 1000/2000	P2	800M	14	5	8
NextSeq 1000/2000	P3	2400M	42	15	24
HiSeq 4000	8 lanes per flow cell	10B	176	68	111
NovaSeq 6000	SP (per flow cell)	1.6B	28	11	17

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

\* Based on 2 x 149 bp paired-end reads.

**Table 34j. Number of multiplexed samples (one DNA and one RNA library for each sample) based on a DNA+RNA panel size (Combined targeted DNA+RNA Enrichment workflow) with 25,600x mean coverage for the DNA library and 5000 reads/primer for the RNA library\***

		Primer no. DNA RNA	QHS-005Z 4149 487	QHS-006Z 11243 665	QHS-009Z 6244 1116
Per DNA library	Input	Coverage (X)	Reads allocated to each DNA library (M) <sup>†</sup>		
MiniSeq	Mid output	16M	N/A	N/A	N/A
MiniSeq	High output	50M	N/A	N/A	N/A
MiSeq	v2	30M	N/A	N/A	N/A
NextSeq 500/550	Mid output	260M	1	N/A	1
NextSeq 500/550	High output	800M	5	2	3
NextSeq 1000/2000	P1	200M	1	N/A	N/A
NextSeq 1000/2000	P2	800M	5	2	3
NextSeq 1000/2000	P3	2400M	15	6	9
HiSeq 4000	8 lanes per flow cell	10B	70	27	44
NovaSeq 6000	SP (per flow cell)	1.6B	11	4	7

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

\* Based on 2 x 149 bp paired-end reads.

# Appendix G: Legacy Multimodal Index Formats

Legacy QIAseq Multimodal Index kits for the Separated Enrichment Workflow (SW) for Target enrichment are listed below (Set A, Table 35, and Set B, Table 36). The layouts for these plates are listed in Table 37 and Table 38. These index kits can still be used for the Separated Enrichment Workflow, but will be discontinued.

**Note:** Index pair 22, 39, or 139 is not recommended to be used on two-color instruments.

**Table 35. Legacy QIAseq Multimodal Index Set A for the Separated Enrichment Workflow**

<b>QIAseq Multimodal Index I Set A SW</b> <b>(2 identical sets of 48 DNA + 48 RNA sample indexes using the Separated Enrichment Workflow for Targeted Enrichment)</b>	
<b>Catalog no.</b>	<b>(96)</b> <b>333985</b>
<b>Number of samples</b>	<b>96</b>
Multimodal N7 Plate Set A (48): MTIN-96ASWK	2
Each plate allows N7 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried N7 index primers for DNA libraries (columns 1–6) and RNA libraries (columns 7–12). The plates can be cut in columns to enable indexing of the desired number of samples.	
Multimodal S5 Plate Set A (48): MTIS-96AK	2
Each plate allows S5 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried S5 indexes for DNA libraries pre-mixed with universal DNA primer (columns 1–6) and dried S5 indexes for RNA libraries pre-mixed with universal RNA primer (columns 7–12).	
UPCR Buffer, 5x	500 µL x 3
DNA qPCR AMP Set	96 µL
RNA qPCR AMP Set	96 µL
QIAseq A Read 1 Primer I (100 µM)	4 x 24 µL
Multimodal Read 2 Primer (100 µM)	4 x 24 µL
Optical Thin-wall 8-cap Strips	48 strips

Table 36. Legacy QIAseq Multimodal Index Set B for the Separated Enrichment Workflow

<b>QIAseq Multimodal Index I Set B SW</b> <b>(2 identical sets of 48 DNA + 48 RNA sample indexes using the Separated Enrichment Workflow for Targeted Enrichment)</b>	
<b>Catalog no.</b>	<b>(96)</b> <b>33995</b>
<b>Number of samples</b>	<b>96</b>
Multimodal N7 Plate Set B SW (48): MTIN-96BSWK	2
Each plate allows N7 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried N7 index primers for DNA libraries (columns 1–6) and RNA libraries (columns 7–12). The plates can be cut in columns to enable indexing of the desired number of samples.	
Multimodal S5 Plate Set B (48): MTIS-96BK	2
Each plate allows S5 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried S5 indexes for DNA libraries pre-mixed with universal DNA primer (columns 1–6) and dried S5 indexes for RNA libraries pre-mixed with universal RNA primer (columns 7–12).	
UPCR Buffer, 5x	500 µL x 3
DNA qPCR AMP Set	96 µL
RNA qPCR AMP Set	96 µL
QIAseq A Read 1 Primer I (100 µM)	4 x 24 µL
Multimodal Read 2 Primer (100 µM)	4 x 24 µL
Optical Thin-wall 8-cap Strips	48 strips

Table 37a. Layout of MTIN-96ASWK

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNAP-M001	DNAP-M009	DNAP-M017	DNAP-M025	DNAP-M033	DNAP-M041	RNAp-M049	RNAp-M057	RNAp-M065	RNAp-M073	RNAp-M081	RNAp-M089
	S1	S9	S17	S25	S33	S41	S1	S9	S17	S25	S33	S41
B	DNAP-M002	DNAP-M010	DNAP-M018	DNAP-M026	DNAP-M034	DNAP-M042	RNAp-M050	RNAp-M058	RNAp-M066	RNAp-M074	RNAp-M082	RNAp-M090
	S2	S10	S18	S26	S34	S42	S2	S10	S18	S26	S34	S42
C	DNAP-M003	DNAP-M011	DNAP-M019	DNAP-M027	DNAP-M035	DNAP-M043	RNAp-M051	RNAp-M059	RNAp-M067	RNAp-M075	RNAp-M083	RNAp-M091
	S3	S11	S19	S27	S35	S43	S3	S11	S19	S27	S35	S43
D	DNAP-M004	DNAP-M012	DNAP-M020	DNAP-M028	DNAP-M036	DNAP-M044	RNAp-M052	RNAp-M060	RNAp-M068	RNAp-M076	RNAp-M084	RNAp-M092
	S4	S12	S20	S28	S36	S44	S4	S12	S20	S28	S36	S44
E	DNAP-M005	DNAP-M013	DNAP-M021	DNAP-M029	DNAP-M037	DNAP-M045	RNAp-M053	RNAp-M061	RNAp-M069	RNAp-M077	RNAp-M085	RNAp-M093
	S5	S13	S21	S29	S37	S45	S5	S13	S21	S29	S37	S45
F	DNAP-M006	DNAP-M014	DNAP-M022	DNAP-M030	DNAP-M038	DNAP-M046	RNAp-M054	RNAp-M062	RNAp-M070	RNAp-M078	RNAp-M086	RNAp-M094
	S6	S14	S22	S30	S38	S46	S6	S14	S22	S30	S38	S46
G	DNAP-M007	DNAP-M015	DNAP-M023	DNAP-M031	DNAP-M039	DNAP-M047	RNAp-M055	RNAp-M063	RNAp-M071	RNAp-M079	RNAp-M087	RNAp-M095
	S7	S15	S23	S31	S39	S47	S7	S15	S23	S31	S39	S47
H	DNAP-M008	DNAP-M016	DNAP-M024	DNAP-M032	DNAP-M040	DNAP-M048	RNAp-M056	RNAp-M064	RNAp-M072	RNAp-M080	RNAp-M088	RNAp-M096
	S8	S16	S24	S32	S40	S48	S8	S16	S24	S32	S40	S48

Table 37b. Layout of MTIS-96AK

	1	2	3	4	5	6	7	8	9	10	11	12
A	SQDIB01	SQDIB09	SQDIB07	SQDIB05	SQDIB03	SQDIB01	SQDIB09	SQDIB07	SQDIB05	SQDIB03	SQDIB01	SQDIB09
	S1	S9	S17	S25	S33	S41	S1	S9	S17	S25	S33	S41
B	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB02	SQDIB00	SQDIB08	SQDIB06	SQDIB04	SQDIB02	SQDIB00	SQDIB08	SQDIB06	SQDIB04	SQDIB02	SQDIB00
C	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB03	SQDIB01	SQDIB09	SQDIB07	SQDIB05	SQDIB03	SQDIB01	SQDIB09	SQDIB07	SQDIB05	SQDIB03	SQDIB01
D	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB04	SQDIB02	SQDIB00	SQDIB08	SQDIB06	SQDIB04	SQDIB02	SQDIB00	SQDIB08	SQDIB06	SQDIB04	SQDIB02
E	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB05	SQDIB03	SQDIB01	SQDIB09	SQDIB07	SQDIB05	SQDIB03	SQDIB01	SQDIB09	SQDIB07	SQDIB05	SQDIB03
F	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB06	SQDIB04	SQDIB02	SQDIB00	SQDIB08	SQDIB06	SQDIB04	SQDIB02	SQDIB00	SQDIB08	SQDIB06	SQDIB04
G	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB07	SQDIB05	SQDIB03	SQDIB01	SQDIB09	SQDIB07	SQDIB05	SQDIB03	SQDIB01	SQDIB09	SQDIB07	SQDIB05
H	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB08	SQDIB06	SQDIB04	SQDIB02	SQDIB00	SQDIB08	SQDIB06	SQDIB04	SQDIB02	SQDIB00	SQDIB08	SQDIB06

Table 38. Layout of MTIN-96BSWK

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNAP-M097	DNAP-M105	DNAP-M113	DNAP-M121	DNAP-M129	DNAP-M137	RNAp-M145	RNAp-M153	RNAp-M161	RNAp-M169	RNAp-M177	RNAp-M185
	S49	S57	S65	S73	S81	S89	S49	S57	S65	S73	S81	S89
B	DNAP-M098	DNAP-M106	DNAP-M114	DNAP-M122	DNAP-M130	DNAP-M138	RNAp-M146	RNAp-M154	RNAp-M162	RNAp-M170	RNAp-M178	RNAp-M186
	S50	S58	S66	S74	S82	S90	S50	S58	S66	S74	S82	S90
C	DNAP-M099	DNAP-M107	DNAP-M115	DNAP-M123	DNAP-M131	DNAP-M139	RNAp-M147	RNAp-M155	RNAp-M163	RNAp-M171	RNAp-M179	RNAp-M187
	S51	S59	S67	S75	S83	S91	S51	S59	S67	S75	S83	S91
D	DNAP-M100	DNAP-M108	DNAP-M116	DNAP-M124	DNAP-M132	DNAP-M140	RNAp-M148	RNAp-M156	RNAp-M164	RNAp-M172	RNAp-M180	RNAp-M188
	S52	S60	S68	S76	S84	S92	S52	S60	S68	S76	S84	S92
E	DNAP-M101	DNAP-M109	DNAP-M117	DNAP-M125	DNAP-M133	DNAP-M141	RNAp-M149	RNAp-M157	RNAp-M165	RNAp-M173	RNAp-M181	RNAp-M189
	S53	S61	S69	S77	S85	S93	S53	S61	S69	S77	S85	S93
F	DNAP-M102	DNAP-M110	DNAP-M118	DNAP-M126	DNAP-M134	DNAP-M142	RNAp-M150	RNAp-M158	RNAp-M166	RNAp-M174	RNAp-M182	RNAp-M190
	S54	S62	S70	S78	S86	S94	S54	S62	S70	S78	S86	S94
G	DNAP-M103	DNAP-M111	DNAP-M119	DNAP-M127	DNAP-M135	DNAP-M143	RNAp-M151	RNAp-M159	RNAp-M167	RNAp-M175	RNAp-M183	RNAp-M191
	S55	S63	S71	S79	S87	S95	S55	S63	S71	S79	S87	S95
H	DNAP-M104	DNAP-M112	DNAP-M120	DNAP-M128	DNAP-M136	DNAP-M144	RNAp-M152	RNAp-M160	RNAp-M168	RNAp-M176	RNAp-M184	RNAp-M192
	S56	S64	S72	S80	S88	S96	S56	S64	S72	S80	S88	S96

Table 38a. Layout of MTIS-96BK

	1	2	3	4	5	6	7	8	9	10	11	12
A	SQDIB097	SQDIB105	SQDIB113	SQDIB121	SQDIB129	SQDIB137	SQDIB145	SQDIB153	SQDIB161	SQDIB169	SQDIB177	SQDIB185
	S49	S57	S65	S73	S81	S89	S49	S57	S65	S73	S81	S89
B	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB098	SQDIB106	SQDIB114	SQDIB122	SQDIB130	SQDIB138	SQDIB146	SQDIB154	SQDIB162	SQDIB170	SQDIB178	SQDIB186
C	S50	S58	S66	S74	S82	S90	S50	S58	S66	S74	S82	S90
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
D	SQDIB099	SQDIB107	SQDIB115	SQDIB123	SQDIB131	SQDIB139	SQDIB147	SQDIB155	SQDIB163	SQDIB171	SQDIB179	SQDIB187
	S51	S59	S67	S75	S83	S91	S51	S59	S67	S75	S83	S91
E	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB100	SQDIB108	SQDIB116	SQDIB124	SQDIB132	SQDIB140	SQDIB148	SQDIB156	SQDIB164	SQDIB172	SQDIB180	SQDIB188
F	S52	S60	S68	S76	S84	S92	S52	S60	S68	S76	S84	S92
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
G	SQDIB101	SQDIB109	SQDIB117	SQDIB125	SQDIB133	SQDIB141	SQDIB149	SQDIB157	SQDIB165	SQDIB173	SQDIB181	SQDIB189
	S53	S61	S69	S77	S85	S93	S53	S61	S69	S77	S85	S93
H	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB102	SQDIB110	SQDIB118	SQDIB126	SQDIB134	SQDIB142	SQDIB150	SQDIB158	SQDIB166	SQDIB174	SQDIB182	SQDIB190
I	S54	S62	S70	S78	S86	S94	S54	S62	S70	S78	S86	S94
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
J	SQDIB103	SQDIB111	SQDIB119	SQDIB127	SQDIB135	SQDIB143	SQDIB151	SQDIB159	SQDIB167	SQDIB175	SQDIB183	SQDIB191
	S55	S63	S71	S79	S87	S95	S55	S63	S71	S79	S87	S95
K	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SQDIB104	SQDIB112	SQDIB120	SQDIB128	SQDIB136	SQDIB144	SQDIB152	SQDIB160	SQDIB168	SQDIB176	SQDIB184	SQDIB192
L	S56	S64	S72	S80	S88	S96	S56	S64	S72	S80	S88	S96
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr

Legacy QIAseq Multimodal Index kits for the Combined Enrichment Workflow (CW) for Target enrichment are listed in the following tables (Set A, Table 39, and Set B, Table 40). The layouts for these plates are listed in Table 41 and Table 42. These index kits can still be used for the Combined Enrichment Workflow, but will be discontinued.

**Table 39. Legacy QIAseq Multimodal Index Set A for the Combined Enrichment Workflow**

<b>QIAseq Multimodal Index I Set A</b> <b>(2 identical sets of 48 DNA + 48 RNA sample indexes for Illumina platforms)</b> <b>Catalog no.</b> <b>Number of samples</b>		<b>(96)</b> <b>333965</b> <b>96</b>
Multimodal N7 Plate Set A (48): MTIN-96AK		2
Each plate allows N7 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried N7 index primers specific for DNA libraries mixed with N7 index primers specific for RNA libraries. The plates can be cut in columns to enable indexing of the desired number of samples.		
Multimodal S5 Plate Set A (48): MTIS-96AK		2
Each plate allows S5 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried S5 indexes for DNA libraries pre-mixed with universal DNA primer (columns 1–6) and dried S5 indexes for RNA libraries pre-mixed with universal RNA primer (columns 7–12).		
UPCR Buffer, 5x		500 µL x 3
DNA qPCR AMP Set		96 µL
RNA qPCR AMP Set		96 µL
QIAseq A Read 1 Primer I (100 µM)		4 x 24 µL
Multimodal Read 2 Primer (100 µM)		4 x 24 µL
Optical Thin-wall 8-cap Strips		48 strips



Table 40. Legacy QIAseq Multimodal Index Set B for the Combined Enrichment Workflow

<b>QIAseq Multimodal Index I Set B</b> <b>(2 identical sets of 48 DNA + 48 RNA sample indexes for Illumina platforms)</b> <b>Catalog no.</b> <b>Number of samples</b>		<b>(96)</b> <b>333975</b> <b>96</b>
Multimodal N7 Plate Set B (48): MTIN-96BK		2
Each plate allows N7 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried N7 index primers specific for DNA libraries mixed with N7 index primers specific for RNA libraries. The plates can be cut in columns to enable indexing of the desired number of samples.		
Multimodal S5 Plate Set B (48): MTIS-96BK		2
Each plate allows S5 indexing of 48 samples: 48 for DNA and 48 for RNA. Each well in the plate is single use and contains dried S5 indexes for DNA libraries pre-mixed with universal DNA primer (columns 1–6) and dried S5 indexes for RNA libraries pre-mixed with universal RNA primer (columns 7–12).		
UPCR Buffer, 5x		500 µL x 3
DNA qPCR AMP Set		96 µL
RNA qPCR AMP Set		96 µL
QIAseq A Read 1 Primer I (100 µM)		4 x 24 µL
Multimodal Read 2 Primer (100 µM)		4 x 24 µL
Optical Thin-wall 8-cap Strips		48 strips

Table 41a. Layout of MTIN-96AK

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNAp-M001 S1	DNAp-M009 S9	DNAp-M017 S17	DNAp-M025 S25	DNAp-M033 S33	DNAp-M041 S41						
	RNAp-M049	RNAp-M057	RNAp-M065	RNAp-M073	RNAp-M081	RNAp-M089						
B	DNAp-M002 S2	DNAp-M010 S10	DNAp-M018 S18	DNAp-M026 S26	DNAp-M034 S34	DNAp-M042 S42						
	RNAp-M050	RNAp-M058	RNAp-M066	RNAp-M074	RNAp-M082	RNAp-M090						
C	DNAp-M003 S3	DNAp-M011 S11	DNAp-M019 S19	DNAp-M027 S27	DNAp-M035 S35	DNAp-M043 S43						
	RNAp-M051	RNAp-M059	RNAp-M067	RNAp-M075	RNAp-M083	RNAp-M091						
D	DNAp-M004 S4	DNAp-M012 S12	DNAp-M020 S20	DNAp-M028 S28	DNAp-M036 S36	DNAp-M044 S44						
	RNAp-M052	RNAp-M060	RNAp-M068	RNAp-M076	RNAp-M084	RNAp-M092						
E	DNAp-M005 S5	DNAp-M013 S13	DNAp-M021 S21	DNAp-M029 S29	DNAp-M037 S37	DNAp-M045 S45						
	RNAp-M053	RNAp-M061	RNAp-M069	RNAp-M077	RNAp-M085	RNAp-M093						
F	DNAp-M006 S6	DNAp-M014 S14	DNAp-M022 S22	DNAp-M030 S30	DNAp-M038 S38	DNAp-M046 S46						
	RNAp-M054	RNAp-M062	RNAp-M070	RNAp-M078	RNAp-M086	RNAp-M094						
G	DNAp-M007 S7	DNAp-M015 S15	DNAp-M023 S23	DNAp-M031 S31	DNAp-M039 S39	DNAp-M047 S47						
	RNAp-M055	RNAp-M063	RNAp-M071	RNAp-M079	RNAp-M087	RNAp-M095						
H	DNAp-M008 S8	DNAp-M016 S16	DNAp-M024 S24	DNAp-M032 S32	DNAp-M040 S40	DNAp-M048 S48						
	RNAp-M056	RNAp-M064	RNAp-M072	RNAp-M080	RNAp-M088	RNAp-M096						

Table 41b. Layout of MTIS-96AK

	1	2	3	4	5	6	7	8	9	10	11	12
A	SGDIB01 S1	SGDIB09 S9	SGDIB07 S17	SGDIB05 S25	SGDIB03 S33	SGDIB01 S41	SGDIB09 S1	SGDIB07 S9	SGDIB05 S17	SGDIB03 S25	SGDIB01 S33	SGDIB09 S41
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
B	SGDIB02 S2	SGDIB00 S10	SGDIB08 S18	SGDIB06 S26	SGDIB04 S34	SGDIB02 S42	SGDIB00 S2	SGDIB08 S10	SGDIB06 S18	SGDIB04 S26	SGDIB02 S34	SGDIB00 S42
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
C	SGDIB03 S3	SGDIB01 S11	SGDIB09 S19	SGDIB07 S27	SGDIB05 S35	SGDIB03 S43	SGDIB01 S3	SGDIB09 S11	SGDIB07 S19	SGDIB05 S27	SGDIB03 S35	SGDIB01 S43
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
D	SGDIB04 S4	SGDIB02 S12	SGDIB00 S20	SGDIB08 S28	SGDIB06 S36	SGDIB04 S44	SGDIB02 S4	SGDIB00 S12	SGDIB08 S20	SGDIB06 S28	SGDIB04 S36	SGDIB02 S44
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
E	SGDIB05 S5	SGDIB03 S13	SGDIB01 S21	SGDIB09 S29	SGDIB07 S37	SGDIB05 S45	SGDIB03 S5	SGDIB01 S13	SGDIB09 S21	SGDIB07 S29	SGDIB05 S37	SGDIB03 S45
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
F	SGDIB06 S6	SGDIB04 S14	SGDIB02 S22	SGDIB00 S30	SGDIB08 S38	SGDIB06 S46	SGDIB04 S6	SGDIB02 S14	SGDIB00 S22	SGDIB08 S30	SGDIB06 S38	SGDIB04 S46
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
G	SGDIB07 S7	SGDIB05 S15	SGDIB03 S23	SGDIB01 S31	SGDIB09 S39	SGDIB07 S47	SGDIB05 S7	SGDIB03 S15	SGDIB01 S23	SGDIB09 S31	SGDIB07 S39	SGDIB05 S47
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
H	SGDIB08 S8	SGDIB06 S16	SGDIB04 S24	SGDIB02 S32	SGDIB00 S40	SGDIB08 S48	SGDIB06 S8	SGDIB04 S16	SGDIB02 S24	SGDIB00 S32	SGDIB08 S40	SGDIB06 S48
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr

Table 42. Layout of MTIN-96BK

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNAp-M097	DNAp-M105	DNAp-M113	DNAp-M121	DNAp-M129	DNAp-M137						
	S49	S57	S65	S73	S81	S89	—	—	—	—	—	—
B	DNAp-M098	DNAp-M106	DNAp-M114	DNAp-M122	DNAp-M130	DNAp-M138						
	S50	S58	S66	S74	S82	S90	—	—	—	—	—	—
C	DNAp-M099	DNAp-M107	DNAp-M115	DNAp-M123	DNAp-M131	DNAp-M139						
	S51	S59	S67	S75	S83	S91	—	—	—	—	—	—
D	DNAp-M100	DNAp-M108	DNAp-M116	DNAp-M124	DNAp-M132	DNAp-M140						
	S52	S60	S68	S76	S84	S92	—	—	—	—	—	—
E	DNAp-M101	DNAp-M109	DNAp-M117	DNAp-M125	DNAp-M133	DNAp-M141						
	S53	S61	S69	S77	S85	S93	—	—	—	—	—	—
F	DNAp-M102	DNAp-M110	DNAp-M118	DNAp-M126	DNAp-M134	DNAp-M142						
	S54	S62	S70	S78	S86	S94	—	—	—	—	—	—
G	DNAp-M103	DNAp-M111	DNAp-M119	DNAp-M127	DNAp-M135	DNAp-M143						
	S55	S63	S71	S79	S87	S95	—	—	—	—	—	—
H	DNAp-M104	DNAp-M112	DNAp-M120	DNAp-M128	DNAp-M136	DNAp-M144						
	S56	S64	S72	S80	S88	S96	—	—	—	—	—	—
	RNAp-M152	RNAp-M160	RNAp-M168	RNAp-M176	RNAp-M184	RNAp-M192						

Table 42a. Layout of MTIS-96BK

	1	2	3	4	5	6	7	8	9	10	11	12
A	SGDIB097	SGDIB105	SGDIB113	SGDIB121	SGDIB129	SGDIB137	SGDIB145	SGDIB153	SGDIB161	SGDIB169	SGDIB177	SGDIB185
	S49	S57	S65	S73	S81	S89	S49	S57	S65	S73	S81	S89
B	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SGDIB098	SGDIB106	SGDIB114	SGDIB122	SGDIB130	SGDIB138	SGDIB146	SGDIB154	SGDIB162	SGDIB170	SGDIB178	SGDIB186
C	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SGDIB099	SGDIB107	SGDIB115	SGDIB123	SGDIB131	SGDIB139	SGDIB147	SGDIB155	SGDIB163	SGDIB171	SGDIB179	SGDIB187
D	S51	S59	S67	S75	S83	S91	S51	S59	S67	S75	S83	S91
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
E	SGDIB100	SGDIB108	SGDIB116	SGDIB124	SGDIB132	SGDIB140	SGDIB148	SGDIB156	SGDIB164	SGDIB172	SGDIB180	SGDIB188
	S52	S60	S68	S76	S84	S92	S52	S60	S68	S76	S84	S92
F	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SGDIB101	SGDIB109	SGDIB117	SGDIB125	SGDIB133	SGDIB141	SGDIB149	SGDIB157	SGDIB165	SGDIB173	SGDIB181	SGDIB189
G	S53	S61	S69	S77	S85	S93	S53	S61	S69	S77	S85	S93
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
H	SGDIB102	SGDIB110	SGDIB118	SGDIB126	SGDIB134	SGDIB142	SGDIB150	SGDIB158	SGDIB166	SGDIB174	SGDIB182	SGDIB190
	S54	S62	S70	S78	S86	S94	S54	S62	S70	S78	S86	S94
I	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
	SGDIB103	SGDIB111	SGDIB119	SGDIB127	SGDIB135	SGDIB143	SGDIB151	SGDIB159	SGDIB167	SGDIB175	SGDIB183	SGDIB191
J	S55	S63	S71	S79	S87	S95	S55	S63	S71	S79	S87	S95
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr
K	SGDIB104	SGDIB112	SGDIB120	SGDIB128	SGDIB136	SGDIB144	SGDIB152	SGDIB160	SGDIB168	SGDIB176	SGDIB184	SGDIB192
	S56	S64	S72	S80	S88	S96	S56	S64	S72	S80	S88	S96
	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uDNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr	uRNA Pr

# Ordering Information

Product	Contents	Cat. no.
QIAseq Multimodal Panel HT (12)*	Kit containing all reagents (except indexes) for multimodal (DNA and RNA) sequencing; fixed small panel for a total of 12 samples: 12 DNA and 12 RNA libraries	334932
QIAseq Multimodal Panel HT (96)*	Kit containing all reagents (except indexes) for multimodal (DNA and RNA) sequencing; fixed small panel for a total of 96 samples: 96 DNA and 96 RNA libraries	334935
QIAseq Multimodal HC Panel HT (12)*	Kit containing all reagents (except indexes) for multimodal (DNA and RNA) sequencing; fixed high content (HC) panel for a total of 12 samples: 12 DNA and 12 RNA libraries	334942
QIAseq Multimodal HC Panel HT (96)*	Kit containing all reagents (except indexes) for multimodal (DNA and RNA) sequencing; fixed high content (HC) panel for a total of 96 samples: 96 DNA and 96 RNA libraries	334945
QIAseq Multimodal Custom Panel HT (96)*	Kit containing all reagents (except indexes) for multimodal (DNA and RNA) sequencing; custom panel for a total of 96 samples: 96 DNA and 96 RNA libraries	334955
QIAseq Multimodal Index I SW (12)*	Box containing oligos, enough to process a total of 12 samples, for indexing up to a total of 12 samples (12 for DNA and 12 for RNA libraries) for multimodal panel sequencing on Illumina platforms <b>Note:</b> For Separated Enrichment Workflow (SW) for Targeted Enrichment	333982
QIAseq Multimodal HT SW 96 UDI*	Box containing oligos, enough to process a total of 96 samples, for indexing up to a total of 96 samples (96 for DNA and 96 for RNA) for multimodal panel sequencing on Illumina platforms <b>Note:</b> For Separated Enrichment Workflow (SW) for Targeted Enrichment	333986
QIAseq Multimodal Index I (12)*	Box containing oligos, enough to process a total of 12 samples, for indexing up to a total of 12 samples (12 for DNA and 12 for RNA libraries) for multimodal panel sequencing on Illumina platforms <b>Note:</b> For Combined Enrichment Workflow (CW) for Targeted Enrichment	333962

Product	Contents	Cat. no.
QIAseq Multimodal HT CW 96 UDI*	Box containing oligos, enough to process a total of 96 samples, for indexing up to a total of 96 samples (96 for DNA and 96 for RNA) for multimodal panel sequencing on Illumina platforms  <b>Note:</b> For Combined Enrichment Workflow (CW) for Targeted Enrichment	333979
<b>Related products</b>		
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
AllPrep DNA/RNA Mini Kit (50)	For 50 minipreps: AllPrep DNA Spin Columns, RNeasy Mini Spin Columns, collection tubes, RNase-free water, and buffers	80204
AllPrep DNA/RNA FFPE Kit (50)	50 RNeasy MinElute Spin Columns, 50 QIAamp MinElute Spin Columns, collection tubes, RNase-free reagents, and buffers	80234
PAXgene Blood DNA Kit (25)	Processing tubes and buffers for 25 preparations	761133
PAXgene Blood RNA Kit (50)	50 PAXgene Spin Columns, 50 PAXgene Shredder Spin Columns, processing tubes, RNase-Free DNase I, RNase-free reagents and buffers	Inquire
GeneRead® DNA FFPE Kit (50)	QIAamp MinElute columns, proteinase K, UNG, collection tubes (2 mL), buffers, deparaffinization solution, RNase A	180134

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# Document Revision History

Date	Description
04/2022	Initial release
07/2025	Added the QIAseq Multimodal Panel HT in the Kit Contents section. Changed “Separated Workflow” and “Combined Workflow” to “Separated Enrichment Workflow” and “Combined Enrichment Workflow”, respectively, throughout the handbook. Changed all occurrences of “QIAseq Multimodal Panels” to “QIAseq Multimodal Panels HT”. Added Appendix A: FFPE Sample Quality and Quantity. Moved Appendix B: Combined Enrichment Workflow for Targeted Enrichment to the Appendix. Updated the following sections: Shipping and Storage, Introduction, Important Notes, Equipment and Reagents to Be Supplied by User, Protocol: RNA Polyadenylation, Protocol: Separated Enrichment Workflow (SW) for Targeted Enrichment, Protocol: qPCR Determination of Universal PCR Cycles, Protocol: Universal PCR, Protocol: Sequencing Setup on Illumina MiSeq and NextSeq 550, and Appendices.

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