April 2022

QIAseq® Multimodal Panel HT Handbook

Consolidated targeted next-generation sequencing of DNA and RNA

Sample to Insight

QIAGEN

Contents

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
QN Taq Polymerase	60 µl	60 µl	450 µl	450 µl	450 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 3	10.2 ml x 3	10.2 ml x 3

Indexing for Separated Workflow (SW) for Targeted Enrichment

Note: Follow "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35.

QIAseq Multimodal Index I SW (12) **(DNA and RNA indexing for 12 samples using the Separated Workflow for Targeted Enrichment)** **Catalog no.** **Number of samples**	**(12)** **333982** **12**
Multimodal N7 Plate SW (12): MTIN-12SWK 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 in columns to enable indexing of the desired number of samples.	1
Multimodal S5 Plate (12): MTIS-12K 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 in columns to enable indexing of the desired number of samples.	1
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

QIAseq Multimodal HT SW 96 UDI* (DNA and RNA indexing for 96 samples using the Separated Workflow for Targeted Enrichment)	**(96)**
Catalog no.	**333986 MTSW-96A MTSW-96X MTSW-96K**
Number of samples	**96**
Multimodal N7 Plate SW (96) for DNA: MTIN-96DNA/K/X 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
Multimodal N7 Plate SW (96) for RNA: MTIN-96RNA/K/X 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
Multimodal S5 Plate (96) for DNA: MTIS-96DNA/K/X 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
Multimodal S5 Plate (96) for RNA: MTIS-96RNA/K/X 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 Workflow (CW) for Targeted Enrichment

Note: Follow "Appendix B: Appendix B: Combined Workflow for Targeted Enrichment", page 60.

QIAseq Multimodal Index I (12) **(DNA and RNA indexing for 12 samples using the Combined Workflow for Targeted Enrichment)**	**(12)**
Catalog no.	**333962**
Number of samples	**12**
Multimodal N7 Plate (12): MTIN-12K 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 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.	1
Multimodal S5 Plate (12): MTIS-12K 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 in columns to enable indexing of the desired number of samples.	1
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

QIAseq Multimodal HT CW 96 UDI* (DNA and RNA indexing for 96 samples using the Separated Workflow for Targeted Enrichment)	**(96)**
Catalog no.	**333979 MTCW-96A MTCW-96X MTCW-96K**
Number of samples	**96**
Multimodal N7 Plate (96): MTIN-96ABA/K/X 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
Multimodal S5 Plate (96) for DNA: MTIS-96DNA/K/X 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
Multimodal S5 Plate (96) for RNA: MTIS-96RNA/K/X 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.

Storage

QIAseq Multimodal Panels (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 to –15°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°C.

QIAseq Multimodal Index kits are shipped on dry ice and should be stored at –30 to –15°C upon arrival.

Intended Use

QIAseq Multimodal Panels 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** 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 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, 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 2 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 have been developed. QIAseq Multimodal Panels 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 indices (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 use a targeted approach to sequencing by enriching specific genomic or transcriptomic regions. This enhances DNA and RNA NGS by enabling users to sequence specific regions of interest, which in turn effectively increases sequencing depth and sample throughput while minimizing cost. More importantly, QIAseq Multimodal Panels 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 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. 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. 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 enable the simultaneous enrichment and library prep of DNA+RNA, with up to 40,000 primers per panel (20,000 DNA + 20,000 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).

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.

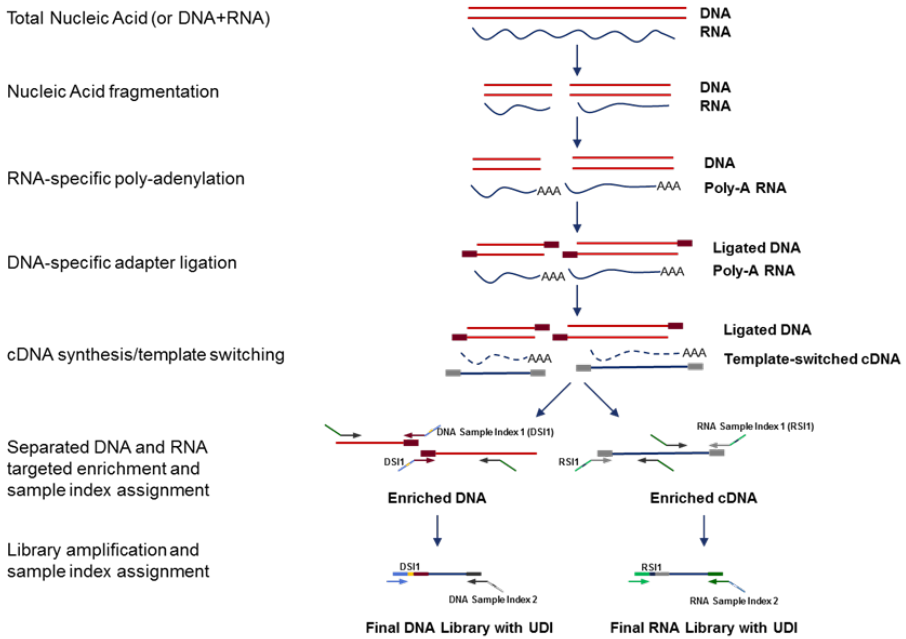


Figure 2. QIAseq Multimodal Panels workflow, using the Separated Workflow (SW) for Targeted Enrichment. Alternatively, the Combined Workflow (CW) for Targeted Enrichment can be performed using "Appendix B: Combined Workflow for Targeted Enrichment".

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 Workflow (SW) for Targeted Enrichment":

- "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35.
- "Appendix B: Combined Workflow (CW) for Targeted Enrichment", page 60.

IMPORTANT: Table 1 outlines important points to consider when choosing between the 2 protocols.

Table 1. Target enrichment options

Choose …	For …	Notes
"Protocol: Separated Workflow (SW) for Targeted Enrichment" **Note:** Samples are split before targeted enrichment, so the input DNA recommendations would be doubled, compared to Combined Workflow (CW) for Targeted Enrichment.	**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 1,000 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 1,000 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 ≥ 12,000
	QIAseq Multimodal Panel UHS-5000Z	QIAseq Multimodal Pan Cancer Panel (UHS-5000Z) is designed exclusively for the "Separated Targeted DNA and RNA Enrichment in Separate Tubes" workflow
"Appendix B: Combined 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 1,000 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 a single primer extension (SPE) approach. 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/QIAseqMultimodalPanels**.

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/QIAseqMultimodalPanels**.

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

Next-generation sequencing

QIAseq Multimodal Panels are compatible with Illumina NGS platforms including MiniSeq®, MiSeq®, NextSeq® 500/550, NextSeq 1000/2000, HiSeq® 2500, HiSeq 3000/4000, and NovaSeq™ 6000. The QIAseq Multimodal Panels cannot be used on Illumina's iSeq 100 platform. When using Illumina 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.

Data analysis

Data from QIAseq Multimodal Panels can be analyzed using the QIAGEN CLC Genomics Workbench, which allows you to optimize analysis parameters for your specific panels. The parameters can then be locked for routine use. All detected variants can be further interpreted using QCI-I for QIAseq.

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**.

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 Table 2 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 Blood RNA Kit (cat. no. 762174).

Note: If samples must be harvested from biological samples for which kits are not available, please contact QIAGEN Technical Services (**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, the QIAseq DNA QuantiMIZE kits (cat. no. 333404 or 333414) are strongly recommended for determining the quality of each FFPE sample. Appendix A 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 and the QIAseq Multimodal Index kit, the following are required:

- EvaGreen®, 20x, in water (Biotium, cat. no. 31000-T or 31000)
- 80% ethanol (made fresh daily) *
- Nuclease-free pipette tips and tubes
- 1.5 ml LoBind® tubes (Eppendorf, cat. no. 022431021)
- PCR tubes (0.2 ml individual tubes [VWR, cat. no. 20170-012] or tube strips [VWR, cat. no. 93001-118]) 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)
- Agilent High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626)

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

Protocol: Nucleic Acid Fragmentation, Standard Samples

Important points before starting

- This protocol describes fragmentation of nucleic acids from "standard samples" (i.e., cells or tissue). For fragmentation of FFPE samples, please refer to "Protocol: Nucleic Acid Fragmentation, FFPE Samples", page 24.
- 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 Workflow (SW) for Targeted Enrichment", the recommended amount of DNA is 20–80 ng.
- When performing "Appendix B: Combined Workflow (CW) for Targeted Enrichment", the recommended amount of DNA is 10–40 ng.
- The recommended amount of RNA is 10 ng to 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.
   2a. Thaw Fragmentation Buffer, 10x, and FERA Solution at room temperature (15–25°C).
   2b. Mix by flicking the tube, and then centrifuge briefly.

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

3. On ice, prepare the fragmentation 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 "Important points before starting" section)*	Variable A
RNA (see input recommendation in the "Important points before starting" 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)
Total	**20 µl**

***** 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 "Important points before starting" section)*	Variable A	Variable A
RNA (see input recommendation in the "Important points before starting" 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
Total	**20 µl**	**20 µl**

***** 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	Incubation temperature	Incubation time for standard sample	Incubation time for cfDNA
1	4°C	1 min	1 min
2	32°C	24 min	14 min
3	72°C	30 min	30 min
4	4°C	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", page 27.

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" (i.e., cells or tissue), please refer to "Protocol: Nucleic Acid Fragmentation, Standard Samples", page 21.
- 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", page 58). 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.
   2a. Thaw Fragmentation Buffer, 10x, and FERA Solution at room temperature.
   2b. Mix by flicking the tube, and centrifuge briefly.

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

3. On ice, prepare the fragmentation 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 "Important points before starting" section)*	Variable A
RNA (see input recommendation in the "Important points before starting" 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)
Total	**16 µl**

***** 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 µl 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	Incubation temperature	Incubation time
1	4°C	1 min
2	32°C	14 min
3	72°C	30 min
4	4°C	Hold

7. 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.

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°C.
10. Place the samples on ice, and immediately proceed to “Protocol: RNA Polyadenylation”, page 27.

Protocol: RNA Polyadenylation

Important points before starting

- The product from "Protocol: Nucleic Acid Fragmentation, Standard Samples", page 21, or "Protocol: Nucleic Acid Fragmentation, FFPE Samples", page 24, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure

1. Prepare the reagents required for the polyadenylation.
   1a. Thaw PAP Dilution Buffer, 10x, and ATP Solution on ice.
   1b. Mix by flicking the tube, and then centrifuge briefly.
   Note: T4 Polynucleotide Kinase and PAP Enzyme should be removed from the freezer just before use and placed on ice. After use, immediately return the enzymes to the freezer.
2. Prepare 1x PAP Dilution Buffer by diluting 2 µl of the 10x PAP Dilution Buffer with 18 µl Nuclease-free Water.
3. Use the 1x PAP Dilution Buffer to dilute an aliquot of the PAP Enzyme from 5 U/µl to 2 U/µl. 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
Fragmentation reaction (already in tube)	20 µl
ATP Solution	1.25 µl
T4 Polynucleotide Kinase	1 µl
Diluted PAP Enzyme (2 U/µl)*	1 µl
Nuclease-free Water	1.75 µl
Total	**25 µl**

* Ensure PAP Enzyme has been diluted from its stock 5U/µl concentration to 2U/µl using 1x PAP Dilution Buffer.

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	Incubation temperature	Incubation time
1	4°C	1 min
2	30°C	10 min
3	4°C	Hold

6. Upon completion, place the reactions on ice and proceed to "Protocol: DNA Ligation", page 29.

Protocol: DNA Ligation

Important points before starting

- The product from "Protocol: RNA Polyadenylation", page 27, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- **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 the DNA ligation.

   1a. Thaw DNA Ligation Adapter; UPH Ligation Buffer, 2.5x, at room temperature.

   1b. Mix by flicking the tube, and then centrifuge briefly.

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

2. Prepare the 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
RNA polyadenylation reaction (already in tube)	25 µl
UPH Ligation Buffer, 2.5x	24 µl
DNA Ligation Adapter	3.36 µl
DNA Ligase	6 µl
Nuclease-free Water	1.64 µl
Total	**60 µl**

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

4. Add 40 µl of Nuclease-free Water to bring each sample to 100 µl.
5. Add 130 µl QIAseq Beads, and then mix by vortexing.
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).

   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.

   Tip: For plates, the following may improve performance. After 8 min, remove 130 µl supernatant. Leave it on the magnetic stand for 2 min and remove 90 µl supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1min, and use a 10 µl pipette to remove the remaining supernatant.

8. Add 80 µl of Nuclease-free Water to resuspend the beads and then 128 µl 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.
   Tip: For plates, the following may improve performance. After 8 min, remove 108µl supernatant. Leave it on the magnetic stand for 2 min and remove 90 µl supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1min, and use a 10 µl pipette to remove the remaining supernatant.
10. With the beads still on the magnetic stand, add 200 µl 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.
11. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µl pipette tip first, spin down briefly and then use a 10 µl 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 µl Nuclease-free Water. Mix well by pipetting.
14. Return the tube/plate to the magnetic rack until the solution has cleared.
15. Transfer 16.62 µl of the supernatant to clean tubes/plate.

    Proceed to “Protocol: Reverse Transcription”, page 32. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Reverse Transcription

Important points before starting

- The 16.62 µl product from "Protocol: DNA Ligation", page 29, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- **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 the reverse transcription.
   1a. Thaw the Multimodal RT Primer; Multimodal RT Buffer, 5x; and Multimodal RT Enhancer at room temperature.
   1b. Mix by flicking the tube, and then centrifuge briefly.

   Note: The RNase Inhibitor and EZ Reverse Transcriptase should be removed from the freezer just before use and placed on ice. 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
Sample (from "Protocol: DNA Ligation", page 29)	16.62 µl
Multimodal RT Primer	1 µl
Multimodal RT Buffer, 5x	5 µl
Multimodal RT Enhancer	0.5 µl
RNase Inhibitor	0.63 µl
EZ Reverse Transcriptase	1.25 µl
Total	**25 µl**

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	Incubation temperature	Incubation time
1	4°C	1 min
2	25°C	10 min
3	42°C	45 min
4	70°C	15 min
5	4°C	Hold

4. Add 75 µl of Nuclease-free Water to bring each sample to 100 µl.
5. Add 130 µl 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.

Tip: For plates, the following may improve performance. After 8 min, remove 130 µl supernatant. Leave it on the magnetic stand for 2 min and remove 90 µl supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10 µl pipette to remove the remaining supernatant.

8. With the beads still on the magnetic stand, add 200 µl 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 µl pipette tip first, spin down briefly, and then use a 10 µl 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 µl Nuclease-free Water.

    Important: If performing the Combined Workflow (CW) for Targeted Enrichment (Appendix B, page 60), elute by adding 15 µl Nuclease-free Water.
12. Return the tube/plate to the magnetic rack until solution the solution has cleared.
13. Transfer 10.2 µl of the eluate to each of 2 clean tubes/plate wells per sample.

    Important: If performing the Combined Workflow (CW) for Targeted Enrichment (Appendix B, page 60), transfer 12.4 µl of the eluate to clean tubes/plate wells and proceed to the Appendix B protocol.
14. Proceed to "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Separated Workflow (SW) for Targeted Enrichment

Important points before starting

- Two 10.2 µl aliquots of the product from "Protocol: Reverse Transcription", page 32, 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.

  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**
- **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.
- **Important**: 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 15)

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

   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 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	Separate RNA
Sample (from "Protocol: Reverse Transcription", page 32)	10.2 µl	10.2 µl
TEPCR Buffer, 5x	4 µl	4 µl
Multimodal DHS Panel (DNA)	5 µl	0 µl
Multimodal VHS Panel (RNA)	0 µl	4 µl
QN Taq Polymerase	0.8 µl	0.8 µl
Nuclease-free Water	0 µl	1 µl
Total	**20 µl**	**20 µl**

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

Important: Put any unused plate(s) back in the foil back and keep in –30 to –15°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 15), 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
A	DNAp-M001 S1	DNAp-M009 S9	Empty	Empty	Empty	Empty	RNAp-M049 S1	RNAp-M057 S9	Empty	Empty	Empty	Empty
B	DNAp-M002 S2	DNAp-M010 S10	Empty	Empty	Empty	Empty	RNAp-M050 S2	RNAp-M058 S10	Empty	Empty	Empty	Empty
C	DNAp-M003 S3	DNAp-M011 S11	Empty	Empty	Empty	Empty	RNAp-M051 S3	RNAp-M059 S11	Empty	Empty	Empty	Empty
D	DNAp-M004 S4	DNAp-M012 S12	Empty	Empty	Empty	Empty	RNAp-M052 S4	RNAp-M060 S12	Empty	Empty	Empty	Empty
E	DNAp-M005 S5	Empty	Empty	Empty	Empty	Empty	RNAp-M053 S5	Empty	Empty	Empty	Empty	Empty
F	DNAp-M006 S6	Empty	Empty	Empty	Empty	Empty	RNAp-M054 S6	Empty	Empty	Empty	Empty	Empty
G	DNAp-M007 S7	Empty	Empty	Empty	Empty	Empty	RNAp-M055 S7	Empty	Empty	Empty	Empty	Empty
H	DNAp-M008 S8	Empty	Empty	Empty	Empty	Empty	RNAp-M056 S8	Empty	Empty	Empty	Empty	Empty

Table 15. Layout of QIAseq Multimodal N7 index plates (a) MTIN-96DNA/K/X and (b) MTIN-96RNA/K/X

(a)

	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**	DNAp-M097 **S49**	DNAp-M105 **S57**	DNAp-M113 **S65**	DNAp-M121 **S73**	DNAp-M129 **S81**	DNAp-M137 **S89**
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**
C	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**
D	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**
E	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**
F	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**
G	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**
H	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**

(b)

	1	2	3	4	5	6	7	8	9	10	11	12
A	RNAp-M049 **S1**	RNAp-M057 **S9**	RNAp-M065 **S17**	RNAp-M073 **S25**	RNAp-M081 **S33**	RNAp-M089 **S41**	RNAp-M0145 **S49**	RNAp-M153 **S57**	RNAp-M161 **S65**	RNAp-M169 **S73**	RNAp-M177 **S81**	RNAp-M185 **S89**
B	RNAp-M050 **S2**	RNAp-M058 **S10**	RNAp-M066 **S18**	RNAp-M074 **S26**	RNAp-M082 **S34**	RNAp-M090 **S42**	RNAp-M146 **S50**	RNAp-M154 **S58**	RNAp-M162 **S66**	RNAp-M170 **S74**	RNAp-M178 **S82**	RNAp-M186 **S90**
C	RNAp-M051 **S3**	RNAp-M059 **S11**	RNAp-M067 **S19**	RNAp-M075 **S27**	RNAp-M083 **S35**	RNAp-M091 **S43**	RNAp-M147 **S51**	RNAp-M155 **S59**	RNAp-M163 **S67**	RNAp-M171 **S75**	RNAp-M179 **S83**	RNAp-M187 **S91**
D	RNAp-M052 **S4**	RNAp-M060 **S12**	RNAp-M068 **S20**	RNAp-M076 **S28**	RNAp-M084 **S36**	RNAp-M092 **S44**	RNAp-M148 **S52**	RNAp-M156 **S60**	RNAp-M164 **S68**	RNAp-M172 **S76**	RNAp-M180 **S84**	RNAp-M188 **S92**
E	RNAp-M053 **S5**	RNAp-M061 **S13**	RNAp-M069 **S21**	RNAp-M077 **S29**	RNAp-M085 **S37**	RNAp-M093 **S45**	RNAp-M149 **S53**	RNAp-M157 **S61**	RNAp-M165 **S69**	RNAp-M173 **S77**	RNAp-M181 **S85**	RNAp-M189 **S93**
F	RNAp-M054 **S6**	RNAp-M062 **S14**	RNAp-M070 **S22**	RNAp-M078 **S30**	RNAp-M086 **S38**	RNAp-M094 **S46**	RNAp-M150 **S54**	RNAp-M158 **S62**	RNAp-M166 **S70**	RNAp-M174 **S78**	RNAp-M182 **S86**	RNAp-M190 **S94**
G	RNAp-M055 **S7**	RNAp-M063 **S15**	RNAp-M071 **S23**	RNAp-M077 **S31**	RNAp-M087 **S39**	RNAp-M095 **S47**	RNAp-M151 **S55**	RNAp-M159 **S63**	RNAp-M167 **S71**	RNAp-M175 **S79**	RNAp-M183 **S87**	RNAp-M191 **S95**
H	RNAp-M056 **S8**	RNAp-M064 **S16**	RNAp-M072 **S24**	RNAp-M080 **S32**	RNAp-M088 **S40**	RNAp-M096 **S48**	RNAp-M152 **S56**	RNAp-M160 **S64**	RNAp-M168 **S72**	RNAp-M176 **S80**	RNAp-M184 **S88**	RNAp-M192 **S96**

3. 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.

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

Table 16a. Cycling conditions for target enrichment if the number of primers is <3000

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

Table 16b. Cycling conditions for target enrichment if the number of primers is ≥3000/tube

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

5. Place the target enrichment reaction in the thermal cycler and start the run.
6. Once the run has finished, add 80 µl of Nuclease-free Water to bring each sample to 100 µl.
7. Add 80 µl QIAseq Beads for standard/FFPE sample or 100 µl QIAseq Beads for cfDNA sample and mix by vortexing or by pipetting up and down several times.
8. 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).

   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: For FFPE samples or cfDNA/RNA, the following may improve performance. Add 65 µl of Nuclease-free Water to resuspend beads, and then add 65 µl of QIAseq Bead Binding Buffer. Mix by vortexing or pipetting up and down. Repeat steps 8 and 9.

Tip: For plates, the following may improve performance. After 8 min, remove 100 µl 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 µl pipette to remove the residues.

10. With the beads still on the magnetic stand, add 200 µl 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.
11. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol after this second wash. Remove the ethanol with a 200 µl pipette first, spin down briefly, and then use a 10 µl pipette 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. Ethanol carryover to the next universal PCR step will affect PCR efficiency.
13. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 14 µl Nuclease-free Water. Mix well by pipetting.
14. Return the tube/plate to the magnetic rack until the solution has cleared.
15. Transfer 12 µl of the supernatant to clean tubes/plate.
16. Proceed to "Protocol: qPCR Determination of Universal PCR Cycles", page 41. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: qPCR Determination of Universal PCR Cycles

Important points before starting

- 2 µl of the product from "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35, or "Appendix B: Combined Workflow (CW) for Targeted Enrichment", page 60, is the starting material for each of the reaction mixes.
- **Important**: EvaGreen, 20x in water, is required for this procedure and must be purchased from Biotium (cat. nos. 31000-T, 31000)
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure

1. Prepare the reagents required for the qPCR.
   1a. Thaw UPCR Buffer, 5x; DNA qPCR AMP Set; and RNA qPCR AMP. Set at room temperature.
   1b. Mix by flicking the tube, and then centrifuge briefly.
   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 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
DNA Sample [from "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35] or Sample [from "Appendix B: Combined Workflow (CW) for Targeted Enrichment", page 60]	2 µl
UPCR Buffer, 5x	2 µl
Nuclease-free Water	4.1 µl
DNA qPCR AMP Set	1 µl
QN Taq Polymerase	0.4 µl
EvaGreen, 20x in water*	0.5 µl
Total	**10 µl**

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

Table 18. Reaction mix for qPCR of RNA library

Component	Volume/reaction
RNA Sample [from "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35] or Sample [from "Appendix B: Combined Workflow (CW) for Targeted Enrichment", page 60]	2 µl
UPCR Buffer, 5x	2 µl
Nuclease-free Water	4.1 µl
RNA qPCR AMP Set	1 µl
QN Taq Polymerase	0.4 µl
EvaGreen, 20x in water*	0.5 µl
Total	**10 µl**

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

3. Program a qPCR instrument using the cycling conditions in Table 19.

   Note: No melting curve is required.

Table 19. Reaction mix for qPCR of RNA library

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

* 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.

   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.

5. Once the amplification cycles for universal PCR have been determined, proceed to "Protocol: Universal PCR", page 44.

Protocol: Universal PCR

Important points before starting

- 9 ul of the product from "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35, or "Appendix B: Combined Workflow (CW) for Targeted Enrichment, page 60, is the starting material for each of the reaction mixes.
- The number of cycles required for amplification are determined in "Protocol: qPCR Determination of Universal PCR Cycles", page 39.
- 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 23)
  - 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 23)
  - **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.

- **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**
- **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 universal PCR.
   1a. Thaw UPCR Buffer, 5x, and bring the QIAseq Multimodal S5 Plate to room temperature.
   1b. Mix by flicking the tube, and then centrifuge briefly.
   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 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
DNA Sample [from "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35] or Sample [from "Appendix B: Combined Workflow (CW) for Targeted Enrichment", page 60]	9 µl
UPCR Buffer, 5x	5 µl
Nuclease-free Water	10 µl
QN Taq Polymerase	1 µl
Total	**25 µl**

Table 21. Reaction mix for universal PCR of RNA library

Component	Volume/reaction
RNA Sample [from "Protocol: Separated Workflow (SW) for Targeted Enrichment", page 35] or Sample [from "Appendix B: Combined Workflow (CW) for Targeted Enrichment", page 60]	9 µl
UPCR Buffer, 5x	5 µl
Nuclease-free Water	10 µl
QN Taq Polymerase	1 µl
Total	**25 µl**

3. To the QIAseq Multimodal S5 index plate (Table 22 or Table 23) 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 23), 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
A	SQDIB001 **S1** uDNA Pr	SQDIB009 **S9** uDNA Pr	Empty	Empty	Empty	Empty	SQDIB049 **S1** uRNA Pr	SQDIB057 **S9** uRNA Pr	Empty	Empty	Empty	Empty
B	SQDIB002 **S2** uDNA Pr	SQDIB010 **S10** uDNA Pr	Empty	Empty	Empty	Empty	SQDIB050 **S2** uRNA Pr	SQDIB058 **S10** uRNA Pr	Empty	Empty	Empty	Empty
C	SQDIB003 **S3** uDNA Pr	SQDIB011 **S11** uDNA Pr	Empty	Empty	Empty	Empty	SQDIB051 **S3** uRNA Pr	SQDIB059 **S11** uRNA Pr	Empty	Empty	Empty	Empty
D	SQDIB004 **S4** uDNA Pr	SQDIB012 **S12** uDNA Pr	Empty	Empty	Empty	Empty	SQDIB052 **S4** uRNA Pr	SQDIB060 **S12** uRNA Pr	Empty	Empty	Empty	Empty
E	SQDIB005 **S5** uDNA Pr	Empty	Empty	Empty	Empty	Empty	SQDIB053 **S5** uRNA Pr	Empty	Empty	Empty	Empty	Empty
F	SQDIB006 **S6** uDNA Pr	Empty	Empty	Empty	Empty	Empty	SQDIB054 **S6** uRNA Pr	Empty	Empty	Empty	Empty	Empty
G	SQDIB007 **S7** uDNA Pr	Empty	Empty	Empty	Empty	Empty	SQDIB055 **S7** uRNA Pr	Empty	Empty	Empty	Empty	Empty
H	SQDIB008 **S8** uDNA Pr	Empty	Empty	Empty	Empty	Empty	SQDIB056 **S8** uRNA Pr	Empty	Empty	Empty	Empty	Empty

Table 23. Layout of QIAseq Multimodal S5 index plates (a) MTIS-96DNA/K/X and (b) MTIS-96RNA/K/X

(a)

	1	2	3	4	5	6	7	8	9	10	11	12
A	SQDIB001 **S1** uDNA Pr	SQDIB009 **S9** uDNA Pr	SQDIB017 **S17** uDNA Pr	SQDIB025 **S25** uDNA Pr	SQDIB033 **S33** uDNA Pr	SQDIB041 **S41** uDNA Pr	SQDIB097 **S49** uDNA Pr	SQDIB105 **S57** uDNA Pr	SQDIB113 **S65** uDNA Pr	SQDIB121 **S73** uDNA Pr	SQDIB129 **S81** uDNA Pr	SQDIB137 **S89** uDNA Pr
B	SQDIB002 **S2** uDNA Pr	SQDIB010 **S10** uDNA Pr	SQDIB018 **S18** uDNA Pr	SQDIB026 **S26** uDNA Pr	SQDIB034 **S34** uDNA Pr	SQDIB042 **S42** uDNA Pr	SQDIB098 **S50** uDNA Pr	SQDIB106 **S58** uDNA Pr	SQDIB114 **S66** uDNA Pr	SQDIB122 **S74** uDNA Pr	SQDIB130 **S82** uDNA Pr	SQDIB138 **S90** uDNA Pr
C	SQDIB003 **S3** uDNA Pr	SQDIB011 **S11** uDNA Pr	SQDIB019 **S19** uDNA Pr	SQDIB027 **S27** uDNA Pr	SQDIB035 **S35** uDNA Pr	SQDIB043 **S43** uDNA Pr	SQDIB099 **S51** uDNA Pr	SQDIB107 **S59** uDNA Pr	SQDIB115 **S67** uDNA Pr	SQDIB123 **S75** uDNA Pr	SQDIB131 **S83** uDNA Pr	SQDIB764 **S91** uDNA Pr
D	SQDIB004 **S4** uDNA Pr	SQDIB012 **S12** uDNA Pr	SQDIB020 **S20** uDNA Pr	SQDIB028 **S28** uDNA Pr	SQDIB036 **S36** uDNA Pr	SQDIB044 **S44** uDNA Pr	SQDIB100 **S52** uDNA Pr	SQDIB108 **S60** uDNA Pr	SQDIB116 **S68** uDNA Pr	SQDIB124 **S76** uDNA Pr	SQDIB132 **S84** uDNA Pr	SQDIB140 **S92** uDNA Pr
E	SQDIB005 **S5** uDNA Pr	SQDIB013 **S13** uDNA Pr	SQDIB021 **S21** uDNA Pr	SQDIB029 **S29** uDNA Pr	SQDIB037 **S37** uDNA Pr	SQDIB045 **S45** uDNA Pr	SQDIB101 **S53** uDNA Pr	SQDIB109 **S61** uDNA Pr	SQDIB117 **S69** uDNA Pr	SQDIB125 **S77** uDNA Pr	SQDIB133 **S85** uDNA Pr	SQDIB141 **S93** uDNA Pr
F	SQDIB006 **S6** uDNA Pr	SQDIB014 **S14** uDNA Pr	SQDIB761 **S22** uDNA Pr	SQDIB030 **S30** uDNA Pr	SQDIB038 **S38** uDNA Pr	SQDIB046 **S46** uDNA Pr	SQDIB102 **S54** uDNA Pr	SQDIB110 **S62** uDNA Pr	SQDIB118 **S70** uDNA Pr	SQDIB126 **S78** uDNA Pr	SQDIB134 **S86** uDNA Pr	SQDIB142 **S94** uDNA Pr
G	SQDIB007 **S7** uDNA Pr	SQDIB015 **S15** uDNA Pr	SQDIB023 **S23** uDNA Pr	SQDIB031 **S31** uDNA Pr	SQDIB763 **S39** uDNA Pr	SQDIB047 **S47** uDNA Pr	SQDIB103 **S55** uDNA Pr	SQDIB111 **S63** uDNA Pr	SQDIB119 **S71** uDNA Pr	SQDIB127 **S79** uDNA Pr	SQDIB135 **S87** uDNA Pr	SQDIB143 **S95** uDNA Pr
H	SQDIB008 **S8** uDNA Pr	SQDIB016 **S16** uDNA Pr	SQDIB024 **S24** uDNA Pr	SQDIB032 **S32** uDNA Pr	SQDIB040 **S40** uDNA Pr	SQDIB048 **S48** uDNA Pr	SQDIB104 **S56** uDNA Pr	SQDIB112 **S64** uDNA Pr	SQDIB120 **S72** uDNA Pr	SQDIB128 **S80** uDNA Pr	SQDIB136 **S88** uDNA Pr	SQDIB144 **S96** uDNA Pr

(b)

	1	2	3	4	5	6	7	8	9	10	11	12
A	SQDIB049 **S1** uRNA Pr	SQDIB057 **S9** uRNA Pr	SQDIB065 **S17** uRNA Pr	SQDIB073 **S25** uRNA Pr	SQDIB081 **S33** uRNA Pr	SQDIB089 **S41** uRNA Pr	SQDIB0145 **S49** uRNA Pr	SQDIB153 **S57** uRNA Pr	SQDIB161 **S65** uRNA Pr	SQDIB169 **S73** uRNA Pr	SQDIB177 **S81** uRNA Pr	SQDIB185 **S89** uRNA Pr
B	SQDIB050 **S2** uRNA Pr	SQDIB058 **S10** uRNA Pr	SQDIB066 **S18** uRNA Pr	SQDIB074 **S26** uRNA Pr	SQDIB082 **S34** uRNA Pr	SQDIB090 **S42** uRNA Pr	SQDIB146 **S50** uRNA Pr	SQDIB154 **S58** uRNA Pr	SQDIB162 **S66** uRNA Pr	SQDIB170 **S74** uRNA Pr	SQDIB178 **S82** uRNA Pr	SQDIB186 **S90** uRNA Pr
C	SQDIB051 **S3** uRNA Pr	SQDIB059 **S11** uRNA Pr	SQDIB067 **S19** uRNA Pr	SQDIB075 **S27** uRNA Pr	SQDIB083 **S35** uRNA Pr	SQDIB091 **S43** uRNA Pr	SQDIB147 **S51** uRNA Pr	SQDIB155 **S59** uRNA Pr	SQDIB163 **S67** uRNA Pr	SQDIB171 **S75** uRNA Pr	SQDIB179 **S83** uRNA Pr	SQDIB187 **S91** uRNA Pr
D	SQDIB052 **S4** uRNA Pr	SQDIB060 **S12** uRNA Pr	SQDIB068 **S20** uRNA Pr	SQDIB076 **S28** uRNA Pr	SQDIB084 **S36** uRNA Pr	SQDIB092 **S44** uRNA Pr	SQDIB148 **S52** uRNA Pr	SQDIB156 **S60** uRNA Pr	SQDIB164 **S68** uRNA Pr	SQDIB172 **S76** uRNA Pr	SQDIB180 **S84** uRNA Pr	SQDIB188 **S92** uRNA Pr
E	SQDIB053 **S5** uRNA Pr	SQDIB061 **S13** uRNA Pr	SQDIB069 **S21** uRNA Pr	SQDIB077 **S29** uRNA Pr	SQDIB085 **S37** uRNA Pr	SQDIB093 **S45** uRNA Pr	SQDIB149 **S53** uRNA Pr	SQDIB157 **S61** uRNA Pr	SQDIB165 **S69** uRNA Pr	SQDIB173 **S77** uRNA Pr	SQDIB181 **S85** uRNA Pr	SQDIB189 **S93** uRNA Pr
F	SQDIB054 **S6** uRNA Pr	SQDIB062 **S14** uRNA Pr	SQDIB070 **S22** uRNA Pr	SQDIB078 **S30** uRNA Pr	SQDIB086 **S38** uRNA Pr	SQDIB094 **S46** uRNA Pr	SQDIB150 **S54** uRNA Pr	SQDIB158 **S62** uRNA Pr	SQDIB166 **S70** uRNA Pr	SQDIB174 **S78** uRNA Pr	SQDIB182 **S86** uRNA Pr	SQDIB190 **S94** uRNA Pr
G	SQDIB055 **S7** uRNA Pr	SQDIB063 **S15** uRNA Pr	SQDIB071 **S23** uRNA Pr	SQDIB077 **S31** uRNA Pr	SQDIB087 **S39** uRNA Pr	SQDIB095 **S47** uRNA Pr	SQDIB151 **S55** uRNA Pr	SQDIB159 **S63** uRNA Pr	SQDIB167 **S71** uRNA Pr	SQDIB175 **S79** uRNA Pr	SQDIB183 **S87** uRNA Pr	SQDIB191 **S95** uRNA Pr
H	SQDIB056 **S8** uRNA Pr	SQDIB064 **S16** uRNA Pr	SQDIB072 **S24** uRNA Pr	SQDIB080 **S32** uRNA Pr	SQDIB088 **S40** uRNA Pr	SQDIB096 **S48** uRNA Pr	SQDIB152 **S56** uRNA Pr	SQDIB160 **S64** uRNA Pr	SQDIB168 **S72** uRNA Pr	SQDIB176 **S80** uRNA Pr	SQDIB184 **S88** uRNA Pr	SQDIB192 **S96** uRNA Pr

4. Program a thermal cycler using the cycling conditions in Table 24.

Table 24. Cycling conditions for universal PCR

Step	Time	Temperature
Hold	2 min	98°C
2-step cycling		
Denaturation	15 s	98°C
Annealing/Extension	30 s	62°C
Cycle number	**Based on "Protocol: qPCR Determination of Universal PCR Cycles", page 39**	
Hold	∞	4°C

5. After the reaction is complete, add 75 µl of Nuclease-free Water to bring each sample to 100 µl.
6. Add 80 µl QIAseq Beads for standard/FFPE sample or 100 µl 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.

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

9. With the beads still on the magnetic stand, add 200 µl 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 µl pipette tip first, spin down briefly, and then use a 10 µl 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 µl Nuclease-free Water. Mix well by pipetting.

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

14. Transfer 18 µl of the supernatant to clean tubes/plate.

15. Proceed to “Recommendations: Library QC and Quantification”, page 50. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Recommendations: Library QC and Quantification

NGS library QC

QC can be performed with the 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).

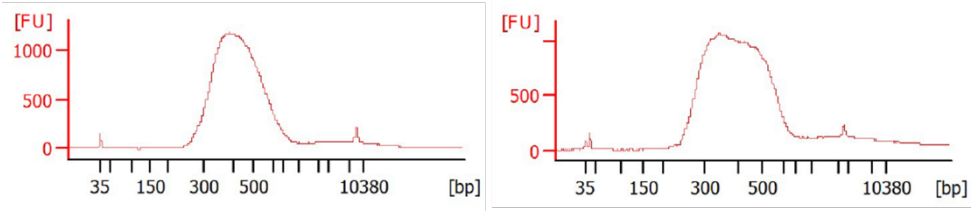


Figure 3. QIAseq Multimodal Targeted DNA (left) and Targeted RNA (right) libraries.

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 library.

Protocol: Sequencing Setup on Illumina MiSeq and NextSeq

Important points before starting

- **Important**: Recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System.
- **Important**: 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.
- **Important**: 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
  - **Important**: Multimodal Read 2 Primer (the Custom Read 2 Sequencing Primer) goes into the following specific reagent cartridge positions:
    MiSeq position #20
  - NextSeq position #8
- **Important**: 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.

Sequencing preparations for MiSeq

1. Download the appropriate template from the "Resource" tab of the QIAseq Multimodal Panel:
   - Sample Sheet Multimodal 96 UDI: **www.qiagen.com/PROM-20735 (also used for Indexes 1–12)**
2. On the template:
   2a. 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.
   2b. Delete any unused index pairs and save the sample sheet for uploading.
   2c. 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 2 or 4 nM for MiSeq. Then combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.
   Note: If combining libraries with the same number of primers, pool equal volumes of the individual libraries at 4 nM together. If the libraries have different primer numbers, then combine the libraries at volume ratios according to their number of primers. For example, Library A has 5000 primers at 4 nM, and Library B has 600 primers at 4 nM; combining 50 µl Library A with 6 µl Library B will result in similar coverage depth for both libraries A and B in the same sequencing run.
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 µl HT1 (hybridization buffer) from the Illumina Sequencing Kit to dilute 3 µl of QIAseq A Read 1

Primer I (provided) to obtain a final concentration of 0.5 µM. Load 600 µl of the diluted QIAseq A Read 1 Primer I to position 18 of the MiSeq reagent cartridge (Figure 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 µl HT1 (hybridization buffer) from the Illumina Sequencing Kit to dilute 3 µl of Multimodal Read 2 Primer (provided) to obtain a final concentration of 0.5 µM. Load 600 µl 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*.

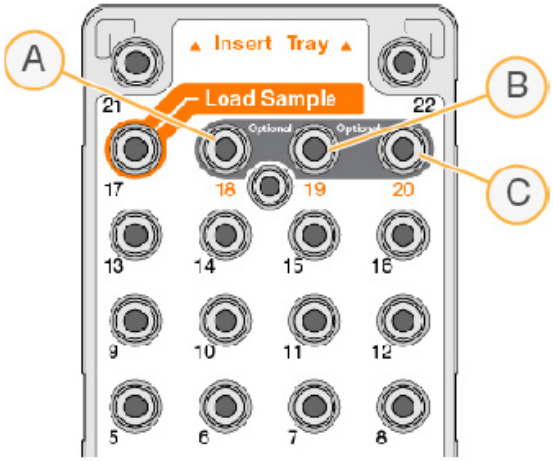
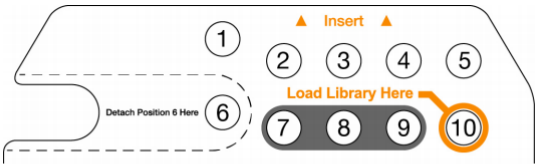


Figure 4. MiSeq reagent cartridge. A: position 18 for Read 1 Custom Primer; **C**: position 20 for Read 2 Custom Primer**.**

Sequencing preparations for NextSeq

1. **Sample dilution and pooling**: Dilute libraries to 0.5, 1, 2, or 4 nM for NextSeq. Then combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.
   Note: Recommendations for library dilution concentrations are based on the QIAseq Library Quant System.
   Note: If combining libraries with the same number of primers, pool equal volumes of the individual libraries at 4 nM together. If the libraries have different primer numbers, then combine the libraries at volume ratios according to their number of primers. For example, Library A has 5000 primers at 4 nM, and Library B has 600 primers at 4 nM; combining 50 µl Library A with 6 µl Library B will result in similar coverage depth for both libraries A and B in the same sequencing run.
2. **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 µl HT1 (hybridization buffer) from the Illumina Sequencing Kit to dilute 6 µl of QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.3 µM. Load 2 ml of the diluted QIAseq A Read 1 Primer I to position 7 of the NextSeq reagent cartridge (Figure 5).
   Note: All other steps refer to run setup workflow as described in the *NextSeq 500 System Guide* (part # 15046563) or *NextSeq 550 System Guide* (part # 15069765-02).

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



Position #	Custom Primer
7	Custom Read 1 primer
8	Custom Read 2 primer

Figure 5. NextSeq reagent cartridge.

5. When working with the QIAseq Multimodal custom UDIs, use Local Run Manager (LRM) V2 on the instrument to upload sample sheet (see page 66 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**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low library yield	
a) Suboptimal reaction conditions due to low 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 (100uM) **must** be used when sequencing on any Illumina platform.
Variant detection issues	
Known variants not detected	Variant detection sensitivity is directly related to the input DNA and read depth. Check Table 32 (page 68), Table 33 (page 70), and Table 34 (page 74) to determine if the required input DNA, UMI numbers, and read depth are met for the specific variant detection application.

References

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

Appendix A: 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 ≤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).

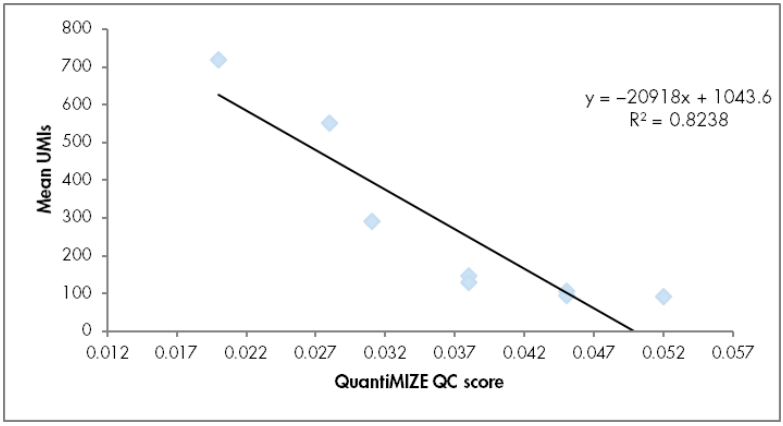


Figure 6. Correlation between QIAseq QuantiMIZE QC Score and the number of UMIs.

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 Workflow for Targeted Enrichment

Important points before starting

- The 12.4 µl product from "Protocol: Reverse Transcription", page 32, 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.
- **Important**: 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-reaciton 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**
- **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.
   1a. Thaw TEPCR Buffer, 5x; Multimodal DHS Panel (DNA); and Multimodal VHS Panel (RNA); and bring QIAseq Multimodal N7 index plate to room temperature.
   1b. 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
Sample (from "Protocol: Reverse Transcription", page 32)	12.4 µl
TEPCR Buffer, 5x	8 µl
Multimodal DHS Panel (DNA)	10 µl
Multimodal VHS Panel (RNA)	8 µl
QN Taq Polymerase	1.6 µl
Total	**40 µl**

3. Add the 40 µl 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 to –15°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
A	DNAp-M001 **S1** RNAp-M049	DNAp-M009 **S9** RNAp-M057	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	DNAp-M002 **S2** RNAp-M050	DNAp-M010 **S10** RNAp-M058	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	DNAp-M003 **S3** RNAp-M051	DNAp-M011 **S11** RNAp-M059	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
D	DNAp-M004 **S4** RNAp-M052	DNAp-M012 **S12** RNAp-M060	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	DNAp-M005 **S5** RNAp-M053	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
F	DNAp-M006 **S6** RNAp-M054	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
G	DNAp-M007 **S7** RNAp-M055	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
H	DNAp-M008 **S8** RNAp-M056	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

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
A	DNAp-M001 **S1** RNAp-M049	DNAp-M009 **S9** RNAp-M057	DNAp-M017 **S17** RNAp-M065	DNAp-M025 **S25** RNAp-M073	DNAp-M033 **S33** RNAp-M081	DNAp-M041 **S41** RNAp-M089	DNAp-M097 **S49** RNAp-M145	DNAp-M105 **S57** RNAp-M153	DNAp-M113 **S65** RNAp-M161	DNAp-M121 **S73** RNAp-M169	DNAp-M129 **S81** RNAp-M177	DNAp-M137 **S89** RNAp-M185
B	DNAp-M002 **S2** RNAp-M050	DNAp-M010 **S10** RNAp-M058	DNAp-M018 **S18** RNAp-M066	DNAp-M026 **S26** RNAp-M074	DNAp-M034 **S34** RNAp-M082	DNAp-M042 **S42** RNAp-M090	DNAp-M098 **S50** RNAp-M146	DNAp-M106 **S58** RNAp-M154	DNAp-M114 **S66** RNAp-M162	DNAp-M122 **S74** RNAp-M170	DNAp-M130 **S82** RNAp-M178	DNAp-M138 **S90** RNAp-M186
C	DNAp-M003 **S3** RNAp-M051	DNAp-M011 **S11** RNAp-M059	DNAp-M019 **S19** RNAp-M067	DNAp-M027 **S27** RNAp-M075	DNAp-M035 **S35** RNAp-M083	DNAp-M043 **S43** RNAp-M091	DNAp-M099 **S51** RNAp-M147	DNAp-M107 **S59** RNAp-M155	DNAp-M115 **S67** RNAp-M163	DNAp-M123 **S75** RNAp-M171	DNAp-M131 **S83** RNAp-M179	DNAp-M139 **S91** RNAp-M187
D	DNAp-M004 **S4** RNAp-M052	DNAp-M012 **S12** RNAp-M060	DNAp-M020 **S20** RNAp-M068	DNAp-M028 **S28** RNAp-M076	DNAp-M036 **S36** RNAp-M084	DNAp-M044 **S44** RNAp-M092	DNAp-M100 **S52** RNAp-M1148	DNAp-M108 **S60** RNAp-M156	DNAp-M116 **S68** RNAp-M164	DNAp-M124 **S76** RNAp-M172	DNAp-M132 **S84** RNAp-M180	DNAp-M140 **S92** RNAp-M188
E	DNAp-M005 **S5** RNAp-M053	DNAp-M013 **S13** RNAp-M061	DNAp-M021 **S21** RNAp-M069	DNAp-M029 **S29** RNAp-M077	DNAp-M037 **S37** RNAp-M085	DNAp-M045 **S45** RNAp-M093	DNAp-M101 **S53** RNAp-M149	DNAp-M109 **S61** RNAp-M157	DNAp-M117 **S69** RNAp-M165	DNAp-M125 **S77** RNAp-M173	DNAp-M133 **S85** RNAp-M181	DNAp-M141 **S93** RNAp-M189
F	DNAp-M006 **S6** RNAp-M054	DNAp-M014 **S14** RNAp-M062	DNAp-M022 **S22** RNAp-M070	DNAp-M030 **S30** RNAp-M078	DNAp-M038 **S38** RNAp-M086	DNAp-M046 **S46** RNAp-M094	DNAp-M102 **S54** RNAp-M150	DNAp-M110 **S62** RNAp-M158	DNAp-M118 **S70** RNAp-M166	DNAp-M126 **S78** RNAp-M174	DNAp-M134 **S86** RNAp-M182	DNAp-M142 **S94** RNAp-M190
G	DNAp-M007 **S7** RNAp-M055	DNAp-M015 **S15** RNAp-M063	DNAp-M023 **S23** RNAp-M071	DNAp-M031 **S31** RNAp-M079	DNAp-M039 **S39** RNAp-M087	DNAp-M047 **S47** RNAp-M095	DNAp-M103 **S55** RNAp-M151	DNAp-M111 **S63** RNAp-M159	DNAp-M119 **S71** RNAp-M167	DNAp-M127 **S79** RNAp-M175	DNAp-M135 **S87** RNAp-M183	DNAp-M143 **S95** RNAp-M191
H	DNAp-M008 **S8** RNAp-M056	DNAp-M016 **S16** RNAp-M064	DNAp-M024 **S24** RNAp-M072	DNAp-M032 **S32** RNAp-M080	DNAp-M040 **S40** RNAp-M088	DNAp-M048 **S48** RNAp-M096	DNAp-M104 **S56** RNAp-M152	DNAp-M112 **S64** RNAp-M160	DNAp-M120 **S72** RNAp-M168	DNAp-M128 **S80** RNAp-M176	DNAp-M136 **S88** RNAp-M184	DNAp-M144 **S96** 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
Initial denaturation	2 min	98°C
8 cycles	15 s	98°C
	2 min	68°C
Hold	∞	4°C

Table 28b. Cycling conditions for target enrichment if number of primers ≥3000/tube

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

6. Place the target enrichment reaction in the thermal cycler and start the run.
7. Once the run has finished, add 60 µl of Nuclease-free Water to bring each sample to 100 µl.
8. Add 80 µl QIAseq Beads for standard/FFPE sample or 100 µl 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 µl of Nuclease-free Water to resuspend beads, and then, add 100 µl of QIAseq Bead Binding Buffer. Mix by vortexing or pipetting up and down. Repeat steps 10 and 11.

    Tip: For plates, the following may improve performance. After 8 min, remove 100 µl supernatant. Leave it on the magnetic stand for 2 min and remove 90 µl supernatant.

Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10 µl pipette to remove the remaining supernatant.

11. With the beads still on the magnetic stand, add 200 µl 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 µl pipette tip first, spin down briefly, and then use a 10 µl 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 µl Nuclease-free Water. Mix well by pipetting.
15. Return the tube/plate to the magnetic rack until the solution has cleared.
16. Transfer 24 µl 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”, page 41. Alternatively, the samples can be stored at –30 to –15°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.

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	Volume of booster panel to combine
1–2000	50 µl	5 µl
2001–4000	50 µl	3.75 µl
4001–12000	50 µl	2.5 µl
12001–20000	50 µl	1.25 µl

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	Volume of booster panel to combine
1–2000	40 µl	5 µl
2001–4000	40 µl	3.75 µl
4001–12000	40 µl	2.5 µl
12001–20000	40 µl	1.25 µl

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 artifactual variants can be greatly reduced by calling variants across all reads within a unique UMI instead of picking up variants at the original read level.

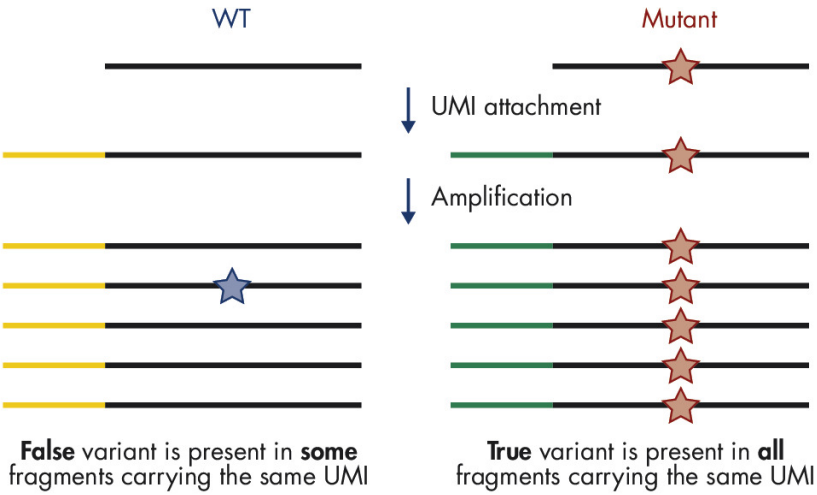


Figure 7. Principle of variant detection with UMIs. Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while false positives are mutations present in only one or a few reads within a UMI. Description of the variant calling algorithm can be found and downloaded from **doi.org/10.1186/s12864-016-3425-4** (1).

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/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†	4	7200x
5%	40†	2	3640x
1%	80†	4	25,600x

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

† If performing Appendix B: Combined Workflow (CW) for Targeted Enrichment, use one-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 (UHS-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)

		Primer Number	UHS-005Z	UHS-009Z	UHS-006Z	UHS-5000Z*
		DNA primer #	4149	6244	11243	19995
		RNA primer #	487	1116	665	2571
Per DNA library	**Input**	**Coverage (X)**	**Reads allocated to each DNA library (M)†**			
5% VAF	20 ng‡	7200	30	45	81	144
5% VAF	40 ng‡	3640	15	23	41	73
1% VAF	80 ng‡	25600	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.

† Reads allocated to each library (M) = Coverage X Primer number/10^6

‡ Since samples are split before targeted enrichment, the recommend 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 Number	UHS-005Z	UHS-009Z	UHS-006Z
		DNA primer #	4149	6244	11243
		RNA primer #	487	1116	665
Per DNA library	**Input**	**Coverage (X)**	**Reads allocated to each DNA library (M)***		
5% VAF	10 ng	7200	33	53	86
5% VAF	20 ng	3640	17	27	43
1% VAF	40 ng	25600	119	188	305
			Reads allocated to each RNA library (M)*		
Per RNA library		5000	23	37	60

* Reads allocated to each library (M) = Coverage X Primer number/10^6

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 33a through Table 33d) or the Combined Targeted DNA+RNA Enrichment workflow (Table 34a through Table 34d). Fine-tuning the read depth is possible after the first run. See read allocation and sample multiplexing template for the custom panel on **www.qiagen.com/PROM-16466**.

Table 33a. 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*

			UHS-005Z	UHS-009Z	UHS-006Z	UHS-5000Z
		DNA primer #	**4149**	**6244**	**11243**	**19995**
		RNA primer #	**487**	**1116**	**665**	**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	91	89	35
NextSeq 1000/2000	P1	200M	44	22	22	8
NextSeq 1000/2000	P2	800M	177	91	89	35
NextSeq 1000/2000	P3	2400M	531	273	267	105
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	266	137	134	52
HiSeq 3000	8 lanes per flow cell	5B	1108	574	558	218
HiSeq 4000	8 lanes per flow cell	10B	2217	1149	1117	437
NovaSeq 6000	SP (per flow cell)	1.6B	354	183	178	70

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

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

Table 33b. 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*

			UHS-005Z	UHS-009Z	UHS-006Z	UHS-5000Z
		DNA primer #	**4149**	**6244**	**11243**	**19995**
		RNA primer #	**487**	**1116**	**665**	**2571**
Instrument	**Version**	**Capacity(paired-end reads)**				
MiniSeq	Mid output	16M	N/A	N/A	N/A	N/A
MiniSeq	High output	50M	2	1	1	N/A
MiSeq	v2	30M	1	1	N/A	N/A
NextSeq 500/550	Mid output	260M	14	9	5	3
NextSeq 500/550	High output	800M	45	28	18	9
NextSeq 1000/2000	P1	200M	11	7	4	2
NextSeq 1000/2000	P2	800M	45	28	18	9
NextSeq 1000/2000	P3	2400M	135	84	54	27
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	68	42	27	14
HiSeq 3000	8 lanes per flow cell	5B	285	176	112	58
HiSeq 4000	8 lanes per flow cell	10B	570	353	225	116
NovaSeq 6000	SP (per flow cell)	1.6B	91	56	36	18

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

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

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 7200x mean coverage for DNA library and 5000 reads/primer for the RNA library*

			UHS-005Z	UHS-009Z	UHS-006Z	UHS-5000Z
		DNA primer #	**4149**	**6244**	**11243**	**19995**
		RNA primer #	**487**	**1116**	**665**	**2571**
Instrument	**Version**	**Capacity (paired-end reads)**				
MiniSeq	Mid output	16M	N/A	N/A	N/A	N/A
MiniSeq	High output	50M	1	N/A	N/A	N/A
MiSeq	v2	30M	N/A	N/A	N/A	N/A
NextSeq 500/550	Mid output	260M	8	5	3	1
NextSeq 500/550	High output	800M	24	15	9	5
NextSeq 1000/2000	P1	200M	6	3	2	1
NextSeq 1000/2000	P2	800M	24	15	9	5
NextSeq 1000/2000	P3	2400M	72	45	27	15
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	37	23	14	7
HiSeq 3000	8 lanes per flow cell	5B	154	98	59	31
HiSeq 4000	8 lanes per flow cell	10B	309	197	118	63
NovaSeq 6000	SP (per flow cell)	1.6B	49	31	18	10

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 25,600x mean coverage for DNA library and 5000 reads/primer for the RNA library*

			UHS-005Z	UHS-009Z	UHS-006Z	UHS-5000Z
		DNA primer #	**4149**	**6244**	**11243**	**19995**
		RNA primer #	**487**	**1116**	**665**	**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	1	N/A	N/A
NextSeq 500/550	High output	800M	7	4	2	1
NextSeq 1000/2000	P1	200M	1	1	N/A	N/A
NextSeq 1000/2000	P2	800M	7	4	2	1
NextSeq 1000/2000	P3	2400M	21	12	6	3
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	11	7	4	2
HiSeq 3000	8 lanes per flow cell	5B	46	30	17	9
HiSeq 4000	8 lanes per flow cell	10B	92	60	34	19
NovaSeq 6000	SP (per flow cell)	1.6B	14	9	5	3

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

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

Table 34a. 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*

			UHS-005Z	UHS-009Z	UHS-006Z
		DNA primer #	**4149**	**6244**	**11243**
		RNA primer #	**487**	**1116**	**665**
Instrument	**Version**	**Capacity (paired-end reads)**			
MiniSeq	Mid output	16M	N/A	N/A	N/A
MiniSeq	High output	50M	1	1	N/A
MiSeq	v2	30M	1	N/A	N/A
NextSeq 500/550	Mid output	260M	10	6	3
NextSeq 500/550	High output	800M	31	19	12
NextSeq 1000/2000	P1	200M	7	4	3
NextSeq 1000/2000	P2	800M	31	19	12
NextSeq 1000/2000	P3	2400M	93	57	36
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	47	29	18
HiSeq 3000	8 lanes per flow cell	5B	196	123	76
HiSeq 4000	8 lanes per flow cell	10B	392	247	152
NovaSeq 6000	SP (per flow cell)	1.6B	62	39	24

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

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

Table 34b. 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*

			UHS-005Z	UHS-009Z	UHS-006Z
		DNA primer #	4149	6244	11243
		RNA primer #	487	1116	665
Instrument	**Version**	**Capacity (paired-end reads)**			
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	4	2
NextSeq 500/550	High output	800M	19	12	7
NextSeq 1000/2000	P1	200M	4	3	1
NextSeq 1000/2000	P2	800M	19	12	7
NextSeq 1000/2000	P3	2400M	57	36	21
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	29	18	11
HiSeq 3000	8 lanes per flow cell	5B	124	78	48
HiSeq 4000	8 lanes per flow cell	10B	249	157	97
NovaSeq 6000	SP (per flow cell)	1.6B	39	25	15

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

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

Table 34c. 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*

			UHS-005Z	UHS-009Z	UHS-006Z
		DNA primer #	4149	6244	11243
		RNA primer #	487	1116	665
Instrument	**Version**	**Capacity (paired-end reads)**			
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	2	1
NextSeq 500/550	High output	800M	14	8	5
NextSeq 1000/2000	P1	200M	3	2	1
NextSeq 1000/2000	P2	800M	14	8	5
NextSeq 1000/2000	P3	2400M	42	24	15
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	21	13	8
HiSeq 3000	8 lanes per flow cell	5B	88	55	34
HiSeq 4000	8 lanes per flow cell	10B	176	111	68
NovaSeq 6000	SP (per flow cell)	1.6B	28	17	11

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

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

Table 34d. 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*

			UHS-005Z	UHS-009Z	UHS-006Z
		DNA primer #	4149	6244	11243
		RNA primer #	487	1116	665
Instrument	**Version**	**Capacity (paired-end reads)**			
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	1	N/A
NextSeq 500/550	High output	800M	5	3	2
NextSeq 1000/2000	P1	200M	1	N/A	N/A
NextSeq 1000/2000	P2	800M	5	3	2
NextSeq 1000/2000	P3	2400M	15	9	6
HiSeq 2500 rapid run	Dual Flowcell v2	1200M	8	5	3
HiSeq 3000	8 lanes per flow cell	5B	35	22	13
HiSeq 4000	8 lanes per flow cell	10B	70	44	27
NovaSeq 6000	SP (per flow cell)	1.6B	11	7	4

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 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 Workflow, but will be discontinued.

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

QIAseq Multimodal Index I Set A SW (Two identical sets of 48 DNA + 48 RNA sample indexes using the Separated Workflow for Targeted Enrichment)	**(96) 333985**
Catalog no.	**96**
Number of samples	
Multimodal N7 Plate Set A (48): MTIN-96ASWK 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.	2
Multimodal S5 Plate Set A (48): MTIS-96AK 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).	2
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 Workflow

QIAseq Multimodal Index I Set B SW (Two identical sets of 48 DNA + 48 RNA sample indexes using the Separated Workflow for Targeted Enrichment)	**(96)**
Catalog no.	**333995**
Number of samples	**96**
Multimodal N7 Plate Set B SW (48): MTIN-96BSWK 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.	2
Multimodal S5 Plate Set B (48): MTIS-96BK 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).	2
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 37. Layout of (a) MTIN-96ASWK and (b) MTIS-96AK

(a)

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

(b)

	1	2	3	4	5	6	7	8	9	10	11	12
A	SQDIB001 S1 uDNA Pr	SQDIB009 S9 uDNA Pr	SQDIB017 S17 uDNA Pr	SQDIB025 S25 uDNA Pr	SQDIB033 S33 uDNA Pr	SQDIB041 S41 uDNA Pr	SQDIB049 S1 uRNA Pr	SQDIB057 S9 uRNA Pr	SQDIB065 S17 uRNA Pr	SQDIB073 S25 uRNA Pr	SQDIB081 S33 uRNA Pr	SQDIB089 S41 uRNA Pr
B	SQDIB002 S2 uDNA Pr	SQDIB010 S10 uDNA Pr	SQDIB018 S18 uDNA Pr	SQDIB026 S26 uDNA Pr	SQDIB034 S34 uDNA Pr	SQDIB042 S42 uDNA Pr	SQDIB050 S2 uRNA Pr	SQDIB058 S10 uRNA Pr	SQDIB066 S18 uRNA Pr	SQDIB074 S26 uRNA Pr	SQDIB082 S34 uRNA Pr	SQDIB090 S42 uRNA Pr
C	SQDIB003 S3 uDNA Pr	SQDIB011 S11 uDNA Pr	SQDIB019 S19 uDNA Pr	SQDIB027 S27 uDNA Pr	SQDIB035 S35 uDNA Pr	SQDIB043 S43 uDNA Pr	SQDIB051 S3 uRNA Pr	SQDIB059 S11 uRNA Pr	SQDIB067 S19 uRNA Pr	SQDIB075 S27 uRNA Pr	SQDIB083 S35 uRNA Pr	SQDIB091 S43 uRNA Pr
D	SQDIB004 S4 uDNA Pr	SQDIB012 S12 uDNA Pr	SQDIB020 S20 uDNA Pr	SQDIB028 S28 uDNA Pr	SQDIB036 S36 uDNA Pr	SQDIB044 S44 uDNA Pr	SQDIB052 S4 uRNA Pr	SQDIB060 S12 uRNA Pr	SQDIB068 S20 uRNA Pr	SQDIB076 S28 uRNA Pr	SQDIB084 S36 uRNA Pr	SQDIB092 S44 uRNA Pr
E	SQDIB005 S5 uDNA Pr	SQDIB013 S13 uDNA Pr	SQDIB021 S21 uDNA Pr	SQDIB029 S29 uDNA Pr	SQDIB037 S37 uDNA Pr	SQDIB045 S45 uDNA Pr	SQDIB053 S5 uRNA Pr	SQDIB061 S13 uRNA Pr	SQDIB069 S21 uRNA Pr	SQDIB077 S29 uRNA Pr	SQDIB085 S37 uRNA Pr	SQDIB093 S45 uRNA Pr
F	SQDIB006 S6 uDNA Pr	SQDIB014 S14 uDNA Pr	SQDIB022 S22 uDNA Pr	SQDIB030 S30 uDNA Pr	SQDIB038 S38 uDNA Pr	SQDIB046 S46 uDNA Pr	SQDIB054 S6 uRNA Pr	SQDIB062 S14 uRNA Pr	SQDIB070 S22 uRNA Pr	SQDIB078 S30 uRNA Pr	SQDIB086 S38 uRNA Pr	SQDIB094 S46 uRNA Pr
G	SQDIB007 S7 uDNA Pr	SQDIB015 S15 uDNA Pr	SQDIB023 S23 uDNA Pr	SQDIB031 S31 uDNA Pr	SQDIB039 S39 uDNA Pr	SQDIB047 S47 uDNA Pr	SQDIB055 S7 uRNA Pr	SQDIB063 S15 uRNA Pr	SQDIB071 S23 uRNA Pr	SQDIB077 S31 uRNA Pr	SQDIB087 S39 uRNA Pr	SQDIB095 S47 uRNA Pr
H	SQDIB008 S8 uDNA Pr	SQDIB016 S16 uDNA Pr	SQDIB024 S24 uDNA Pr	SQDIB032 S32 uDNA Pr	SQDIB040 S40 uDNA Pr	SQDIB048 S48 uDNA Pr	SQDIB056 S8 uRNA Pr	SQDIB064 S16 uRNA Pr	SQDIB072 S24 uRNA Pr	SQDIB080 S32 uRNA Pr	SQDIB088 S40 uRNA Pr	SQDIB096 S48 uRNA Pr

Table 38. Layout of (a) MTIN-96BSWK and (b) MTIS-96BK

(a)

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

(b)

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

Legacy QIAseq Multimodal Index kits for the Combined Workflow (CW) for Target enrichment are listed below (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 Workflow, but will be discontinued.

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

QIAseq Multimodal Index I Set A (Two identical sets of 48 DNA + 48 RNA sample indexes for Illumina platforms)	**(96)**
Catalog no.	**333965**
Number of samples	**96**
Multimodal N7 Plate Set A (48): MTIN-96AK 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.	2
Multimodal S5 Plate Set A (48): MTIS-96AK 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).	2
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 Workflow

QIAseq Multimodal Index I Set B (Two identical sets of 48 DNA + 48 RNA sample indexes for Illumina platforms)	**(96)**
Catalog no.	**333975**
Number of samples	**96**
Multimodal N7 Plate Set B (48): MTIN-96BK 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.	2
Multimodal S5 Plate Set B (48): MTIS-96BK 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).	2
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 41. Layout of (a) MTIN-96AK and (b) MTIS-96AK

(a)

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

(b)

	1	2	3	4	5	6	7	8	9	10	11	12
A	SQDIB001 **S1** uDNA Pr	SQDIB009 **S9** uDNA Pr	SQDIB017 **S17** uDNA Pr	SQDIB025 **S25** uDNA Pr	SQDIB033 **S33** uDNA Pr	SQDIB041 **S41** uDNA Pr	SQDIB049 **S1** uRNA Pr	SQDIB057 **S9** uRNA Pr	SQDIB065 **S17** uRNA Pr	SQDIB073 **S25** uRNA Pr	SQDIB081 **S33** uRNA Pr	SQDIB089 **S41** uRNA Pr
B	SQDIB002 **S2** uDNA Pr	SQDIB010 **S10** uDNA Pr	SQDIB018 **S18** uDNA Pr	SQDIB026 **S26** uDNA Pr	SQDIB034 **S34** uDNA Pr	SQDIB042 **S42** uDNA Pr	SQDIB050 **S2** uRNA Pr	SQDIB058 **S10** uRNA Pr	SQDIB066 **S18** uRNA Pr	SQDIB074 **S26** uRNA Pr	SQDIB082 **S34** uRNA Pr	SQDIB090 **S42** uRNA Pr
C	SQDIB003 **S3** uDNA Pr	SQDIB011 **S11** uDNA Pr	SQDIB019 **S19** uDNA Pr	SQDIB027 **S27** uDNA Pr	SQDIB035 **S35** uDNA Pr	SQDIB043 **S43** uDNA Pr	SQDIB051 **S3** uRNA Pr	SQDIB059 **S11** uRNA Pr	SQDIB067 **S19** uRNA Pr	SQDIB075 **S27** uRNA Pr	SQDIB083 **S35** uRNA Pr	SQDIB091 **S43** uRNA Pr
D	SQDIB004 **S4** uDNA Pr	SQDIB012 **S12** uDNA Pr	SQDIB020 **S20** uDNA Pr	SQDIB028 **S28** uDNA Pr	SQDIB036 **S36** uDNA Pr	SQDIB044 **S44** uDNA Pr	SQDIB052 **S4** uRNA Pr	SQDIB060 **S12** uRNA Pr	SQDIB068 **S20** uRNA Pr	SQDIB076 **S28** uRNA Pr	SQDIB084 **S36** uRNA Pr	SQDIB092 **S44** uRNA Pr
E	SQDIB005 **S5** uDNA Pr	SQDIB013 **S13** uDNA Pr	SQDIB021 **S21** uDNA Pr	SQDIB029 **S29** uDNA Pr	SQDIB037 **S37** uDNA Pr	SQDIB045 **S45** uDNA Pr	SQDIB053 **S5** uRNA Pr	SQDIB061 **S13** uRNA Pr	SQDIB069 **S21** uRNA Pr	SQDIB077 **S29** uRNA Pr	SQDIB085 **S37** uRNA Pr	SQDIB093 **S45** uRNA Pr
F	SQDIB006 **S6** uDNA Pr	SQDIB014 **S14** uDNA Pr	SQDIB022 **S22** uDNA Pr	SQDIB030 **S30** uDNA Pr	SQDIB038 **S38** uDNA Pr	SQDIB046 **S46** uDNA Pr	SQDIB054 **S6** uRNA Pr	SQDIB062 **S14** uRNA Pr	SQDIB070 **S22** uRNA Pr	SQDIB078 **S30** uRNA Pr	SQDIB086 **S38** uRNA Pr	SQDIB094 **S46** uRNA Pr
G	SQDIB007 **S7** uDNA Pr	SQDIB015 **S15** uDNA Pr	SQDIB023 **S23** uDNA Pr	SQDIB031 **S31** uDNA Pr	SQDIB039 **S39** uDNA Pr	SQDIB047 **S47** uDNA Pr	SQDIB055 **S7** uRNA Pr	SQDIB063 **S15** uRNA Pr	SQDIB071 **S23** uRNA Pr	SQDIB077 **S31** uRNA Pr	SQDIB087 **S39** uRNA Pr	SQDIB095 **S47** uRNA Pr
H	SQDIB008 **S8** uDNA Pr	SQDIB016 **S16** uDNA Pr	SQDIB024 **S24** uDNA Pr	SQDIB032 **S32** uDNA Pr	SQDIB040 **S40** uDNA Pr	SQDIB048 **S48** uDNA Pr	SQDIB056 **S8** uRNA Pr	SQDIB064 **S16** uRNA Pr	SQDIB072 **S24** uRNA Pr	SQDIB080 **S32** uRNA Pr	SQDIB088 **S40** uRNA Pr	SQDIB096 **S48** uRNA Pr

Table 42. Layout of (a) MTIN-96BK and (b) MTIS-96BK

(a)

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNAp-M097 **S49** RNAp-M145	DNAp-M105 **S57** RNAp-M153	DNAp-M113 **S65** RNAp-M161	DNAp-M121 **S73** RNAp-M169	DNAp-M129 **S81** RNAp-M177	DNAp-M137 **S89** RNAp-M185	Empty	Empty	Empty	Empty	Empty	Empty
B	DNAp-M098 **S50** RNAp-M146	DNAp-M106 **S58** RNAp-M154	DNAp-M114 **S66** RNAp-M162	DNAp-M122 **S74** RNAp-M170	DNAp-M130 **S82** RNAp-M178	DNAp-M138 **S90** RNAp-M186	Empty	Empty	Empty	Empty	Empty	Empty
C	DNAp-M099 **S51** RNAp-M147	DNAp-M107 **S59** RNAp-M155	DNAp-M115 **S67** RNAp-M163	DNAp-M123 **S75** RNAp-M171	DNAp-M131 **S83** RNAp-M179	DNAp-M139 **S91** RNAp-M187	Empty	Empty	Empty	Empty	Empty	Empty
D	DNAp-M100 **S52** RNAp-M1148	DNAp-M108 **S60** RNAp-M156	DNAp-M116 **S68** RNAp-M164	DNAp-M124 **S76** RNAp-M172	DNAp-M132 **S84** RNAp-M180	DNAp-M140 **S92** RNAp-M188	Empty	Empty	Empty	Empty	Empty	Empty
E	DNAp-M101 **S53** RNAp-M149	DNAp-M109 **S61** RNAp-M157	DNAp-M117 **S69** RNAp-M165	DNAp-M125 **S77** RNAp-M173	DNAp-M133 **S85** RNAp-M181	DNAp-M141 **S93** RNAp-M189	Empty	Empty	Empty	Empty	Empty	Empty
F	DNAp-M102 **S54** RNAp-M150	DNAp-M110 **S62** RNAp-M158	DNAp-M118 **S70** RNAp-M166	DNAp-M126 **S78** RNAp-M174	DNAp-M134 **S86** RNAp-M182	DNAp-M142 **S94** RNAp-M190	Empty	Empty	Empty	Empty	Empty	Empty
G	DNAp-M103 **S55** RNAp-M151	DNAp-M111 **S63** RNAp-M159	DNAp-M119 **S71** RNAp-M167	DNAp-M127 **S79** RNAp-M175	DNAp-M135 **S87** RNAp-M183	DNAp-M143 **S95** RNAp-M191	Empty	Empty	Empty	Empty	Empty	Empty
H	DNAp-M104 **S56** RNAp-M152	DNAp-M112 **S64** RNAp-M160	DNAp-M120 **S72** RNAp-M168	DNAp-M128 **S80** RNAp-M176	DNAp-M136 **S88** RNAp-M184	DNAp-M144 **S96** RNAp-M192	Empty	Empty	Empty	Empty	Empty	Empty

(b)

	1	2	3	4	5	6	7	8	9	10	11	12
A	SQDIB097 **S49** uDNA Pr	SQDIB105 **S57** uDNA Pr	SQDIB113 **S65** uDNA Pr	SQDIB121 **S73** uDNA Pr	SQDIB129 **S81** uDNA Pr	SQDIB137 **S89** uDNA Pr	SQDIB145 **S49** uRNA Pr	SQDIB153 **S57** uRNA Pr	SQDIB161 **S65** uRNA Pr	SQDIB169 **S73** uRNA Pr	SQDIB177 **S81** uRNA Pr	SQDIB185 **S89** uRNA Pr
B	SQDIB098 **S50** uDNA Pr	SQDIB106 **S58** uDNA Pr	SQDIB114 **S66** uDNA Pr	SQDIB122 **S74** uDNA Pr	SQDIB130 **S82** uDNA Pr	SQDIB138 **S90** uDNA Pr	SQDIB146 **S50** uRNA Pr	SQDIB154 **S58** uRNA Pr	SQDIB162 **S66** uRNA Pr	SQDIB170 **S74** uRNA Pr	SQDIB178 **S82** uRNA Pr	SQDIB186 **S90** uRNA Pr
C	SQDIB099 **S51** uDNA Pr	SQDIB107 **S59** uDNA Pr	SQDIB115 **S67** uDNA Pr	SQDIB123 **S75** uDNA Pr	SQDIB131 **S83** uDNA Pr	SQDIB139 **S91** uDNA Pr	SQDIB147 **S51** uRNA Pr	SQDIB155 **S59** uRNA Pr	SQDIB163 **S67** uRNA Pr	SQDIB171 **S75** uRNA Pr	SQDIB179 **S83** uRNA Pr	SQDIB187 **S91** uRNA Pr
D	SQDIB100 **S52** uDNA Pr	SQDIB108 **S60** uDNA Pr	SQDIB116 **S68** uDNA Pr	SQDIB124 **S76** uDNA Pr	SQDIB132 **S84** uDNA Pr	SQDIB140 **S92** uDNA Pr	SQDIB148 **S52** uRNA Pr	SQDIB156 **S60** uRNA Pr	SQDIB164 **S68** uRNA Pr	SQDIB172 **S76** uRNA Pr	SQDIB180 **S84** uRNA Pr	SQDIB188 **S92** uRNA Pr
E	SQDIB101 **S53** uDNA Pr	SQDIB109 **S61** uDNA Pr	SQDIB117 **S69** uDNA Pr	SQDIB125 **S77** uDNA Pr	SQDIB133 **S85** uDNA Pr	SQDIB141 **S93** uDNA Pr	SQDIB149 **S53** uRNA Pr	SQDIB157 **S61** uRNA Pr	SQDIB165 **S69** uRNA Pr	SQDIB173 **S77** uRNA Pr	SQDIB181 **S85** uRNA Pr	SQDIB189 **S93** uRNA Pr
F	SQDIB102 **S54** uDNA Pr	SQDIB110 **S62** uDNA Pr	SQDIB118 **S70** uDNA Pr	SQDIB126 **S78** uDNA Pr	SQDIB134 **S86** uDNA Pr	SQDIB142 **S94** uDNA Pr	SQDIB150 **S54** uRNA Pr	SQDIB158 **S62** uRNA Pr	SQDIB166 **S70** uRNA Pr	SQDIB174 **S78** uRNA Pr	SQDIB182 **S86** uRNA Pr	SQDIB190 **S94** uRNA Pr
G	SQDIB103 **S55** uDNA Pr	SQDIB111 **S63** uDNA Pr	SQDIB119 **S71** uDNA Pr	SQDIB127 **S79** uDNA Pr	SQDIB135 **S87** uDNA Pr	SQDIB143 **S95** uDNA Pr	SQDIB151 **S55** uRNA Pr	SQDIB159 **S63** uRNA Pr	SQDIB167 **S71** uRNA Pr	SQDIB175 **S79** uRNA Pr	SQDIB183 **S87** uRNA Pr	SQDIB191 **S95** uRNA Pr
H	SQDIB104 **S56** uDNA Pr	SQDIB112 **S64** uDNA Pr	SQDIB120 **S72** uDNA Pr	SQDIB128 **S80** uDNA Pr	SQDIB136 **S88** uDNA Pr	SQDIB144 **S96** uDNA Pr	SQDIB152 **S56** uRNA Pr	SQDIB160 **S64** uRNA Pr	SQDIB168 **S72** uRNA Pr	SQDIB176 **S80** uRNA Pr	SQDIB184 **S88** uRNA Pr	SQDIB192 **S96** 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
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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)* **Note**: For Separated Workflow (SW) for Targeted Enrichment	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	333982

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QIAseq Multimodal HT CW 96 UDI **Note**: For Combined Workflow (CW) for Targeted Enrichment	Box containing oligos, enough to process a total of 96 samples, for indexing up to a total of 96 libraries (96 for DNA and 96 for RNA) for multimodal panel sequencing on Illumina platforms	333979
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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

Product	Contents	Cat. no.
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
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Document Revision History

Date	Changes
04/2022	Initial release

Notes

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