June 2024

QIAseq® Multimodal DNA/RNA Lib Kit Handbook

Multiomic WGS and WTS library prep

Sample to Insight

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Kit Contents

QIAseq Multimodal DNA/RNA Lib Kit	(24)	(96)
Catalog no.	334842	334845
Number of samples	24	96
10x FX Buffer	48 µL	225 µL
5X WGS FX Mix	96 µL	475 µL
QIAseq FastSelect™ –rRNA HMR (8)	12 µL	12 µL
Side Reaction Reducer	48 µL	192 µL
FG Solution	170 µL	170 µL
ATP Solution	36 µL	290 µL
PAP Enzyme	96 µL	96 µL
PAP Dilution Buffer	192 µL	192 µL
T4 Polynucleotide Kinase	125 µL	125 µL
non-UMI Adapter	60 µL	275 µL
UMI Adapter	60 µL	275 µL
DNA Ligase	120 µL x 2	1100 µL
UPH Ligation Buffer, 2.5x	1152 µL	1500 µL x 3
MM RNA RT Primer	24 µL	115 µL
MM RNA TSO Primer	24 µL	115 µL
Buffer GE2 gDNA Elimination Buffer	72 µL	250 µL
US RT Buffer, 5X	150 µL	450 µL
100 mM DTT	20 µL	80 µL
10 mM dNTP	55 µL	235 µL
EZ Reverse Transcriptase	36 µL	96 µL x 2
RNase Inhibitor	12 µL	96 µL
Nuclease-free Water	10 mL	10 mL
QIAseq Beads	10 mL	38.4 mL

QIAseq Multimodal DNA/RNA UDI (24) **(DNA and RNA indexing for 24 samples)**	**(24)**
Catalog no.	**334852**
MDNA24X (QIAseq Multimodal MDNA Index Plate 24) Each plate allows indexing of 24 DNA samples. Each well in the plate is single use and contains 9 µL of UDI index pairs for DNA libraries.	1
MRNA-24X (QIAseq Multimodal MRNA Index Plate 24) Each plate allows indexing of 24 RNA samples. Each well in the plate is single use and contains 9 µL of UDI index pairs for RNA libraries.	1
HiFi Ultra Buffer	384 µL
HiFi Ultra Polymerase	192 µL
MM F-R Primer Mix	77 µL

QIAseq Multimodal DNA/RNA UDI Set A **(DNA and RNA indexing for 96 samples)**	**(96)**
Catalog nos.	**334855**
MDNA-96AX (QIAseq Multimodal MDNA Index 96 Set A) Each plate allows indexing of 96 DNA samples. Each well in the plate is single use and contains 9 µL of UDI index pairs for DNA libraries.	1
MRNA-96AX (QIAseq Multimodal MRNA Index 96 Set A) Each plate allows indexing of 96 RNA samples. Each well in the plate is single use and contains 9 µL of UDI index pairs for RNA libraries.	1
HiFi Ultra Buffer	1536 µL
HiFi Ultra Polymerase	850 µL
MM F-R Primer Mix	340 µL

Shipping and Storage

QIAseq Multimodal DNA/RNA Lib Kit (except QIAseq Beads and Nuclease-free Water) is shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. QIAseq Beads and Nuclease-free Water are shipped in a separate box on cold packs and should be stored immediately upon receipt at 2–8°C.

QIAseq Multimodal DNA/RNA index kits are shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer.

Intended Use

QIAseq Multimodal DNA/RNA Lib kits and QIAseq Multimodal DNA/RNA 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 QIAseq Multimodal DNA/RNA Lib Kit and QIAseq Multimodal DNA/RNA index kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Next-Generation Sequencing (NGS) revolutionizes the analysis of various genomic features, including single nucleotide variants (SNVs), Insertions-deletions (indels), copy number variants (CNVs), fusions, and gene expression levels. However, existing solutions present a limitation: users are typically required to employ separate workflows for DNA and RNA analysis, particularly for whole genome sequencing (WGS) and whole transcriptome sequencing (WTS). This not only increases complexity but also poses challenges, especially when dealing with low-yield samples. As a result, there is a growing need for integrated solutions that streamline both DNA and RNA analyses within a single workflow. Such integration would not only simplify the analysis process but also improve efficiency, particularly for samples with limited yields. By consolidating DNA and RNA inputs and analyses, researchers can more effectively explore the comprehensive genomic landscape, leading to deeper insights and discoveries in genomics research and clinical applications.

QIAseq Multimodal DNA/RNA Lib Kit enables construction of WGS and WTS libraries from total nucleic acid or separately isolated DNA and RNA. Workflows are provided to tag all DNA and RNA molecules with unique molecular indexes (UMIs). Resulting libraries can be sequenced directly or hybrid captured; for hybrid capture, the libraries are compatible with both QIAseq xHYB catalog and custom panels as well as third-party offerings. QIAseq Multimodal DNA/RNA Lib Kit facilitates sensitive DNA variant detection as well as fusions and gene expression detection from RNA on a genome- and transcriptome-wide scale from cells, tissue, and biofluids.

The QIAseq Multimodal DNA/RNA Lib Kit is part of a Sample to Insight solution for DNA and RNA analysis using NGS (Figure 1).

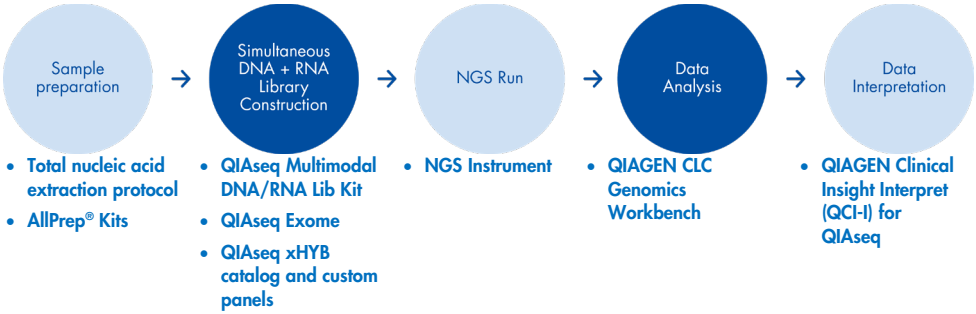

Figure 1. Overview of the Sample to Insight NGS workflow with QIAseq Multimodal DNA/RNA Lib Kit. The complete Sample to Insight procedure begins with total nucleic acid or AllPrep® (separate DNA and RNA) extractions. Next is library construction with the QIAseq Multimodal DNA/RNA Lib Kit and optional hybrid capture with QIAseq Exome or xHYB catalog/custom panels. Following NGS, data analysis is performed using the QIAseq Multimodal 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 DNA/RNA Lib Kit enables construction of WGS and WTS libraries. For DNA, the recommended input range is 10–100 ng for fresh samples or 50–250 ng for FFPE samples. For RNA, the recommended amount input range is 20–200 ng for fresh samples or 100–500 ng for FFPE samples. 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: input for DNA could be as low as 100 pg and RNA could be reduced to 10 ng; however, for the preparation of indexed WGS or WTS libraries to be used for hybridization capture, we recommend using 50 ng of input DNA or 100 ng of input RNA. The following reactions occur in a streamlined, single-tube workflow (Figure 2, next page).

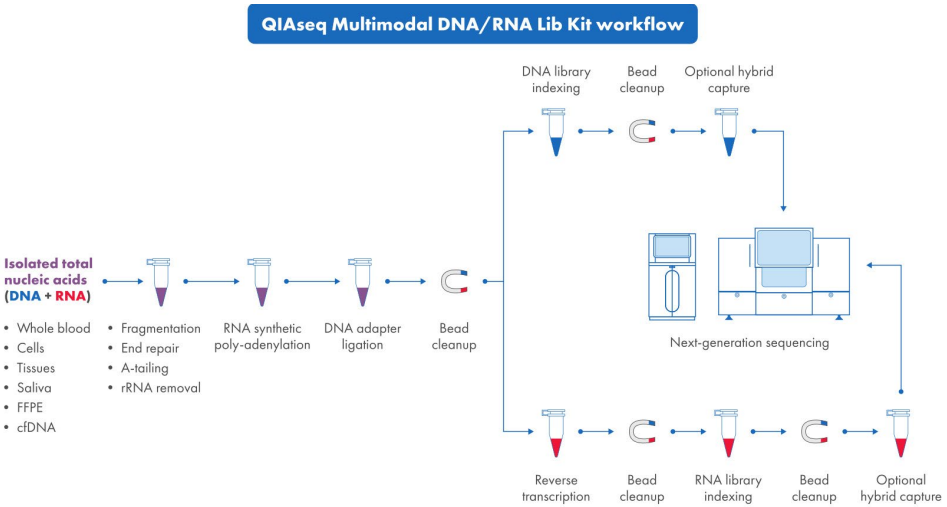

Figure 2. QIAseq Multimodal DNA/RNA Lib Kit workflow.

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. Built into the reaction is the elimination of human, mouse, and rat ribosomal RNA (rRNA) using QIAseq FastSelect –rRNA HMR. QIAseq FastSelect –Globin can also be added to eliminate globin mRNA when using samples derived from whole blood.

RNA polyadenylation

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

DNA ligation

Specific to DNA, two adapter options are provided. The non-UMI Adapter does not contain a UMI, while the UMI Adapter includes a 14-base fully random sequence UMI sequence and is additionally phased for optimal base diversity during sequencing. When hybrid capture is considered, the DNA adapters contain the TruSeq® adapter sequence. The non-UMI Adapter is recommended for the preparation of WGS libraries to be used for hybridization capture for QIAseq Human Exome or third-party vendor human Exome kits. For hybrid capture with QIAseq xHYB Custom Panel for somatic mutation detection applications, the use of the UMI Adapter is recommended.

Reverse transcription and template switching

Specific to RNA, reverse transcription and template switching are performed. For reverse transcription, the anchored oligo-dT primer is both phased and contains a 10-base fully random UMI sequence, allowing each RNA molecule to be tagged with a UMI. As the heat fragmentation is random, and each random fragment has been poly-adenylated, whole transcriptome library prep is achieved. When hybrid capture is considered, the reverse transcription and template switching oligos contain the Nextera® adapter sequence. QIAseq N-blockers are available (purchased separately) for effectively binding and blocking Nextera-compatible NGS adapters.

Library amplification/indexing

Universal PCR reactions, carried out separately on DNA and RNA libraries, enable optimal amplification of each library as well as unique dual indexing. Collectively, DNA and RNA libraries for a given sample have discrete unique dual indexes (UDIs). The UDI pairs are listed in Appendix A: DNA and RNA Index Layouts, page 45.

Hybrid Capture (purchased separately)

While QIAseq Multimodal DNA/RNA libraries are WGS and WTS, they are additionally compatible with the following hybrid capture solutions. The prepared DNA libraries include TruSeq® adapter sequence, while the RNA libraries include Nextera adapter sequence. As a result, specific N-blockers are required (and discussed in "Recommendations: Hybrid Capture").

- QIAseq Human Exome: Selectively captures exonic regions of interest, allowing for comprehensive analysis of protein-coding genes in the genome. Researchers can efficiently identify and analyze genetic variations such as single nucleotide variants (SNVs), insertions-deletions (indels), and other genomic alterations associated with diseases or traits of interest.
- QIAseq xHYB Human Hybrid Capture Panels: Curated genome-wide content including Carrier, Actionable Exome, and Mitochondrial Panels.
- QIAseq xHYB Custom Panels: Fully customizable panels, specifically for individual research needs.
- Third-party human exome vendors that were tested include: Integrated DNA Technologies (IDT), KAPA (Roche® Group), Agilent® (Agilent Technologies, Inc.), and Twist Bioscience.

Next-generation sequencing

QIAseq Multimodal DNA/RNA libraries are compatible with Illumina NGS platforms including iSeq® 100, MiniSeq®, MiSeq®, NextSeq® 500/550, NextSeq 1000/2000, HiSeq® 2500, HiSeq 3000/4000, NovaSeq® 6000, and NovaSeq X Plus. Additionally, QIAseq Multimodal DNA/RNA libraries are directly compatible with the Element AVITI™ benchtop sequencer.

With an added conversion step, QIAseq Multimodal DNA/RNA libraries are compatible with Complete Genomics/MGI, Singular Genomics, and Ultima Genomics sequencers.

Data analysis

Data from QIAseq Multimodal DNA/RNA Lib Kit 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 library preparation.

It is important to remove all cations and chelators from DNA or RNA preparations, therefore, make sure DNA or RNA is eluted in QIAGEN's Buffer EB or H_2O, not 1XTE buffer containing 1mM EDTA.

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 1 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 1. 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
QIAamp® ccfDNA/RNA Kit	Plasma and serum	55184

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.

The following are required:

- 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)
- QIAxcel® Connect System: QIAxcel DNA High Resolution Kit

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

Protocol: Nucleic Acid Fragmentation

Important points before starting

- This protocol describes fragmentation of nucleic acids samples.
- Recommended starting amounts of nucleic acid:

  The recommended starting amount of DNA is 10–100 ng. For samples where hybrid-capture will be performed, the minimal recommendation is 50ng. The recommended amount of RNA is 20–200 ng total RNA. When working with total nucleic acid samples, we recommend basing the input on the amount of quantified DNA.

  For FFPE samples, the recommended amount of FFPE DNA is 50–250 ng. For better results, we recommend that first-time users start with 250 ng FFPE RNA. When working with total nucleic acid samples, we recommend basing the input on the amount of quantified DNA.

  For cfDNA samples, the recommended starting amount is 20 ng.
- **Set up reactions on ice**.
- Unless specifically indicated, do not vortex any reagents or reactions.

Procedure

1. Thaw nucleic acid sample(s) 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 10x FX Buffer, QIAseq FastSelect –RNA HMR (8) at room temperature (15–25°C).
   2b. Mix by flicking the tube, and then centrifuge briefly.

   Note: 5X WGS FX Mix and Side Reaction Reducer should be removed from the freezer and placed on ice just before use. After use, immediately return the enzymes to the freezer.

3. Dilute an aliquot of QIAseq Fastselect –RNA HMR 1:10 (1:50 for FFPE samples) with Nuclease-free Water. Mix by flicking the tube, and then centrifuge briefly.
4. On ice, prepare the fragmentation mix according to Table 2 (standard samples) or Table 3 (cfDNA samples). Briefly centrifuge, mix by pipetting up and down 20 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 2. Reaction mix for Standard or FFPE samples

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
10x FX Buffer	2 µL
5X WGS FX Mix	4 µL
QIAseq FastSelect –rRNA HMR (diluted 1:10)	1 µL
Side Reaction Reducer	1.6 µL
Nuclease-free Water	11.4 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 3. Reaction mix for nucleic acid fragmentation of cfDNA samples

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
10x FX Buffer	2 µL
5x WGS FX Mix	4 µL
FG Solution	1.25 µL
QIAseq FastSelect –rRNA HMR (diluted 1:10)	1 µL
Nuclease-free Water	11.75 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.

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

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

Table 4. Incubation conditions for nucleic acid fragmentation

Step	Incubation temperature (°C)	Incubation time for standard sample (min)	Incubation time for cfDNA or FFPE (min)
1	4	1	1
2	32	16	12
3	65	30	30
4	60	2	2
5	55	2	2
6	37	5	5
7	25	5	5
8	4	Hold	Hold

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".

Protocol: RNA Polyadenylation

Important points before starting

- This product from "Protocol: Nucleic Acid Fragmentation" 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 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 and placed on ice just before use. After use, immediately return the enzymes to the freezer.
2. Dilute PAP Enzyme from 5 U/µL to 1 U/µL as follows:
   2a. Prepare 1x PAP Dilution Buffer by diluting 2 µL of the PAP Dilution Buffer with 18 µL Nuclease-free Water.
   2b. Use the 1x PAP Dilution Buffer to dilute an aliquot of the PAP Enzyme from 5 U/µL to 1 U/µL. Briefly centrifuge, mix by pipetting up and down 20 times, and then briefly centrifuge again.
3. Prepare the RNA polyadenylation mix according to Table 5. Briefly centrifuge, mix by pipetting up and down 20 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 RNA polyadenylation

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

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

4. Incubate the reactions in a thermal cycler according to Table 6. Use the instrument's heated lid.

Table 6. Incubation conditions for RNA polyadenylation

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

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

Protocol: DNA Ligation

Important points before starting

- The product from "Protocol: RNA Polyadenylation" is the starting material for this protocol.
- In this protocol, there is the option to use the non-UMI Adapter (that does not contain a UMI) or the UMI Adapter (that contains a UMI). Depending on the experiment of choice, choose one adapter or the other.
- The non-UMI Adapter is recommended for the preparation of WGS libraries to be used for hybridization capture for QIAseq Human Exome or third-party vendor human Exome kits.
- **Set up reactions on ice.**
- Do not vortex any reagents or reactions.
- **Note**: 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 non-UMI Adapter *or* UMI Adapter and UPH Ligation Buffer, 2.5x, at room temperature.

   Note: Non-UMI Adapter does not contain a UMI while the UMI Adapter contains a UMI.

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

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

2. Prepare the DNA ligation mix according to Table 7. Briefly centrifuge, mix by pipetting up and down 20 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 DNA ligation

Component	Volume/reaction (µL)
RNA polyadenylation reaction (already in tube)	25
UPH Ligation Buffer, 2.5x	40
Non-UMI Adapter *or* UMI Adapter*	2.5
DNA Ligase	10
Nuclease-free Water	22.5
Total	**100**

* Non-UMI Adapter does not contain a UMI while the UMI Adapter contains a UMI

Table 8. Adapter dilution factors for low input

Standard DNA amount	FFPE samples	Adapter dilution
100–999 pg		1:100
1–9 ng		1:10
	50 ng< input <100 ng	1:10

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

   Important: Do not use the heated lid.

Table 9. Incubation conditions for DNA ligation

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

4. Add 90 µL QIAseq Beads and mix by vortexing.
5. Incubate for 5 min at room temperature.
6. 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 1 min, and use a 10 µL pipette to remove the remaining supernatant.
7. 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.
8. 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.
9. 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.
10. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 52 µL Nuclease-free Water. Mix well by pipetting.
11. Return the tube/plate to the magnetic rack until the solution has cleared.
12. Transfer 50 µL of the supernatant to clean tubes/plate wells.
13. Add 55 µL QIAseq Beads and mix by vortexing.
14. Incubate for 5 min at room temperature.
15. 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 1 min, and use a 10 µL pipette to remove the remaining supernatant.

16. 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.
17. 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.
18. 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.
19. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 18 µL Nuclease-free Water. Mix well by pipetting.
20. Return the tube/plate to the magnetic rack until the solution has cleared.
21. For each sample, aliquot 7.5 µL of the eluate into two separate clean tubes/plate wells.
    Aliquot 1: Proceed to “Protocol: DNA Library Indexing”, page 26.
    Aliquot 2: Proceed to “Protocol: RNA Reverse Transcription”, page 30.
    Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: DNA Library Indexing

Important points before starting

- The starting material is a 7.5 µL sample aliquot from "Protocol: DNA Ligation".
- **Set up reactions on ice.**
- Do not vortex any reagents or reactions.
- **Use MDNA index plates, either MDNA-24X or MDNA-96AX.**
- **Note**: 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 DNA library indexing.
   1a. Thaw HiFi Ultra Buffer, 5x; MM F-R Primer Mix, and MDNA-24X or MDNA-96AX index plate at room temperature.
   1b. Mix by either flicking the tube or vortexing the index plate, and then centrifuge briefly. Plate should be centrifuged at 1000 x *g* for 1 min.
   Note: HiFi Ultra Polymerase should be removed from the freezer and placed on ice just before use. After use, immediately return the enzyme to the freezer.
2. Prepare the reactions according to Table 10. Briefly centrifuge, mix by pipetting up and down 20 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 10. Reaction mix for DNA indexing

Component	Volume/reaction (µL)
Sample aliquot (from Protocol: DNA Ligation)	7.5
HiFi Ultra Buffer, 5x	8
MM F-R Primer Mix	1.6
Nuclease-free Water	16.4
Well from MDNA-24X or MDNA-96AX index plate*	2.5
HiFi Ultra Polymerase	4
Total	**40**

* Ensure proper technique to prevent cross-contamination. Additionally ensure that every sample has a unique index and that no well is used twice.

3. Program a thermal cycler as described in Table 11, using cycle numbers described in Table 12.

   Important: Set up the ramp rate of the thermal cycler at ≤2°C/s.

Table 11. Cycling conditions for DNA indexing

Step	Time	Temperature (°C)
Hold	2 min	98
2-step cycling		
Denaturation	20 s	98
Annealing/Extension	1 min	60
Cycle number	See Table 12.	
Hold	3 min	72
Hold	∞	4

Table 12. Cycle number recommendations for DNA indexing, based on original sample input

DNA Input	Standard Sample (cycles)	FFPE sample (cycles)
100 pg	19	–
1 ng	16	–
10 ng	13	–
100 ng	10	13
250 ng	–	12

4. After the reaction is complete, add 36 µL QIAseq Beads, and then mix by vortexing or pipetting up and down several times.
5. Incubate for 5 min at room temperature.
6. 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.
7. 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.
8. Repeat the ethanol wash twice, for a total of 3 washes.

   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.
9. 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.

10. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 24 µL Nuclease-free Water. Mix well by pipetting.
11. Return the tube/plate to the magnetic rack until the solution has cleared.
12. Transfer 22 µL of the supernatant to clean tubes/plate wells.
13. The library is now ready for sequencing or hybrid capture. Proceed to "Recommendations: Library QC & Quantification". Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: RNA Reverse Transcription

Important points before starting

- The starting material is a 7.5 µL sample aliquot from "Protocol: DNA Ligation".
- **Set up reactions on ice.**
- Do not vortex any reagents or reactions.
- **Note**: 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 sample pre-treatment.

   Note: Buffer GE2 gDNA elimination buffer should be removed from the freezer and placed on ice just before use. After use, immediately return the tube to the freezer.

2. Prepare the sample pretreatment mix according to Table 13. Briefly centrifuge, mix by pipetting up and down 20 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 sample pretreatment

Component	Volume/reaction (µL)
Sample aliquot (from "Protocol: DNA Ligation)	7.5
Buffer GE2 gDNA elimination buffer	2
Total	**9.5**

3. Incubate the reactions in a thermal cycler according to Table 14. Use the instrument's heated lid.

Table 14. Incubation conditions for sample pretreatment

Step	Incubation temperature (°C)	Incubation time (min)
1	42	5
2	75	10
3	4	Hold

4. Prepare the reagents required for reverse transcription.
   4a. Thaw US RT Buffer, 5X; DTT (100 mM); dNTP (10 mM); MM RNA RT Primer; and MM RNA TSO at room temperature.
   4b. Mix by flicking the tubes and then centrifuge briefly.
   Note: The RNase Inhibitor and EZ Reverse Transcriptase should be removed from the freezer and placed on ice just before use. After use, immediately return the enzymes to the freezer.
5. To the treated sample, prepare the reverse transcription mix according to Table 15, next page. Briefly centrifuge, mix by pipetting up and down 20 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 15. Reaction mix for reverse transcription

Component	Volume/reaction (µL)
Treated Sample	9.5
US RT Buffer, 5X	4
DTT (100 mM)	0.5
dNTP (10 mM)	2
MM RNA RT Primer	1
MM RNA TSO	1
RNase Inhibitor	0.5
EZ Reverse Transcriptase	1.5
Total	**20**

6. Incubate the reactions in a thermal cycler according to Table 16. Use the instrument's heated lid.

Table 16. Incubation conditions for reverse transcription

Step	Incubation temperature (°C)	Incubation time (min)
1	4	1
2	42	90
3	70	10
4	4	1
5	4	Hold

7. Add 22 µL QIAseq Beads 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.

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.

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 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. 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 22 µL Nuclease-free Water.
14. Return the tube/plate to the magnetic rack until solution the solution has cleared.
15. Transfer 20 µL of the eluate to clean tubes/plate wells.
16. Add 22 µL QIAseq Beads and mix by vortexing or by pipetting up and down several times.
17. Incubate for 5 min at room temperature.
18. 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.

19. 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.
20. 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.
21. 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.
22. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 22 µL Nuclease-free Water.
23. Return the tube/plate to the magnetic rack until solution the solution has cleared.
24. Transfer 20 µL of the eluate to clean tubes/plate wells.
25. Proceed to "Protocol: RNA Library Indexing". Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: RNA Library Indexing

Important points before starting

- The starting material is the 20 µL sample from "Protocol: RNA Reverse Transcription".
- **Set up reactions on ice.**
- Do not vortex any reagents or reactions.
- **Use MRNA index plates, either MRNA-24X or MRNA-96AX.**
- **Note**: 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 RNA library indexing.
   1a. Thaw HiFi Ultra Buffer, 5x; MM F-R Primer Mix, and MRNA-24X or mRNA-96AX index plate at room temperature.
   1b. Mix by either flicking the tube or vortexing the index plate, and then centrifuge briefly. Plate should be centrifuged at 1000 x *g* for 1 min.

   Note: HiFi Ultra Polymerase should be removed from the freezer and placed on ice just before use. After use, immediately return the enzyme to the freezer.
2. Prepare the reactions according to Table 17. Briefly centrifuge, mix by pipetting up and down 20 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 RNA indexing

Component	Volume/reaction (µL)
Sample aliquot (from Protocol: RNA Reverse Transcription)	20
HiFi Ultra Buffer, 5x	8
MM F-R Primer Mix	1.6
Nuclease-free Water	3.9
Well from MRNA-24X or MRNA-96AX index plate*	2.5
HiFi Ultra Polymerase	4
Total	**40**

* Ensure proper technique to prevent cross-contamination. Additionally ensure that every sample has a unique index and that no well is used twice.

3. Program a thermal cycler as described in Table 18, using cycle numbers described in Table 19.

   Important: Set up the ramp rate of the thermal cycler at ≤2°C/s.

Table 18. Cycling conditions for RNA indexing

Step	Time	Temperature (°C)
Hold	2 min	98
2-step cycling		
Denaturation	20 s	98
Annealing/Extension	1 min	60
Cycle number	See Table 19.	
Hold	3 min	72
Hold	∞	4

Table 19. Cycle number recommendations for RNA indexing, based on original sample input

RNA Input (ng)	Standard Sample (cycle)	FFPE sample (cycle)
10	24	–
20	23	–
100	21	24
200	20	23
500	–	22

4. After the reaction is complete, add 36 µL QIAseq Beads, and then mix by vortexing or pipetting up and down several times.
5. Incubate for 5 min at room temperature.
6. 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.
7. 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.
8. Repeat the ethanol wash twice, for a total of 3 washes.

   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.
9. 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.

10. Remove the beads from the magnetic stand, and then elute the DNA from the beads by adding 24 µL Nuclease-free Water. Mix well by pipetting.
11. Return the tube/plate to the magnetic rack until the solution has cleared.
12. Transfer 22 µL of the supernatant to clean tubes/plate.
13. The library is now ready for sequencing or hybrid capture. Proceed to "Recommendations: Library QC & Quantification". Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Recommendations: Library QC & Quantification

NGS library QC

Perform QC with the QIAxcel Connect or similar instrument. 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).

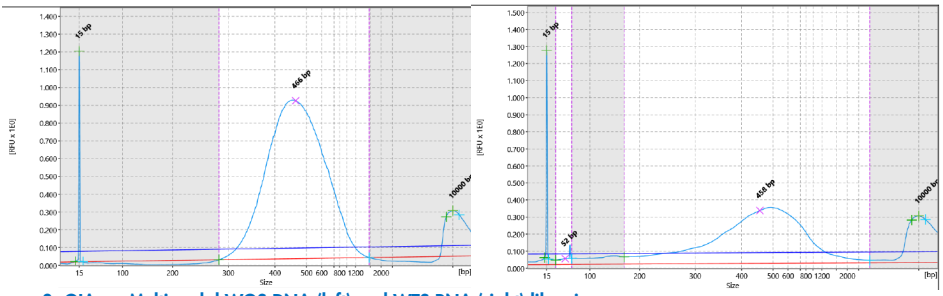

Figure 3. QIAseq Multimodal WGS DNA (left) and WTS RNA (right) libraries.

Preferred library quantification method

Quantify the library using Nanodrop® or QIAexpert if proceeding to "Hybrid Capture" or qPCR-based method if proceeding to sequencing. Once quantified, proceed to either "Recommendations: Hybrid Capture" or "Recommendations: NGS".

Recommendations: Hybrid Capture

While QIAseq Multimodal DNA/RNA libraries are WGS and WTS, they are additionally compatible with hybrid capture solutions (see Ordering Information). The prepared DNA libraries include TruSeq adapter sequence, while the RNA libraries include Nextera adapter sequence. As a result, when WTS are proceeded to hybridization, QIAseq One-4-All Blocking Oligos are required to be replaced with QIAseq N Blocking Oligos (cat. no. 334871).

Please refer to *QIAseq Human Exome Kit Handbook* or *QIAseq xHYB Human Hybrid Capture Panel Handbook* for human exome or capture panels (including Actionable Exome Panel, Carrier Panel, Mitochondrial Spike-in panel, or Custom Panels).

Determine the per-library amount (ng) according to the desired pool size referring to the guidelines in Table 20 or follow the recommendations in Table 21. Make sure to not exceed 3200 ng total input per capture pool.

Table 20. Guidelines for pooling indexed libraries for hybridization capture

Library pooling guidelines	
Recommended number of libraries per capture pool	8
Maximum total input per capture pool	3200 ng
Minimal input per library	200 ng

Table 21. Recommended library pooling strategy for hybridization capture

Number of indexed samples per pool	Amount of each indexed library per pool (ng)
1	200–500
2	200–500
3	200–500
4	200–500
5	200–500
6	200–500
7	200–500
8	200–400*

* Do not exceed a total DNA input of 3200 ng per pool.

Note: To maintain equal representation of libraries during sequencing, use the same input amount for all libraries. Using less than 200 ng input per library may result in reduced complexity.

Recommendations: NGS

Important points before starting

- **Note**: Recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System.
- **Note**: Paired-end sequencing **should** be used for QIAseq Multimodal libraries on Illumina platforms.
  - Read 1: 149 bp
  - Read 2: 149 bp
  - Index 1: 10 bp
  - 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.
- If only RNA libraries are sequenced, spike in 20% PhiX to increase diversity.

Recommended loading concentrations for sequencers

Table 22. Recommended loading concentrations for main sequencers

Instrument	Final Loading Concentration (pM)
iSeq	75
MiSeq	10
NextSeq 500/550	1.2
NextSeq 1000/2000	650
NovaSeq 6000	300
iSeq	75

General read allocation recommendations for WGS and WTS

For WGS library sequencing, 50M reads is recommended.

For WTS library sequencing, refer to Table 23 for read allocation:

Table 23. Recommended read allocation for WTS library sequencing

Total RNA input (ng)	Number of reads per WTS library (M)
20	20
50	40
100	50
200	60

For WTS library from FFPE sample, 20–40M reads is recommended.

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 in 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 or protocols in this handbook (for contact information, visit **support.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 PCR amplification	QIAseq beads need to be completely dried before elution. Ethanol carryover will affect PCR reaction efficiency.
Unexpected signal peaks	
a) Short peaks <200 bp	These are primer–dimers from PCR amplification reaction (<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 PCR amplification, library fragments are larger than the intended peak and can be a PCR artifact due to overamplification of the DNA or RNA library. Overamplification of the library won't affect the sequencing performance as long as qPCR-based method is used for library quantification. Decreasing the number of 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.

Appendix A: DNA and RNA Index Layouts

Sample sheets including index sequences for MDNA and MRNA can be downloaded from the "Resources" section of the product page.

Table 24. Layout of MDNA-24X (QIAseq Multimodal MDNA Index Plate 24)

	1	2	3	4	5	6	7	8	9	10	11	12
A	MDNA-001	MDNA-009	MDNA-017	–	–	–	–	–	–	–	–	–
B	MDNA-002	MDNA-010	MDNA-018	–	–	–	–	–	–	–	–	–
C	MDNA-003	MDNA-011	MDNA-019	–	–	–	–	–	–	–	–	–
D	MDNA-004	MDNA-012	MDNA-020	–	–	–	–	–	–	–	–	–
E	MDNA-005	MDNA-013	MDNA-021	–	–	–	–	–	–	–	–	–
F	MDNA-006	MDNA-014	MDNA-022	–	–	–	–	–	–	–	–	–
G	MDNA-007	MDNA-015	MDNA-023	–	–	–	–	–	–	–	–	–
H	MDNA-008	MDNA-016	MDNA-024	–	–	–	–	–	–	–	–	–

Table 25. Layout of MDNA-96AX (QIAseq Multimodal MDNA Index 96 Set A)

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

Table 26. Layout of MRNA-24X (QIAseq Multimodal MRNA Index Plate 24)

	1	2	3	4	5	6	7	8	9	10	11	12
A	MMRN-001	MMRN -009	MMRN -017	–	–	–	–	–	–	–	–	–
B	MMRN -002	MMRN -010	MMRN -018	–	–	–	–	–	–	–	–	–
C	MMRN -003	MMRN -011	MMRN -019	–	–	–	–	–	–	–	–	–
D	MMRN -004	MMRN -012	MMRN -020	–	–	–	–	–	–	–	–	–
E	MMRN -005	MMRN -013	MMRN -021	–	–	–	–	–	–	–	–	–
F	MMRN -006	MMRN -014	MMRN -022	–	–	–	–	–	–	–	–	–
G	MMRN -007	MMRN -015	MMRN -023	–	–	–	–	–	–	–	–	–
H	MMRN -008	MMRN -016	MMRN -024	–	–	–	–	–	–	–	–	–

Table 27. Layout of MRNA-96AX (QIAseq Multimodal MRNA Index 96 Set A)

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

Ordering Information

Product	Contents	Cat. no.
QIAseq Multimodal DNA/RNA Lib Kit (24)	Kit containing all reagents (except indexes) for multimodal (DNA and RNA) library preparation of 24 samples: 24 DNA and 24 RNA libraries	334842
QIAseq Multimodal DNA/RNA Lib Kit (96)	Kit containing all reagents (except indexes) for multimodal (DNA and RNA) library preparation of 96 samples: 96 DNA and 96 RNA libraries	334845
QIAseq Multimodal DNA/RNA UDI (24)	Kit containing all reagents required for sample indexing of 24 samples: 24 DNA and 24 RNA libraries	334852
QIAseq Multimodal DNA/RNA UDI Set A (96)	Kit containing all reagents required for sample indexing of 96 samples: 96 DNA and 96 RNA libraries.	334855
One-4-All Blocking Oligos (12rxn)	One-4-All Blocking Oligos (12rxn) contains oligos designed to bind and block all Illumina TruSeq®-compatible NGS adapters for 12 reactions of hybridization capture	334861
QIAseq N Blocking Oligos (12rxn)	QIAseq N Blocking Oligos (12rxn) contains oligos designed to bind and block all Illumina Nextera®-compatible NGS adapters for 12 reactions of hybridization capture	334871
QIAseq Multimodal Blocking Oligos(24rxn)	QIAseq Multimodal DNA/RNA UDI (96) contains One-4-All Blocking Oligos (12rxn) and QIAseq N Blocking Oligos (12rxn) - 24 reactions in total	334881

Product	Contents	Cat. no.
QIAseq Human Exome Kit (24)	For 3 hybridization reactions: Buffers, blocking reagents, Human Exome Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 24 whole genome libraries	333937
QIAseq Human Exome Kit (96)	For 12 hybridization reactions: Buffers, blocking reagents, Human Exome Probe Set, Streptavidin beads, wash buffers, and post capture enrichment reagents for hybrid capture of up to 96 whole genome libraries	333939
QIAseq xHYB Actionable Exome Panel (24)	For 3 hybridization reactions: Buffers, blocking reagents, Actionable Exome Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 24 whole genome libraries	333372
QIAseq xHYB Actionable Exome Panel (96)	For 12 hybridization reactions: Buffers, blocking reagents, Actionable Exome Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 96 whole genome libraries	333375
QIAseq xHYB Carrier Panel (24)	For 3 hybridization reactions: Buffers, blocking reagents, Carrier Screening Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 24 whole genome libraries	333362
QIAseq xHYB Carrier Panel (96)	For 12 hybridization reactions: Buffers, blocking reagents, Carrier Screening Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 96 whole genome libraries	333365

Product	Contents	Cat. no.
QIAseq xHYB Mitochondrial Panel (24)	Mitochondrial Probe Set for spike-in for 3 hybridization reactions	333382
QIAseq xHYB Mitochondrial Panel (96)	Mitochondrial Probe Set for spike-in for 12 hybridization reactions	333385
QIAseq xHYB Automation Kit	Excess volume of Streptavidin beads, wash buffers, and Post Capture Binding Buffer for automation purposes	333430
QIAseq xHYB Custom Human Panel	Only includes the custom probe set(s) needed for hybridization capture as well as 12 hybrid capture reactions, which are typically sufficient for 96 samples.	333175
QIAseq xHYB Human Reagent Kit	Contains all reagents required for hybrid capture-based target enrichment and must be purchased in addition to QIAseq xHYB Custom Human Panels. The kit contains 12 hybrid capture reactions, which are typically sufficient for 96 samples.	333195
Related products		
QIAamp® DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, collection tubes (2 mL), reagents and buffers	51304
QIAamp Circulating Nucleic Acid Kit (50)	For 50 DNA preps: QIAamp Mini Columns, Tube Extenders (20 mL), QIAGEN Proteinase K, carrier RNA, buffers, VacConnectors, and collection tubes (1.5 mL and 2 mL)	55114
AllPrep DNA/RNA Mini Kit (50)	For 50 minipreps: AllPrep DNA Spin Columns, RNeasy Mini Spin Columns, collection tubes, RNase-free water, and buffers	80204

Product	Contents	Cat. no.
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	762164

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

Document Revision History

Date	Changes
06/2024	Initial release

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