November 2021

QIAseq® miRNA UDI Library Kit Handbook

Precision small RNA library prep for Illumina® NGS systems using unique dual indexes (UDIs)

Sample to Insight

QIAGEN

Contents

Kit Contents

Note: To successfully build a miRNA library for sequencing, you must use both a QIAseq miRNA library Kit (Column A) and a QIAseq miRNA Index Kit with Unique Dual Indexes (Column B).

Column A: QIAseq miRNA Library Kits		**Column B: QIAseq miRNA Index Kits with Unique Dual Index Kits**	
331502	QIAseq miRNA Library Kit (12)	331601	QIAseq miRNA 12 Index Kit IL UDI (12)
331505	QIAseq miRNA Library Kit (96)	331615	QIAseq miRNA 96 Index Kit IL UDI-A (96)
		331625	QIAseq miRNA 96 Index Kit IL UDI-B (96)
		331635	QIAseq miRNA 96 Index Kit IL UDI-C (96)
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		331727	QIAseq miRNA 96 Index Kit UDI E–H (384)
		331738	QIAseq miRNA 96 Index Kit UDI A–H (768)

QIAseq miRNA Library Kit	(12)	(96)
Catalog no.	331502	331505
Number of reactions	12	96
Box 1 of 2		
QIAseq miRNA 3' Adapter	12 µl	96 µl
QIAseq miRNA 3' Buffer	24 µl	192 µl
QIAseq miRNA 3' Ligase	12 µl	96 µl
QIAseq miRNA RI	36 µl	288 µl
Nuclease-free Water	1 x 1.5 ml	2 x 1.5 ml
QIAseq miRNA 5′ Adapter	12 µl	96 µl
QIAseq miRNA 5' Buffer	24 µl	192 µl
QIAseq miRNA 5' Ligase	12 µl	96 µl
QIAseq miRNA NGS RT Initiator	24 µl	192 µl
QIAseq miRNA RT Primer	24 µl	192 µl
QIAseq miRNA RT Buffer	144 µl	1152 µl
QIAseq miRNA RT Enzyme	12 µl	96 µl
QIAseq miRNA Library Buffer	192 µl	1536 µl
HotStarTaq® DNA Polymerase	36 µl	288 µl
QIAseq miRNA 3C Primer Assay	240 µl	240 µl
QIAseq miRNA 5C Primer Assay	240 µl	240 µl
QIAseq miRNA RTC Primer Assay	240 µl	240 µl
Box 2 of 2		
Ligation Activator	120 µl	2 x 600 µl
QIAseq Beads	10 ml	38.4 ml
QIAseq Bead Binding Buffer	7 ml	54 ml

QIAseq miRNA 12 Index and QIAseq miRNA 96 Index Kits are sold separately and are necessary for library completion with unique dual indexes. These Index kits contain a QIAseq UDI Index plate with unique dual-index adapters. To multiplex more than 96 libraries in a single sequencing run, combine kits with different UDI adapter plates. For example, combining the QIAseq UDI Library UDI-A (or B or C or D) (96) Kit will allow the generation of 384 libraries with different sample indices for 384-plex sequencing.

QIAseq miRNA 12 Index Kit IL UDI	**(12)**
Catalog no.	**331601**
Number of reactions	**12**
96 well plate with 12 UDI sample indexes	1 plate
UDI 5' Adapter (96)	96 ul
UDI RT Initiator (96)	192 ul

QIAseq miRNA 96 Index Kit IL UDI	**UDI-A (96)**	**UDI-B (96)**	**UDI-C (96)**	**UDI-D (96)**
Catalog no.	**331615**	**331625**	**331635**	**331645**
Number of reactions	**96**	**96**	**96**	**96**
96 well plate with 96 UDI sample indexes	1 plate RUDI-96AA	1 plate RUDI-96BA	1 plate RUDI-96CA	1 plate RUDI-96DA
UDI 5' Adapter (96)	96 µl	96 µl	96 µl	96 µl
UDI RT Initiator (96)	192 µl	192 µl	192 µl	192 µl

QIAseq miRNA 96 Index Kit IL UDI	**UDI-E (96)**	**UDI-F (96)**	**UDI-G (96)**	**UDI-H (96)**
Catalog no.	**331655**	**331665**	**331675**	**331685**
Number of reactions	**96**	**96**	**96**	**96**
96 well plate with 96 UDI sample indexes	1 plate RUDI-96EA	1 plate RUDI-96FA	1 plate RUDI-96GA	1 plate RUDI-96HA
UDI 5' Adapter (96)	96 µl	96 µl	96 µl	96 µl
UDI RT Initiator (96)	192 µl	192 µl	192 µl	192 µl

QIAseq miRNA 96 Index Kit IL UDI Catalog no. Number of reactions	UDI A-D (384) 331717 384	UDI E-H (384) 331727 384	UDI A-H (768) 331738 768
96 well plate with 96 UDI sample indexes	1 plate each: RUDI-96AA RUDI-96BA RUDI-96CA RUDI-96DA	1 plate each: RUDI-96EA RUDI-96FA RUDI-96GA RUDI-96HA	1 plate each: RUDI-96AA RUDI-96BA RUDI-96CA RUDI-96DA RUDI-96EA RUDI-96FA RUDI-96GA RUDI-96HA
UDI 5' Adapter (96)	4 x 96 µl	4 x 96 µl	8 x 96 µl
UDI RT Initiator (96)	4 x 192 µl	4 x 192 µl	8 x 192 µl

QIAseq miRNA index plate layouts:

Each well contains 9 µl of pre-mixed sample indexing oligos. The plates have been sealed with a pierceable foil seal and each well is intended for single use. For more information on QIAseq UDI Adapter Plates and plate layouts, please refer to “Appendix C: QIAseq miRNA Unique Dual Indexes”.

Shipping and Storage

The QIAseq miRNA UDI Library Kit is shipped in 2 boxes.

- Box 1 is shipped on dry ice or blue ice. Upon receipt, all components in Box 1 should be stored immediately at –30 to –15°C in a constant-temperature freezer.
- Box 2 is shipped on blue ice. This should be stored immediately at 2–8°C. The Ligation Activator included in Box 2 should be stored at 2–8°C or stored at –30 to –15°C in a constant-temperature freezer.

When stored correctly, the QIAseq miRNA UDI Library Kit is good until the expiration date printed on the kit box.

QIAseq Index Kits are sold separately and are shipped on dry ice or blue ice. Upon receipt, all components in each box should be stored immediately at –30 to –15°C in a constant-temperature freezer.

Intended Use

All QIAseq miRNA products 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 miRNA UDI Library Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

QIAseq miRNA enables Sample to Insight®, precision next-generation sequencing (NGS) of mature miRNAs on Illumina NGS instruments. This highly optimized solution facilitates both enhanced differential expression analysis using integrated unique molecular indices (UMIs) and novel discovery of miRNA from cells, tissues, and biofluids. The required amount of template for a single QIAseq miRNA sequencing reaction can range from 500 ng to as little as 1 ng of purified total RNA.

In recent years, NGS has emerged as a highly advanced research tool for both high-throughput miRNA expression analysis and novel miRNA discovery. Among commercially available solutions, QIAseq miRNA defines a new generation of small RNA sequencing products and includes several distinct features not found in other sequencing kits. The standard QIAseq miRNA procedure does not require gel purification, excision, and elution, which substantially reduces the required hands-on time and noticeably shortens the length of the whole workflow. Proprietary methodology utilizing modified oligonucleotides efficiently prevents adapter–dimerization in the sequencing library and the highly optimized reaction chemistry virtually eliminates biases and background contaminants, facilitating the preparation of robust, miRNA-specific libraries. The kit also integrates UMIs into the reverse transcription process, enabling unbiased and accurate miRNome-wide quantification of mature miRNAs by NGS. Should a library fail presequencing quality control (QC), in-line controls are included in the library generation procedure to allow the use of real-time PCR for fast and efficient troubleshooting. Both primary and secondary data analysis solutions have been developed to facilitate rapid and robust UMI counting, miRNA mapping, and differential expression analysis. Overall, QIAseq miRNA offers an unrivaled Sample to Insight solution for differential expression analysis and discovery of novel miRNAs using NGS (Figure 1).

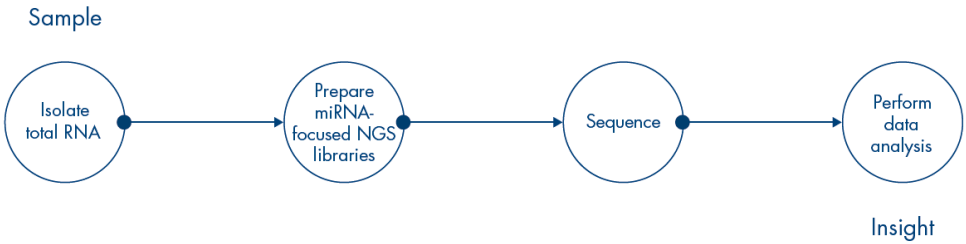


Figure 1. QIAGEN's Sample to Insight QIAseq miRNA workflow.

Principle and procedure

Mature miRNAs are naturally occurring, 22-nucleotide, noncoding RNAs that mediate post-transcriptional gene regulation. Unlike most cellular RNAs, mature miRNAs possess both a 3' hydroxyl group and a 5' phosphate group. This allows adapters to be specifically ligated to both the 3' end and 5' end of miRNAs enabling universal reverse transcription and library preparation of mature miRNAs, while minimizing the background from other RNA species. In addition, the QIAseq miRNA UDI Library Kit enables library preparation and multiplexing of up to 384 samples with unique dual indexes. QIAseq UDI Adapters use a fixed combination of two unique index barcode motives per adapter molecule. Therefore, each single-index motive is only used once on any UDI Adapter plate. Usage of UDI adapters effectively mitigates the risk of read misassignment due to index hopping. This is enabled by filtering misassigned reads during the demultiplexing of individual samples, thus generating highly accurate output data.

Universal cDNA synthesis and library preparation of miRNA

In an unbiased reaction, adapters are ligated sequentially to the 3' and 5' ends of miRNAs. Subsequently, universal cDNA synthesis with UMI assignment, cDNA cleanup, library amplification, and library cleanup are performed. Proprietary methodology using modified oligonucleotides virtually eliminates the presence of adapter–dimers in the sequencing library, effectively removing a major contaminant often observed during sequencing. Additionally, the kit is designed to also minimize the presence of hY4 Y RNA, which is often observed in high levels in serum and plasma samples.

The following reactions are part of the workflow (Figure 2):

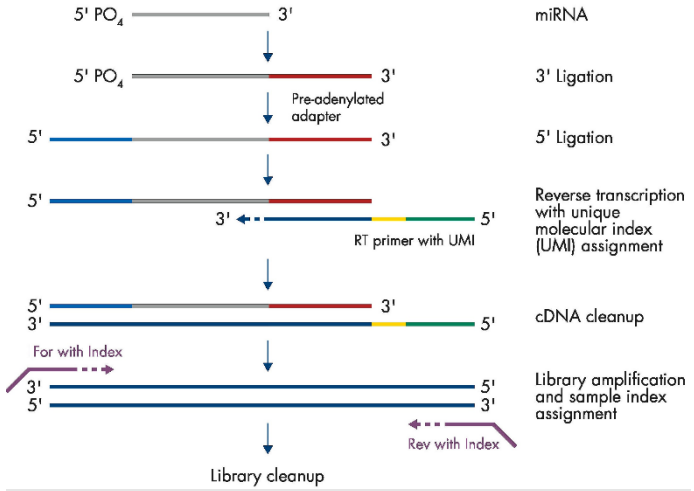


Figure 2. miRNA sequencing library preparation using the QIAseq miRNA Library Kit. Specially designed 3' and 5' adapters are ligated to mature miRNAs. The ligated miRNAs are then reverse transcribed to cDNA using a reverse transcription (RT) primer with a UMI. No libraries are prepared from adapter–dimers. Following cDNA cleanup, library amplification occurs with a forward primer that has a unique index and the reverse primer has another unique index for dual indexing. Following a final library cleanup, the miRNA library is then ready for QC and subsequent NGS.

- **3' ligation**: A preadenylated DNA adapter is ligated to the 3' ends of all miRNAs. The QIAseq miRNA 3' Ligase is highly optimized for efficient ligation as well as prevention of undesired side products.
- **5' ligation**: An RNA adapter is ligated to the 5' end of mature miRNAs.
- **cDNA synthesis**: The reverse transcription (RT) primer contains an integrated UMI. The RT primer binds to a region of the 3' adapter and facilitates conversion of the 3'/5' ligated miRNAs into cDNA while assigning a UMI to every miRNA molecule. During reverse transcription, a universal sequence is also added that is recognized by the sample indexing primers during library amplification.
- **cDNA cleanup**: After reverse transcription, a cleanup of the cDNA is performed using a streamlined magnetic bead-based method.

- **Library amplification**: Library amplification is accomplished by using an aliquot from a single well of the plate from the QIAseq miRNA 12 or QIAseq miRNA 96 Index Kit. In each well, a unique i5 index is premixed with a unique i7 index. The premixed oligos will be used in a PCR reaction to assign each sample a unique dual index. The unbiased amplification of all miRNAs in a single reaction ensures that sufficient target is present for NGS.
- **Library cleanup**: After library amplification, a cleanup of the miRNA library is performed using a streamlined magnetic bead-based method.

NGS on Illumina NGS systems

miRNA sequencing libraries prepared with the QIAseq miRNA UDI Library Kit can be sequenced using an Illumina NGS system (iSeq®, MiSeq®, MiniSeq®, NextSeq® 500/550, HiSeq® 3000, HiSeq 4000, and NovaSeq® 6000). QIAseq miRNA UDI Library Kit derived libraries require 72 bp single reads with 10 bp dual indexing. It is recommended to allocate 5–10 million reads per sample. A 50 bp single read protocol can be used if there is no desire to include the UMIs. If a 50 bp single read protocol is used, data analysis cannot be performed using the QIAseq miRNA Data Analysis pipelines on GeneGlobe.

Data Analysis using RNA-seq Analysis Portal

QIAseq miRNA Library kits include access to online NGS analysis tools through GeneGlobe Analyze at **www.qiagen.com**. These online tools allow researchers to upload sequencing files and perform alignment to commonly used reference databases, read mapping, UMI counting, and calculate differential expression of miRNAs with statistics. The RNA-seq analysis portal includes modules for visualization and filtering of data, access to the QIAGEN knowledge base for upstream, downstream and pathway mapping, and prioritization and filtering of data for further verification by digital PCR or real-time PCR. For a preview of data and capabilities, please visit **https://geneglobe.qiagen.com/analyze**.

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.

- Nuclease-free pipette tips and tubes
- Microfuge tubes (1.5–2 ml)
- PCR tubes (0.2 ml individual tubes or tubes strips) (VWR cat. no. 20170-012 or 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 cat. no. 12331D)
- **Library QC Option 1**:
  - 2100 Bioanalyzer® (Agilent)
  - Agilent High Sensitivity DNA Kit (Agilent cat. no. 5067-4626)
- **Library QC Option 2**:
  - PAGE gel-related equipment and consumables to prepare and run a 6% PAGE TBE gel
- **Library Concentration Readings**:
  - Qubit™ Fluorometer (Thermo Fisher Scientific)
  - Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific cat. no. Q32854)
  - Qubit Assay Tubes (Thermo Fisher Scientific cat. no. Q32856)

Important Notes

- The QIAseq miRNA UDI Library Kit has been optimized to prepare miRNA (and other similarly sized RNAs with a 3' hydroxyl group and a 5' phosphate group such as piRNA) sequencing libraries for use with Illumina sequencers.

  Total RNA containing miRNA is the required starting material for the QIAseq miRNA UDI Library Kit. It is not necessary to enrich for small RNA. QIAGEN provides a range of solutions for the purification of total RNA including miRNA (Table 1).

Table 1. Recommended kits for purification of total RNA containing miRNA

Kit	Cat. no.	Starting material
miRNeasy Micro Kit	217084	Small amounts of cells and tissue
miRNeasy Mini Kit	217004	Animal/human tissues and cells
miRNeasy 96 Kit	217061	Animal/human tissues and cells
miRNeasy FFPE Kit	217504	FFPE tissue samples
miRNeasy Serum/Plasma Kit	217184	Animal and human plasma and serum
miRNeasy Serum/Plasma Advanced Kit	217204	Animal and human plasma and serum
QIAamp ccfDNA/RNA Kit	55184	Animal and human plasma and serum
exoRNeasy Midi Kit	77144	Animal and human plasma and serum
exoRNeasy Maxi Kit	77164	Animal and human plasma and serum

- Ensure that total RNA samples are of high quality relative to their sample type. For additional information, please see "Appendix B: General Remarks on Handling RNA".
  RNA quantification: Determine the concentration and purity of total RNA isolated from cells and fresh/frozen tissues by measuring the absorbance in a spectrophotometer. As the spectral properties of nucleic acids are highly dependent on pH, we recommend preparing dilutions and measure absorbance in 10 mM Tris·Cl, pH 7.5, instead of RNase-free water. Pure RNA has an A_{260}:A_{280} ratio of 1.9–2.1 in 10 mM Tris·Cl, pH 7.5. It is not useful to assess the concentration and purity of total RNA derived from fluids and/or exosomes.

  RNA integrity: The integrity and size distribution of total RNA from cells and fresh/frozen tissue can be confirmed using an automated analysis system (such as the QIAxcel® Advanced System or the Agilent 2100 Bioanalyzer) that assesses RNA integrity using an RNA integrity score (RIS) or RNA integrity number (RIN). Although the RIN should ideally

be ≥8, successful miRNA library prep is still possible with samples whose RIN values are ≤8. However, for samples with low RIN values, the sequencing reads allocated per sample should be increased to allow for RNA degradation products. This is also the case with FFPE-derived RNA samples, which typically have low RIN values. It is not useful to assess the RNA integrity of total RNA derived from fluids and/or exosomes.

- When working with cell and tissue samples, the recommended starting amount of total RNA is 100 ng. The protocol can be used with 1–500 ng of total RNA.
- When working with serum and plasma samples, the recommended starting amount of total RNA is 5 µl of the RNA eluate when 200 µl of serum/plasma has been processed using the miRNeasy Serum/Plasma Kit or miRNeasy Serum/Plasma Advanced Kit.
- When working with exosome samples prepared from serum and plasma samples, the recommended starting amount of total RNA is 5 µl of the RNA eluate when 1 ml of serum/plasma has been processed using the exoRNeasy kits.
- Ensure reaction components are added in the order listed.
- Ensure reactions are thoroughly mixed, as well as prepared and incubated at the recommended temperatures. Due to the viscosity of the ligation reactions, correct preparation is crucial for a successful experiment.
- If the workflow is not expected to be completed in one day, convenient stopping points are indicated at the end of particular sections, including "Protocol: cDNA Cleanup" and "Protocol: Library Sample Indexing and Amplification Using QIAseq miRNA 12 Index Kit or QIAseq miRNA 96 Index Kits".
- During setup of the sequencing run, select **FASTQ Only**. To make use of the UMIs, the recommended protocol is 72 bp single read with 10 bp dual indexing. A 50 bp single read protocol can be used if there is no desire to include the UMIs, but the QIAseq miRNA primary data analysis pipeline cannot be used.

Protocol: 3' Ligation

Important points before starting

- When working with cell and tissues samples, the recommended starting amount of total RNA is 100 ng.
- When working with serum and plasma samples, the recommended starting amount of total RNA is 5 µl of the RNA eluate when 200 µl of serum/plasma have been processed using either the miRNeasy Serum/Plasma Advanced Kit or miRNeasy Serum/Plasma Kit. The recommended starting amount of total RNA is 5 µl of the RNA eluate when 1 ml of serum/plasma has been processed using the exoRNeasy kits.
- When working with low total RNA inputs amounts or serum/plasma samples, the QIAseq miRNA 3' Adapter must be diluted according to Table 2.
- Set up the 3' ligation reactions on ice, adding the components in the order listed.
- The 3' ligation reactions are very viscous. To mix, pipet slowly and thoroughly (pipet up and down at least 15–20 times).
- Do not vortex QIAseq miRNA RI, QIAseq miRNA 3' Ligase, template RNA, or the 3' ligation reactions.
- Upon completion of the 3' ligation reactions, proceed immediately to "Protocol: 5' Ligation".

Procedure

1. Thaw template RNA on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and return to ice.
2. Prepare reagents required for the 3' ligation reactions. Thaw QIAseq miRNA 3' Adapter, QIAseq miRNA 3' Buffer, Ligation Activator, and Nuclease-free Water at room temperature (15–25°C). Mix each solution by flicking the tubes. Centrifuge the tubes briefly to collect any residual liquid from the sides of the tubes and keep at room temperature.

   Remove QIAseq miRNA RI and QIAseq miRNA 3' RNA Ligase from the –30 to –15°C freezer just before use, and place on ice. Return both enzymes to the freezer immediately after use.

3. If working with low RNA inputs or serum/plasma samples, dilute the QIAseq miRNA 3' Adapter using nuclease-free water according to Table 2. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly again.

Table 2. Dilution of the QIAseq miRNA 3' Adapter

Template RNA input (total RNA)	Adapter dilution
500 ng	Use undiluted
100 ng	Use undiluted
10 ng	Dilute 1:5
1 ng	Dilute 1:20
Serum/Plasma	Dilute 1:5

4. On ice, prepare the 3' ligation reaction according to Table 3. Briefly centrifuge, mix by pipetting up and down 15–20 times, and centrifuge briefly again.

   Important: Pipet slowly when mixing the reaction. Ligation Activator is very viscous.

   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 3. Setup of 3' ligation reactions

Component	Volume/reaction
Nuclease-free Water	Variable
QIAseq miRNA 3' Adapter*	1 µl
QIAseq miRNA RI	1 µl
QIAseq miRNA 3' Ligase	1 µl
QIAseq miRNA 3' Buffer	2 µl
Ligation Activator	10 µl
Template RNA (added in step 5)	Variable†‡
Total volume	**20 µl**

* For low input and serum/plasma RNA, the QIAseq miRNA 3' Adapter must be diluted according to Table 2.

† For cell and tissue samples, the recommended starting amount of total RNA is 100 ng.

‡ For serum/plasma samples, the recommended starting amount of total RNA is 5 µl of the RNA eluate when 200 µl of serum/plasma has been processed using either the miRNeasy Serum/Plasma Advanced Kit or the miRNeasy Serum/Plasma Kit. The recommended starting amount of total RNA is 5 µl of the RNA eluate when 1 ml of serum/plasma has been processed using the exoRNeasy kits.

5. Add template RNA to each tube containing the 3' ligation Master Mix. Briefly centrifuge, mix by pipetting up and down 15–20 times, and centrifuge briefly again.

   Important: Pipet slowly to mix. The reaction mix is very viscous.

6. Incubate for 1 h at 28°C.
7. Incubate for 20 min at 65°C.
8. Hold at 4°C.

   Important: Hold at 4°C for at least 5 min.

9. Proceed immediately to "Protocol: 5' Ligation".

Protocol: 5' Ligation

Important points before starting

- The UDI 5′ Adapter is provided with the QIAseq miRNA 12 Index Kit IL UDI and QIAseq miRNA 96 Index Kit IL UDI.

  Important: Do not use the QIAseq miRNA 5′ Adapter provided with the QIAseq miRNA Library Kit.
- The entire 20 µl 3' ligation reaction completed in "Protocol: 3' Ligation" is the starting material for the 5' ligation reaction.
- The 5' ligation components are added directly to the tube containing the completed 3' ligation reaction.
- When working with low RNA inputs or serum/plasma samples, the UDI 5' Adapter must be diluted according to Table 4.
- Set up the 5' ligation reactions on ice, adding the components in the order listed.
- The 5' ligation reactions are very viscous. Pipet slowly and thoroughly (pipet up and down 15–20 times) to mix the reaction.
- Do not vortex the QIAseq miRNA RI, QIAseq miRNA 5' Ligase, or 5' ligation reactions.
- Upon completion of the 5' ligations reactions, proceed immediately to "Protocol: Reverse Transcription".

Procedure

1. Prepare reagents required for the 5' ligation reactions. Thaw UDI 5' Adapter (supplied in the QIAseq miRNA 12 Index Kit IL UDI and QIAseq miRNA 96 Index Kit IL UDI Kits) and QIAseq miRNA 5' Buffer at room temperature. Mix by flicking the tube. Centrifuge the tube briefly to collect residual liquid from the sides of the tube and keep at room temperature.

Remove QIAseq miRNA RI and QIAseq miRNA 5' Ligase from the –30 to –15°C freezer just before preparation of the Master Mix, and place on ice. Return both enzymes to the freezer immediately after use.

2. If working with low RNA inputs or serum/plasma samples, dilute the UDI 5' Adapter using nuclease-free water according to Table 4. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly again.

Table 4. Dilution of the UDI 5' Adapter

Template RNA Input (total RNA)	Adapter dilution
500 ng	Use undiluted
100 ng	Use undiluted
10 ng	Dilute 1:2.5
1 ng	Dilute 1:10
Serum/Plasma	Dilute 1:2.5

3. On ice, prepare the 5' ligation reaction according to Table 5, adding the components in the order listed. Briefly centrifuge, mix by pipetting up and down 10–15 times, and centrifuge briefly again.

   Important: Pipet slowly when mixing the reaction. The reaction mix is very viscous.

   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. Setup of 5' ligation reactions

Component	Volume/reaction
3' ligation reaction (already in tube)	20 µl
Nuclease-free water	15 µl
QIAseq miRNA 5' Buffer	2 µl
QIAseq miRNA RI	1 µl
QIAseq miRNA 5' Ligase	1 µl
UDI 5' Adapter*	1 µl
Total volume	**40 µl**

* For low input and serum/plasma RNA, the UDI 5' Adapter must be diluted according to Table 4.

4. Incubate for 30 min at 28°C.
5. Incubate for 20 min at 65°C.
6. Hold at 4°C.
7. Proceed immediately to “Protocol: Reverse Transcription”.

Protocol: Reverse Transcription

Important points before starting

- The entire 40 µl 5' ligation reaction completed in "Protocol: 5' Ligation" is the starting material for the reverse transcription reaction.
- The reverse transcription components are added directly to the tube containing the completed 5' ligation reaction.
- When working with low RNA inputs or serum/plasma samples, the QIAseq miRNA RT Primer must be diluted according to Table 7.
- Set up reverse transcription reactions on ice.
- Do not vortex the QIAseq miRNA RI, QIAseq miRNA RT Enzyme, or reverse transcription reactions.
- Be certain to use the UDI RT Initiator (provided with the QIAseq miRNA 12 Index Kit IL UDI or QIAseq miRNA 96 Index Kit IL UDI).

  Important: Do not use the QIAseq miRNA NGS RT Initiator provided with the QIAseq miRNA Library Kit.
- Upon completion of the reverse transcription reactions, proceed immediately to "Protocol: Preparation of QIAseq miRNA Beads (QMN Beads)".

  Note: This protocol can be performed while the reverse transcription reactions are incubating.

Procedure

1. Prepare reagents required for the reverse transcription reactions. Thaw UDI RT Initiator (supplied in the QIAseq miRNA 12 Index Kit IL UDI and QIAseq miRNA 96 Index Kit IL UDI Kits), QIAseq miRNA RT Buffer, and QIAseq miRNA RT Primer at room temperature. Mix by flicking the tube. Centrifuge the tubes briefly to collect residual liquid from the sides of the tubes, and keep at room temperature.

Remove QIAseq miRNA RI and QIAseq miRNA RT Enzyme from the –30 to –15°C freezer just before preparation of the Master Mix, and place on ice. Return both enzymes to the freezer immediately after use.

2. Add 2 µl UDI RT Initiator to each tube. Briefly centrifuge, mix by pipetting up and down 15–20 times, and centrifuge briefly again.
3. Incubate the tubes as described in Table 6.

Table 6. Incubation of tubes with UDI RT Initiator

Time	Temperature
2 min	75°C
2 min	70°C
2 min	65°C
2 min	60°C
2 min	55°C
5 min	37°C
5 min	25°C
∞*	4°C

* Hold until setup of the RT reaction.

4. If working with low RNA inputs or serum/plasma samples, dilute the QIAseq miRNA RT Primer using nuclease-free water according to Table 7.

Table 7. Dilution of the QIAseq miRNA RT Primer

Template RNA input (total RNA)	RT Primer dilution
500 ng	Use undiluted
100 ng	Use undiluted
10 ng	Dilute 1:5
1 ng	Dilute 1:20
Serum/Plasma	Dilute 1:5

5. On ice, prepare the reverse transcription reaction according to Table 8. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly 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 8. Setup of reverse transcription reactions

Component	Volume/reaction
5' ligation reaction + QIAseq miRNA RT Initiator (already in tube)	42 µl
QIAseq miRNA RT Primer*	2 µl
Nuclease-free Water	2 µl
QIAseq miRNA RT Buffer	12 µl
QIAseq miRNA RI	1 µl
QIAseq miRNA RT Enzyme	1 µl
Total volume	**60 µl**

* For low input and serum/plasma RNA, the QIAseq miRNA RT Primer must be diluted according to Table 7.

6. Incubate for 1 h at 50°C.
7. Incubate for 15 min at 70°C.
8. Hold at 4°C.

   Important: Hold at 4°C for at least 5 min.

9. Proceed to "Protocol: Preparation of QIAseq miRNA Beads (QMN Beads)".

Protocol: Preparation of QIAseq miRNA Beads (QMN Beads)

Important points before starting

- This protocol prepares the QIAseq miRNA Beads, hereafter referred to as QMN Beads. QIAseq Beads are rebuffered with QIAseq Bead Binding Buffer to create QMN Beads.
- **Important**: QIAseq Beads and the subsequently prepared QMN Beads need to be homogenous. This necessitates working quickly and resuspending the beads thoroughly immediately before use. If a delay in the protocol occurs, simply vortex the beads again.
- **Important**: After preparation, the QMN Beads need to be placed on ice.

Procedure

1. Thoroughly vortex QIAseq Beads and QIAseq Bead Binding Buffer to ensure that the beads are in suspension and homogenously distributed. Do not centrifuge the reagents.

   Important: QIAseq Beads need to be homogenous. This necessitates working quickly and thoroughly resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads again.

2. Carefully add 400 µl of QIAseq Beads (bead storage buffer is viscous) to a 2 ml microfuge tube. This amount of beads is sufficient to perform "Protocol: cDNA Cleanup" and the cleanup associated with library amplification for one sample. Briefly centrifuge and immediately separate beads on a magnet stand.

   Note: Beads for up to 4 samples (1.6 ml) can be prepared at one time in a single 2 ml tube. If beads for multiple samples are processed together, simply scale up the amounts of QIAseq Beads and QIAseq Bead Binding Buffer added below.

3. When beads have fully migrated, carefully remove and discard the supernatant.

   Note: At this step, it is acceptable to leave a small amount of supernatant in the tube.

4. Remove the tube from the magnet stand, and carefully pipet (buffer is viscous) 150 µl of QIAseq Bead Binding Buffer onto the beads. Thoroughly vortex to completely resuspend the bead pellet. Briefly centrifuge and immediately separate the beads on a magnet stand.

5. When beads have fully migrated, carefully remove and discard the supernatant.

   Note: Without disturbing the beads, ensure that as much supernatant as possible has been removed.

6. Remove the tube from the magnet stand and carefully pipet 400 µl of QIAseq Bead Binding Buffer onto the beads (buffer is viscous). Thoroughly vortex to completely resuspend the bead pellet.

   Preparation of the QMN Beads is now complete. If the beads will not be used immediately, store beads on ice or at 2–8°C.

   Note: QMN Beads can be stored at 2–8°C for up to one week.

7. Proceed to “Protocol: cDNA Cleanup“.

Protocol: cDNA Cleanup

Important points before starting

- The entire 60 µl cDNA synthesis completed in "Protocol: Reverse Transcription" is the starting material for the cleanup procedure.
- The QMN Beads prepared in "Protocol: Preparation of QIAseq miRNA Beads (QMN Beads)" are required for the cleanup procedure.
- Beads cleanups can be performed in tubes or plates. When working with plates, perform brief centrifugations at 2000 rpm for 2 min.
- Prepare fresh 80% ethanol using nuclease-free water.
- **Important**: Following ethanol washes, beads must be completely dried. Specific recommendations are given to remove excess ethanol.

Procedure

1. Ensure that the QMN 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 again.
2. Centrifuge the tubes/plates containing the cDNA reactions.
3. Add 143 µl of QMN Beads to tubes/plates containing the cDNA reactions. Vortex for 3 s and centrifuge briefly.

   Note: When working with plates, centrifuge at 2000 rpm for 2 min.

   Note: If plates are warped, transfer mixtures to new plates.
4. Incubate for 5 min at room temperature.
5. Place the tubes/plates on a magnet stand for ~4 min or until the beads have fully migrated.

   Note: Ensure that the beads have fully migrated before proceeding.

6. Discard the supernatant and keep the beads.

   Note: Do not remove the tubes/plates from the magnet stand.

7. With the beads still on the magnet stand, add 200 µl of 80% ethanol. Immediately remove and discard the ethanol wash.

8. Repeat the wash by adding 200 µl of 80% ethanol. Immediately remove and discard the second ethanol wash.

   Important: Completely remove all traces of ethanol after the second wash. Briefly centrifuge (centrifuge plates at 2000 rpm) and return the tubes/plates to the magnetic stand. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.

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

   Note: Visually inspect that the pellets are completely dry and that all residual ethanol has evaporated. Residual ethanol can hinder amplification efficiency in the subsequent library amplification reactions. Depending on humidity, extended drying time may be required.

10. With the beads still on the magnetic stand, elute the DNA by adding 17 µl of nuclease-free water to the tubes/plates. Subsequently close/cover and remove the tubes/plates from the magnetic stand.

11. Carefully pipet up and down until all the beads are thoroughly resuspended, briefly centrifuge, and incubate at room temperature for 2 min.

12. Return the tubes/plates to the magnetic stand for ~2 min or until the beads have fully migrated.

    Note: Ensure that the beads have fully migrated before proceeding.

13. Transfer 15 µl of eluted DNA to new tubes/plates.

14. Proceed to "Protocol: Library Sample Indexing and Amplification Using QIAseq miRNA 12 Index Kit or QIAseq miRNA 96 Index Kits". Alternatively, the completed cDNA cleanup product can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Library Sample Indexing and Amplification Using QIAseq miRNA 12 Index Kit or QIAseq miRNA 96 Index Kits

Important points before starting

- This library amplification protocol uses plate UDIs from QIAseq miRNA 12 Index Kit or QIAseq miRNA 96 Index Kits.
- 15 µl of the product from "Protocol: cDNA Cleanup" is the starting material for the library amplification procedure.
- Set up library amplification reactions on ice.
- Do not vortex the HotStarTaq DNA Polymerase or library amplification reactions.
- **Important**: During bead cleanups, beads must be completely dried following the ethanol washing step. Specific recommendations are given to remove excess ethanol.

Procedure

1. Prepare reagents required for the library amplification reactions. Thaw QIAseq miRNA Library Buffer, and required index plates. Mix by flicking the tube or plate, and centrifuge the tube or plate briefly to collect residual liquid from the sides of the tube and plate.

   Remove HotStarTaq DNA Polymerase from the –30 to –15°C freezer just before preparation of the Master Mix, and place on ice. Return HotStarTaq DNA Polymerase to the freezer immediately after use.

2. Open the QIAseq RUDI Index plate and pierce the wells required for amplification to assign each sample a unique index.

   Note: This is a pierceable plate that contains both a i5 and i7 UDI pair in every well. The layout is described in Table 13 to Table 21.

   Note: During the reaction setup in step 3, components from QIAseq RUDI are added directly to the plate.

3. On ice, prepare the library amplification reaction according to Table 9. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly again.

   Note: Reaction components are added first with the addition of the indexes from plate QIAseq RUDI added last to assign each sample a unique dual index.

   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. Setup of library amplification reactions when using plate indices

Component	Volume/reaction
Product from "Protocol: cDNA Cleanup"	15 µl
QIAseq miRNA Library Buffer	10 µl
HotStarTaq DNA Polymerase	1.5 µl
Sample index from 1 well of QIAseq miRNA 12 or QIAseq miRNA 96 UDI Index Plate *	2 µl
Nuclease-free water	21.5 µl
Total volume	**50 µl**

* Up to 768 unique QIAseq miRNA UDI indexes are available for use.

4. Program the thermal cycler according to Table 10. The correct number of cycles depends on the original RNA input and is shown in Table 11.

Table 10. Library amplification protocol

Step	Time	Temperature
Hold	15 min	95°C
3-step cycling (see Table 11 for number of cycles)		
Denaturation	15 s	95°C
Annealing	30 s	60°C
Extension	15 s	72°C
Hold	2 min	72°C
Hold	∞*	4°C

* Hold at 4°C for at least 5 min.

Table 11. Cycles of library amplification

Original RNA input (total RNA)	Cycle number
500 ng	13
100 ng	16
10 ng	19
1 ng	24
Serum/Plasma	22

5. Place the library amplification reaction in the thermal cycler and start the run.

   Important: Upon completion of the protocol, hold at 4°C for at least 5 min.

6. Briefly centrifuge the 50 µl library amplification reactions, tubes/plates, upon thermal cycler completion and add 47 µl of QMN Beads to each library amplification reaction.

   Note: Ensure the QMN 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.

   Note: When working with plates, centrifuge at 2000 rpm.

   Note: If plates are warped, transfer mixtures to new plates.

7. Vortex for 3 s and briefly centrifuge.

8. Incubate for 5 min at room temperature.
9. Place tubes/plates on a magnet stand for approximately 4 min or until the beads have fully migrated.

   Note: Ensure that the beads have fully migrated before proceeding.
10. Keep the supernatant, and transfer 92 µl of the supernatant to new tubes/plates. Discard the tubes containing the beads.

    Important: Do not discard the supernatant at this step.
11. To the 92 µl supernatant, add 83 µl of QMN Beads. Vortex for 3 s and briefly centrifuge.
12. Incubate at room temperature for 5 min.
13. Place the tubes/plates on a magnet stand until beads have fully migrated.

    Note: Ensure that the beads have fully migrated before proceeding.
14. Discard the supernatant and keep the beads.

    Note: Do not remove the tubes/plates from the magnet stand.
15. With the beads still on the magnet stand, add 200 µl of 80% ethanol. Immediately remove and discard the ethanol wash.
16. Repeat the wash by adding 200 µl of 80% ethanol. Immediately remove and discard the second ethanol wash.

    Note: It is important to completely remove all traces of the ethanol wash after the second wash. Briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.
17. With the beads still on the magnetic stand, air-dry at room temperature for 10 min.

    Note: Visually inspect that the pellet is completely dry and that all residual ethanol has evaporated. Depending on humidity, extended drying time may be required.
18. With the beads still on the magnetic stand, elute the DNA by adding 17 µl of nuclease-free water to the tubes. Subsequently close and remove the tubes from the magnetic stand.

19. Carefully pipet up and down until all beads are thoroughly resuspended; briefly centrifuge and incubate at room temperature for 2 min.
20. Place the tubes/plates on the magnetic stand for ~2 min (or until beads have cleared).

    Note: Ensure that the beads have fully migrated before proceeding.
21. Transfer 15 µl of eluted DNA to new tubes. This is the miRNA Sequencing Library.
22. Proceed to "Protocol: miRNA Library Presequencing QC". Alternatively, the completed miRNA Sequencing Library can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: miRNA Library Presequencing QC

Important points before starting

- A portion of the 15 µl miRNA Sequencing Library from "Protocol: Library Sample Indexing and Amplification Using QIAseq miRNA 12 Index Kit or QIAseq miRNA 96 Index Kits" is the starting material for the library QC. When not in use, store the miRNA Sequencing Library on ice.
- Performing 1 of 2 options is recommended for library QC. "Procedure: Option 1" involves the use of an Agilent Bioanalyzer 2100. "Procedure: Option 2" involves use of PAGE gel electrophoresis.

Procedure: Option 1 (Agilent Bioanalyzer 2100)

1. Analyze 1 µl of the miRNA Sequencing Library on an Agilent Bioanalyzer or TapeStation using a High Sensitivity DNA chip according to the manufacturer's instructions. A miRNA-sized library is approximately 200 bp, and a piRNA-sized library is approximately 198 bp. Typical miRNA-sized library results are shown in Figure 3.

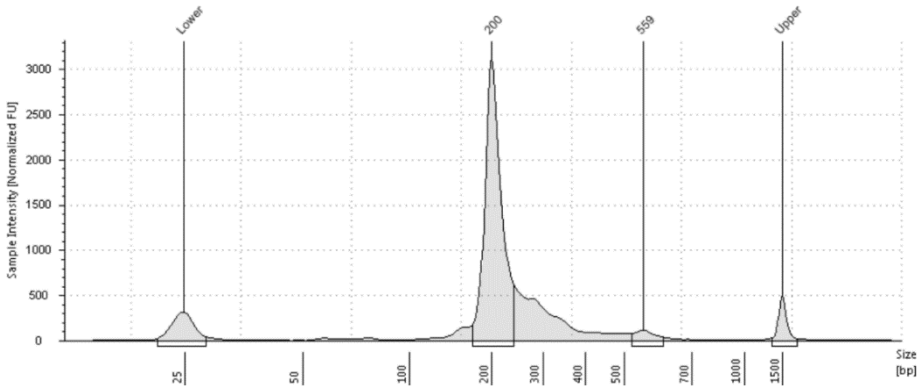


Figure 3. TapeStation trace of miRNA-sized library prepared with the QIAseq miRNA Library Kit.

2. If a large peak (greater than 25% of the height of the miRNA peak) is observed at approximately 157 bp (adapter–dimer), or if other undesired bands are noted, gel excision on the remainder of the miRNA Sequencing Library is recommended to select the specific library of interest (see "Appendix A: Gel Size Selection of Library").

   Note: To prevent adapter–dimerization, use 1 ng or more of total RNA and ensure that all reaction components have been added in the order listed.

3. Proceed to "Protocol: Determining Library Concentration and Read Allocation per Sample".

Procedure: Option 2 (PAGE gel electrophoresis)

1. Prepare a 6% PAGE TBE gel.
2. Load 3 µl of the library cleanup product on the gel; use a 25 bp DNA ladder for size reference.
3. Run the gel at 120V for approximately 1 h or until the dye front has reached the bottom of the cassette.
4. Take an image of the gel. A miRNA-sized library is approximately 173 bp, and a piRNA-sized library is approximately 181 bp. Typical results are shown in Figure 4.

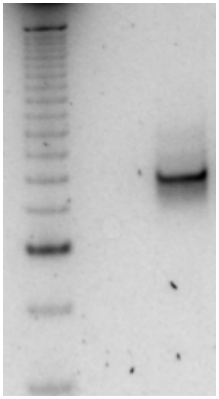


Figure 4. PAGE gel of miRNA-sized library prepared with the QIAseq miRNA Library Kit.

5. If a prominent band is observed at approximately 150 bp (adapter–dimer), or if other undesired bands are observed, perform gel excision on the remainder of the miRNA Sequencing Library to select the specific library of interest (see "Appendix A: Gel Size Selection of Library").

   Note: To prevent adapter–dimerization, use 1 ng or more of total RNA and ensure that all reaction components have been added in the order listed.

6. Proceed to "Protocol: Determining Library Concentration and Read Allocation per Sample".

Protocol: Determining Library Concentration and Read Allocation per Sample

Important points before starting

- A portion of the 15 µl miRNA Sequencing Library from "Protocol: Library Sample Indexing and Amplification Using QIAseq miRNA 12 Index Kit or QIAseq miRNA 96 Index Kits" is the starting material for the library QC. When not in use, store the library on ice.
- A Qubit Fluorimeter is recommended to determine the library concentration.

Procedure

1. Determine the concentration of 2 µl of the miRNA Sequencing Library on a Qubit Fluorimeter according to the manufacturer's instructions.
2. Determine the molarity of each sample (in nM) using the following equation. The equation is for a miRNA-sized library.

$$(X\ ng/\mu l)(10^6)/(112450) = Y\ nM$$

3. Dilute individual libraries to 4 nM using nuclease-free water.
4. If multiplexing, combine libraries in equimolar amounts and mix well.
   Important: It is recommended to allocate 5–10 million reads per sample.

Protocol: Preparation for Sequencing

Important points before starting

- The diluted individual or multiplexed 4 nM library from "Protocol: Determining Library Concentration and Read Allocation per Sample" is the starting material for sequencing.
- For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific Illumina documents.
- QIAseq miRNA UDIs use custom 10 bp unique dual sample indices.

Generation of sample sheets for Illumina Instruments

Index sequences for QIAseq RUDI Adapters are available for download at **www.qiagen.com**. To make sequencing preparation more convenient, ready-to-use templates that include sample sheets containing all QIAseq RUDI Adapter index sequences are available for the different sequencing instruments, MiSeq, MiniSeq, NextSeq, HiSeq, and NovaSeq at **www.qiagen.com**. These can be imported and edited using the Illumina Local Run Manager, or any text editor. Make sure to download the appropriate sample sheet for the Illumina systems depending on whether you are using Local Run Manager or manually configuring the sequencing run.

All Illumina Instruments

1. Go to **www.qiagen.com/shop/sequencing/qiaseq-mirna-ngs** and select **Product Resources** > **Instrument Technical Documents** to find and download the appropriate QIAseq miRNA UDI template depending on your experimental setup.
2. The sample sheet already contains all relevant information to use with the instrument.
3. Open the CSV file, delete any UDI indices that will not be used in the experiment, and save the file with a new name.

4. Copy the file into the "Sample Sheet" folder on the MiSeq instrument or upload the "Sample Sheet" into Local Run Manager for Illumina instruments: MiSeq, MiniSeq, and NextSeq.
5. When ready to perform run, select the file.
6. **Sample dilution and pooling**: Dilute individual libraries to 4 nM, except for the NovaSeq; dilute the individual libraries to 10 nM. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.

   Note: For the NovaSeq, the final pooled library concentration recommendation is between 1.0 – 1.5 nM yielding a final loading concentration of between 200 – 300 pM on the NovaSeq.
7. **Library preparation and loading**: Prepare and load the pooled library on an Illumina instrument according to the specific Illumina instrument guide. Dilute the denatured library pool a second time, to obtain a final library concentration as stated below in Table 12.

Table 12. Recommended final library loading concentrations for Illumina instruments

Illumina sequencing instrument	Illumina specific documentation	Final library concentration (pM)
iSeq	*iSeq 100 System Guide*	75
MiSeq	*MiSeq System Guide*	10
MiniSeq	*MiniSeq System Guide*	1.2
NextSeq 500/550	*NextSeq 500 System Guide or NextSeq 550 System Guide*	1.2
NextSeq 1000/2000	*nextseq-1000-2000-denature-dilute-1000000139235-03*	75
NovaSeq 6000	*NovaSeq 6000 Sequencing System Guide*	200–300

8. **Sequencing run setup**: Select **FASTQ Only**.

   The recommended protocol is 72 bp single read with 10 bp dual indexing. A 50 bp single read protocol can be used if there is no desire to include the UMIs.
9. Upon completion of the sequencing run, proceed with "Protocol: Data Analysis".

Protocol: Data Analysis Using GeneGlobe Web-Based Analysis Tools

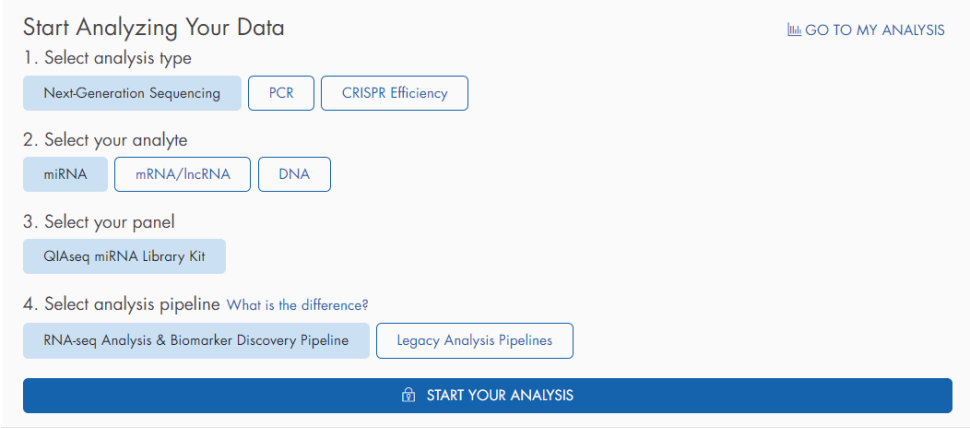
Important points before starting

- Primary and secondary analysis tools are available at **geneglobe.qiagen.com.**
- The RNA-seq Analysis & Biomarker Discovery Pipeline uses QIAGEN CLC Biomedical Workbench for read alignment, UMI counting and differential expression, and Ingenuity Pathway Analysis to return the top hits from the QIAGEN knowledge base for canonical pathways, upstream regulators, and diseases.
- Using the RNA-seq Analysis & Biomarker Discovery Pipeline, FASTQ files can be uploaded, miRNA sequences are aligned, and UMIs are counted. Differential miRNA expression will be calculated and visualized using interactive volcano plots. Differentially expressed miRNAs will be queried against the QIAGEN knowledge base for canonical pathways, upstream regulators, and diseases and biological functions. Important microRNAs can then be identified, and digital PCR and qPCR assays are easily found for biological verification.
- For each alignment, a credit is deducted from your account. Credits for using the RNA-seq Analysis & Biomarker Discovery Pipeline are included with QIAseq library kits. Credits can also be purchased for using the RNA-seq Analysis portal with non-QIAGEN kits through **www.qiagen.com.**
- The legacy analysis pipelines contain online tools for read alignment, UMI counting, and differential expression. These pipelines for data analysis must have all of the data uploaded simultaneously, and the read alignment and UMI counting must be completed in one session.

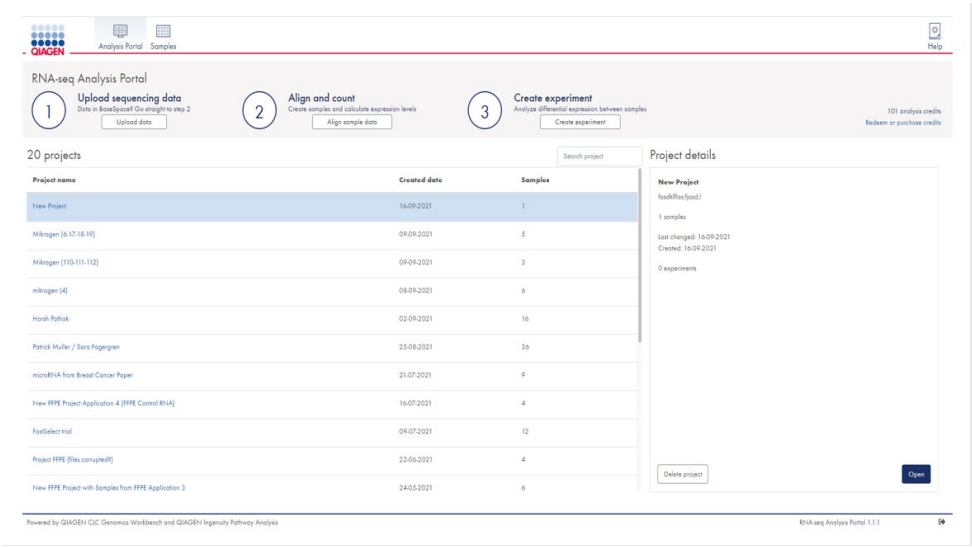
Data analysis steps

1. Go to **geneglobe.qiagen.com/analyze**.

   Log into GeneGlobe with your username and password.



2. Select **Next-Generation Sequencing, miRNA**, **QIAseq miRNA Library Kit**, **RNA-seq Analysis & Biomarker Discovery Pipeline**.
3. Click **Start Analysis**.
4. Follow the 3 steps to Upload sequencing data, Align and count, and Create an experiment. Directions on how to use the RNA-seq analysis portal can be accessed by clicking **Help** at the upper-right corner.


Analysis Portal
Samples
Help
RNA-seq Analysis Portal
1
Upload sequencing data
Data in BaseSpace® Go straight to step 2
Upload data
2
Align and count
Create samples and calculate expression levels
Align sample data
3
Create experiment
Analyze differential expression between samples
Create experiment
101 analysis credits
Redeem or purchase credits
20 projects
Search project
Project name
Created date
Samples
New Project 16-09-2021 1
Mikrogen (6-17-18-19) 09-09-2021 5
Mikrogen (110-111-112) 09-09-2021 3
mikrogen (4) 08-09-2021 6
Harsh Pathak 02-09-2021 16
Patrick Muller / Sara Fagergren 25-08-2021 36
microRNA from Breast Cancer Paper 21-07-2021 9
New FFPE Project Application 4 (FFPE Control RNA) 16-07-2021 4
FastSelect trial 09-07-2021 12
Project FFPE (files corrupted!) 22-06-2021 4
New FFPE Project with Samples from FFPE Application 3 24-05-2021 6
Project details
New Project
fasdklflas;fjasd;l
1 samples
Last changed: 16-09-2021
Created: 16-09-2021
0 experiments
Delete project
Open
Powered by QIAGEN CLC Genomics Workbench and QIAGEN Ingenuity Pathway Analysis
RNA-seq Analysis Portal 1.1.1

Troubleshooting Guide

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

	Comments and suggestions
During cDNA cleanup or library cleanup, not enough sample can be pipetted	
Excess evaporation may have occurred during the previous reaction, or sample may not have been centrifuged prior to cleanup	Check that caps on tubes have a secure fit and that samples are centrifuged prior to cDNA cleanup.
During cDNA cleanup or library cleanup, the supernatant does not completely clear after 4–6 minutes	
This is not a significant issue	Sometimes, samples do not completely clarify. This is possibly due to the cold temperature of the buffer. Simply proceed with the cleanup.
During library prep QC, no library is observed	
a) 3' ligation reaction has not been properly mixed	Once all components have been added to the reaction, briefly centrifuge, mix by pipetting up and down 15–20 times, and centrifuge briefly again.
b) Excess ethanol from the cDNA cleanup has been carried over to the amplification reaction	After the second wash, briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.
During library prep QC, prominent adapter–dimer band is observed at 150 bp (greater than 25%)	
a) Ensure that the UDI RT Initiator has been added as indicated, between the 5' ligation and RT reactions, and the correct temperature profile has been set up for the initiation	Double check the RT reaction setup.

		Comments and suggestions
b)	Ensure that 3' ligation and 5' ligation components were added to their respective reactions in the order listed	Double check 3' ligation and 5' ligation reaction setup.

During library prep QC, a prominent product of approximately 225 bp is observed

a)	QIAseq Beads were not rebuffered with QIAseq Bead Binding Buffer to produce QMN Beads	Rebuffer QIAseq Beads with QIAseq Bead Binding Buffer to produce QMN Beads.
b)	Each reaction was not held at 4°C for 5 min.	At the end of each reaction (3' ligation, reverse transcription, and library amplification), hold at 4°C for at least 5 min.

miRNA Sequencing Library concentrations are too low to obtain a 4 nM library

Not necessarily a problem	If Library QC suggests the library is of good quality and simply low in concentration, use 2 nM library instead, or sequence the maximum amount possible of that library (either individually or in multiplex with other samples). At the same time, keep all libraries being multiplexed at comparable concentrations.

During primary data analysis, Unique Molecular Indices (UMIs) are not present

A read length shorter than 72 bp may have been performed	Resequence and ensure that 72 bp single reads with 10 bp dual indices are performed.

Appendix A: Gel Size Selection of Library

This protocol describes excision of a library from a 6% TBE PAGE gel.

Important points before starting

- The miRNA Sequencing Library from "Protocol: Library Sample Indexing and Amplification Using QIAseq miRNA 12 Index Kit or QIAseq miRNA 96 Index Kits" is the starting material for gel excision.
- PAGE-gel-related equipment and consumables to prepare and run a 6% PAGE TBE gel are required.
- 5x GelPilot® DNA Loading Dye (cat. no. 239901) or equivalent is required.
- 25 bp DNA Ladder (Thermo Fisher Scientific cat. no. 10597-011) or equivalent is required.
- SYBR® Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific cat. no. S11494) is required.
- Gel Breaker Tubes (Fisher Scientific cat. no. NC0462125) are required.
- Corning® Costar® Spin-X® Centrifuge Tube Filters (Fisher Scientific cat. no. 07-200-387) are required.
- 3 M NaOAc, pH 5.2, is required.
- Linear Acrylamide is required.

Procedure

1. Prepare a 6% PAGE TBE gel.
2. Adjust the volume of the miScript® Sequencing Library to 24 µl using nuclease-free water. Add 6 µl of 5x GelPilot DNA Loading Dye and mix thoroughly.
3. Distribute the mixture across 3 lanes of the 6% PAGE TBE gel.
4. Run the gel at 120V for 1 h or until the dye front has reached the bottom of the cassette.

5. Remove the gel from the cassette and stain with 1x SYBR Gold for 10 min.
6. Excise the library of choice.

   Note: A miRNA-sized library is approximately 173 bp, and a piRNA-sized library is approximately 181 bp.
7. Place each excised band in a 0.5 ml Gel Breaker tube in a 2 ml tube and centrifuge at max speed for 2 min.
8. Soak the debris in 250 µl 0.3 M sodium acetate.
9. Rotate at room temperature for at least 2 h.
10. Transfer eluate and gel debris to a Corning Costar Spin-X Centrifuge Tube Filter column and centrifuge for 2 min at max speed.
11. Recover eluate and add 1 µl of Linear Acrylamide and 750 µl of 100% ethanol.
12. Vortex and incubate at –80°C for at least 1 h.
13. Centrifuge at 14,000 x *g* for 30 min at 4°C.
14. Remove supernatant without disturbing the pellet.
15. Wash the pellet with 500 µl of 80% ethanol.
16. Centrifuge at 14,000 x *g* for 30 min at 4°C.
17. Remove alcohol and air-dry the pellet at 37°C for 10 min.
18. Resuspend pellet in 15 µl water.

Appendix B: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to degrade RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice.

To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipettes and electrophoresis tanks), general laboratory reagents can be used. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water, or rinse with chloroform* if the plasticware is chloroform resistant. To decontaminate electrophoresis tanks, clean with detergent* (e.g., 0.5% SDS), rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol resistant), and allow to dry.

* 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.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed and oven baked at 240°C for 4 hours or more (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), as described in "Solutions" below.

Solutions

Note: QIAGEN solutions, such as the components found in the miScript Single Cell qPCR Kit, are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong but not absolute inhibitor of RNases. DEPC is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated, and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers.* DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA–RNA or RNA–RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

* 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.

Appendix C: QIAseq miRNA Unique Dual Indexes

Generation of sample sheets for Illumina instruments

Index sequences for QIAseq miRNA Unique Dual Indexes are available for download at **www.qiagen.com**. Sequencing on the NextSeq, HiSeq X™, or HiSeq 3000/4000 system follows a different dual-indexing workflow than other Illumina systems. If you are manually creating sample sheets for these instruments, enter the reverse complement of the i5 index adapter sequence. If using Illumina Experiment Manager, BaseSpace®, or Local Run Manager for run planning, the software will automatically reverse complement index sequences when necessary.

Ready-to-use sample sheets containing all QIAseq miRNA Unique Dual Indexes are available for MiSeq, NextSeq, MiniSeq, HiSeq, and NovaSeq instruments. These can be conveniently downloaded from **www.qiagen.com**. These can be imported and edited using the Illumina Experiment Manager Software, Illumina Local Run Manager, or any text editor. Make sure to download the appropriate sample sheet for NextSeq, HiSeq X, or HiSeq 3000/4000 systems depending on whether you are using Local Run Manager or manually configuring the sequencing run.

QIAseq miRNA UDI Layouts

The layouts of the single-use QIAseq miRNA UDI plates are shown in Table 13 to Table 21. The index motives used in the QIAseq Unique Dual Index Kits are listed at **www.qiagen.com**. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments on **www.qiagen.com**.

Table 13. QIAseq miRNA UDI Set A (96) (cat. no. 331615) layout: RUDI-96AA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI001	iRUDI009	iRUDI017	iRUDI025	iRUDI033	iRUDI041	iRUDI049	iRUDI057	iRUDI065	iRUDI073	iRUDI081	iRUDI089
B	iRUDI002	iRUDI010	iRUDI018	iRUDI026	iRUDI034	iRUDI042	iRUDI050	iRUDI058	iRUDI066	iRUDI074	iRUDI082	iRUDI090
C	iRUDI003	iRUDI011	iRUDI019	iRUDI027	iRUDI035	iRUDI043	iRUDI051	iRUDI059	iRUDI067	iRUDI075	iRUDI083	iRUDI091
D	iRUDI004	iRUDI012	iRUDI020	iRUDI028	iRUDI036	iRUDI044	iRUDI052	iRUDI060	iRUDI068	iRUDI076	iRUDI084	iRUDI092
E	iRUDI005	iRUDI013	iRUDI021	iRUDI029	iRUDI037	iRUDI045	iRUDI053	iRUDI061	iRUDI069	iRUDI077	iRUDI085	iRUDI093
F	iRUDI006	iRUDI014	iRUDI022	iRUDI030	iRUDI038	iRUDI046	iRUDI054	iRUDI062	iRUDI070	iRUDI078	iRUDI086	iRUDI094
G	iRUDI007	iRUDI015	iRUDI023	iRUDI031	iRUDI039	iRUDI047	iRUDI055	iRUDI063	iRUDI071	iRUDI079	iRUDI087	iRUDI095
H	iRUDI008	iRUDI016	iRUDI024	iRUDI032	iRUDI040	iRUDI048	iRUDI056	iRUDI064	iRUDI072	iRUDI080	iRUDI088	iRUDI096

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 14. QIAseq miRNA UDI Set B (96) (cat. no. 331625) layout: RUDI-96BA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI097	iRUDI105	iRUDI113	iRUDI121	iRUDI129	iRUDI137	iRUDI145	iRUDI153	iRUDI161	iRUDI169	iRUDI177	iRUDI185
B	iRUDI098	iRUDI106	iRUDI114	iRUDI122	iRUDI130	iRUDI138	iRUDI146	iRUDI154	iRUDI162	iRUDI170	iRUDI178	iRUDI186
C	iRUDI099	iRUDI107	iRUDI115	iRUDI123	iRUDI131	iRUDI139	iRUDI147	iRUDI155	iRUDI163	iRUDI171	iRUDI179	iRUDI187
D	iRUDI100	iRUDI108	iRUDI116	iRUDI124	iRUDI132	iRUDI140	iRUDI148	iRUDI156	iRUDI164	iRUDI172	iRUDI180	iRUDI188
E	iRUDI101	iRUDI109	iRUDI117	iRUDI125	iRUDI133	iRUDI141	iRUDI149	iRUDI157	iRUDI165	iRUDI173	iRUDI181	iRUDI189
F	iRUDI102	iRUDI110	iRUDI118	iRUDI126	iRUDI134	iRUDI142	iRUDI150	iRUDI158	iRUDI166	iRUDI174	iRUDI182	iRUDI190
G	iRUDI103	iRUDI111	iRUDI119	iRUDI127	iRUDI135	iRUDI143	iRUDI151	iRUDI159	iRUDI167	iRUDI175	iRUDI183	iRUDI191
H	iRUDI104	iRUDI112	iRUDI120	iRUDI128	iRUDI136	iRUDI144	iRUDI152	iRUDI160	iRUDI168	iRUDI176	iRUDI184	iRUDI192

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 15. QIAseq miRNA UDI Set C (96) (cat. no. 331635) layout: RUDI-96CA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI193	iRUDI201	iRUDI209	iRUDI217	iRUDI225	iRUDI233	iRUDI241	iRUDI249	iRUDI257	iRUDI265	iRUDI273	iRUDI281
B	iRUDI194	iRUDI202	iRUDI210	iRUDI218	iRUDI226	iRUDI234	iRUDI242	iRUDI250	iRUDI258	iRUDI266	iRUDI274	iRUDI282
C	iRUDI195	iRUDI203	iRUDI211	iRUDI219	iRUDI227	iRUDI235	iRUDI243	iRUDI251	iRUDI259	iRUDI267	iRUDI275	iRUDI283
D	iRUDI196	iRUDI204	iRUDI212	iRUDI220	iRUDI228	iRUDI236	iRUDI244	iRUDI252	iRUDI260	iRUDI268	iRUDI276	iRUDI284
E	iRUDI197	iRUDI205	iRUDI213	iRUDI221	iRUDI229	iRUDI237	iRUDI245	iRUDI253	iRUDI261	iRUDI269	iRUDI277	iRUDI285
F	iRUDI198	iRUDI206	iRUDI214	iRUDI222	iRUDI230	iRUDI238	iRUDI246	iRUDI254	iRUDI262	iRUDI270	iRUDI278	iRUDI286
G	iRUDI199	iRUDI207	iRUDI215	iRUDI223	iRUDI231	iRUDI239	iRUDI247	iRUDI255	iRUDI263	iRUDI271	iRUDI279	iRUDI287
H	iRUDI200	iRUDI208	iRUDI216	iRUDI224	iRUDI232	iRUDI240	iRUDI248	iRUDI256	iRUDI264	iRUDI272	iRUDI280	iRUDI288

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 16. QIAseq miRNA UDI Set D (96) (cat. no. 331645) layout: RUDI-96DA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI289	iRUDI297	iRUDI305	iRUDI313	iRUDI321	iRUDI329	iRUDI337	iRUDI345	iRUDI353	iRUDI361	iRUDI369	iRUDI377
B	iRUDI290	iRUDI298	iRUDI306	iRUDI314	iRUDI322	iRUDI330	iRUDI338	iRUDI346	iRUDI354	iRUDI362	iRUDI370	iRUDI378
C	iRUD291	iRUDI299	iRUDI307	iRUDI315	iRUDI323	iRUDI331	iRUDI339	iRUDI347	iRUDI355	iRUDI363	iRUDI371	iRUDI379
D	iRUDI292	iRUDI300	iRUDI308	iRUDI316	iRUDI324	iRUDI332	iRUDI340	iRUDI348	iRUDI356	iRUDI364	iRUDI372	iRUDI380
E	iRUDI293	iRUDI301	iRUDI309	iRUDI317	iRUDI325	iRUDI333	iRUDI341	iRUDI349	iRUDI357	iRUDI365	iRUDI373	iRUDI381
F	iRUDI294	iRUDI302	iRUDI310	iRUDI318	iRUDI326	iRUDI334	iRUDI342	iRUDI350	iRUDI358	iRUDI366	iRUDI374	iRUDI382
G	iRUDI295	iRUDI303	iRUDI311	iRUDI319	iRUDI327	iRUDI335	iRUDI343	iRUDI351	iRUDI359	iRUDI367	iRUDI375	iRUDI383
H	iRUDI296	iRUDI304	iRUDI312	iRUDI320	iRUDI328	iRUDI336	iRUDI344	iRUDI352	iRUDI360	iRUDI368	iRUDI376	iRUDI384

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 17. QIAseq miRNA UDI Set E (96) (cat. no. 331655) layout: RUDI-96EA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI385	iRUDI393	iRUDI401	iRUDI409	iRUDI417	iRUDI425	iRUDI433	iRUDI441	iRUDI449	iRUDI457	iRUDI465	iRUDI473
B	iRUDI386	iRUDI394	iRUDI402	iRUDI410	iRUDI418	iRUDI426	iRUDI434	iRUDI442	iRUDI450	iRUDI458	iRUDI466	iRUDI474
C	iRUDI387	iRUDI395	iRUDI403	iRUDI411	iRUDI419	iRUDI427	iRUDI435	iRUDI443	iRUDI451	iRUDI459	iRUDI467	iRUDI475
D	iRUDI388	iRUDI396	iRUDI404	iRUDI412	iRUDI420	iRUDI428	iRUDI436	iRUDI444	iRUDI452	iRUDI460	iRUDI468	iRUDI476
E	iRUDI389	iRUDI397	iRUDI405	iRUDI413	iRUDI421	iRUDI429	iRUDI437	iRUDI445	iRUDI453	iRUDI461	iRUDI469	iRUDI477
F	iRUDI390	iRUDI398	iRUDI406	iRUDI414	iRUDI422	iRUDI430	iRUDI438	iRUDI446	iRUDI454	iRUDI462	iRUDI470	iRUDI478
G	iRUDI391	iRUDI399	iRUDI407	iRUDI415	iRUDI423	iRUDI431	iRUDI439	iRUDI447	iRUDI455	iRUDI463	iRUDI471	iRUDI479
H	iRUDI392	iRUDI400	iRUDI408	iRUDI416	iRUDI424	iRUDI432	iRUDI440	iRUDI448	iRUDI456	iRUDI464	iRUDI472	iRUDI480

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 18. QIAseq miRNA UDI Set F (96) (cat. no. 331665) layout: RUDI-96FA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI481	iRUDI489	iRUDI497	iRUDI505	iRUDI513	iRUDI521	iRUDI529	iRUDI537	iRUDI545	iRUDI553	iRUDI561	iRUDI569
B	iRUDI482	iRUDI490	iRUDI498	iRUDI506	iRUDI514	iRUDI522	iRUDI530	iRUDI538	iRUDI546	iRUDI554	iRUDI562	iRUDI570
C	iRUDI483	iRUDI491	iRUDI499	iRUDI507	iRUDI515	iRUDI523	iRUDI531	iRUDI539	iRUDI547	iRUDI555	iRUDI563	iRUDI571
D	iRUDI484	iRUDI492	iRUDI500	iRUDI508	iRUDI516	iRUDI524	iRUDI532	iRUDI540	iRUDI548	iRUDI556	iRUDI564	iRUDI572
E	iRUDI485	iRUDI493	iRUDI501	iRUDI509	iRUDI517	iRUDI525	iRUDI533	iRUDI541	iRUDI549	iRUDI557	iRUDI565	iRUDI573
F	iRUDI486	iRUDI494	iRUDI502	iRUDI510	iRUDI518	iRUDI526	iRUDI534	iRUDI542	iRUDI550	iRUDI558	iRUDI566	iRUDI574
G	iRUDI487	iRUDI495	iRUDI503	iRUDI511	iRUDI519	iRUDI527	iRUDI535	iRUDI543	iRUDI551	iRUDI559	iRUDI567	iRUDI575
H	iRUDI488	iRUDI496	iRUDI504	iRUDI512	iRUDI520	iRUDI528	iRUDI536	iRUDI544	iRUDI552	iRUDI560	iRUDI568	iRUDI576

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 19. QIAseq miRNA UDI Set G (96) (cat. no. 331675) layout: RUDI-96GA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI577	iRUDI585	iRUDI593	iRUDI601	iRUDI609	iRUDI617	iRUDI625	iRUDI633	iRUDI641	iRUDI649	iRUDI657	iRUDI665
B	iRUDI578	iRUDI586	iRUDI594	iRUDI602	iRUDI610	iRUDI618	iRUDI626	iRUDI634	iRUDI642	iRUDI650	iRUDI658	iRUDI666
C	iRUDI579	iRUDI587	iRUDI595	iRUDI603	iRUDI611	iRUDI619	iRUDI627	iRUDI635	iRUDI643	iRUDI651	iRUDI659	iRUDI667
D	iRUDI580	iRUDI588	iRUDI596	iRUDI604	iRUDI612	iRUDI620	iRUDI628	iRUDI636	iRUDI644	iRUDI652	iRUDI660	iRUDI668
E	iRUDI581	iRUDI589	iRUDI597	iRUDI605	iRUDI613	iRUDI621	iRUDI629	iRUDI637	iRUDI645	iRUDI653	iRUDI661	iRUDI669
F	iRUDI582	iRUDI590	iRUDI598	iRUDI606	iRUDI614	iRUDI622	iRUDI630	iRUDI638	iRUDI646	iRUDI654	iRUDI662	iRUDI670
G	iRUDI583	iRUDI591	iRUDI599	iRUDI607	iRUDI615	iRUDI623	iRUDI631	iRUDI639	iRUDI647	iRUDI655	iRUDI663	iRUDI671
H	iRUDI584	iRUDI592	iRUDI600	iRUDI608	iRUDI616	iRUDI624	iRUDI632	iRUDI640	iRUDI648	iRUDI656	iRUDI664	iRUDI672

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 20. QIAseq miRNA UDI Set H (96) (cat. no. 331685) layout: RUDI-96HA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI673	iRUDI681	iRUDI689	iRUDI697	iRUDI705	iRUDI713	iRUDI721	iRUDI729	iRUDI737	iRUDI745	iRUDI753	iRUDI761
B	iRUDI674	iRUDI682	iRUDI690	iRUDI698	iRUDI706	iRUDI714	iRUDI722	iRUDI730	iRUDI738	iRUDI746	iRUDI754	iRUDI762
C	iRUDI675	iRUDI683	iRUDI691	iRUDI699	iRUDI707	iRUDI715	iRUDI723	iRUDI731	iRUDI739	iRUDI747	iRUDI755	iRUDI763
D	iRUDI676	iRUDI684	iRUDI692	iRUDI700	iRUDI708	iRUDI716	iRUDI724	iRUDI732	iRUDI740	iRUDI748	iRUDI756	iRUDI764
E	iRUDI677	iRUDI685	iRUDI693	iRUDI701	iRUDI709	iRUDI717	iRUDI725	iRUDI733	iRUDI741	iRUDI749	iRUDI757	iRUDI765
F	iRUDI578	iRUDI686	iRUDI694	iRUDI702	iRUDI710	iRUDI718	iRUDI726	iRUDI734	iRUDI742	iRUDI750	iRUDI758	iRUDI766
G	iRUDI679	iRUDI687	iRUDI695	iRUDI703	iRUDI711	iRUDI719	iRUDI727	iRUDI735	iRUDI743	iRUDI751	iRUDI759	iRUDI767
H	iRUDI680	iRUDI688	iRUDI696	iRUDI704	iRUDI712	iRUDI720	iRUDI728	iRUDI736	iRUDI744	iRUDI752	iRUDI760	iRUDI768

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Table 21. QIAseq miRNA 12 Index Kit IL UDI (12) (cat. no. 331601) layout: RUDI-12A

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI001	iRUDI009	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	iRUDI002	iRUDI010	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	iRUDI003	iRUDI011	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
D	iRUDI004	iRUDI012	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	iRUDI005	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
F	iRUDI006	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
G	iRUDI007	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
H	iRUDI008	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

Unique i5 and i7 Index pairs are wet single-use plates. During reaction setup, components are aliquoted into another plate.

Ordering Information

Product	Contents	Cat. no.
QIAseq miRNA Library Kit (12)	For 12 sequencing prep reactions: 3' ligation, 5' ligation, reverse transcription, cDNA cleanup, library amplification, and library cleanup reagents; quality control primers	331502
QIAseq miRNA Library Kit (96)	For 96 sequencing prep reactions: 3' ligation, 5' ligation, reverse transcription, cDNA cleanup, library amplification, and library cleanup reagents; quality control primers	331505
QIAseq miRNA 12 Index Kit IL UDI (12)	Indexing for 12 samples; includes a 96 well, foil covered plate with 12 pre-mixed unique dual indexes, UDI 5' Adapter (96), and UDI RT Initiator (96)	331601
QIAseq miRNA 96 Index Kit IL UDI-A to UDI-H (96)	Indexing for 96 samples; includes a 96 well, foil covered plate with 96 pre-mixed unique dual indexes, UDI 5' Adapter (96), and UDI RT Initiator (96) Each catalog number contains a different set of 96 unique dual indexing sample indices.	331615 331625 331635 331645 331655 331665 331675 331685
QIAseq miRNA 96 Index Kit UDI A–D (384)	Indexing for 384 samples; includes 4 x 96 well, foil covered plate with 96 pre-mixed unique dual indexes, UDI 5' Adapter (96), and UDI RT Initiator (96)	331717
QIAseq miRNA 96 Index Kit UDI E–H (384)	Indexing for 384 samples; includes 4 x 96 well, foil covered plate with 96 pre-mixed unique dual indexes, UDI 5' Adapter (96), and UDI RT Initiator (96)	331727

QIAseq miRNA 96 Index Kit UDI A–H (768)	Indexing for 768 samples; includes 8 x 96 well, foil covered plate with 96 pre-mixed unique dual indexes, UDI 5' Adapter (96), and UDI RT Initiator (96)	331738
QuantiTect® SYBR Green PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect SYBR Green PCR Master Mix, 2 x 2 ml RNase-Free Water	204143
QuantiTect SYBR Green PCR Kit (1000)	For 1000 x 50 µl reactions: 25 ml 2x QuantiTect SYBR Green PCR Master Mix, 20 ml RNase-Free Water	204145

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Document Revision History

Date	Changes
11/2021	Initial revision

Notes

Limited License Agreement for QIAseq miRNA UDI Library Kit, Illumina NGS Systems

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HB-2983-001 11/2021