September 2023

QIAseq® FastSelect™ RNA Library Kit

Stranded RNA-seq library preparation with integrated rRNA removal for complete transcriptome or 3′ RNA-seq from 1 ng to 1000 ng of Total RNA

Sample to Insight

Contents

Kit Contents

The QIAseq FastSelect RNA library kits ship in multiple boxes. It is very important to review the storage temperature of each box and promptly place refrigerated and frozen items into the appropriate storage areas.

			Kit contents			
Kit	**Kit size**	**Catalog number**	**Component**	**Component size**	**Catalog number**	**Quantity**
QIAseq FastSelect RNA Lib HMR Kit	(24) (96)	334232 334235	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333221 333220	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (24) 2 (96)
			QIAseq FastSelect –rRNA HMR Kit	(24) (96)	334386 334387	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq FastSelect RNA Lib HMR Kit	(384) (768)	334237 334238	QIAseq RNA Lib Enzymes & Buffers	(96)	334785	4 (384) 8 (768)
			NGS 2x Hi-Fi MasterMix Kit	(2.8 mL)	333220	4 (384) 8 (768)
			QIAseq Beads	(55 mL)	333903	1 (384) 2 (768)
			QIAseq FastSelect –rRNA HMR Kit	(384)	334388	1 (384) 2 (768)
			QIAseq Advanced Analysis	(96)	333785	4 (384) 8 (768)

			Kit contents			
Kit	**Kit size**	**Catalog number**	**Component**	**Component size**	**Catalog number**	**Quantity**
QIAseq FastSelect RNA Lib Blood Kit	(24) (96)	334222 334225	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333220 333221	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (24) 2 (96)
			QIAseq FastSelect –rRNA HMR Kit	(24) (96)	334386 334387	1
			QIAseq FastSelect –Globin Kit	(24) (96)	334376 334377	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq FastSelect RNA Lib Blood Kit	(384) (768)	334227 334228	QIAseq RNA Lib Enzymes & Buffers	(96)	334785	4 (384) 8 (768)
			NGS 2x Hi-Fi MasterMix Kit	(2.8 mL)	333220	4 (384) 8 (768)
			QIAseq Beads	(55 mL)	333903	1 (384) 2 (768)
			QIAseq FastSelect –rRNA HMR Kit	(384)	334388	1 (384) 2 (768)
			QIAseq FastSelect –Globin Kit	(384)	334378	1 (384) 2 (768)
			QIAseq Advanced Analysis	(96)	333785	4 (384) 8 (768)

			Kit contents			
Kit	**Kit size**	**Catalog number**	**Component**	**Component size**	**Catalog number**	**Quantity**
QIAseq FastSelect RNA Lib Epi. Kit	(24) (96)	334242 334245	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333221 333220	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (only 96)
			QIAseq FastSelect –rRNA HMR Kit	(24) (96)	334386 334387	1
			QIAseq FastSelect –5S/16S/23S Kit	(24) (96)	335925 335927	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq FastSelect RNA Lib Epi. Kit	(384) (768)	334247 334248	QIAseq RNA Lib Enzymes & Buffers	(96)	334785	4 (384) 8 (768)
			QIAseq 2x Hi-Fi MasterMix Kit	(2.8 mL)	333220	4 (384) 8 (768)
			QIAseq Beads	(55 mL)	333903	1 (384) 2 (768)
			QIAseq FastSelect –rRNA HMR Kit	(384)	334388	1 (384) 2 (768)
			QIAseq FastSelect –5S/16S/23S Kit	(384)	335929	1 (384) 2 (768)
			QIAseq Advanced Analysis	(96)	333785	4 (384) 8 (768)
QIAseq FastSelect RNA Lib Bac. Kit	(24) (96)	334262 334265	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333220 333221	1 (24) 1 (96)

			Kit contents			
Kit	**Kit size**	**Catalog number**	**Component**	**Component size**	**Catalog number**	**Quantity**
			QIAseq Beads	(10 mL)	333923	1 (only 96)
			QIAseq FastSelect –5S/16S/23S Kit	(24) (96)	335925 335927	1
QIAseq FastSelect RNA Lib Plant Kit	(24) (96)	334252 334255	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333221 333220	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (24) 2 (96)
			QIAseq FastSelect –rRNA Plant	(24) (96)	334315 334317	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq FastSelect RNA Lib Fish Kit	(24) (96)	334272 334275	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333221 333220	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (24) 2 (96)
			QIAseq FastSelect –rRNA Fish Kit (24), (96)	(24) (96)	333252 333255	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq FastSelect RNA Lib Worm Kit	(24) (96)	334292 334295	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1

			Kit contents			
Kit	**Kit size**	**Catalog number**	**Component**	**Component size**	**Catalog number**	**Quantity**
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333221 333220	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (24) 2 (96)
			QIAseq FastSelect –rRNA Worm Kit	(24) (96)	333242 333245	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq FastSelect RNA Lib Yeast Kit	(24) (96)	334282 334285	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333221 333220	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (24) 2 (96)
			QIAseq FastSelect –rRNA Yeast	(24) (96)	334215 334217	1
QIAseq FastSelect RNA Lib Fly Kit	(24) (96)	334302 334305	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			NGS 2x Hi-Fi MasterMix Kit	(0.7 mL) (2.8 mL)	333221 333220	1 (24) 1 (96)
			QIAseq Beads	(10 mL)	333923	1 (24) 2 (96)
			QIAseq FastSelect –rRNA Fly Kit	(24) (96)	333262 333265	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1

QIAseq RNA Lib Enzymes & Buffers Catalog no. Number of reactions	(24) 334782 24	(96) 334785 96
US RT Buffer, 5x	150 µL	580 µL
DTT (100 mM)	20 µL	80 µL
dNTP Mix (10 mM)	55 µL	235 µL
N6-T RT Primer	30 µL	120 µL
ODT-T RT Primer	30 µL	120 µL
RNase Inhibitor	20 µL	96 µL
EZ Reverse Transcriptase	2 tubes x 36 µL	2 tubes x 150 µL
Nuclease-Free Water	1 tube	2 tubes
QIAseq 2x HiFi MM	100 µL	400 µL
Optical Thin-wall 8-cap Strips (12/bag)	1 bag	2 bags
SIDT-24A	1 plate	–
SIDT-96A	–	1 plate

Kit QIAseq Beads Catalog no. Volume of reagents	333923 (10 mL)	333903 (55 mL)
QIAseq Beads	1 bottle	1 bottle

Kit QIAseq Advanced Analysis Catalog no. Number of analysis credits	333782 (24)	333785 (96)
Analysis credits for GeneGlobe® RNA-seq Analysis Portal	24	96
QIAseq Advanced Analysis cards	1 card	1 card

QIAseq FastSelect –rRNA HMR Kit Catalog no. Number of reactions	(24) 334386 24	(96) 334387 96	(384) 334388 384
QIAseq FastSelect –rRNA HMR	3 x 12 µL	120 µL	4 x 120 µL

QIAseq FastSelect –Globin Kit Catalog no. Number of reactions	(24) 334376 24	(96) 334377 96	(384) 334378 384
QIAseq FastSelect –Globin	3 x 12 µL	120 µL	4 x 120 µL

QIAseq FastSelect –5S/16S/23S Kit Catalog no. Number of reactions	(24) 335925 24	(96) 335927 96	(384) 335929 384
FastSelect 5S/16S/23S	3 x 8 µL	96 µL	4 x 96 µL
FastSelect FH Buffer	3 x 12 µL	144 µL	4 x 144 µL
Nuclease-Free Water	1 tube	1 tube	1 tube
QIAseq Beads	10 mL	10 mL	10 mL
QIAseq Bead Binding Buffer	10.2 mL	10.2 mL	10.2 mL

QIAseq FastSelect –rRNA Plant Catalog no. Number of reactions	(24) 334315 24	(96) 334317 96
QIAseq FastSelect –rRNA Plant	3 x 12 µL	120 µL

QIAseq FastSelect –rRNA Fish Kit Catalog no. Number of reactions	(24) 333252 24	(96) 333255 96
QIAseq FastSelect –rRNA Fish	3 x 12 µL	120 µL

QIAseq FastSelect –rRNA Worm Kit Catalog no. Number of reactions	(24) 333242 24	(96) 333245 96
QIAseq FastSelect –rRNA Worm	3 x 12 µL	120 µL

QIAseq FastSelect –rRNA Yeast Catalog no. Number of reactions	(24) 334215 24	(96) 334217 96
QIAseq FastSelect –rRNA Yeast	3 x 12 µL	120 µL

QIAseq FastSelect –rRNA Fly Kit Catalog no. Number of reactions	(24) 333262 24	(96) 333265 96
QIAseq FastSelect –rRNA Fly	3 x 12 µL	120 µL

NGS 2x Hi-Fi MasterMix Kit Catalog no. Component no. Number of reactions	(0.7 mL) 333221 1122082 24	(2.8 mL) 333220 1122083 96
QIAseq 2x Hi-Fi MM	720 µL	2 x 1.440 µL

QIAseq UX Index Kits IL UDI (sold separately)

QIAseq UX Index IL UDI Kits (see table below) are required for library amplification/indexing of RNA-seq libraries made with QIAseq FastSelect RNA library kits.

QIAseq UX 12 Index Kit IL UDI Catalog no. Number of Indexes	(12) 331801 12
Index plate* name	RUDI-12A

* Twelve wells of each hard plastic 96-well plate contains 9 µL pre-mixed indexes. The plate has a pierceable foil seal and is sealed in a silver-foil bag. Each index is intended for single use.

QIAseq UX 96 Index Kit IL UDI	–A (96)	–B (96)	–C (96)	–D (96)	–E (96)	–F (96)	–G (96)	–H (96)
Catalog no.	331815	331825	331835	331845	331855	331865	331875	331885
Number of Indexes	96	96	96	96	96	96	96	96
Index plate* name	MUDI-96AA	MUDI-96BA	MUDI-96CA	MUDI-96DA	MUDI-96EA	MUDI-96FA	MUDI-96GA	MUDI-96HA

* Each hard plastic 96-well plate contains 9 µL pre-mixed indexes. The plate has a pierceable foil seal and is sealed in a silver-foil bag. Each index is intended for single use.

QIAseq UX 96 Index Kit IL UDI Catalog no. Number of Indexes	A–D (384) 331817 384	E–H (384) 331857 384	A–H (768) 331818 768
Index plate* names	MUDI-96AA MUDI-96BA MUDI-96CA MUDI-96DA	MUDI-96EA MUDI-96FA MUDI-96GA MUDI-96HA	MUDI-96AA MUDI-96BA MUDI-96CA MUDI-96DA MUDI-96EA MUDI-96FA MUDI-96GA MUDI-96HA

* Each hard plastic 96-well plate contains 9 µL pre-mixed indexes. The plate has a pierceable foil seal and is sealed in in a silver-foil bag. Each index is intended for single use.

Shipping and Storage

As described in "Kit Contents" (starting on page 4), QIAseq FastSelect RNA library kits are shipped in several boxes and may arrive on separate days or in different shipments. Even though 2 kits have the same number of components, they might be shipped in different number of boxes.

- QIAseq RNA Lib Enzymes & Buffers is shipped on dry ice. Upon receipt, all components should be stored immediately at –30°C to –15°C in a constant-temperature freezer.
- NGS 2x Hi-Fi MasterMix Kit is shipped on dry ice. Upon receipt, all components should be stored immediately at –30°C to –15°C in a constant-temperature freezer.
- QIAseq FastSelect –rRNA HMR, QIAseq FastSelect –Globin, QIAseq FastSelect –rRNA Plant, QIAseq FastSelect –rRNA Fish, QIAseq FastSelect –rRNA Worm, QIAseq FastSelect –rRNA Yeast, and QIAseq FastSelect –rRNA Fly kits are shipped on dry ice and should be stored at –30°C to –15°C in a constant-temperature freezer.
- QIAseq FastSelect –5S/16S/23S is shipped on blue ice. Upon receipt, the FastSelect 5S/16S/23S tube should be immediately stored at –30°C to –15°C in a constant-temperature freezer. All remaining components should immediately be stored in a refrigerator at 2–8°C.
- QIAseq Beads are shipped at 4°C and upon receipt should be stored at 2–8°C in a refrigerator.
  Warning: QIAseq Beads are damaged by freezing.
- QIAseq Advanced Analysis is a paper card that contains a unique code for data analysis using the GeneGlobe RNA-seq Analysis Portal. The card ships at any temperature and should be stored in a safe location at room temperature (15–25°C).
- QIAseq UX index kits (sold separately) are shipped on dry ice and should be stored at –30°C to –15°C upon receipt.

When stored correctly, the QIAseq FastSelect library kits and QIAseq UX index kits can be used until the expiration date printed on the kit box lid. Under these conditions, the components

are stable, without showing any reduction in performance and quality, until the date indicated on the label.

Intended Use

All QIAseq FastSelect RNA library kits and UX products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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 FastSelect RNA library kits and QIAseq UX index kits are tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAseq FastSelect RNA library kits enable one-day, sample-to-sequencer next-generation sequencing (NGS) library prep using Illumina® NGS instruments. This RNA-library kit features cDNA barcoding and several different workflows, which enables either complete transcription or 3′ RNA-seq, simply by varying the reverse transcription reaction and/or the RNA sample. In addition, the kits include QIAseq Beads for fast and efficient reaction cleanup between protocol steps. By using the QIAseq UX Index IL UDI Index Kits, up to 768 samples can be multiplexed.

The QIAseq FastSelect RNA library kits and corresponding data analysis are intended for library construction and gene expression analysis of purified total RNA (1 ng – 1 µg) or enriched mRNA. The kit presents 2 innovative advantages compared to other protocols.

First, the inclusion of QIAseq FastSelect in the workflow enables rapid and efficient removal of ribosomal RNA during the preparation of the NGS RNA library. In one step, QIAseq FastSelect removes up to 99% of all unwanted rRNA – even when starting with difficult samples or instances where the RNA is already degraded, such as when using formalin-fixed paraffin embedded (FFPE) samples. This significantly increases the number of usable reads during sequencing.

Second, during reverse transcription, a unique sample ID is incorporated into each transcript. This barcodes each sample's RNA at the first reaction step, preventing sample mix-ups.

During library amplification/indexing, up to 768 different unique dual indexes (UDIs) can be used, allowing the number of samples to be scaled up significantly for one experiment.

The QIAseq FastSelect RNA library kits are supported with online, cloud-based pipelines through QIAGEN's GeneGlobe RNA-seq Analysis Portal, as well as with on-site software through QIAGEN CLC Genomics Workbench. QIAseq FastSelect RNA library kit data analysis

includes sample de-multiplexing, primary mapping, differential expression, and sample sequencing quality control.

The Sample to Insight® workflow of the QIAseq FastSelect RNA library kits defines a new generation of NGS technologies for gene expression analysis from eukaryotic cells and isolated RNA samples (Figure 1). The versatile RNA-library kit allows multiple types of RNA-seq libraries to be constructed when starting from total RNA or poly-A enriched RNA.

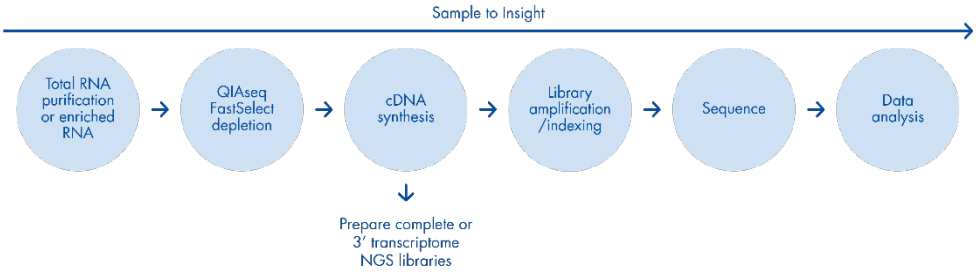

Figure 1. QIAGEN's Sample to Insight QIAseq FastSelect RNA library kit workflow.

Principle and procedure

The QIAseq FastSelect RNA library kit workflow is described in Figure 2. There are 2 magnetic bead-based cleanup steps using QIAseq beads (included with the kit).

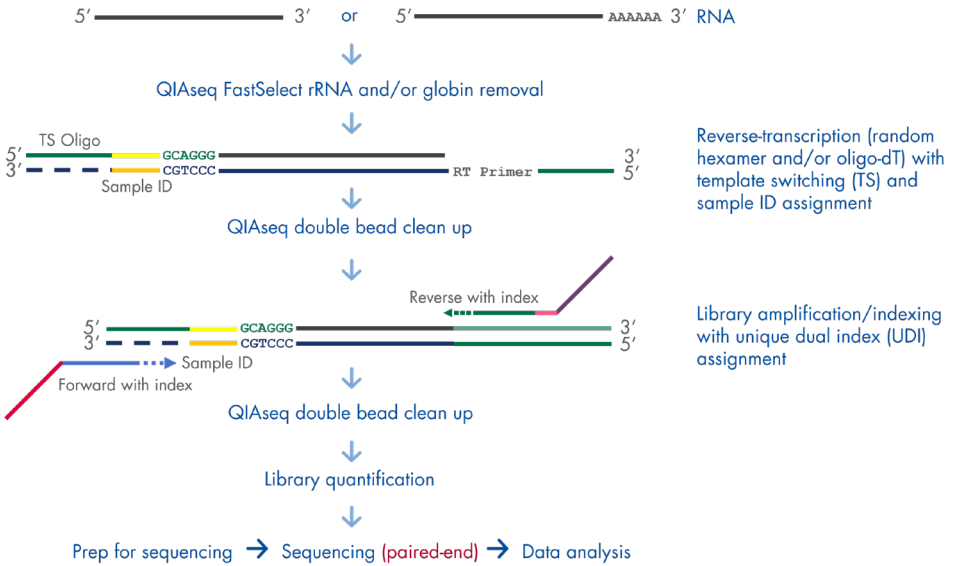

Figure 2. QIAseq FastSelect RNA library kit workflow.

- Start with purified total RNA, or enriched mRNA: 1 ng – 1 µg of purified total RNA can be used for each sample; 100 ng of total RNA (or greater) is recommended.
- **FastSelect rRNA depletion**: The FastSelect rRNA reagent is directly combined with the RNA and the US RT Buffer, 5x, enabling a rapid rRNA removal reaction. FastSelect prevents cDNA synthesis of rRNA. Specific heat fragmentation of the RNA is not necessary, as the reverse transcription chemistry is tuned to synthesize a specific range of cDNA sizes, regardless of starting sample quality.
- **cDNA synthesis**: cDNA synthesis can be performed using either the N6-T RT Primer (random hexamer) either alone or in combination with the ODT-T RT Primer (oligo-dT primer) for complete transcriptome analysis or the ODT-T RT Primer exclusively for 3′ transcriptome analysis.

  The SID-TS-24S RT Plate (Table 1) or SID-TS-96S RT Plate (Table 2) contain 24 or 96 lyophilized template switching oligos, respectively, that facilitate 10 bp sample

barcoding during cDNA synthesis; if not all wells are used in an experiment, unused wells can be covered using provided strip caps.

Table 1. QIAseq FastSelect RNA library kit sample Index (SID-TS-24s) RT Plate. Layout of SID-TS-24S plate for 24 samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A01	A02	A03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	B01	B02	B03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	C01	C02	C03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
D	D01	D02	D03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	E01	E02	E03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
F	F01	F02	F03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
G	G01	G02	G03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
H	H01	H02	H03	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

Table 2. QIAseq FastSelect RNA library kit sample Index (SID-TS-96S) RT Plate. Layout of SID-TS-96S Plate for 96 samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12
B	B01	B02	B03	B04	B05	B06	B07	B08	B09	B10	B11	B12
C	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12
D	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12
E	E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12
F	F01	F02	F03	F04	F05	F06	F07	F08	F09	F10	F11	F12
G	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12
H	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11	H12

- **Library amplification/indexing**: Library amplification using QIAseq UX Index Kits introduces 10-base, UDIs into the library. Up to 768 UDIs are available.
- **NGS**: The QIAseq FastSelect RNA library kits are compatible with Illumina NGS systems (MiniSeq®, MiSeq®, NextSeq® 500/550, NextSeq 2000, HiSeq® 1000, HiSeq 1500, HiSeq 2000, HiSeq 2500, HiSeq 3000/4000, NovaSeq® 6000, and other Illumina-based sequencing instruments that support paired-end sequencing. For standard expression analysis, 74 bp paired-end sequencing with dual 10 bp indexes should be used. For fusion analysis along with standard expression analysis, 149 bp paired-end sequencing with dual 10 bp indexes should be used. Recommendations for read allocation are found in Table 3; this should be used as a starting point, as read allocation is ultimately dependent on both the application and sample type.

Table 3. Read allocation recommendations per sample

	Number of reads per sample	
Total RNA input per sample	**Complete Transcriptome**	**3′ RNA-seq**
500 pg RNA	1,000,000	250,000
1 ng RNA	5,000,000	2,000,000
10 ng RNA	20,000,000	5,000,000
100 ng RNA	50,000,000	10,000,000

- **Data analysis**: The QIAseq FastSelect RNA library kits are supported through 2 analysis pipelines. The RNA-seq Analysis & Biomarker Discovery Pipeline is a cloud-based RNA-seq pipeline available in the QIAGEN GeneGlobe Analysis Portal. Additionally, the QIAGEN CLC Genomics Workbench can be used. The pipelines automatically perform all steps necessary for primary mapping, sample cluster analysis, and differential expression for bulk sequencing applications.

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 SDSs available from the product supplier.

- Nuclease-free pipette tips and tubes
- Microcentrifuge tubes (2 mL)
- PCR tubes (0.2 mL individual tubes or tubes strips) (VWR cat. no. 20170-012 or 93001-118)
- Ice
- Microcentrifuge
- Thermal cycler
- Magnet for QIAseq Bead Cleanups — MagneSphere® Technology Magnetic Separation Stand (Promega cat. no. Z5342)
- 100% ethanol, ACS-grade

Library QC methods

- QIAxcel® Connect (QIAGEN, cat. no. 9003110)
- 2100 Bioanalyzer® (Agilent, cat. no. varies)
- Agilent® High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626)
- Agilent High Sensitivity D5000 ScreenTape (Agilent, cat. no. 5067-5592)
- Library concentration readings:
  - Qubit® Fluorometer (Thermo Fisher Scientific, cat. no. varies)
  - Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, cat. no. Q32854)
  - Qubit Assay Tubes (Thermo Fisher Scientific, cat. no. Q32856)

Optional RNA spike-in

- ERCC ExFold RNA Spike-In Mixes (Thermo Fisher Scientific, cat. no. 4456739)

Optional control total RNA samples for process optimization

- Human XpressRef Universal Total RNA (cat. no. 338112)
- Mouse XpressRef Universal Total RNA (cat. no. 338114)
- Rat XpressRef Universal Total RNA (cat. no. 338116)

Important Notes

- DNase treatment (on-column and in-solution) of total RNA samples is highly recommended.
- When starting with isolated RNA, 1 ng – 1 µg of purified total RNA can be used, with 100 ng total RNA (or greater) being the preferred starting amount. QIAGEN provides a range of solutions for purification of total RNA from different amounts of sample (Table 4).

Table 4. Recommended kits for purification of total RNA

Kit	Cat. no.	Starting material
RNeasy® Micro Kit	74004	Small amounts of cells and tissue
RNeasy Mini Kit	74104 and 74106	Animal and human tissues and cells
RNeasy 96 Kit	74181 and 74182	Animal and human tissues and cells
RNeasy FFPE Kit	73504	FFPE tissue samples
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 RNA samples are of high quality and free of inhibitors that would compromise a reverse transcription or PCR. For more information about recommended laboratory procedures, please consult the handbook with your QIAGEN isolation kit.
- **RNA quantification**: Determine the concentration and purity of total RNA isolated from cells and fresh or 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 measuring 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 purified from cells and fresh/frozen tissue can be confirmed using an automated analysis system (such as the

QIAxcel Connect 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 NGS library construction is still possible with samples whose RIN values are ≤8.

- Ensure reactions are thoroughly mixed and that they are prepared and incubated at the recommended temperatures.
- If the workflow is not expected to be completed in one day, convenient stopping points are indicated at the end of the relevant sections.

Protocol: QIAseq FastSelect Library Construction

FastSelect rRNA depletion procedure

Important points before starting

- This protocol can be used with low amounts (1 ng – 1 µg) of purified RNA. The recommended starting amount is 100 ng (or greater).
- DNase treatment (on-column or in-solution) of total RNA samples is highly recommended.
- ERCC Control RNA (see "Equipment and Reagents to be Supplied by User") can be added according to the concentrations specified by the manufacturer. If added, the total reaction volume should remain 11 µL.
- Gently yet thoroughly vortex reactions or reagents unless instructed otherwise. Ensure all reactions are mixed thoroughly and handled at the temperatures recommended in the protocol.
- Use a thermal cycler with a heated lid.

Procedure

1. Thaw template RNA on ice. Gently yet thoroughly mix, then briefly centrifuge to collect residual liquid from the sides of the tubes and return to ice.
2. Prepare the reagents required for FastSelect rRNA depletion.
   2a. Thaw the appropriate tube(s) of QIAseq FastSelect, US RT Buffer, 5x, and Nuclease-Free Water at room temperature (15–25°C). Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.
   2b. Dilute an aliquot for each FastSelect tube to 0.1x (as an example: using 2 µL FastSelect tube + 18 µL Nuclease-Free Water). Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
   Note: For 1 ng samples, potential experimental optimization is to dilute FastSelect to 0.005x.
3. Prepare the FastSelect rRNA depletion reaction on as described in Table 5. Briefly centrifuge, gently yet thoroughly vortex to mix, and 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. FastSelect rRNA depletion reaction

Component	Volume/reaction
RNA (1 ng – 1 µg)	Variable
QIAseq FastSelect (0.1x)*	1 µL
ERCC Control†	Optional
US RT Buffer, 5x	4 µL
Nuclease-Free Water	Bring total reaction volume to 11 µL
Total volume	**11 µL**

* Any QIAseq FastSelect RNA Removal reagent or combination can be supplemented or substituted here. If additional QIAseq FastSelect RNA Removal reagents are used, the total reaction volume should remain 11 µL. If using QIAseq FastSelect –5S/16S/23S, which is used to remove bacterial rRNA, refer to Appendix C (page 51). If using QIAseq FastSelect Custom refer to the *QIAseq FastSelect Custom Handbook*.

† ERCC Control RNA (see "Equipment and Reagents to be Supplied by User") can be added according to the concentrations specified by the manufacturer. If added, the total reaction volume should remain 11 µL.

4. Incubate as described in Table 6 using a thermal cycler with a heated lid.
5. Proceed to “cDNA synthesis procedure”.

Table 6. FastSelect rRNA depletion incubation

Step	Time	Temperature
1	2 min	75°C
2	2 min	70°C
3	2 min	65°C
4	2 min	60°C
5	2 min	55°C
6	2 min	37°C
7	2 min	25°C
8	2 min	4°C
9	Hold	4°C

cDNA synthesis procedure

Important points before starting

- Set up the reactions on ice.
- Use a thermal cycler with a heated lid.
- The SID-TS-96S or SID-TS-24S is a 96-well single-use sample ID RT plate. Each well of the SID-TS-96S and only 24 wells of the SID-TS-24S Plate contain pre-dispensed sample ID template switching oligos required for the cDNA synthesis reaction.
  Important: If, during the setup of the cDNA synthesis reactions, only some of the wells in the 96-well plate will be used, add 2.5 µL Nuclease-Free Water to each well intended to be used, vortex the plate, centrifuge briefly, and incubate for 10 min at room temperature (15–25°C) to fully dissolve the primer. Then transfer the dissolved content of the wells that are going to be used to a new plate. Unused wells should be sealed with provided strip caps.

- Equilibrate the QIAseq Beads to room temperature for 20–30 min before use.
- Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color. If a delay in the protocol occurs, simply vortex the beads.
- **Important**: Prepare fresh 80% ethanol before performing the procedure.

Procedure

1. Prepare the reagents required for cDNA synthesis.
   1a. Thaw DTT (100 mM), dNTP (10 mM), Nuclease-Free Water, N6-T RT Primer, and/or ODT-T RT Primer at room temperature (15–25°C). Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.
   1b. Keep RNase Inhibitor and EZ Reverse Transcriptase on ice. Mix by flicking the tubes, briefly centrifuge, and return to ice. After use, immediately return the enzymes to the freezer.
   1c. Process the SID-TS-96S or SID-TS-24S plate as described in "Important points before starting" (previous page) if not using all the wells in the plate at one time.
2. Prepare the cDNA synthesis reaction on ice according to Table 7. Briefly centrifuge, gently yet thoroughly vortex to mix, and 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. cDNA synthesis reaction

Component	Volume per well
rRNA-depleted sample from previous step	11 µL
DTT (100 mM)	0.5 µL
dNTP (10 mM)	2 µL
Nuclease-Free Water*	2.5 µL
SID-TS-96S or SID-TS-24S RT Plate Dried*	–
N6-T RT Primer†	1 µL
ODT-T RT Primer†	1 µL
EZ Reverse Transcriptase	1.5 µL
RNase Inhibitor	0.5 µL
Total volume	**20 µL**

* If not using all the wells in the SID-TS-96S Plate, reduce the 2.5 µL volume of Nuclease-Free Water to 0 µL in the cDNA synthesis mix, as 2.5 µL of Nuclease-Free Water will be used to resuspend the sample ID template switching oligos that will be used.

† N6-T RT Primer can be used alone or in combination with the ODT-T RT Primer. If exclusively using the N6-T RT Primer, replace the ODT-RT primer with an additional 1 µL of Nuclease-Free Water. For 3' transcriptome analysis, only use the ODT-T RT primer, and replace the N6-T RT primer with an additional 1 µL of Nuclease-Free Water.

3. Add the cDNA synthesis mix to the corresponding wells in the SID-TS-96S plate. Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.

   Important: A unique sample ID must be used for each sample.

4. Incubate as described in Table 8.

   Note: The cDNA generated from each well of a SID-TS-96S RT Plate contains a barcoded, specific sample ID.

Table 8. cDNA synthesis incubation

Step	Time	Temperature
1	1 min	4°C
2	5 min	25°C
3	90 min	42°C
4	10 min	70°C
5	1 min	4°C
6	∞	4°C

5. Add 22 µL (1.1x the reaction volume) of QIAseq Beads to the completed cDNA synthesis reactions. Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.

   Note: Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.

6. Incubate for 5 min at room temperature.
7. Centrifuge the tube until the beads are pelleted (2 min), and then place the plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.

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

   Tip: It may be valuable to discard the supernatant twice. The contents settle after the first discard.

8. With the tube still on the magnetic stand, add 200–300 µL freshly prepared 80% ethanol. Wait 2 min and carefully remove and discard the wash. Repeat the wash for a total of 2 ethanol washes. Remove as much excess ethanol as possible.

   Note: When decided between 200 µL or 300 µL, simply ensure that enough is added to submerge the beads.

   Important: It is vital to completely remove all traces of ethanol after the second wash. Remove the ethanol with a 200 µL pipette first, briefly centrifuge the tubes immediately,

and return the tubes to the magnetic stand. Then use a 10 µL pipette to remove any residual ethanol. This step should be performed quickly.

9. With the tube (cap open) still on the magnetic stand, air-dry at room temperature for 5–10 min (until the beads start to crack and pellet loses its shine).

   Note: Visually inspect that the pellet is completely dry.

10. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 22 µL Nuclease-Free Water. Gently yet thoroughly vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min.
11. Centrifuge the tube until the beads are pelleted (2 min), and then return the tube to the magnetic rack until the solution has cleared.
12. Transfer 20 µL of the supernatant to a clean tube.
13. Add 22 µL of QIAseq Beads (1.1x the reaction volume) to the supernatant. Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.

    Note: Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.

14. Incubate for 5 min at room temperature.
15. Centrifuge the tube until the beads are pelleted (2 min), and then place the plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.

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

    Tip: It may be useful to discard the supernatant twice. The contents settle after the first discard.

16. With the tube still on the magnetic stand, add 200 µL freshly prepared 80% ethanol. Wait 2 min and carefully remove and discard the wash. Repeat the wash for a total of 2 ethanol washes. Remove excess ethanol as much as possible.

    Important: It is vital to completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette first, briefly centrifuge the tubes immediately,

and return the tubes to the magnetic stand. Then use a 10 µL pipette to remove any residual ethanol. This step should be performed quickly.

17. With the tube (cap open) still on the magnetic stand, air-dry at room temperature for 5–10 min (until the beads start to crack and pellet loses its shine).

    Note: Visually inspect that the pellet is completely dry.

18. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 25 µL Nuclease-Free Water. Gently yet thoroughly vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min.
19. Centrifuge the tube until the beads are pelleted (2 min), and then return the tube to the magnetic rack until the solution has cleared.
20. Transfer 23 µL of the supernatant to a clean tube.

    Proceed with "Library amplification/indexing procedure" (below). Alternatively, the samples can be stored at –30°C to –15°C.

Library amplification/indexing procedure

Important points before starting

- The QIAseq UX index plates have pierceable foil seals, and the indexes must be pipetted from the plate into separate reaction plates. To prevent cross-contamination, each well is single use.
- A precipitate might be present in the QIAseq 2x HiFi MM. If so, bring the reagent to room temperature for 5 min, and dissolve the precipitate by mixing with pipettor and/or by gentle vortexing.
- Set up the reactions on ice.
- Use a thermal cycler with a heated lid.
- Equilibrate the QIAseq Beads to room temperature (15–25°C) for 20–30 min before use.
- Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color. If a delay in the protocol occurs, simply vortex the beads.
- **Important**: Prepare fresh 80% ethanol before performing this procedure.

Procedure

1. Prepare reagents required for library amplification or indexing.
   1a. Thaw the QIAseq UX index plates at room temperature (15–25°C). Gently yet thoroughly vortex to mix, and then centrifuge briefly.
   Note: The layout and use of QIAseq UX index plates is described in "Appendix A: QIAseq UX Index Plates" (page 44). During the reaction setup in step 2, indexes are removed to a new plate.
   1b. Thaw the QIAseq 2x HiFi MM on ice. Mix by gently but thoroughly vortexing the tube, and then centrifuge briefly.
2. On ice, prepare the library amplification/indexing reaction according to Table 9. Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.

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

Table 9. Library amplification/indexing reaction

Component	Volume/reaction
cDNA synthesis bead cleanup supernatant	23 µL
QIAseq 2x HiFi MM	25 µL
Index from QIAseq UX index plate	2 µL
Total volume	**50 µL**

3. In a thermal cycler, perform the cycling program described in Table 10 with the number of cycles determined from Table 11.

Table 10. Library amplification or indexing cycling program

Step	Time	Temperature	Number of cycles
Initial denaturation	30 s	98°C	1
3-step cycling			
Denaturation	5 s	98°C	
Annealing	10 s	55°C	See Table 9
Extension	20 s	72°C	
Final extension	2 min	72°C	1
Hold	1 min	4°C	1
	∞	4°C	Hold

Table 11. PCR cycle number recommendation, based on total RNA input and number of samples pooled

Total RNA input	Number of amplification cycles
1 ng	27
10 ng	24
100 ng	20
1 µg	17
Enriched poly A^+	27

4. Upon completion of library-amplification or indexing reactions, add 40 µL QIAseq Beads (0.8x the reaction volume). Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.

   Note: Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.

5. Incubate for 5 min at room temperature.

6. Centrifuge the tube or plate until the beads are pelleted (2 min), and then place the tubes or plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.

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

   Tip: It may be useful to discard the supernatant twice. The contents settle after the first discard.

7. With the tube still on the magnetic stand, add 200 µL freshly prepared 80% ethanol. Wait 2 min and carefully remove and discard the wash. Repeat the wash, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.

   Important: It is vital to completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette first, briefly centrifuge the tubes immediately and return the tubes to the magnetic stand. Then use a 10 µL pipette to remove any residual ethanol. This step should be performed quickly.

8. With the tube (cap open) still on the magnetic stand, air-dry at room temperature for 5–10 min (until the beads start to crack and pellet loses its shine).

   Note: Visually inspect that the pellet is completely dry.

9. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 22 µL Nuclease-Free Water. Gently yet thoroughly vortex to mix, briefly centrifuge, and incubate for 2 min.

10. Centrifuge the tube until the beads are pelleted (2 min), and then return the tube to the magnetic rack until the solution has cleared.

11. Transfer 20 µL of the supernatant to a clean tube.

12. Add 16 µL (0.8x the reaction volume) of QIAseq Beads to the supernatant. Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.

    Note: Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.

13. Incubate for 5 min at room temperature.

14. Centrifuge the tube until the beads are pelleted (2 min), and then place the plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.

    Important: Do not discard the beads, as they contain the DNA of interest.
    Tip: It may be valuable to discard the supernatant twice. The contents settle after the first discard.

15. With the tube still on the magnetic stand, add 200 µL freshly prepared 80% ethanol. Wait 1 min and carefully remove and discard the wash. Repeat the wash, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
16. **Important**: It is vital to completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette first, briefly centrifuge the tubes immediately and return the tubes to the magnetic stand. Then use a 10 µL pipette to remove any residual ethanol. This step should be performed quickly.
17. With the tube (cap open) still on the magnetic stand, air-dry at room temperature for 5–10 min (until the beads start to crack and pellet loses its shine).

    Note: Visually inspect that the pellet is completely dry.
18. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 24 µL Nuclease-Free Water. Gently yet thoroughly vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min.
19. Centrifuge the tube until the beads are pelleted (2 min), and then return the tube to the magnetic rack until the solution has cleared.
20. Transfer 22 µL of the supernatant to a clean tube. If not proceeding immediately, the sample can be stored at –30°C to –15°C.
21. Assess the quality of the library using a QIAxcel Connect, Bioanalyzer, TapeStation®, or Fragment Analyzer. Check for the expected size distribution of library fragments. An example library is shown in Figure 3.

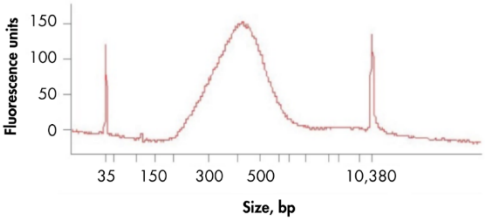

Figure 3. TapeStation trace of library prepared with the QIAseq FastSelect RNA library kit.

22. Quantify and normalize the individual libraries.

    Option 1: Quantification of the libraries using Qubit.

    Option 2: The library yield measurements of Qubit or Nanodrop or the Bioanalyzer and TapeStation systems use fluorescence dyes, which intercalate into DNA or RNA and cannot discriminate between cDNA that have and do not have adapter sequences. Real-time PCR-based methods provide an accurate quantification of complete RNA-seq libraries with full adapter sequences. As a result, QIAGEN's QIAseq Library Quant Array Kit (cat. no. 333304) or Assay Kit (cat. no. 333314), which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library. Please consult the handbook for QIAseq library quant array or assay kits for directions.

23. Proceed to "Protocol: Sequencing Setup", page 37. Alternatively, the purified FastSelect RNA library can be safely stored at –30°C to –15°C until ready to use for sequencing.

Protocol: Sequencing Setup

QIAseq FastSelect RNA libraries are compatible with Illumina NGS platforms, including iSeq® 100, MiniSeq, MiSeq, NextSeq 500/550, NextSeq 1000/2000, HiSeq 2500, HiSeq 3000/4000, and NovaSeq 6000.

Important points before starting

- To make sequencing preparation convenient, download Illumina-compatible sample sheets for your sequencing instruments at **www.qiagen.com**, and refer to Appendix A, page 44.
- For standard expression analysis, 74 bp paired-end sequencing with dual 10 bp indexes should be used.
- For fusion analysis along with standard expression analysis, 149 bp paired-end sequencing with dual 10 bp indexes should be used.
- Ensure that PhiX is included in the sequencing run. Refer to the table below for recommended PhiX amounts. If the system is not listed, refer to the system-specific Illumina documents for recommended PhiX amounts.
- For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific Illumina documents.
- Sample dilution, pooling, and sequencing:
  - Dilute the individual libraries to a concentration of 4 nM, and then combine libraries with different sample indexes in equimolar amounts. The recommended starting final loading concentration of the pooled FastSelect RNA libraries to load onto a MiSeq is 10 pM, or 1.6 pM on a MiniSeq, or 1.6 pM on a NextSeq instrument.
  - Dilute the individual FastSelect RNA libraries to a concentration of 10 nM, then combine libraries with different sample indexes in equimolar amounts. The recommended final loading concentration of the pooled FastSelect RNA libraries to load onto a NovaSeq instrument is between 175 pM and 265 pM.

Generation of sample sheets for Illumina instruments

Sequences for QIAseq UX UDI indexes are available at **www.qiagen.com** for download. To make sequencing preparation more convenient, ready-to-use templates that include sample sheets containing all QIAseq UX UDI index sequences are available at **www.qiagen.com** for different sequencing instruments.

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 **qiagen.com/qiaseq-fastselect-rna-library-kit** and select **Product Resources** then **Instrument Technical Documents** to find and download the appropriate QIAseq FastSelect RNA library kit template. Different templates might be available, depending on the instrument.
2. The sample sheet already contains all relevant information to use with the instrument.
3. Open the CSV file, delete any UDI indexes 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 or NextSeq instrument or upload the **Sample Sheet** into Local Run Manager for MiSeq, MiniSeq, and NextSeq Illumina instruments.
5. When ready to perform the run, select the file.

6. **Sample dilution and pooling**: Dilute individual libraries to 4 nM unless using for the NovaSeq, in which case, dilute the individual libraries to 10 nM. Then, combine libraries with different sample indexes in equimolar amounts if a similar sequencing depth is needed for each library.

   Note: For the NovaSeq, the recommended final pooled library concentration is 1.0–1.5 nM, which will yield a final loading concentration of 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 as described in Table 12 to obtain the final library concentration.

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	*NextSeq 500 System Guide or NextSeq 550 System Guide*	1.2
NovaSeq 6000	*NovaSeq 6000 Sequencing System Guide*	200–300

8. PhiX Control v3 Spike-in (Illumina, cat. no. FC-110-3001) recommendations are indicated in Table 13, page 40. Follow Illumina guidelines on how to denature, dilute, and combine a PhiX control library with your own pool of libraries. Make sure to use a fresh, reliable stock of PhiX control library. Spike-in 10% PhiX or more of the total library pool.
9. Sequencing run setup: Select **FASTQ Only**.
10. The recommended protocol is 74 bp paired end read with 10 bp dual indexing.
11. Upon completion of the sequencing run, proceed with "Protocol: Data Analysis", page 41.

Table 13. Summary of sequencing recommendations for Illumina NGS instruments

Illumina instrument	Flow cell	Recommended sequencing setup	Recommended read 1*	Recommended read 2*	Recommended dual index read	PhiX
MiSeq	V3 150 cycle	Paired end	74	74	10	10%
NextSeq 500	Mid/high-output 150 cycle	Paired end	74	74	10	10%
NextSeq 1000/2000	200 cycle P2	Paired end	74	74	10	10%

* The read-length is a recommendation. The read-length can be adjusted, based on the number of cycles of a given flow cell. Additionally, for fusion analysis along with standard expression analysis, 149 bp paired-end sequencing should be used.

Protocol: Data Analysis

RNA-seq Analysis & Biomarker Discovery pipeline

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, and differential expression. QIAGEN Ingenuity® Pathway Analysis (IPA®) returns 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, and RNA sequences aligned. Differential RNA expression will be calculated and visualized using interactive volcano plots. Differentially expressed RNAs will be queried against the QIAGEN Knowledge Base for canonical pathways, upstream regulators, and diseases and biological functions. Important RNAs can then be identified, and digital PCR and qPCR assays 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 at **www.qiagen.com**.

Procedure

1. Go to **GeneGlobe.QIAGEN.com**.
2. Click **Analyze** in the top menu.

3. Under **Start Analyzing Your Data**:
    - Select analysis type: **Next-Generation Sequencing**
    - Select your analyte: **mRNA/lncRNA**
    - Select your kit: **QIAseq FastSelect**
4. Click **Start Your Analysis**.

Data Analysis using QIAGEN CLC Genomics Workbench

QIAGEN CLC Genomics Workbench (cat. no. 832021) is available for installation on local desktop computers or servers on a subscription basis. QIAGEN CLC Genomics Workbench is a powerful solution that works for everyone, no matter the workflow. Cutting-edge technology, unique features, and algorithms widely used by scientific leaders in industry and academia make it easy to overcome challenges associated with data analysis.

QIAseq FastSelect RNA analysis is supported by downloading the Biomedical Genomics Analysis plug-in, which provides tools and workflows for NGS panel data analysis, WES, WGS, and RNA-seq.

Troubleshooting Guide

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

	Comments and suggestions
Low yield	
a) Using the multi-use Sample ID RT Plate, primers were not properly reconstituted	Prior to use, add 2.5 µL Nuclease-Free Water into each well, vortex the plate, centrifuge briefly, and incubate for 10 min at room temperature (15–25°C) to fully dissolve the primer.
b) Improper reaction setup	Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.
c) Excess ethanol not removed during bead cleanup steps	After each second ethanol wash, ensure that excess ethanol is removed. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then with a 10 µL pipette to remove any residual ethanol. In addition, allow beads to dry for the appropriate amount of time.
Sequencing issues	
a) Cluster density that is too low or too high	Accurate library quantification is the key for optimal cluster density on any sequencing instrument. A 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: QIAseq UX Index Plates

QIAseq UX index plate layouts

The layouts of the single-use QIAseq UX index plates are shown in Table 14 to Table 22. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments at **www.qiagen.com**.

Table 14. QIAseq UX 96 Index Kit IL UDI-A (96) (cat. no. 331815) layout: MUDI-96AA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 001	iMUDI 009	iMUDI 017	iMUDI 025	iMUDI 033	iMUDI 041	iMUDI 049	iMUDI 057	iMUDI 065	iMUDI 073	iMUDI 081	iMUDI 089
B	iMUDI 002	iMUDI 010	iMUDI 018	iMUDI 026	iMUDI 034	iMUDI 042	iMUDI 050	iMUDI 058	iMUDI 066	iMUDI 074	iMUDI 082	iMUDI 090
C	iMUDI 003	iMUDI 011	iMUDI 019	iMUDI 027	iMUDI 035	iMUDI 043	iMUDI 051	iMUDI 059	iMUDI 067	iMUDI 075	iMUDI 083	iMUDI 091
D	iMUDI 004	iMUDI 012	iMUDI 020	iMUDI 028	iMUDI 036	iMUDI 044	iMUDI 052	iMUDI 060	iMUDI 068	iMUDI 076	iMUDI 084	iMUDI 092
E	iMUDI 005	iMUDI 013	iMUDI 021	iMUDI 029	iMUDI 037	iMUDI 045	iMUDI 053	iMUDI 061	iMUDI 069	iMUDI 077	iMUDI 085	iMUDI 093
F	iMUDI 006	iMUDI 014	iMUDI 769	iMUDI 030	iMUDI 038	iMUDI 046	iMUDI 054	iMUDI 062	iMUDI 070	iMUDI 078	iMUDI 086	iMUDI 094
G	iMUDI 007	iMUDI 015	iMUDI 023	iMUDI 031	iMUDI 039	iMUDI 047	iMUDI 055	iMUDI 063	iMUDI 071	iMUDI 079	iMUDI 087	iMUDI 095
H	iMUDI 008	iMUDI 016	iMUDI 024	iMUDI 032	iMUDI 040	iMUDI 048	iMUDI 056	iMUDI 064	iMUDI 072	iMUDI 080	iMUDI 088	iMUDI 096

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

Table 15. QIAseq UX 96 Index Kit IL UDI-B (96) (cat. no. 331825) layout — MUDI-96BA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 097	iMUDI 105	iMUDI 113	iMUDI 121	iMUDI 129	iMUDI 137	iMUDI 145	iMUDI 153	iMUDI 161	iMUDI 169	iMUDI 177	iMUDI 185
B	iMUDI 098	iMUDI 106	iMUDI 114	iMUDI 122	iMUDI 130	iMUDI 138	iMUDI 146	iMUDI 154	iMUDI 162	iMUDI 170	iMUDI 178	iMUDI 186
C	iMUDI 099	iMUDI 107	iMUDI 115	iMUDI 123	iMUDI 131	iMUDI 139	iMUDI 147	iMUDI 155	iMUDI 163	iMUDI 171	iMUDI 179	iMUDI 187
D	iMUDI 100	iMUDI 108	iMUDI 116	iMUDI 124	iMUDI 132	iMUDI 140	iMUDI 148	iMUDI 156	iMUDI 164	iMUDI 172	iMUDI 180	iMUDI 188
E	iMUDI 101	iMUDI 109	iMUDI 117	iMUDI 125	iMUDI 133	iMUDI 141	iMUDI 149	iMUDI 157	iMUDI 165	iMUDI 173	iMUDI 181	iMUDI 189
F	iMUDI 102	iMUDI 110	iMUDI 118	iMUDI 126	iMUDI 134	iMUDI 142	iMUDI 150	iMUDI 158	iMUDI 166	iMUDI 174	iMUDI 182	iMUDI 770
G	iMUDI 103	iMUDI 111	iMUDI 119	iMUDI 127	iMUDI 135	iMUDI 143	iMUDI 151	iMUDI 159	iMUDI 167	iMUDI 175	iMUDI 183	iMUDI 191
H	iMUDI 104	iMUDI 112	iMUDI 120	iMUDI 128	iMUDI 136	iMUDI 144	iMUDI 152	iMUDI 160	iMUDI 168	iMUDI 176	iMUDI 184	iMUDI 192

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

Table 16. QIAseq UX 96 Index Kit IL UDI-C (96) (cat. no. 331835) layout — MUDI-96CA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 193	iMUDI 201	iMUDI 209	iMUDI 217	iMUDI 225	iMUDI 233	iMUDI 241	iMUDI 249	iMUDI 257	iMUDI 265	iMUDI 273	iMUDI 281
B	iMUDI 194	iMUDI 202	iMUDI 210	iMUDI 218	iMUDI 226	iMUDI 234	iMUDI 242	iMUDI 250	iMUDI 258	iMUDI 266	iMUDI 274	iMUDI 282
C	iMUDI 195	iMUDI 203	iMUDI 211	iMUDI 219	iMUDI 227	iMUDI 235	iMUDI 243	iMUDI 251	iMUDI 259	iMUDI 267	iMUDI 275	iMUDI 283
D	iMUDI 196	iMUDI 204	iMUDI 212	iMUDI 220	iMUDI 228	iMUDI 236	iMUDI 244	iMUDI 252	iMUDI 260	iMUDI 268	iMUDI 276	iMUDI 284
E	iMUDI 197	iMUDI 205	iMUDI 213	iMUDI 221	iMUDI 229	iMUDI 237	iMUDI 245	iMUDI 253	iMUDI 261	iMUDI 269	iMUDI 277	iMUDI 285
F	iMUDI 198	iMUDI 206	iMUDI 214	iMUDI 222	iMUDI 230	iMUDI 238	iMUDI 246	iMUDI 254	iMUDI 262	iMUDI 270	iMUDI 278	iMUDI 286
G	iMUDI 199	iMUDI 207	iMUDI 215	iMUDI 223	iMUDI 231	iMUDI 239	iMUDI 247	iMUDI 255	iMUDI 263	iMUDI 271	iMUDI 279	iMUDI 287
H	iMUDI 200	iMUDI 208	iMUDI 216	iMUDI 224	iMUDI 232	iMUDI 240	iMUDI 248	iMUDI 256	iMUDI 264	iMUDI 272	iMUDI 280	iMUDI 288

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

Table 17. QIAseq UX 96 Index Kit IL UDI-D (96) (cat.no. 331845) layout — MUDI-96DA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 289	iMUDI 297	iMUDI 305	iMUDI 313	iMUDI 321	iMUDI 329	iMUDI 337	iMUDI 345	iMUDI 353	iMUDI 361	iMUDI 369	iMUDI 377
B	iMUDI 290	iMUDI 298	iMUDI 306	iMUDI 314	iMUDI 322	iMUDI 330	iMUDI 338	iMUDI 346	iMUDI 354	iMUDI 362	iMUDI 370	iMUDI 378
C	iRUD2 91	iMUDI 299	iMUDI 307	iMUDI 315	iMUDI 323	iMUDI 331	iMUDI 339	iMUDI 347	iMUDI 355	iMUDI 363	iMUDI 371	iMUDI 379
D	iMUDI 292	iMUDI 300	iMUDI 308	iMUDI 316	iMUDI 324	iMUDI 332	iMUDI 340	iMUDI 348	iMUDI 356	iMUDI 364	iMUDI 372	iMUDI 380
E	iMUDI 293	iMUDI 301	iMUDI 309	iMUDI 317	iMUDI 325	iMUDI 333	iMUDI 341	iMUDI 349	iMUDI 357	iMUDI 365	iMUDI 373	iMUDI 772
F	iMUDI 294	iMUDI 302	iMUDI 310	iMUDI 318	iMUDI 326	iMUDI 334	iMUDI 342	iMUDI 350	iMUDI 358	iMUDI 366	iMUDI 374	iMUDI 382
G	iMUDI 295	iMUDI 303	iMUDI 311	iMUDI 319	iMUDI 327	iMUDI 335	iMUDI 343	iMUDI 351	iMUDI 359	iMUDI 367	iMUDI 375	iMUDI 383
H	iMUDI 296	iMUDI 304	iMUDI 312	iMUDI 320	iMUDI 328	iMUDI 336	iMUDI 344	iMUDI 352	iMUDI 360	iMUDI 368	iMUDI 771	iMUDI 384

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

Table 18. QIAseq UX 96 Index Kit IL UDI-E (96) (cat. no. 331855) layout — MUDI-96EA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 385	iMUDI 393	iMUDI 401	iMUDI 409	iMUDI 417	iMUDI 425	iMUDI 433	iMUDI 441	iMUD I774	iMUDI 457	iMUDI 465	iMUDI 473
B	iMUDI 386	iMUDI 394	iMUDI 402	iMUDI 410	iMUDI 418	iMUDI 426	iMUDI 434	iMUDI 442	iMUDI 450	iMUDI 458	iMUDI 775	iMUDI 474
C	iMUDI 387	iMUDI 395	iMUDI 403	iMUDI 411	iMUDI 419	iMUDI 427	iMUDI 435	iMUDI 443	iMUDI 451	iMUDI 459	iMUDI 467	iMUDI 475
D	iMUDI 388	iMUDI 396	iMUDI 404	iMUDI 412	iMUDI 420	iMUDI 428	iMUDI 436	iMUDI 444	iMUDI 452	iMUDI 460	iMUDI 468	iMUDI 476
E	iMUDI 389	iMUDI 397	iMUDI 405	iMUDI 413	iMUDI 421	iMUDI 429	iMUDI 437	iMUDI 445	iMUDI 453	iMUDI 461	iMUDI 469	iMUDI 477
F	iMUDI 390	iMUDI 398	iMUDI 406	iMUDI 414	iMUDI 422	iMUDI 430	iMUDI 438	iMUDI 446	iMUDI 454	iMUDI 462	iMUDI 470	iMUDI 478
G	iMUDI 391	iMUDI 399	iMUD I773	iMUDI 415	iMUDI 423	iMUDI 431	iMUDI 439	iMUDI 447	iMUDI 455	iMUDI 463	iMUDI 471	iMUDI 479
H	iMUDI 392	iMUDI 400	iMUDI 408	iMUDI 416	iMUDI 424	iMUDI 432	iMUDI 440	iMUDI 448	iMUDI 456	iMUDI 464	iMUDI 472	iMUDI 480

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

Table 19. QIAseq UX 96 Index Kit IL UDI-F (96) (cat. no. 331865) layout — MUDI-96FA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 481	iMUDI 489	iMUDI 497	iMUDI 505	iMUDI 513	iMUDI 521	iMUDI 529	iMUDI 777	iMUDI 545	iMUDI 553	iMUDI 561	iMUDI 569
B	iMUDI 482	iMUDI 490	iMUDI 498	iMUDI 506	iMUDI 514	iMUDI 522	iMUDI 530	iMUDI 538	iMUDI 546	iMUDI 554	iMUDI 562	iMUDI 570
C	iMUDI 483	iMUDI 491	iMUDI 499	iMUDI 507	iMUDI 515	iMUDI 523	iMUDI 531	iMUDI 539	iMUDI 547	iMUDI 555	iMUDI 563	iMUDI 571
D	iMUDI 484	iMUDI 492	iMUDI 500	iMUDI 508	iMUDI 516	iMUDI 524	iMUDI 532	iMUDI 540	iMUDI 548	iMUDI 556	iMUDI 564	iMUDI 572
E	iMUDI 485	iMUDI 493	iMUDI 501	iMUDI 509	iMUDI 517	iMUDI 525	iMUDI 533	iMUDI 541	iMUDI 549	iMUDI 557	iMUDI 565	iMUDI 573
F	iMUDI 486	iMUDI 494	iMUDI 502	iMUDI 776	iMUDI 518	iMUDI 526	iMUDI 534	iMUDI 542	iMUDI 550	iMUDI 558	iMUDI 566	iMUDI 574
G	iMUDI 487	iMUDI 495	iMUDI 503	iMUDI 511	iMUDI 519	iMUDI 527	iMUDI 535	iMUDI 543	iMUDI 551	iMUDI 559	iMUDI 567	iMUDI 575
H	iMUDI 488	iMUDI 496	iMUDI 504	iMUDI 512	iMUDI 520	iMUDI 528	iMUDI 536	iMUDI 544	iMUDI 552	iMUDI 560	iMUDI 568	iMUDI 576

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

Table 20. QIAseq UX 96 Index Kit IL UDI-G (96) (cat. no. 331875) layout — MUDI-96GA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 577	iMUDI 585	iMUDI 593	iMUDI 601	iMUDI 609	iMUDI 617	iMUDI 625	iMUDI 633	iMUDI 641	iMUDI 649	iMUDI 657	iMUDI 665
B	iMUDI 778	iMUDI 586	iMUDI 594	iMUDI 602	iMUDI 610	iMUDI 779	iMUDI 626	iMUDI 634	iMUDI 642	iMUDI 650	iMUDI 658	iMUDI 666
C	iMUDI 579	iMUDI 587	iMUDI 595	iMUDI 603	iMUDI 611	iMUDI 780	iMUDI 627	iMUDI 635	iMUDI 643	iMUDI 651	iMUDI 659	iMUDI 667
D	iMUDI 580	iMUDI 588	iMUDI 596	iMUDI 604	iMUDI 612	iMUDI 620	iMUDI 628	iMUDI 636	iMUDI 644	iMUDI 652	iMUDI 660	iMUDI 668
E	iMUDI 581	iMUDI 589	iMUDI 597	iMUDI 605	iMUDI 613	iMUDI 621	iMUDI 629	iMUDI 637	iMUDI 645	iMUDI 653	iMUDI 661	iMUDI 669
F	iMUDI 582	iMUDI 590	iMUDI 598	iMUDI 606	iMUDI 614	iMUDI 622	iMUDI 630	iMUDI 638	iMUDI 646	iMUDI 654	iMUDI 662	iMUDI 670
G	iMUDI 583	iMUDI 591	iMUDI 599	iMUDI 607	iMUDI 615	iMUDI 623	iMUDI 631	iMUDI 639	iMUDI 647	iMUDI 655	iMUDI 663	iMUDI 671
H	iMUDI 584	iMUDI 592	iMUDI 600	iMUDI 608	iMUDI 616	iMUDI 624	iMUDI 632	iMUDI 640	iMUDI 648	iMUDI 656	iMUDI 664	iMUDI 672

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

Table 21. QIAseq UX 96 Index Kit IL UDI-H (96) (cat. no. 331885) layout — MUDI-96HA

	1	2	3	4	5	6	7	8	9	10	11	12
A	iMUDI 673	iMUDI 681	iMUDI 689	iMUDI 697	iMUDI 705	iMUDI 713	iMUDI 721	iMUDI 729	iMUDI 737	iMUDI 745	iMUDI 753	iMUDI 761
B	iMUDI 781	iMUDI 682	iMUDI 690	iMUDI 698	iMUDI 706	iMUDI 714	iMUDI 722	iMUDI 730	iMUDI 738	iMUDI 746	iMUDI 754	iMUDI 762
C	iMUDI 675	iMUDI 683	iMUDI 691	iMUDI 699	iMUDI 707	iMUDI 715	iMUDI 723	iMUDI 731	iMUDI 739	iMUDI 747	iMUDI 755	iMUDI 763
D	iMUDI 676	iMUDI 684	iMUDI 692	iMUDI 700	iMUDI 708	iMUDI 716	iMUDI 724	iMUDI 732	iMUDI 740	iMUDI 748	iMUDI 756	iMUDI 764
E	iMUDI 677	iMUDI 685	iMUDI 693	iMUDI 701	iMUDI 709	iMUDI 717	iMUDI 725	iMUDI 733	iMUDI 741	iMUDI 749	iMUDI 757	iMUDI 765
F	iMUDI 678	iMUDI 686	iMUDI 694	iMUDI 702	iMUDI 710	iMUDI 718	iMUDI 726	iMUDI 734	iMUDI 742	iMUDI 750	iMUDI 758	iMUDI 766
G	iMUDI 679	iMUDI 687	iMUDI 695	iMUDI 703	iMUDI 711	iMUDI 719	iMUDI 727	iMUDI 735	iMUDI 743	iMUDI 751	iMUDI 759	iMUDI 767
H	iMUDI 680	iMUDI 688	iMUDI 696	iMUDI 704	iMUDI 712	iMUDI 720	iMUDI 728	iMUDI 736	iMUDI 744	iMUDI 752	iMUDI 760	iMUDI 768

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

Table 22. QIAseq UX 12 Index Kit IL UDI (12) (cat. no. 331801) layout — RUDI-12A

	1	2	3	4	5	6	7	8	9	10	11	12
A	iRUDI 001	iRUDI 009	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	iRUDI 002	IRUDI 010	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	iRUDI 003	IRUDI 011	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
D	iRUDI 004	iRUD I012	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	iRUD I005	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
F	iRUDI 006	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
G	iRUDI 007	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
H	iRUD I008	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.

Appendix B: mRNA Enrichment using RNeasy Pure mRNA Bead Kit

Below is a brief protocol for mRNA enrichment. For further information, visit **www.qiagen.com/HB-1783** for the RNeasy Pure mRNA Bead Handbook.

Important points before starting

- This protocol is for mRNA enrichment, and the starting material is 100 ng of total RNA.
- Vortex the bottle containing Pure mRNA Beads for 3 min (before first use) or 1 min (before subsequent uses) to ensure that the magnetic particles are fully resuspended.
- Heat a heating block to 70°C, and heat Buffer OEB to 70°C.
- Unless otherwise indicated, all protocol steps – including centrifugation – should be performed at 20–30°C. Steps 5–8 are processed using a magnetic rack.

Procedure

1. Determine the amount of starting RNA. Pipette 100 ng total RNA into an RNase-free 1.5 mL tube and adjust the volume with RNase-free Water (if necessary) to a volume of 250 µL.
2. Add 1 µL RNase Inhibitor (4 U/µL), 250 µL Buffer mRBB and 25 µL Pure mRNA Beads, and vortex.
3. Incubate the sample for 3 min at 70°C in a heating block. This step disrupts the secondary structure of RNA.
4. Remove the sample for the heating block and place at room temperature (15–25°C) for 10 min. This step allows hybridization between the oligo-dT of the Pure mRNA Beads and the poly-A tail of the mRNA.

5. Briefly pellet the mRNA–bead complex by centrifugation for 2 min at maximum speed, and place the tube on a magnetic rack. Wait for 1 min, making sure the bead separation is complete, and remove the supernatant.

   Note: Save the supernatant until certain that satisfactory binding and elution of poly A+ mRNA has occurred.

6. Resuspend the mRNA–bead pellet in 400 µL Buffer OW2 by vortexing and pipette the solution into a 1.5 mL Eppendorf® tube. Briefly centrifuge the tube to pellet the mRNA–bead pellet prior to placing on a magnetic rack. Wait 1 min until the bead separation has been completed and remove the supernatant.
7. Appy another 400 µL Buffer OW2, mix by vortexing and pipette the solution into a 1.5 mL Eppendorf tube. Briefly centrifuge the tube to pellet the mRNA–bead pellet prior to placing on a magnetic rack. Wait 1 min until the bead separation has been completed and remove the supernatant.
8. Pipette 10 µL hot (70°C) Buffer OEB into the 1.5 mL tube containing mRNA–bead and pipette up and down 3–4 times to resuspend the beads thoroughly. Briefly centrifuge and place the tube on a magnetic rack and wait 1 min until bead separation has been completed. Remove the clear supernatant and transfer it to a new 1.5 mL Eppendorf tube as this contains the enriched mRNA.
9. With this enriched poly A+ mRNA, proceed to “FastSelect rRNA depletion procedure”, page 24.

Appendix C: QIAseq FastSelect –5S/16S/23S with the QIAseq FastSelect library kit

Important points before starting

- This protocol is for incorporation of QIAseq FastSelect –5S/16S/23S rRNA removal with the QIAseq FastSelect RNA library kit. The starting material is 10 ng to 1 µg of total RNA; using less than 10 ng input is not recommended when using QIAseq FastSelect –5S/16S/23S.
- We highly recommend DNase treatment (on-column and in-solution) of total RNA samples.
- ERCC Control RNA (see "Equipment and Reagents to be Supplied by User") can be added according to the concentrations specified by the manufacturer. If added, the total reaction volume should remain 11 µL.
- Equilibrate FastSelect 5S/16S/23S, FastSelect FH Buffer, and Nuclease-Free Water to room temperature (15–25°C).

  Important: Only for QIAseq FastSelect –5S/16S/23S, incubate the tube at 37°C for 5 min, and then vortex to dissolve the precipitate.

- Dilute an aliquot for each FastSelect tube to 0.1x using 2 µL FastSelect tube and 18 µL Nuclease-Free Water. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.

Procedure

1. Thaw template RNA on ice. Gently yet thoroughly mix, then briefly centrifuge to collect residual liquid from the sides of the tubes and return to ice.
2. Prepare the reagents required for FastSelect rRNA depletion.

2a. Thaw the appropriate tube(s) of QIAseq FastSelect, FastSelect FH Buffer, and Nuclease-Free Water at room temperature (15–25°C). Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.

Note: For QIAseq FastSelect –5S/16S/23S, incubate the tube at 37°C for 5 min, and then vortex to dissolve the precipitate.

2b. Prepare a 1:10 dilution of QIAseq FastSelect –5S/16S/23S tube (as an example: using 2 µL FastSelect tube and 18 µL Nuclease-Free Water). Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.

3. Prepare the FastSelect rRNA depletion reaction on as described in Table 23. Briefly centrifuge, gently yet thoroughly vortex to mix, 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 23. Preparation of FastSelect –5S/16S/23S depletion reaction

Component	Volume/reaction
RNA (10 ng – 1 µg)	Variable
QIAseq FastSelect –5S/16S/23S (0.1x)*	1 µL
ERCC Control†	Optional
FastSelect FH Buffer	1.5 µL
Nuclease-Free Water	Bring total reaction volume to 15 µL
Total volume	**15 µL**

* Any QIAseq FastSelect RNA Removal reagent or combination can be supplemented or substituted here. If additional QIAseq FastSelect RNA Removal reagents are used, the total reaction volume should remain 15 µL.

† ERCC Control RNA (see "Equipment and Reagents to be Supplied by User", page 20) can be added according to the concentrations specified by the manufacturer. If added, the total reaction volume should remain 15 µL.

4. Incubate as described in Table 24 (next page) using a thermal cycler with a heated lid.

Table 24. FastSelect –5S/16S/23S rRNA depletion incubation

Step	Time	Temperature
1	2 min	75°C
2	2 min	70°C
3	2 min	65°C
4	2 min	60°C
5	2 min	55°C
6	2 min	37°C
7	2 min	25°C
8	2 min	4°C
9	Hold	4°C

5. Upon completion of the rRNA depletion reaction, add 19.5 µL (1.3x volume) QIAseq Beads to the 15 µL reaction. Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.
   Note: Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.
6. Incubate for 5 min at room temperature.
7. Centrifuge the tube or plate until the beads are pelleted (2 min), and then place the plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.
   Important: Do not discard the beads, as they contain the DNA of interest.
   Tip: It may be useful to discard the supernatant twice. The contents settle after the first discard.
8. Add 15 µL of Nuclease-Free Water and 19.5 µL QIAseq Bead Binding Buffer (prewarmed to room temperature and mixed by vortexing). Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.
9. Incubate for 5 min at room temperature.

10. Centrifuge the tube or plate until the beads are pelleted (2 min), and then place the tube or plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.

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

    Tip: It may be useful to discard the supernatant twice. The contents settle after the first discard.

11. With the tube still on the magnetic stand, add 200 µL freshly prepared 80% ethanol. Wait 2 min and carefully remove and discard the wash. Repeat the wash, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.

12. **Important**: It is vital to completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette first, briefly centrifuge the tubes immediately and return the tubes to the magnetic stand. Then use a 10 µL pipette to remove any residual ethanol. This step should be performed quickly.

13. With the tube (cap open) still on the magnetic stand, air-dry at room temperature for 5–10 min (until the beads start to crack and pellet loses its shine).

    Note: Visually inspect that the pellet is completely dry.

14. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 9 µL Nuclease-Free Water. Gently yet thoroughly vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min.

15. Centrifuge the tube until the beads are pelleted (2 min), and then return the tube to the magnetic rack until the solution has cleared.

16. Transfer 7 µL of the supernatant to a clean tube.

17. Thaw the US RT Buffer, 5x and add 4 µL to the 7 µL supernatant. Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.

18. Proceed to “cDNA Synthesis Procedure”, page 26. Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Ordering Information

Product	Contents	Cat. no.
QIAseq FastSelect RNA Lib Kit (24)	For 24 reactions: contains all buffers and reagents for either whole or 3' transcriptome library prep of 24 RNA samples	Varies
QIAseq FastSelect RNA Lib Kit (96)	For 96 reactions: contains all buffers and reagents for either whole or 3' transcriptome library prep of 96 RNA samples	Varies
QIAseq FastSelect RNA Lib Kit (384)	For 384 reactions: contains all buffers and reagents for either whole or 3' transcriptome library prep of 384 RNA samples	Varies
QIAseq FastSelect RNA Lib Kit (768)	For 768 reactions: contains all buffers and reagents for either whole or 3' transcriptome library prep of 768 RNA samples	Varies
QIAseq FastSelect Custom RNA Removal Kits	Includes QIAseq FastSelect Custom RNA Removal Kit for 1536 standard samples	Varies
QIAseq UX 12 Index Kit IL UDI (12)	Sample Index Kit for 12 libraries using unique dial indexes on Illumina NGS instruments; indexes are supplied as liquid in a 96-well plate with a pierceable foil seal	331801
QIAseq UX 96 Index Kit IL UDI A–H	Sample Index Kits sold in sets of 96 libraries using unique dual indexes on Illumina NGS instruments; indexes are supplied as liquid in a 96-well plate with	Varies

Product	Contents	Cat. no.
	a pierceable foil seal. 8 different sets of 96 indexes are available for a total of 768 UDIs.	
Related products		
QIAseq Library Quant Array Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent® platforms; array format	333304
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; assay format	333314
Human XpressRef Universal Total RNA	2 tubes, each with 100 µg of total RNA prepared from 20 different human adult and fetal normal major organs.	338112
Mouse XpressRef Universal Total RNA	2 tubes, each with 100 µg of total RNA prepared from several male and female mice (Balb/c strain), whole bodies without fur	338114
Rat XpressRef Universal Total RNA	2 tubes, each with 100 µg of total RNA prepared from several male and female rats (SD Wistar strain), whole bodies without fur	338116
RNeasy Micro Kit (50)	50 RNeasy MinElute® Spin Columns, collection tubes (1.5 mL and 2 mL), RNase-free DNase I, carrier RNA, RNase-free reagents, and buffers	74004
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, collection tubes (1.5 mL and 2 mL), RNase-free reagents, and buffers	74104

Product	Contents	Cat. no.
RNeasy 96 Kit (4)*	For 4 x 96 total and cytoplasmic RNA preps: 4 RNeasy 96 Plates, Elution Microtubes CL, caps, S-Blocks, AirPore tape sheets, RNase-free reagents, and buffers	74181
QIAGEN CLC Genomics Workbench	Comprehensive analysis package for the analysis and visualization of data from all major next-generation sequencing (NGS) platforms. The workbench supports and seamlessly integrates into a typical NGS workflow. CLC Genomics Workbench is available for Windows, Mac OS X, and Linux platforms	832021

* Larger kit sizes available; visit **www.qiagen.com**.

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Document Revision History

Revision	Description
November 2022	Initial release
September 2023	Corrected the error where the catalog numbers of 2.8 mL and 0.7 mL of NGS 2x Hi-Fi MasterMix Kit in the Kit Contents section were interchanged. Updated index plate component information for material numbers 331815, 331825, 331835, 331845, 331855, 331865, 331875, 331885, 331817, 331857, and 331818 by changing “RUDI indexes” to “MUDI indexes”. Changed the description for Table 13 and the contents of third row. Updated the footnotes for Table 23 by correcting the total reaction volume from 11 µL to 15 µL.

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