March 2025

QIAseq® FastSelect™ RNA Library Kit Handbook

QIAseq FastSelect RNA Library Kit

For stranded RNA-seq library preparation with integrated rRNA removal for complete transcriptome or 3′ RNA-seq

QIAseq Low Input RNA Library Kit

For stranded RNA-seq library preparation from previously enriched mRNA or rRNA depleted total RNA for complete transcriptome or 3′ RNA-seq

QIAseq UX 12/96 Index Kits IL UDI

For amplification and sample indexing of libraries made using QIAseq FastSelect RNA Library Kit and QIAseq Low Input RNA Library Kit

QIAseq Stranded mRNA Enrichment Kit

For enrichment of mRNA from total RNA using oligo-dT magnetic beads

Sample to Insight

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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

Kit	Kit size	Catalog number	Kit contents: Component	Component size	Catalog number	Quantity
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)
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

Kit	Kit size	Catalog number	Kit contents: Component	Component size	Catalog number	Quantity
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)
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

Kit	Kit size	Catalog number	Kit contents			
			Component	Component size	Catalog number	Quantity
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)
			QIAseq Beads	(10 mL)	333923	1 (only 96)
			QIAseq FastSelect – 5S/16S/23S Kit	(24) (96)	335925 335927	1

Kit	Kit size	Catalog number	Kit contents: Component	Component size	Catalog number	Quantity
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

Kit	Kit size	Catalog number	Kit contents: Component	Component size	Catalog number	Quantity
QIAseq FastSelect RNA Lib Worm Kit	(24) (96)	334292 334295	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 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

Kit	Kit size	Catalog number	Kit contents: Component	Component size	Catalog number	Quantity
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 Low Input RNA Library Kit	(24) (96) (384) (768)	334202 334205 334207 334208	QIAseq RNA Lib Enzymes & Buffers	(24) (96) (384) (768)	334782 334785 334785 334785	1 1 4 8

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

QIAseq RNA Lib Enzymes & Buffers Catalog no. Number of reactions	(24) 334782 24	(96) 334785 96
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	(24)	(96)
Catalog no.	333262	333265
Number of reactions	24	96
QIAseq FastSelect –rRNA Fly	3 x 12 µL	120 µL

QIAseq FastSelect –Human Mitochondrial Genes	(24)
Catalog no.	333282
Number of reactions	24
QIAseq FastSelect –Human Mitochondrial Genes	1 tube

NGS 2x Hi-Fi MasterMix Kit	(0.7 mL)	(2.8 mL)
Catalog no.	333221	333220
Component no.	1122082	1122083
Number of reactions	24	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 the 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	(12)
Catalog no.	331801
Number of Indexes	12
Index plate* name	RUDI-12A

* The hard plastic 96-well plate contains 9 µL pre-mixed indexes in 12 wells of the plate. 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 (96) 331815 96	–B (96) 331825 96	–C (96) 331835 96	–D (96) 331845 96	–E (96) 331855 96	–F (96) 331865 96	–G (96) 331875 96	–H (96) 331885 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 in each well. 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.

QIAseq Stranded mRNA Enrichment Kit Catalog no. Volume of mRNA enrichments	(24) 1105688 24	(96) 1105689 96
Pure mRNA Beads	2 x 600 µL	6 x 600 µL
Buffer mRBB (binding buffer)	2 x 8 mL	4 x 8 mL
Buffer OW2 (wash buffer)	2 x 19 mL	7 x 19 mL
RNase-Free Water	1 x 10 mL	3 x 10 mL
Buffer OEB (elution buffer)	2 x 1.5 mL	5 x 1.5 mL

Shipping and Storage

As described in "Kit Contents" (starting on page 4), the QIAseq FastSelect RNA Library Kits are shipped in several boxes and may arrive on separate days or in different shipments.

- QIAseq RNA Lib Enzymes & Buffers are 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, QIAseq FastSelect Mitochondrial Genes, 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.
- For the QIAseq Stranded mRNA Enrichment Kits (sold separately), the Buffers mRBB and Pure mRNA Beads should be stored at 2–8°C (do not freeze). Buffer OW2 (wash buffer), RNase-Free Water, and Buffer OEB (elution buffer) should be stored at room temperature (15–25°C).

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 1 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 enable either complete transcription of RNA molecules or 3′ RNA-seq, simply by varying the primer in the reverse transcription reaction. In addition, the kits include QIAseq Beads for fast and efficient reaction cleanup between protocol steps. By using the QIAseq UX Index IL UDI Kits, up to 768 samples can be multiplexed in one sequencing run.

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, optimal range: 10–500 ng) 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 degraded RNA 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 sequencing quality control, primary mapping, differential expression, and pathway analysis.

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.

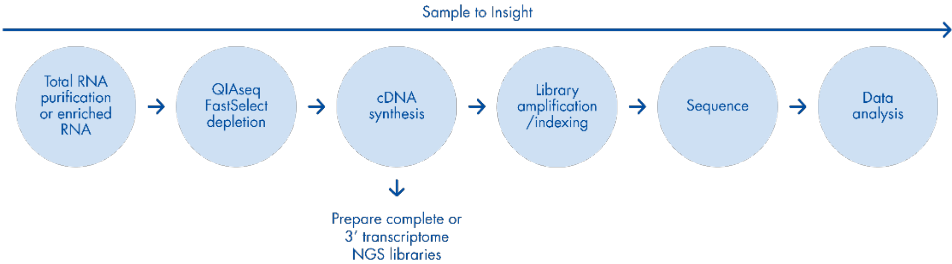

Figure 1. QIAGEN's Sample to Insight QIAseq FastSelect RNA Library Kit workflow.

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).

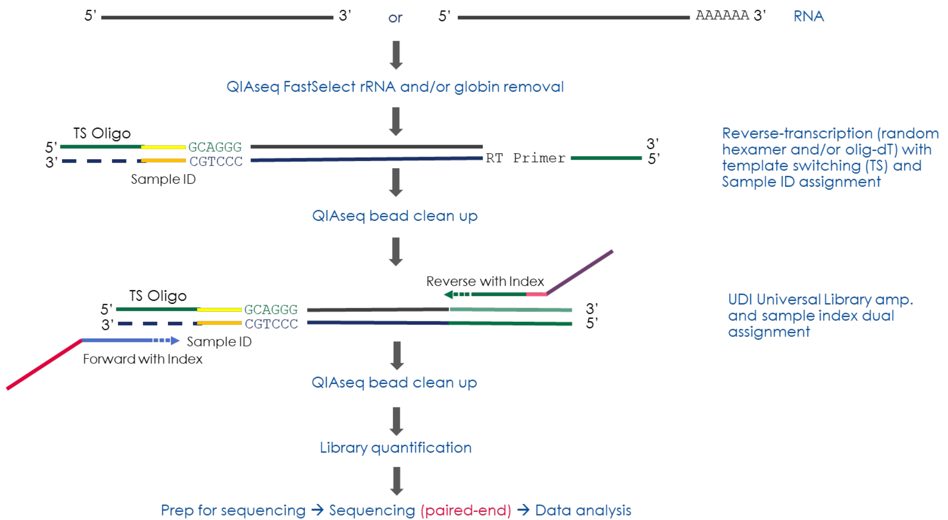

Figure 2. QIAseq FastSelect RNA Library Kit workflow.

- **Start with purified total RNA or enriched mRNA**: 1 ng – 1 µg (optimal range 10–500 ng)of purified total RNA can be used for each sample; mRNA enriched from 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 is adjusted depending on the RNA-input, RNA-integrity, and primers used for reverse transcription reaction.

- **cDNA synthesis**: cDNA synthesis can be performed using a random hexamer (N6-T RT Primer) for complete transcriptome analysis or with an oligo-dT primer (ODT-T RT Primer) for 3′ RNA-seq analysis. Both primers may also be combined if desired.

  The SID-TS-24S RT Plate (Table 1) and 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 will be used in an experiment, unused wells can be covered using the provided strip caps. We recommend resuspending the oligos as needed or directly using the entire plate in your experiment. The unused wells must be covered prior resuspension to avoid any cross contamination with the ones in use.

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®, MiSeq i100, NextSeq® 500/550, NextSeq 2000, NovaSeq® 6000, NovaSeq X, and other Illumina-based sequencing instruments that support paired-end sequencing. For standard expression analysis, 75 bp paired-end or 100 bp paired-end sequencing with dual 10 bp indexes should be used. For fusion analysis along with standard expression analysis, 150 bp paired-end or 200 bp paired-end sequencing with dual 10 bp indexes should be used. Depending on the instrument and sequencing kit used the sequencing length can vary. In Table 14, the sequencing length for different instruments and sequencing kit according to Illumina's documentation is summarized. Recommendations for read allocation are found in Table 3; this should be used as a starting point, as read allocation ultimately depends on both the application and sample type.

Table 3. Read allocation recommendations per sample

Total RNA input per sample	rRNA removal Complete Transcriptome	Number of reads per sample	
		mRNA-seq	3′ RNA-seq
1 ng RNA	5,000,000	1,000,000	1,000,000
10 ng RNA	10,000,000	5,000,000	5,000,000
100 ng RNA	20,000,000	15,000,000	10,000,000
1000 ng RNA	60,000,000	25,000,000	20,000,000

- **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 tube strips) (VWR cat. no. 20170-012 or 93001-118)
- Ice
- Microcentrifuge
- Thermal cycler; recommended with heated lid and maximal heating and cooling rate 2.5°C/s. Temperature uniformity ±0.4°C well-to-well within 10 s of arrival at 90°C.
- Magnet for QIAseq Bead Cleanups — MagneSphere® Technology Magnetic Separation Stand (Promega, cat. no. Z5342), DynaMag-2 magnetic rack (Thermo Fisher Scientific, cat. no. 12321D), DynaMag™-96 Side (Thermo Fisher Scientific, cat. no. 12331D), or similar.
- 100% ethanol, ACS-grade

Library QC methods

- QIAxcel® Connect (QIAGEN, cat. no. 9003110)
- QIAxcel DNA High Sensitivity Kit (QIAGEN, cat. no. 929012)
- 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) and D1000 ScreenTape (Agilent, cat. no. 5067-5582)
- 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). ERCC controls are recommended when performing benchmarking/ comparisons studies. These controls should be added to the RNA samples before cDNA synthesis.

Optional control total RNA samples for process optimization

- Human XpressRef Universal Total RNA (cat. no. 338112)
- Mouse XpressRef Universal Total RNA (cat. no. 338114)
- Bacterial RNA reference: *E.coli* total RNA (Thermo Fisher Scientific, cat.no. AM7940)

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 (optimal range: 10–500 ng) of purified total RNA can be used, with 100 ng total RNA (or greater) being the preferred starting amount for workflow evaluation. 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	Tissues and cells of animal and human
RNeasy 96 Kit	74181 and 74182	Tissues and cells of animal and human
RNeasy FFPE Kit	73504	Total RNA from formalin-fixed, paraffin-embedded tissue sections
QIAamp® ccfDNA/RNA Kit	55184	Animal and human plasma and serum
QIAwave RNA Mini Kit	74534 and 74536	Eco-friendly standard kit for extracting total RNA from cells, tissues, and yeast
RNeasy Plus Mini Kit (50)	74134	Small amounts of animal, human tissues, and cells Includes genomic DNA removal columns
RNeasy Plus Micro Kit (50)	74034	Animal, human tissues, and cells Includes genomic DNA removal columns
RNeasy Plus 96 Kit (12)	74192	Animal, human tissues, and cells. Includes genomic DNA removal plates.
QIAwave RNA Plus Mini Kit	74634 and 74636	Eco-friendly standard kit for small amounts of animal, human tissues, and cells with genomic DNA removal columns

Table 4. Recommended kits for purification of total RNA (continued)

Kit	Cat. no.	Starting material
RNeasy Plant Mini Kit (50)	74904	Plant and fungal samples including leaves or needles, flowers, buds, roots, cultured cells, and fruit
RNeasy PowerPlant® Kit	13500-50	Most difficult plant types, including strawberry leaf, cotton leaf, cotton seeds, and pine needles with inhibitor Removal Technology removes 100% of polyphenolics and polysaccharides
RNeasy Plus Universal Mini Kit (50)	73404	Includes QIAzol® lysis for difficult to lyse tissues
RNeasy 96 Universal Tissue Kit (4)	74881	Includes QIAzol lysis for difficult to lyse tissues
RNeasy Fibrous Tissue Mini Kit	74704	Skeletal muscle, heart and aorta, tissues difficult to lyse due to the abundance of contractile proteins, connective tissue, and collagen; includes DNase for DNA removal
RNeasy PowerBiofilm® Kit (50)	25000-50	Biofilms, dental plaques, and microbial mats
RNeasy PowerWater® Kit	14700-50-NF	RNA from bacteria (Gram +/-), algae, and fungi from turbid water samples
RNeasy PowerSoil® Total RNA Kit	12866-25	Start with up to 2 g of soil samples, including compost, sediment, and manure
RNeasy PowerMax® Soil Pro Kit	47082	5–15 g of soil samples including compost, sediment, and manure
RNeasy PowerFecal® Pro Kits	78404	Stool and gut samples, sludge, or wastewater
QIAamp RNA Blood Mini Kit	52304	Purification of cellular RNA from up to 1.5 mL of fresh, whole human blood stabilized with any common anticoagulant, such as citrate, heparin, or EDTA; includes genomic DNA removal columns
exoRNeasy Midi Kit	77144	Plasma and serum of animal and human
exoRNeasy Maxi Kit	77164	Plasma and serum of animal and human

- 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.

  We recommend a minimum DV200 value of 35%.

- Ensure that 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.
- Use calibrated thermocyclers to ensure the correct reaction temperatures.

Protocol: QIAseq FastSelect Library Construction

RNA fragmentation and FastSelect RNA removal procedure

Important points before starting

- This protocol can be used with low amounts (1 ng – 1 µg; optimal range: 10–500 ng) of purified total RNA.
- For enriched mRNA, see Appendix B: mRNA Enrichment Using RNeasy Pure mRNA Bead Kit or Appendix D: mRNA Enrichment in 200 µL Plates using QIAseq Stranded mRNA Enrichment Kit.
- When using the QIAseq Low Input RNA Library Kit, replace FastSelect with water. The RNA fragmentation and FastSelect RNA removal procedure should still be performed even without FastSelect rRNA removal reagent to ensure RNA is fragmented prior to library preparation.
- 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" section) 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.
- Prepare the SID-TS RT Primer for the reverse transcription reaction by resuspending the dried oligo in the SID-TS Plate.

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.
   a. Thaw the appropriate tube(s) of QIAseq FastSelect, 5 x US RT Buffer, and Nuclease-Free Water at room temperature (15–25°C). Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.
   b. Dilute an aliquot for each FastSelect tube to 0.1x (e.g., using 2 µL FastSelect tube + 18 µL Nuclease-Free Water) and for FastSelect Mitochondrial Genes to 0.5x (e.g., using 10 µL FastSelect tube + 10 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, a potential experimental optimization is to dilute FastSelect to 0.005x. The dilution of FastSelect can be optimized depending on the species used for total-RNA isolation and input of total RNA in the experiment.
3. Prepare the FastSelect rRNA depletion reaction as described in Table 5. Any QIAseq FastSelect RNA Removal reagent or combination can be used. 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.

   Note: If using QIAseq Low Input RNA Library Kits for applications without FastSelect rRNA removal reagent, replace QIAseq FastSelect with water to maintain 11 µL total reaction volume.

   Note: When working with the QIAseq FastSelect –5S/16S/23S and the QIAseq FastSelect Epidemiology kits, do not add the N6-T RT primer and the ODT-T RT primer during this step.

After the QIAseq FastSelect depletion, proceed with Sample Cleanup using the QIAseq Beads as described in Appendix C (page 69) in this handbook, or in the *QIAseq FastSelect–5S/16S/23S Handbook* and the *QIAseq FastSelect Epidemiology Handbook*.

Note: If using QIAseq FastSelect Custom, refer to the *QIAseq FastSelect Custom Handbook*.

Table 5. RNA fragmentation and FastSelect rRNA depletion reaction

	Volume/reaction			
Component	**N6-T primer**	**ODT-T primer**	**N6-T RT and ODT-T RT primer**	**No FastSelect Depletion**
RNA (1 ng – 1 µg)	Variable	Variable	Variable	Variable
QIAseq FastSelect (0.1x)*	1 µL	1 µL	1 µL	–
ERCC Control†	Optional	Optional	Optional	Optional
N6-T RT Primer‡	1 µL	–	1 µL	1 µL
ODT-T RT Primer§	–	1 µL	1 µL	1 µL
US RT Buffer, 5x	4 µL	4 µL	4 µL	4 µL
Nuclease-Free Water	Bring total reaction volume to 11 µL	Bring total reaction volume to 11 µL	Bring total reaction volume to 11 µL	Bring total reaction volume to 11 µL
Total volume	11 µL	11 µl	11 µL	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 QIAseq FastSelect RNA Removal reagent is not used, replace with water to maintain 11 µL final reaction volume.
† 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.
‡ N6-T RT Primer can be used alone or in combination with the ODT-T RT Primer. In case only one type of primer is being used, replace 1 µL of the missing primer by 1 µL of Nuclease-Free Water.
§ For 3′ transcriptome analysis, only use the ODT-T RT primer.

4. Incubate as described in RNA fragmentation and FastSelect rRNA depletion incubation using a thermal cycler with a heated lid.

Table 6. RNA fragmentation and FastSelect rRNA depletion incubation

Step	Temperature (°C)	Time
1	94*	Varies, see Table 7 on the facing page
2	75	2 min
3	70	2 min
4	65	2 min
5	60	2 min
6	55	2 min
7	37	2 min
8	25	2 min
9	4	2 min
10	4	Hold

* Fragmentation time may need to be adjusted depending on the input amount, quality of RNA and primer used in Reverse Transcription.

Table 7. Recommended RNA fragmentation times

RNA quality	Input (ng)	Fragmentation time at 94°C	
		N6-T RT Primer only or N6-T RT + ODT-T primer (min)	ODT-T primer only (min)
RIN <3	<10	–	1
	10–250	1	2
	>250	2	3
RIN 3–5	<10	1	3
	10–250	3	3
	>250	3	5
RIN 5–8	<10	2	3
	10–250	3	3
	>250	3	5
RIN >8	<10	3	3
	10–250	3	5
	>250	5	5

Note: Fragmentation times are based on total RNA resuspended in Buffer EB or Nuclease-Free Water from QIAGEN isolation kits. The presence of Mg^{2+}, EDTA, EGTA, other salts, and divalent ion chelators in the RNA sample will affect fragmentation times. These times are a guide and should be optimized based on laboratory procedures and will result in a library fragment size of approximately 400–450 bp. In case that another fragment size is required, we recommend adjusting the fragmentation time by 2 min intervals to achieve library fragment length that fits best for your application.

5. Proceed with resuspending the SID-TS-24S RT Primer in the SID-TS Plate and "cDNA synthesis procedure".

   Note: If the cDNA synthesis will take place outside of the SID-TS-96S or SID-TS-24S plate, resuspend each SID-TS RT primer in 2.5 µL Nuclease-Free Water, centrifuge briefly, and incubate for 10 min at room temperature (15–25°C) to fully dissolve the primer.

Important: 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. The unused wells must be closed with the included cups before resuspending the SID-TS RT primer that will be used in the reverse transcription.

cDNA synthesis procedure

Important points before starting

- Set up the reactions on ice.
- Use a thermal cycler with a heated set to 75°C.
- **Important**: If the cDNA reaction will take place outside of the SID-TS-96S or SID-TS-24S plate, ensure that the reconstituted SID-TS RT primers have been sufficiently resuspended by incubating them for 10 min at room temperature (15–25°C) to fully dissolve the primer.
- 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 have 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.
   a. Thaw DTT (100 mM), dNTP (10 mM), and Nuclease-Free Water at room temperature (15–25°C). Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.
   b. 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.
   c. Process the SID-TS-96S or SID-TS-24S plate as described in step 5 of the previous procedure. Transfer the dissolved content of the wells that are going to be used to a new plate. Unused wells should be closed with caps before resuspension to avoid any cross contamination.
2. Prepare the cDNA synthesis reaction on ice according to Table 8. 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 8. cDNA synthesis reaction

	Volume per reaction	
Component	**RT reaction in SIDT-TS plate using lyophilized SID-TS RT primer**	**RT reaction in user provided plate or tube using resuspended SID-TS RT primer**
rRNA-depleted sample from previous step	11 µL	11 µL
DTT (100 mM)	0.5 µL	0.5 µL
dNTP (10 mM)	2 µL	2 µL
Nuclease-Free Water	4.5 µL	2 µL
SID-TS RT Primer	Lyophilized pellet	2.5 µL
EZ Reverse Transcriptase	1.5 µL	1.5 µL
RNase Inhibitor	0.5 µL	0.5 µL
Total volume	20 µL	20 µL

3. Prepare a cDNA master mix containing DTT, dNTP, nuclease-free water, EZ reverse transcriptase, and RNAse inhibitor. If setting up more than one reaction, prepare a cDNA master mix 10% greater than what is required for the total number of reactions.
    a. If working with lyophilized SID-TS RT Primer, add the cDNA synthesis mix and the rRNA-depleted sample to the corresponding wells in the SID-TS plate containing the lyophilized pellets.
    b. If working with single resuspended SID-TS primer, add 2.5 µL of the resuspended SID-TS and the cDNA synthesis mix to the rRNA-depleted samples. Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.

Important: A unique SID-TS RT primer must be used for each sample.

4. Incubate as described in Table 9. Set the cycler heated lid to 75°C.

   Note: The cDNA generated from each well of a SID-TS RT Plate contains a unique sample ID that can be used to track each sample through library construction.

Table 9. cDNA synthesis incubation

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

5. After completion of the cDNA synthesis proceed with the clean-up of the reaction. The purification can be performed either in PCR plates or in 2 mL tubes.

   Important: QIAseq Beads must be equilibrated at room temperature. Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should have a homogenous brown color.

6. Add 24 µL (0.8x the sample volume) of QIAseq Beads into a 2 mL tube or into a well of a 96-well plate.

7. Add 11 µL Nuclease-Free Water to each tube or well containing the QIAseq Beads.

8. Add 19 µL of the cDNA synthesis reactions to each tube or well containing the QIAseq Beads. Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.

   Note: Adding 19 µL out of the 20 µL cDNA synthesis reaction will ensure that the clean-up conditions will be equal for all samples and will not be affected any variations in the volume of the reverse transcription reaction. Increasing the volume of clean up by adding Nuclease-Free Water simplifies the bead handling and increases robustness.

9. Incubate for 5 min at room temperature.
10. Spin down the tube until the beads are pelleted (approx. 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 cDNA of interest.

    Note: It may be valuable to discard the supernatant twice to completely remove the supernatant.

11. With the tube or plate still on the magnetic stand, add 200 µL in the PCR plate or 300 µL in the 2 mL tubes of 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 deciding either 200 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.

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

    Note: Visually inspect that the pellet is completely dry.

13. Remove the tube (or plate) from the magnetic stand and elute the DNA from the beads by adding 32 µL Nuclease-Free Water. Gently yet thoroughly vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min at room temperature.
14. Return the tube to the magnetic rack until the solution has cleared.
15. Transfer 30 µL of the supernatant to a clean tube.
16. Add 24 µL of QIAseq Beads (0.8x the sample 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 have a homogenous brown color.

17. Incubate for 5 min at room temperature.
18. Pulse 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 cDNA of interest.

    Note: It may be useful to discard the supernatant twice to completely remove the supernatant.

19. 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 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.

20. 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.

21. 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.

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

23. Transfer 23 µL of the supernatant to a clean tube.

    Proceed with "Library amplification/indexing procedure" section (hereafter). 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. Use a fresh pipette tip after each index pipetting step.
- 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 have 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.
   a. 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 58). During the reaction setup in step 2, indexes are transferred to a new plate.

   b. 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 10. 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 10. Library amplification/indexing reaction

Component	Volume/reaction at RNA input < 25 ng	Volume/reaction at RNA input > 25 ng
cDNA synthesis bead cleanup supernatant	23 µL	23 µL
QIAseq 2x HiFi MM	25 µL	25 µL
Index from QIAseq UX index plate	1 µL	2 µL
RNAseq Free Water	1 µL	–
Total volume	50 µL	50 µL

3. In a thermal cycler, perform the cycling program described in Table 11 with the number of cycles determined from Table 12.

Table 11. Library amplification or indexing cycling program

Step	Time	Temperature (°C)	Number of cycles
Initial denaturation	3 min	98	1
3-step cycling			See Table 12
Denaturation	5 s	98	
Annealing	10 s	55	
Extension	20 s	72	
Final extension	2 min	72	1
Hold	1	4	1
	∞	4	Hold

Table 12. PCR cycle number recommendation, based on total RNA input

Total RNA input	Number of amplification cycles
1 ng	27
10 ng	23
100 ng	20
1 µg	17
Enriched poly A^+ from 100 ng of Total RNA	22

Note: If working with QIAseq FastSelect –5S/16S/23S, or QIAseq FastSelect Epidemiology, you can reduce the total number of cycles by 1–2.

4. Upon completion of library-amplification and indexing, add 40 µL QIAseq Beads (0.8x the reaction volume) in a fresh plate well of 2 mL tube.

   Note: Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should have a homogenous brown color. The QIAseq Beads should be equilibrated at room temperature.

5. Add 50 µL of the amplified library to the beads and mix. If less than 50 µL, complement the missing volume with Nuclease-Free Water.

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

6. Incubate for 5 min at room temperature.

7. Pulse centrifuge the tube or plate until the beads are pelleted, and then place the plate or tubes 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.

   Note: It may be useful to discard the supernatant twice to completely remove the supernatant.

8. 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 ethanol.

   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) or plate 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 24 µL Nuclease-Free Water. Gently yet thoroughly vortex to mix, briefly centrifuge, and incubate for 2 min.

11. Pulse centrifuge the plate or tube until the beads are pelleted, and then return the plate or tube to the magnetic rack until the solution has cleared.

12. 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.

13. Assess the quality of the library using the QIAxcel Connect, Agilent BioAnalyzer, TapeStation®, or Fragment Analyzer. Check for the expected size distribution of library fragments. An example library is shown in Figure 3.

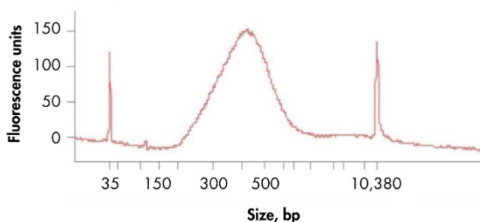

Figure 3. BioAnalyzer trace of library prepared with the QIAseq FastSelect RNA Library Kit.

14. Quantify and normalize the individual libraries.

    Option 1: Quantification of the libraries using Qubit. Qubit should be used only in combination with a capillary electrophoresis device to be able to monitor the adapter dimer yield.

    Option 2: The library yield measurements of Qubit, Nanodrop, 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.

15. Proceed to "Protocol: Sequencing Setup", page 49. 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, MiSeq i100, NextSeq 500/550, NextSeq 1000/2000, NovaSeq 6000, and NovaSeqX. The libraries can be also sequenced on Element Aviti instruments. For sequencing on this platform, follow Element Biosciences' recommendation for third party libraries.

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 58.
- For standard expression analysis, 74 bp paired-end or 100 bp paired-end sequencing with dual 10 bp indexes should be used.
- For fusion analysis along with standard expression analysis, 150 bp paired-end or 200 bp paired-end sequencing with dual 10 bp indexes should be used.
- Depending on the instrument and sequencing kit used, the sequencing length may vary. In Table 14, the sequencing length for different instruments and sequencing kit according to Illumina's documentation is summarized (**How many cycles of SBS chemistry are in my kit? | Illumina Knowledge**).
- Recommendations for read allocation are found in Table 3; this should be used as a starting point, as read allocation ultimately depends on both the application and sample type.
- Ensure that PhiX is included in the sequencing run. Refer to the system-specific Illumina documents for recommended PhiX amounts. In-house best practices with different instruments and Phix library input are summarized in Table 14.

- 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.
  - 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 an Illumina flow cell is summarized in Table 13.

Note that loading of Novaseq instruments requires optimization to achieve a good correlation between Occupancy and Pass Filter reads. Illumina recommends centering titration experiments at approximately 30% of the optimized loading concentration for the NovaSeq 6000 standard onboard workflow, and using a wide range of loading concentrations for the initial titration (use increments of 50 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.

Alternatively, a run setup can be generated in Base space Illumina and the sample sheet can be exported and used to start the sequencing run.

All Illumina instruments

1. Go to **qiagen.com/qiaseq-fastselect-rna-library-kits** and select **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.

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 13 to obtain the final library concentration.

Table 13. 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	1.4
NovaSeq 6000	NovaSeq 6000 Sequencing System Guide	150–180
Novaseq X	NovaSeq X Series Product Documentation	110–120

8. PhiX Control v3 Spike-in (Illumina, cat. no. FC-110-3001) should be used. 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.
9. Sequencing run setup: Select FASTQ Only.
10. The recommended sequencing protocol for 150 cycle and for 200 cycle kits is summarized in Table 14.

11. Upon completion of the sequencing run, proceed with “Protocol: Data Analysis”, page 54.

Table 14. Summary of sequencing recommendations for Illumina NGS instruments

		Recommended conditions				
Illumina instrument	**Flow cell**	**Sequencing setup**	**Sequencing length read 1***	**Sequencing length read 2***	**Dual index read length**	**PhiX (%)**
MiSeq	V3 150 cycles	Paired end	75	75	10	10
MiSeq	V2 300 cycles	Paired end	150	150	10	10
NextSeq 500	Mid/high-output 150 cycles	Paired end	74	74	10	10
NextSeq 1000/2000	P2/P3/P4 200 cycle P2cycles	Paired end	101	101	10	15
NovaSeq 6000	SP/S1/S2/S4200 cycles	Paired end	101	101	10	15
NovaSeq X	200 cycles	Paired end	101	101	10	15

* 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 longer reads should be used to increase unique mapping rates. Single end reads can also be used. Contact QIAGEN Technical Services for other sequencing instrument configurations.

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.
d. Adapter peak removal inefficient	After cDNA synthesis, when SID and SID artifacts will not be completely removed, they will be amplified and result in high adapter dimer artifacts and lower library yields, or even result to overamplification. The adapter peaks can be removed by an additional purification step using 0.8x QIAseq Beads. Alternatively, for low input application of approx. 1 ng, the indexes of the iMUDI plate can be diluted to 0.5x in Nuclease-Free Water and 1 µL can be added in the UPCR.
e. Library overamplification	Shoulders and addition peaks in electrophoretic library traces higher than 700 bp are overamplification peaks. Overamplification may not affect the sequencing run directly. Overamplification will affect the quantification of the library. We recommend using QPCR to quantify the libraries. Alternatively, to resolve the overamplification peak, a reconditioning PCR can be performed. This is a PCR with 1–2 cycles using the universal Illumina primers. You can perform reconditioning PCR using the GeneRead DNA I Amp Kit (100) (QIAGEN, cat. no. 180455).

	Comments and suggestions
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 15 to Table 23. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments at **www.qiagen.com**.

Table 15. 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 16. 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 17. 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 18. 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	iMUDI 291	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	iMUD l328	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 19. 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	iMUDI 774	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	iMUDI4 22	iMUDI 430	iMUDI 438	iMUDI 446	iMUDI 454	iMUDI 462	iMUDI 470	iMUDI 478
G	iMUDI 391	iMUDI 399	iMUDI 773	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	iMUD I472	iMUDI 480

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-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	iMUD I547	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 21. 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 22. 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 23. 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	iRUDI 012	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	iRUDI 005	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	iRUDI 008	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 to 1 µg 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.
- Additional RNase Inhibitor can be ordered using cat. no. Y9240L.

Procedure

1. Determine the amount of starting RNA. Pipet 100 ng to 1 µg of 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 0.5 µL RNase Inhibitor, 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. Apply another 400 µL Buffer OW2, mix by vortexing and pipet 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. Pipet 10 µL hot (70°C) Buffer OEB into the 1.5 mL tube containing mRNA–bead and pipet 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 "RNA fragmentation and FastSelect RNA removal procedure", page 31.

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.
- Prepare the SID-TS Plate used for the next step of cDNA synthesis.
- QIAseq FastSelect –5S/16S/23S rRNA can be used alone or in combination with other QIAseq FastSelect to deplete corresponding host rRNAs or other associated species.

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.
    a. 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.

    b. Prepare a 1:10 dilution of QIAseq FastSelect –5S/16S/23S tube (e.g., 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.

    Note: If working with a FastSelect combination, prepare the additional FastSelect depletion kit according to the corresponding recommendations (e.g., QIAseq FastSelect HMR 1:10 or QIAseq FastSelect mitochondrial rRNA 1:5).

3. Prepare the FastSelect rRNA depletion reaction on as described in Table 24. 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 24. Preparation of FastSelect –5S/16S/23S depletion reaction

Component	Volume/reaction	Volume/reaction
RNA (10 ng – 1 µg)	Variable	Variable
QIAseq FastSelect –5S/16S/23S (0.1x)*	1 µL	1 µL
QIAseq FastSelect HMR 0.1x (or other)	-	1 µL
ERCC Control†	Optional	Optional
FastSelect FH Buffer‡	1.5 µL	1.5 µL
Nuclease-Free Water	Bring total reaction volume to 15 µL	Bring total reaction volume to 15 µL
Total volume	15 µL	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 26) can be added according to the concentrations specified by the manufacturer. If added, the total reaction volume should remain 15 µL.
‡ Additional FH Buffer can be purchased separately (QIAGEN, QIAseq FastSelect Beads and Buffer Kit, cat.no. 333299).

4. Incubate as described in Table 25 using a thermal cycler with a heated lid.

Table 25. FastSelect –5S/16S/23S rRNA depletion incubation

Step	Time	Temperature (°C)
1	See Table 7	94*
2	2 min	75
3	2 min	70
4	2 min	65
5	2 min	60
6	2 min	55
7	2 min	37
8	2 min	25
9	2 min	4
10	Hold	4

* Fragmentation time may need to be adjusted depending on the input amount of RNA.

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 have a homogenous brown color.

6. Incubate for 5 min at room temperature.
7. Pulse centrifuge the tube or plate until the beads are pelleted, 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 RNA of interest.

Note: 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. Pulse centrifuge the tube or plate until the beads are pelleted, 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 RNA of interest.

    Note: 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 RNA 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".
19. If the cDNA reaction will take place outside of the SID-TS-96S or SID-TS-24S plate, resuspend each SID-TS primer in 2.5 µL Nuclease-Free Water, centrifuge briefly, and incubate for 10 min at room temperature (15–25°C) to fully dissolve the primer.

    Important: The SID-TS-96S or SID-TS-24S is a 96-well single-use 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.

cDNA synthesis procedure

Important points before starting

- Set up the reactions on ice.
- Use a thermal cycler with a heated lid.
- Transfer the dissolved content with the SID-TS Primer 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 have 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.
    a. Thaw DTT (100 mM), dNTP (10 mM), and Nuclease-Free Water at room temperature (15–25°C) and the N6 RT primer or ODT RT primer. Briefly centrifuge, gently yet thoroughly vortex to mix, and briefly centrifuge again.
    b. 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.
    c. Process the SID-TS-96S or SID-TS-24S plate as described in "Important points before starting" if not using all the wells in the plate at one time. The unused wells must be closed with the included cups before resuspending the SID-TS that will be used in the reverse transcription.
2. Prepare the cDNA synthesis reaction on ice according to Table 26. 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 required for the total number of reactions.

Table 26. cDNA synthesis reaction

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

* If using the SID-TS-96S or SID-TS-24S Plate directly, replace the volume of Resuspended SID-TS-RT primer with Nuclease-Free Water and setup the reaction directly in the SID-TS-96S or SID-TS-24S plate.
† Use either 1 µL of N6-T RT Primer or 1 µL ODT-T RT primer or 1 µL of each when both are being used.
‡ Use ODT-T RT when 3′-RNAseq analysis is required.

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 27.

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

Table 27. cDNA synthesis incubation

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

5. After completion of cDNA synthesis proceed with the reaction cleanup using QIAseq Beads and the library amplification as described in the protocol steps from step 6 on page 39.

Appendix D: mRNA Enrichment in 200 µL Plates using QIAseq Stranded mRNA Enrichment Kit

This protocol is used to enrich poly(A)+ RNA from total RNA using 200 µL strip tubes or 96-well plates using QIAseq Stranded mRNA Enrichment Kits.

Important point before starting

- The recommended total RNA input is 100 ng to 1 µg.

Things to do before starting

- RNase Inhibitor needs to be ordered separately (cat. no. Y9240L).
- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Vortex the Pure mRNA Beads for 3 min before the first use or for 1 min before subsequent uses.
- Heat a water bath or heating block to 70°C, and heat Buffer OEB to 7°C. Unless otherwise indicated, all protocol steps – including centrifugation – should be performed at room temperature.

Procedure

1. Vortex Pure mRNA Beads for 1 min to thoroughly resuspend.
2. Prepare the enrichment reaction according to Table 28.

   Briefly centrifuge, vortex, and centrifuge briefly again.

Table 28. Setup of enrichment reaction for 200 µL plates

Component	Volume/sample
Total RNA (100ng to 1 µg)	Variable (up to 53 µL)
RNase Inhibitor	1 µL
Buffer mRBB	71 µL
Thoroughly resuspended Pure mRNA Beads	25 µL
Nuclease-Free Water	Add to water to achieve 150 µL
Total volume	150 µL

4. Incubate for 3 min at 70°C, followed by 10 min at room temperature.
5. Briefly centrifuge, and then place tubes onto a magnetic rack. After the solution has cleared (approx. 2 min), discard the supernatant.
6. Add 150 µL Buffer OW2. Vortex, centrifuge briefly, and place the tubes onto a magnetic rack. After the solution has cleared, discard the supernatant.
7. Repeat step 5.
8. Add 50 µL Buffer OEB. Vortex, centrifuge briefly, and incubate at 70°C for 3 min.
9. Remove the sample from 70°C and place at room temperature for 5 min.
10. Add 50 µL of Buffer mRBB and vortex. Briefly centrifuge, and incubate at room temperature for 10 min.

11. Briefly centrifuge, and then place the tubes/plates onto a magnetic rack. After the solution has cleared, carefully discard the supernatant.
12. Add 150 µL of Buffer OW2. Vortex, centrifuge briefly, and place the tubes/plates onto a magnetic rack. After the solution has cleared, discard the supernatant. Do not disturb the bead pellet. Pipette very slowly.
13. Add 10 µL of Buffer OEB heated to 70°C to the bead pellet and vortex.
14. Briefly centrifuge, and place the tubes/plates onto a magnetic rack. After the solution has cleared, transfer 8 µL of the supernatant to a clean tube. The supernatant contains enriched, poly(A)+ RNA.
15. Proceed to "RNA Fragmentation & FastSelect RNA Removal procedure" or store the samples at –90°C to –65°C.

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 a pierceable foil seal. 8 different sets of 96 indexes are available for a total of 768 UDIs.	Varies
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

Product	Contents	Cat. no.
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
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
RNase Inhibitor (20,000U)	For non-competitive inhibition of ribonucleases RNase A, RNase B and RNase C	Y9240L
QIAseq Universal Normalizer Kit (24)/(96)	For efficient and reproducible NGS library normalization	180613 180615
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

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Document Revision History

Date	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.
March 2025	Update product listing to include QIAseq low input kit and QIAseq Stranded mRNA enrichment kits. Updated sequencing recommendations. Incorporated guidance on RNA fragmentation times. Updated sequencing depth table. Added appendix D. Added products to ordering information. Updated QIAseq Beads cleanup and UPCR cycles.

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