January 2024

QIAseq® UPXome RNA Library Kit Handbook

Low-input RNA-seq library preparation for complete transcriptome or 3′ RNA-seq

Sample to Insight

Contents

Kit Contents

The QIAseq UPXome 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 UPXome RNA Lib Kit HMR	(24) (96)	334702 334705	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			QIAseq FastSelect –rRNA HMR Kit	(24) (96)	334386 334387	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq UPXome Dual RNA-seq Lib Kit	(24) (96)	334712 334715	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			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

* 1 bottle is provided in the 24 sample kit; 2 bottles in the 96 sample kit.

Kit	Kit size	Catalog number	Component	Component size	Catalog number	Quantity
QIAseq UPXome RNA Lib Kit Bacteria	(24) (96)	334762 334765	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			QIAseq FastSelect – 5S/16S/23S Kit	(24) (96)	335925 335927	1
QIAseq UPXome RNA Lib Kit Plant	(24) (96)	334722 334725	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			QIAseq FastSelect –rRNA Plant	(24) (96)	334315 334317	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq UPXome RNA Lib Kit Fish	(24) (96)	334732 334735	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			QIAseq FastSelect –rRNA Fish Kit	(24) (96)	333252 333255	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq UPXome RNA Lib Kit Worm	(24) (96)	334752 334755	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			QIAseq FastSelect –rRNA Worm Kit	(24) (96)	333242 333245	1

* 1 bottle is provided in the 24 sample kit; 2 bottles in the 96 sample kit.

			Kit contents			
Kit	**Kit size**	**Catalog number**	**Component**	**Component size**	**Catalog number**	**Quantity**
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq UPXome RNA Lib Kit Yeast	(24) (96)	334742 334745	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			QIAseq FastSelect –rRNA Yeast	(24) (96)	334215 334217	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq UPXome RNA Lib Kit Fly	(24) (96)	334772 334775	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2*
			QIAseq FastSelect –rRNA Fly Kit	(24) (96)	333262 333265	1
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1
QIAseq UPXome RNA Lib Kit HMR	(384)	334707	QIAseq RNA Lib Enzymes & Buffers	(96)	334785	4
			QIAseq Beads	(55 mL)	333903	1
			QIAseq FastSelect –rRNA HMR Kit (96)	(96)	334387	4
			QIAseq Advanced Analysis	(96)	333785	4

* 1 bottle is provided in the 24 sample kit; 2 bottles in the 96 sample kit.

			Kit contents			
Kit	**Kit size**	**Catalog number**	**Component**	**Component size**	**Catalog number**	**Quantity**
QIAseq UPXome RNA Lib Kit HMR	(768)	334708	QIAseq RNA Lib Enzymes & Buffers	(96)	334785	8
			QIAseq Beads	(55 mL)	333903	2
			QIAseq FastSelect –rRNA HMR Kit	(384)	334388	2
			QIAseq Advanced Analysis	(96)	333785	8
QIAseq UPXome RNA Lib Kit	(24) (96)	334692 334695	QIAseq RNA Lib Enzymes & Buffers	(24) (96)	334782 334785	1
			QIAseq Beads	(10 mL)	333923	1 or 2
			QIAseq Advanced Analysis	(24) (96)	333782 333785	1

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

QIAseq RNA Lib Enzymes & Buffers Catalog no. Number of reactions	(24) 334782 24	(96) 334785 96
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 –5S/16S/23S Kit Catalog no. Number of reactions	(24) 335925 24	(96) 335927 96
FastSelect 5S/16S/23S	3 X 8 µL	96 µL
FastSelect FH Buffer	3 x 12 µL	144 µL
Nuclease-free water	1 tube	1 tube
QIAseq Beads	10 mL	10 mL
QIAseq Bead Binding Buffer	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 –rRNAYeast **Catalog no.** **Number of reactions**	**(24)** **334215** **24**	**(96)** **334217** **96**
QIAseq FastSelect –rRNA Yeast	3 x 12 µL	120 µL

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

QIAseq UX Index Kits IL UDI (sold separately)

Following cDNA pooling, QIAseq UX Index IL UDI Kits (see table below) are required for library amplification/indexing of RNA-seq libraries made with QIAseq UPXome RNA library Kits. Following the standard protocol, at least 8 cDNA samples and up to 24 cDNA samples may be pooled into a single library. For certain applications, more than 24 cDNA samples can be pooled into a single library.

The number of sequencing indexes required depends on the cDNA pooling strategy. For example, if 96 samples are used for an experiment with 8 samples per pool, then 12 indexes are necessary. If pooling 24 cDNA samples, only four sequencing indexes would be necessary for these 96 samples (24 cDNAs per sequencing index to make four libraries).

With the QIAseq UX 12 Index IL UDI Kit, a maximum of 288 samples can be sequenced together (corresponding to ultraplex pools of 24 cDNAs x 12 sequencing indexes). Each of the QIAseq UX 96 Index Kits facilitates sequencing of 2304 samples (corresponding to ultraplex pools of 24 cDNAs x 96 sequencing indexes).

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

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

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

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

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

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

Shipping and Storage

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

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

When stored correctly, the QIAseq UPXome RNA Library Kit 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 UPXome 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 UPXome RNA Library Kits and QIAseq UX index kits are tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAseq UPXome RNA Library Kit enables one-day, high-throughput next-generation sequencing (NGS) using Illumina® NGS instruments. This RNA-library kit features ultraplex (UPX) pooling of cDNA and several different workflows, which enables either complete transcription or 3' RNA-seq, simply by varying the reverse transcription reaction and/or the RNA sample. In addition, the kits include QIAseq Beads for fast and efficient reaction cleanup between protocol steps. By using the QIAseq UX Index IL UDI Index Kits, up to 18,432 samples can be sequenced on a single flow cell.

The QIAseq UPXome RNA Library Kits and corresponding data analysis are intended for library construction and gene expression analysis of purified total RNA (500 pg – 100 ng) or enriched mRNA. The kit presents two innovative advantages compared to other protocols.

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

Second, during reverse transcription, a unique sample ID is incorporated into each transcript. Following reverse transcription, cDNAs can be pooled, enabling all subsequent library construction steps to be performed in a reduced number of tubes, potentially even a single tube. This dramatically simplifies RNA-seq library preparation and allows much higher throughput than traditional NGS library preparation methods.

During sample indexing and final library amplification, up to 768 different unique dual indexes (UDIs) can be used, which allows the number of samples to be scaled up significantly for one experiment. When using the standard protocol, up to 24 samples can be pooled for library

amplification and indexing. When combined with the 768 UDIs, up to 18,432 samples can be sequenced together (24 ultraplexed samples x 768 unique dual sequence indexes).

The QIAseq UPXome 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 UPXome data analysis includes sample de-multiplexing, primary mapping, differential expression, and sample sequencing quality control.

The Sample to Insight® workflow of the QIAseq UPXome RNA Library Kit defines a new generation of high-throughput 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.

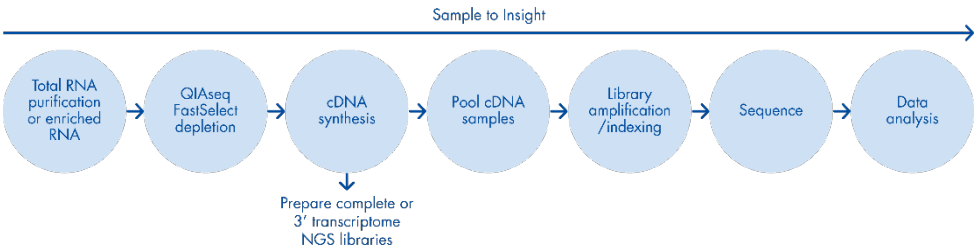

Figure 1. QIAGEN's Sample to Insight QIAseq UPXome RNA Library workflow.

Principle and procedure

The QIAseq UPXome RNA Library Kit workflow is described below (Figure 2 and Figure 3). There are two magnetic bead-based cleanup steps using QIAseq beads (included with the kit).

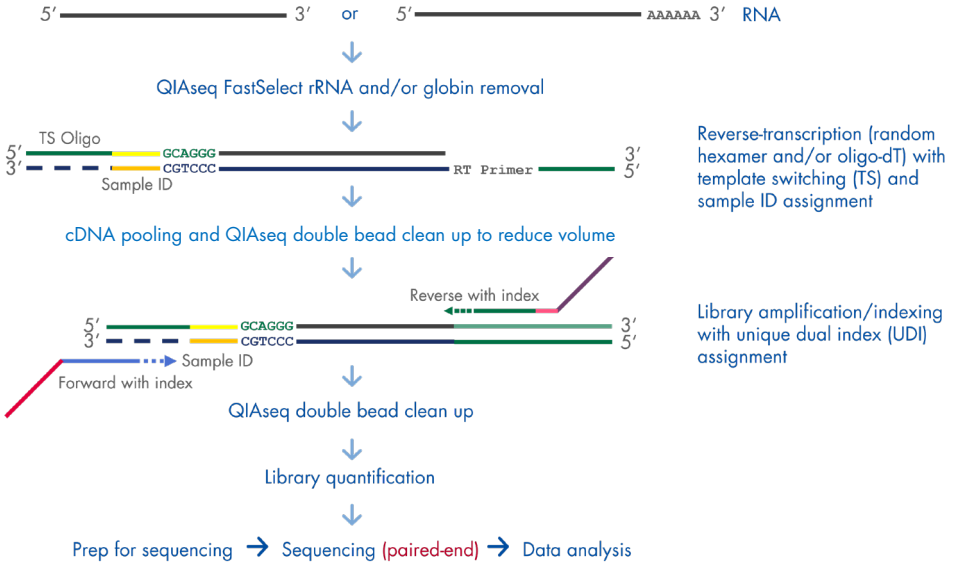

Figure 2. QIAseq UPXome RNA Library Kit workflow.

- Start with purified total RNA, or enriched mRNA: 500 pg – 100 ng of purified total RNA can be used for each sample; 10 ng of total RNA (or greater) is recommended.

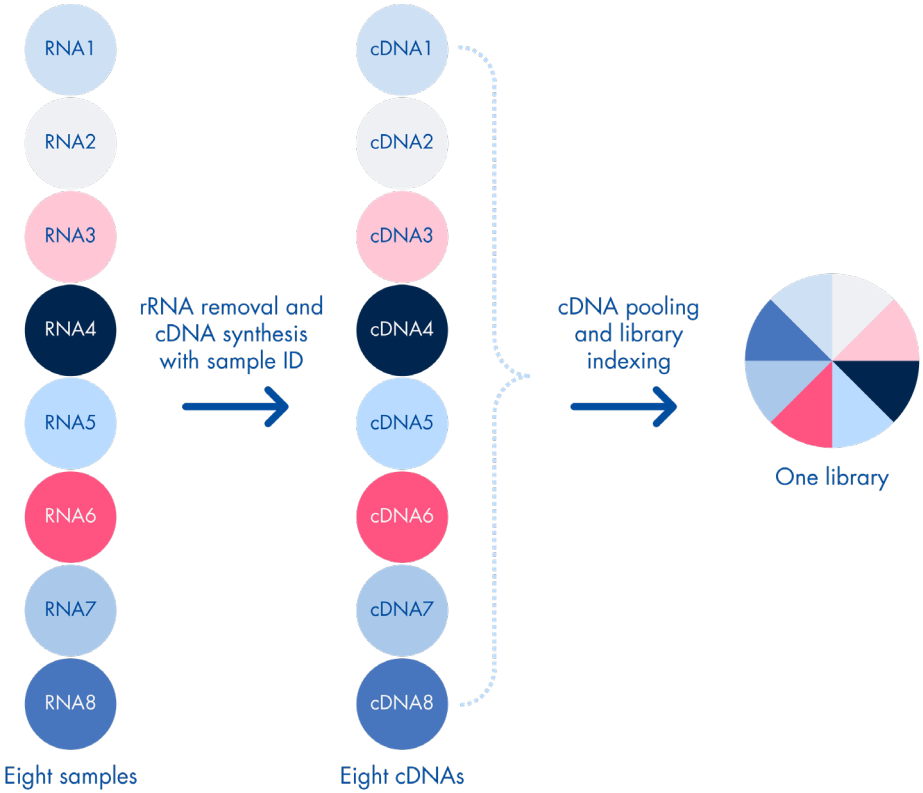

Figure 3. QIAseq UPXome RNA Library Kit workflow. Individual reverse transcription reactions are performed for each sample in the presence of a UPX barcoding oligo, which barcodes each cDNA. During a typical library preparation, up to 24 cDNAs are pooled together into a single library tube. The remaining reactions and library preparation steps associated with those 24 samples are performed in a single tube.

- **FastSelect rRNA depletion**: The FastSelect rRNA reagent is directly combined with the RNA and the US RT Buffer, 5x, enabling a rapid rRNA removal reaction. FastSelect prevents cDNA synthesis of rRNA. Specific heat fragmentation of the RNA is not necessary, as the reverse transcription chemistry is tuned to synthesize a specific range of cDNA sizes, regardless of starting sample quality.
- **cDNA synthesis**: cDNA synthesis can be performed using either the N6-T RT Primer (random hexamer) in combination with the ODT-T RT Primer (oligo-dT primer) for complete

transcriptome analysis or the ODT-T RT Primer exclusively for 3′ transcriptome analysis.

The SID-TS-24S RT Plate (Table 1) or SID-TS-96S RT Plate (Table 2) contain 24 or 96 lyophilized template switching oligos, respectively, that facilitate 10 bp sample barcoding during cDNA synthesis; if not all wells are used in an experiment, unused wells can be covered using provided strip caps. At least 8 samples and no more than 24 samples must be pooled together.

Table 1. QIAseq 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 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 UPXome RNA Library Kit is compatible with Illumina NGS systems (MiniSeq®, MiSeq®, NextSeq® 500/550, NextSeq 2000, HiSeq® 1000, HiSeq 1500, HiSeq 2000, HiSeq 2500, HiSeq 3000/4000, NovaSeq® 6000, and other Illumina-based sequencing instruments that support paired-end sequencing. For standard expression analysis, 74 bp paired-end sequencing with dual 10 bp indexes should be used. For fusion analysis along with standard expression analysis, 149 bp paired-end sequencing with dual 10 bp indexes should be used. Recommendations for read allocation are found in Table 3; this should be used as a starting point, as read allocation is ultimately dependent on both the application and sample type.

Table 3. Read allocation recommendations per sample

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

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

Equipment and Reagents to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate SDSs available from the product supplier.

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

Library QC methods

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

Optional RNA spike-in

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

Optional control total RNA samples for process optimization

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

Important Notes

- DNase treatment (on-column and in-solution) of total RNA samples is highly recommended.
- When starting with isolated RNA, 500 pg – 100 ng of purified total RNA can be used, with 10 ng total RNA (or greater) being the preferred starting amount. QIAGEN provides a range of solutions for purification of total RNA from different amounts of sample (Table 4).

Table 4. Recommended kits for purification of total RNA

Kit	Cat. no.	Starting material
RNeasy® Micro Kit	74004	Small amounts of cells and tissue
RNeasy Mini Kit	74104 and 74106	Animal, human tissues, and cells
RNeasy 96 Kit	74181 and 74182	Animal, human tissues, and cells
RNeasy FFPE Kit	73504	Total RNA from formalin-fixed, paraffin-embedded tissue sections
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
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, polysaccharides
RNeasy Plus Universal Mini Kit (50)	73404	Includes QIAzol lysis for difficuly 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 g to 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
QIAamp® ccfDNA/RNA Kit	55184	Animal ,human plasma, and serum
exoRNeasy Midi Kit	77144	Animal, human plasma, and serum
exoRNeasy Maxi Kit	77164	Animal, human plasma, and serum

- Ensure that RNA samples are of high quality and free of inhibitors that would compromise a reverse transcription or PCR. For more information about recommended laboratory procedures, please consult the handbook with your QIAGEN isolation kit.
- **RNA quantification**: Determine the concentration and purity of total RNA isolated from cells and fresh or frozen tissues by measuring the absorbance in a spectrophotometer. As the spectral properties of nucleic acids are highly dependent on pH, we recommend preparing dilutions and measuring absorbance in 10 mM Tris·Cl, pH 7.5, instead of RNase-free Water. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1 in 10 mM Tris·Cl, pH 7.5. It is not useful to assess the concentration and purity of total RNA derived from fluids and/or exosomes.

- **RNA integrity**: The integrity and size distribution of total RNA purified from cells and fresh/frozen tissue can be confirmed using an automated analysis system (such as the QIAxcel® Connect System or the Agilent 2100 Bioanalyzer) that assesses RNA integrity using an RNA integrity score (RIS) or RNA integrity number (RIN). Although the RIN should ideally be ≥8, successful NGS library construction is still possible with samples whose RIN values are ≤8.
- Ensure reactions are thoroughly mixed and that they are prepared and incubated at the recommended temperatures.
- If the workflow is not expected to be completed in one day, convenient stopping points are indicated at the end of the relevant sections.

Protocol: QIAseq UPXome Library Construction

RNA Fragmentation & FastSelect RNA Removal procedure

Important points before starting

- This protocol can be used with low amounts (500 pg – 100 ng) of purified RNA or enriched mRNA (see Appendix B: mRNA Enrichment).
- The recommended starting amount is 10 ng (or greater) of total RNA or 500pg of enriched mRNA..
- After cDNA synthesis, at least 8 samples and no more than 24 samples must be pooled together.
- DNase treatment (on-column or in-solution) of total RNA samples is highly recommended.
- ERCC Control RNA (see "Equipment and Reagents to be Supplied by User") can be added according to the concentrations specified by the manufacturer. If added, the total reaction volume should remain 11 µL.
- Gently yet thoroughly vortex reactions or reagents unless instructed otherwise. Ensure all reactions are mixed thoroughly and handled at the temperatures recommended in the protocol.
- Use a thermal cycler with a heated lid. Note on PCR instrument ramp rate, etc.

Procedure

1. Thaw template RNA on ice. Gently yet thoroughly mix, then briefly centrifuge to collect residual liquid from the sides of the tubes and return to ice.
2. Prepare the reagents required for FastSelect rRNA depletion.
   2a. Thaw the appropriate tube(s) of QIAseq FastSelect, US RT Buffer, 5x, and Nuclease-free Water at room temperature. Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.
   2b. Dilute an aliquot for each FastSelect tube to 0.1x (as an example: using 2 µL FastSelect tube + 18 µL Nuclease-free Water). Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
   Note: For 500 pg samples, potential experimental optimization is to dilute FastSelect to 0.005x.
3. Prepare the FastSelect rRNA depletion reaction as described in Table 5. 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 5. FastSelect rRNA depletion reaction

Component	Volume/reaction
RNA (500 pg – 100 ng)	Variable
QIAseq FastSelect (0.1x)*	1 µL
ERCC Control†	Optional
US RT Buffer, 5x	4 µL
Nuclease-free Water	Bring total reaction volume to 11 µL
Total volume	**11 µL**

* Any QIAseq FastSelect RNA Removal reagent or combination can be supplemented or substituted here. If additional QIAseq FastSelect RNA Removal reagents are used, the total reaction volume should remain 11 µL. If using QIAseq FastSelect –5S/16S/23S, which is used to remove bacterial rRNA, refer to Appendix C (page 56). If using QIAseq FastSelect Custom refer to the *QIAseq FastSelect Custom Handbook*.

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

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

Table 6. FastSelect rRNA depletion incubation

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

* Omit this step when using strongly degraded samples.

cDNA synthesis procedure

Important points before starting

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

that are going to be used to a new plate. Unused wells should be sealed with provided strip caps.

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

Procedure

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

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

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

† For 3′ transcriptome analysis, only use the ODT-T RT primer, and replace the N6-T RT primer with an additional 1 µL of Nuclease-free Water.

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

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

4. Incubate as described in Table 8.

Table 8. cDNA synthesis incubation

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

5. Upon completion of the cDNA synthesis reactions, pool at least 8 samples and no more than 24 samples together into one 1.7 mL (or 2.0 mL) microcentrifuge tube.

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

6. Add the appropriate volume (1.1x the reaction volume) of QIAseq Beads to the combined cDNA synthesis reactions from Step 5 according to Table 9. Addition of QIAseqBeads for cDNA cleanup. Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.

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

Table 9. Addition of QIAseqBeads for cDNA cleanup

Number of wells combined	Sample volume (µL)	QIAseq Bead volume (µL)
8	160	176
24	480	528

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

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

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

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

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

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

10. 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.

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

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

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

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

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

17. 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 two ethanol washes. Remove excess ethanol as much as possible.

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

18. 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.

19. 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.

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

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

    Note: From this point forward in the protocol, the procedures are written with the assumption that all cDNA wells (either 8 or 24) have been combined into a single tube. In your experiment, you may be using more tubes.

22. Proceed with “Library amplification/indexing procedure” (page 33). Alternatively, the samples can be stored at –30°C to –15°C.

Library amplification/indexing procedure

Important points before starting

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

Procedure

1. Prepare reagents required for library amplification or indexing.
   1a. Thaw the QIAseq UX index plates at room temperature. 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 45). During the reaction setup in step 2, indexes are removed to a new plate.
   1b. Thaw the QIAseq 2X HiFi MM on ice. Mix by gently but thoroughly vortexing the tube, and then centrifuge briefly.

2. On ice, prepare the library amplification/indexing reaction according to Table 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 (µL)
cDNA synthesis bead cleanup supernatant	23
QIAseq 2X HiFi MM	25
Index from QIAseq UX index plate	2
Total volume	**50**

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	Number of cycles
Initial denaturation	30 s	98°C	1
3-step cycling			
Denaturation	5 s	98°C	
Annealing	10 s	55°C	See Table 12
Extension	20 s	72°C	
Final extension	2 min	72°C	1
Hold	1 min	4°C	1
	∞	4°C	Hold

Table 12. PCR cycle number recommendation, based on total RNA input and number of samples pooled

	Number of amplification cycles	
Total RNA input	**cDNA pool of 8 samples**	**cDNA pool of 24 samples**
500 pg	24	23
1 ng	20	19
10 ng	18	17
100 ng	15	14
Enriched poly A+	20	19

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

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

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

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

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

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

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

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

   Note: Visually inspect that the pellet is completely dry.

9. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 24 µL Nuclease-free Water. Gently yet thoroughly vortex to mix, briefly centrifuge, and incubate for 2 min.
10. Centrifuge the tube until the beads are pelleted (2 min), and then return the tube to the magnetic rack until the solution has cleared.
11. Transfer 22 µL of the supernatant to a clean tube. If not proceeding immediately, the sample can be stored at –30°C to –15°C.
12. Assess the quality of the library using a QIAxcel Connect, Bioanalyzer, TapeStation®, or Fragment Analyzer. Check for the expected size distribution of library fragments. An example library is shown in Figure 4.

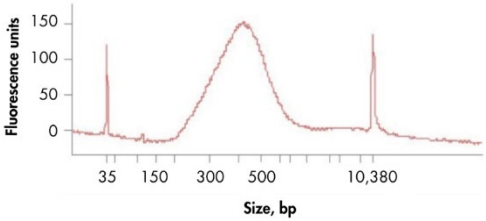

Figure 4. TapeStation trace of library prepared with the QIAseq UPXome RNA Library Kit.

13. Quantify and normalize the individual libraries.

    Option 1: Quantification of the libraries using Qubit.

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

14. Proceed to "Protocol: Sequencing Setup", page 38. Alternatively, the purified UPXome RNA library can be safely stored at –30°C to –15°C until ready to use for sequencing.

Protocol: Sequencing Setup

UPXome libraries are compatible with Illumina NGS platforms, including iSeq100, MiniSeq, MiSeq, NextSeq 500/550, NextSeq 1000/2000, HiSeq 2500, HiSeq 3000/4000, and NovaSeq 6000.

Important points before starting

- To make sequencing preparation convenient, download Illumina-compatible sample sheets for your sequencing instruments on **www.qiagen.com**, and refer to Appendix A, page 45.
- For standard expression analysis, 74 bp paired-end sequencing with dual 10 bp indexes should be used.
- For fusion analysis along with standard expression analysis, 149 bp paired-end sequencing with dual 10 bp indexes should be used.
- Ensure that PhiX is included in the sequencing run. Refer to the table below for recommended PhiX amounts. If the system is not listed, refer to the system-specific Illumina documents for recommended PhiX amounts.
- For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific Illumina documents.
- Sample dilution, pooling, and sequencing:
  - Dilute the individual libraries to a concentration of 4 nM, and then combine libraries with different sample indexes in equimolar amounts. The recommended starting final loading concentration of the pooled UPXome RNA libraries to load onto a MiSeq is 10 pM, or 1.6 pM on a MiniSeq, or 1.6 pM on a NextSeq instrument.
  - Dilute the individual UPXome 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 UPXome RNA libraries to load onto a NovaSeq instrument is between 175 pM and 265 pM.

Generation of sample sheets for Illumina instruments

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

These can be imported and edited using the Illumina Local Run Manager or any text editor. Make sure to download the appropriate sample sheet for the Illumina systems depending on whether you are using Local Run Manager or manually configuring the sequencing run.

All Illumina instruments

1. Go to **qiagen.com/us/products/discovery-and-translational-research/next-generation-sequencing/rna-sequencing/ultraplex/qiaseq-upxome-rna-library-kits/** and select **Product Resources** then **Instrument Technical Documents** to find and download the appropriate QIAseq UPXome RNA Library Kit template. Different templates might be available, depending on the instrument.
2. The sample sheet already contains all relevant information to use with the instrument.
3. Open the CSV file, delete any UDI indexes that will not be used in the experiment, and save the file with a new name.
4. Copy the file into the **Sample Sheet** folder on the MiSeq or NextSeq instrument or upload the **Sample Sheet** into Local Run Manager for MiSeq, MiniSeq, and NextSeq Illumina instruments.
5. When ready to perform the run, select the file.

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

   Note: For the NovaSeq, the recommended final pooled library concentration is 1.0–1.5 nM, which will yield a final loading concentration of 200–300 pM on the NovaSeq.

7. **Library preparation and loading**: Prepare and load the pooled library on an Illumina instrument according to the specific Illumina instrument guide. Dilute the denatured library pool a second time as described in Table 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*	1.2
NovaSeq 6000	*NovaSeq 6000 Sequencing System Guide*	200–300

8. PhiX Control v3 Spike-in (Illumina, cat. no. FC-110-3001) recommendations are indicated in Table 14, page 41. Follow Illumina guidelines on how to denature, dilute, and combine a PhiX control library with your own pool of libraries. Make sure to use a fresh, reliable stock of PhiX control library. Spike-in 10% PhiX or more of the total library pool.
9. Sequencing run setup: Select **FASTQ Only**.
10. The recommended protocol is 74 bp paired end read with 10 bp dual indexing.
11. Upon completion of the sequencing run, proceed with "Protocol: Data Analysis", page 42.

Table 14. Summary of Illumina sequencing recommendations for each NGS instrument tested with QIAseq UPXome Kit

Illumina instrument	Flow cell	Recommended sequencing setup	Recommended read 1*	Recommended read 2*	Recommended dual index read	PhiX (percent)
MiSeq	V3 150 cycle	Paired end	74	74	10	10%
NextSeq 500	Mid/high-output 150 cycle	Paired end	74	74	10	10%
NovaSeq 6000	200 cycle SP	Paired end	74	74	10	10%

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

Protocol: Data Analysis

RNA-seq Analysis & Biomarker Discovery pipeline

Primary and secondary analysis tools are available at **geneglobe.qiagen.com**

The RNA-seq Analysis & Biomarker Discovery Pipeline uses QIAGEN CLC Biomedical Workbench for read alignment, and differential expression. QIAGEN Ingenuity® Pathway Analysis (IPA®) returns the top hits from the QIAGEN Knowledge Base for canonical pathways, upstream regulators, and diseases.

Using the RNA-seq Analysis & Biomarker Discovery Pipeline, FASTQ files can be uploaded, and RNA sequences aligned. Differential RNA expression will be calculated and visualized using interactive volcano plots. Differentially expressed RNAs will be queried against the QIAGEN Knowledge Base for canonical pathways, upstream regulators, and diseases and biological functions. Important RNAs can then be identified, and digital PCR and qPCR assays easily found for biological verification.

For each alignment, a credit is deducted from your account. Credits for using the RNA-seq Analysis & Biomarker Discovery Pipeline are included with QIAseq library kits. Credits can also be purchased for using the RNA-seq Analysis Portal with non-QIAGEN kits at **www.qiagen.com**

Procedure

1. Go to **GeneGlobe.QIAGEN.com**
2. Click **Analyze Data**.
3. Under **Start Analyzing Your Data**; choose the following options:
   - Analysis type: **Next-Generation Sequencing**
   - Analyte: **mRNA/lncRNA**
   - Kit: **QIAseq UPXome RNA Library Kit**
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 UPXome 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) Not enough samples multiplexed per sample index	At least 8 cells must be multiplexed per sample index.
b) 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 to fully dissolve the primer.
c) Improper reaction setup	Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.
d) 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 a 10 µL pipette to remove any residual ethanol. In addition, allow beads to dry for the appropriate amount of time.
Sequencing issues	
a) Cluster density that is too low or too high	Accurate library quantification is the key for optimal cluster density on any sequencing instrument. A PCR-based quantification method is recommended. Other methods may lead to incorrect quantification of the library, especially when there is overamplification.
b) Very low clusters passing filter	Make sure the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument.

Appendix A: QIAseq UX Index Plates

QIAseq UX index plate layouts

The layouts of the single-use QIAseq UX index plates are shown in Table 15 to Table 23. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments on **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	iRUD2 91	iMUDI 299	iMUDI 307	iMUDI 315	iMUDI 323	iMUDI 331	iMUDI 339	iMUDI 347	iMUDI 355	iMUDI 363	iMUDI 371	iMUDI 379
D	iMUDI 292	iMUDI 300	iMUDI 308	iMUDI 316	iMUDI 324	iMUDI 332	iMUDI 340	iMUDI 348	iMUDI 356	iMUDI 364	iMUDI 372	iMUDI 380
E	iMUDI 293	iMUDI 301	iMUDI 309	iMUDI 317	iMUDI 325	iMUDI 333	iMUDI 341	iMUDI 349	iMUDI 357	iMUDI 365	iMUDI 373	iMUDI 772
F	iMUDI 294	iMUDI 302	iMUDI 310	iMUDI 318	iMUDI 326	iMUDI 334	iMUDI 342	iMUDI 350	iMUDI 358	iMUDI 366	iMUDI 374	iMUDI 382
G	iMUDI 295	iMUDI 303	iMUDI 311	iMUDI 319	iMUDI 327	iMUDI 335	iMUDI 343	iMUDI 351	iMUDI 359	iMUDI 367	iMUDI 375	iMUDI 383
H	iMUDI 296	iMUDI 304	iMUDI 312	iMUDI 320	iMUDI 328	iMUDI 336	iMUDI 344	iMUDI 352	iMUDI 360	iMUDI 368	iMUDI 771	iMUDI 384

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

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

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

Table 20. QIAseq UX 96 Index Kit UDI-F (96) (cat. no. 331865) layout — MUDI-96FA

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

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

Table 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

Protocol 1: Using RNeasy Pure mRNA Bead Kit (Cat. no. 180244)

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

Procedure

1. Determine the amount of starting RNA. Pipette 100 ng total RNA into an RNase-free 1.5 mL tube and adjust the volume with RNase-free Water (if necessary) to a volume of 250 µL.
2. Add 1 µL RNase Inhibitor (4U/µL), 250 µL Buffer mRBB, and 25 µL Pure mRNA Beads, and vortex.
3. Incubate the sample for 3 min at 70°C in a heating block. This step disrupts the secondary structure of RNA.
4. Remove the sample from the heating block and place at room temperature 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 it on a magnetic rack. Wait 1 min until the bead separation has been completed and remove the supernatant.
7. Appy another 400 µL Buffer OW2, mix by vortexing, and pipette the solution into a 1.5 mL Eppendorf tube. Briefly centrifuge the tube to pellet the mRNA–bead pellet prior to placing on a magnetic rack. Wait 1 min until the bead separation has been completed and remove the supernatant.
8. Pipette 10 µL hot (70°C) Buffer OEB into the 1.5 mL containing mRNA–bead and pipette up and down 3–4 times to resuspend the beads thoroughly. Briefly centrifuge and place the tube on a magnetic rack and wait 1 min until bead separation has been completed. Remove the clear supernatant and transfer it to a new 1.5 mL Eppendorf tube as this contains the enriched mRNA.
9. With this enriched poly A+ mRNA, proceed to “RNA Fragmentation & FastSelect RNA Removal procedure”, page 25.

Protocol 2: Using QIAseq Stranded mRNA Enrichment Kit (24)/(96) in tubes

Important points before starting

This protocol is optimized for enriching RNA originating from all eukaryotic species with a poly-A tail.

- QIAseq Stranded mRNA Enrichment Kit (24)/(96) is required for this protocol (Cat. nos. 1105688 or 1105689)
- The recommended total RNA input is 100 ng – 1 µg.
- See "Protocol 3: mRNA Enrichment using QIAseq Stranded mRNA Enrichment Kit (24)/(96) in 200 µL plates" for enrichment of mRNA using 200 µL strip tubes or 96-well plates.

Things to do before starting

- 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 70°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 24. Briefly centrifuge, vortex, and centrifuge briefly again.

Table 24. Setup of enrichment reaction.

Component	Volume/reaction (µL)
Total RNA (100 ng – 1 µg)	Variable
RNase Inhibitor	1
Buffer mRBB	250
Thoroughly resuspended Pure mRNA Beads	25
Nuclease-Free Water	Bring total reaction volume to 526
Total volume	**526**

3. Incubate for 3 min at 70°C, followed by 10 min at room temperature.
4. Briefly centrifuge, and then place the tubes onto a magnetic rack. After the solution has cleared (~2 min), discard the supernatant.
5. Add 400 µL of Buffer OW2. Vortex, centrifuge briefly, and place the tubes onto a magnetic rack. After the solution has cleared, discard the supernatant.
6. Repeat step 5.
7. Add 50 µL Buffer OEB. Vortex, centrifuge briefly, and incubate at 70°C for 3 min.
8. Remove the sample from 70°C and place at room temperature for 5 min.
9. Add 50 µL of Buffer mRBB and vortex. Briefly centrifuge, and incubate at room temperature for 10 min.
10. Briefly centrifuge, and then place the tubes onto a magnetic rack. After the solution has cleared, carefully discard the supernatant. Leave any residual liquid in the tube to minimize bead loss.
11. Add 400 µL of Buffer OW2. Vortex, centrifuge briefly, and place tubes onto a magnetic rack. After the solution has cleared, discard the supernatant.
12. Add 29 µL of Buffer OEB heated to 70°C to the bead pellet, and vortex.
13. Briefly centrifuge, and place the tubes onto a magnetic rack. After the solution has cleared, transfer 27 µL of the supernatant to a clean tube. The supernatant contains enriched poly(A)+ RNA.
14. Proceed to "RNA Fragmentation & FastSelect RNA Removal procedure". Alternatively, the samples can be stored at –90°C to –65°C.

Protocol 3: Using QIAseq Stranded mRNA Enrichment Kit (24)/(96) in 200 µL plates

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

Important points before starting

The recommended total RNA input is 100 ng – 1 µg.

Things to do before starting

- QIAseq Stranded mRNA Enrichment Kit (24)/(96) is required for this protocol (Catalog number 1105688 or 1105689)
- 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 15. Briefly centrifuge, vortex, and centrifuge briefly again.

Table 25. Setup of enrichment reaction for 200 µL plates

Component	Volume/reaction
Total RNA (100 ng – 1 µg)	Variable
RNase Inhibitor	1 µL
Buffer mRBB	71 µL
Thoroughly resuspend Pure mRNA Beads	25 µL
Nuclease-Free Water	Bring total reaction volume to 150 µL
Total volume	**150 µL**

3. Incubate for 3 min at 70°C, followed by 10 min at room temperature.
4. Briefly centrifuge, and then place tubes onto a magnetic rack. After the solution has cleared (~2 min), discard the supernatant.
5. Add 150 µL Buffer OW2. Vortex, centrifuge briefly, and place the tubes onto a magnetic rack. After the solution has cleared, discard the supernatant.
6. Repeat step 5.
7. Add 50 µL Buffer OEB. Vortex, centrifuge briefly, and incubate at 70°C for 3 min.
8. Remove the sample from 70°C and place at room temperature for 5 min.
9. Add 50 µL of Buffer mRBB and vortex. Briefly centrifuge, and incubate at room temperature for 10 min.
10. Briefly centrifuge, and then place the tubes/plates onto a magnetic rack. After the solution has cleared, carefully discard the supernatant.
11. 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.
12. Add 29 µL of Buffer OEB heated to 70°C to the bead pellet and vortex.
13. Briefly centrifuge, and place the tubes/plates onto a magnetic rack. After the solution has cleared, transfer 27 µL of the supernatant to a clean tube. The supernatant contains enriched, poly(A)+ RNA.
14. Proceed to "RNA Fragmentation & FastSelect RNA Removal procedure" or store the samples at –90°C to –65°C.

Appendix C: QIAseq FastSelect 5S/16S/23S with the QIAseq UPXome RNA Library Kit

Important points before starting

- This protocol is for incorporation of QIAseq FastSelect –5S/16S/23S rRNA removal with the QIAseq UPXome Library Kit. The starting material is 10 ng to 100 ng total RNA. Using less than 10 ng input is not recommended when using QIAseq FastSelect 5S/16S/23S.
- After cDNA synthesis, at least 8 samples and no more than 24 samples must be pooled together.
- 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.
- Bring FastSelect 5S/16S/23S, FastSelect FH Buffer, and Nuclease-free Water to room temperature (15-25°C).

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

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

Procedure

1. Thaw template RNA on ice. Gently yet thoroughly mix, then briefly centrifuge to collect residual liquid from the sides of the tubes and return to ice.

2. Prepare the reagents required for FastSelect rRNA depletion.

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

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

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

3. Prepare the FastSelect rRNA depletion reaction on as described in Table 26. 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 26. Preparation of FastSelect 5S/16S/23S depletion reaction

Component	Volume/reaction
RNA (10 ng – 100 ng)	Variable
QIAseq FastSelect 5S/16S/23S (0.1x)*	1 µL
ERCC Control†	Optional
FastSelect FH Buffer	1.5 µL
Nuclease-free Water	Bring total reaction volume to 15 µL
Total volume	**15 µL**

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

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

4. Incubate as described in Table 27 using a thermal cycler with a heated lid.

Table 27. FastSelect 5S/16S/23S rRNA depletion incubation

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

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

10. Centrifuge the tube or plate until the beads are pelleted (2 min), and then place the tube or plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.
    Important: do not discard the beads, as they contain the DNA of interest.
    Tip: It may be useful to discard the supernatant twice. The contents settle after the first discard.
11. With the tube still on the magnetic stand, add 200 µL freshly prepared 80% ethanol. Wait 2 min and carefully remove and discard the wash. Repeat the wash, for a total of two ethanol washes. Remove as much excess ethanol as possible.
12. **Important**: It is vital to completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette first, briefly centrifuge the tubes immediately and return the tubes to the magnetic stand. Then use a 10 µL pipette to remove any residual ethanol. This step should be performed quickly.
13. With the tube (cap open) still on the magnetic stand, air-dry at room temperature for 5–10 min (until the beads start to crack and pellet loses its shine).

    Note: visually inspect that the pellet is completely dry.
14. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 9 µL Nuclease-free Water. Gently yet thoroughly vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min.
15. Centrifuge the tube until the beads are pelleted (2 min), and then return the tube to the magnetic rack until the solution has cleared.
16. Transfer 7 µL of the supernatant to a clean tube.
17. Thaw the US RT Buffer, 5x and add 4 µL to the 7 µL supernatant. Briefly centrifuge, gently yet thoroughly vortex to mix, and centrifuge briefly again.
18. Proceed to “cDNA synthesis procedure”, page 27. Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Ordering Information

Product	Contents	Cat. no.
QIAseq UPXome RNA Library Kit (24)	For 24 reactions: Contains all buffers and reagents for either whole or 3' transcriptome library prep of 24 ultra-low input RNA samples	Varies
QIAseq UPXome RNA Library Kit (96)	For 96 reactions: Contains all buffers and reagents for either whole or 3' transcriptome library prep of 96 ultra-low input RNA samples	Varies
QIAseq FastSelect Custom RNA Removal Kits	Includes QIAseq FastSelect Custom RNA Removal Kit for 1536 standard samples	Varies
QIAseq FastSelect –rRNA/Globin Kit (96)	Includes one tube of QIAseq FastSelect reagent for rRNA removal and one tube of QIAseq FastSelect reagent for globin mRNA removal; sufficient for 96 reactions from human, mouse, and rat samples	335377
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

Product	Contents	Cat. no.
Related products		
QIAseq Library Quant Array Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent® platforms; array format	333304
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; assay format	333314
Human XpressRef Universal Total RNA	2 tubes, each with 100 µg of total RNA prepared from 20 different human adult and fetal normal major organs.	338112
Mouse XpressRef Universal Total RNA	2 tubes, each with 100 µg of total RNA prepared from several male and female mice (Balb/c strain), whole bodies without fur	338114
Rat XpressRef Universal Total RNA	2 tubes, each with 100 µg of total RNA prepared from several male and female rats (SD Wistar strain), whole bodies without fur	338116
RNeasy Micro Kit (50)	50 RNeasy MinElute® Spin Columns, collection tubes (1.5 mL and 2 mL), RNase-free DNase I, Carrier RNA, RNase-free reagents, and Buffers	74004
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, collection tubes (1.5 mL and 2 mL), RNase-free Reagents, and buffers	74104
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
QIAseq 2x Hi-Fi MasterMix Kit (0.7 mL)	Includes 1 x 0.72 mL of QIAseq 2x Hi-Fi DNA Mastermix	333221

Product	Contents	Cat. no.
QIAseq 2x Hi-Fi MasterMix Kit (2.8 mL)	Includes 2 x 1.44 mL of QIAseq 2x Hi-Fi DNA Mastermix	333220
QIAseq FastSelect -Globin Kit	Includes QIAseq FastSelect reagent for globin mRNA removal; reactions from human, mouse, and rat samples	Varies
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
QIAseq Stranded mRNA Enrichment Kit (24)	Includes Pure mRNA Beads, binding, wash and elution buffers and RNAse-free water	1105688
QIAseq Stranded mRNA Enrichment Kit (96)	Includes Pure mRNA Beads, binding, wash and elution buffers and RNAse-free water	1105689
RNeasy Pure mRNA Bead Kit	For 48 reactions: Pure mRNA Beads, buffers, RNase-Free Water, Small Spin Columns, and Collection Tubes	180244

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

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

Document Revision History

Revision	Description
July 2022	Initial release
October 2022	Changed QIAseq DIRECT Unique UDIs product name to QIAseq UX Index Kits UDI Table layout changes on Table 12 Change to product catalog numbers on Tables 12–20 Updated procedure steps in Appendix C Updated Ordering Information to add QIAseq UX 12 Index Kit IL UDI (12) and QIAseq UX 96 Index Kit IL UDI A–H (96)
February 2023	Corrected Table numbers and cross-referencing
January 2024	Updated the catalog numbers in “Kit Contents” and “Ordering Information” sections. Added contents in Table 4. Added Step 1 in Table 6. Modified “Library amplification/indexing procedure”. 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”. Added mRNA Enrichment protocols in “Appendix B”. Updated Tables 15,16,17,18,19,20,21, and 22. Updated footnote for Table 24 from 11 µL to 15 µL. Added products in Ordering information table.

Limited License Agreement for QIAseq UPXome RNA Library Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.
6. This product contains PCR enzymes which are licensed for research and commercial use from Bio-Rad Laboratories, Inc., under U.S. Pat. Nos. 6,627,424, 7,541,170, 7,670,808, 7,666,645, and corresponding patents in other countries. No rights are granted for use of the product for Digital PCR or real-time PCR applications, with the exception of quantification in Next Generation Sequencing workflows.

For updated license terms, see www.qiagen.com.

January 2024 HB-3095-004