

December 2021

# QIAseq<sup>®</sup> UPX 3' Targeted RNA Library Handbook

Sample to Insight<sup>®</sup> solution for high-throughput targeted next-generation sequencing from single cells, cell pellets and ultralow amounts of RNA

---

# Contents

Kit Contents .....	3
Shipping and Storage .....	5
Intended Use .....	5
Safety Information .....	6
Quality Control .....	6
Introduction .....	7
<b>Principle and procedure</b> .....	<b>8</b>
Equipment and Reagents to Be Supplied by User .....	13
Important Notes .....	14
Protocol: Cell Lysis .....	16
Protocol: Reverse Transcription of Lysed Cells .....	18
Protocol: Reverse Transcription of RNA .....	23
Protocol: Single Primer Extension .....	28
Optional Protocol: Quantitative Universal PCR .....	31
Protocol: Universal PCR .....	35
Protocol: Library QC and Quantification .....	38
Protocol: Sequencing Setup on Illumina MiSeq and NextSeq .....	40
Troubleshooting Guide .....	48
Appendix A: General remarks on handling RNA .....	50
Ordering Information .....	53
Document Revision History .....	55

# Kit Contents

<b>QIAseq UPX 3' Targeted RNA Panel</b>	<b>(96)</b>	<b>(96-M)</b>	<b>(384)</b>
<b>Catalog no.</b>	<b>333041</b>	<b>333042</b>	<b>333043</b>
<b>Number of reactions</b>	<b>96</b>	<b>96 x 4</b>	<b>384</b>
Cell Lysis Buffer	1 ml	1 ml	1 ml
RNase Inhibitor	96 µl	144 µl	144 µl
Cell Index RT Plate	CID-96S Plate 96-well single-use plate	CID-96M Plate 96-well multi-use plate	CID-384 Plate 384-well single-use plate
3' Tar RT Buffer	150 µl	600 µl	600 µl
EZ Reverse Transcriptase	36 µl	150 µl	150 µl
Nuclease-free Water	1 tube	1 tube	1 tube
UPX AMP Primer	100 µl	100 µl	100 µl
SPE Buffer	120 µl	480 µl	120 µl
Tar uQuant Buffer	80 µl	300 µl	80 µl
HotStarTaq® DNA Polymerase	60 µl	240 µl	60 µl
UPX 3' uPCR Buffer	80 µl	300 µl	80 µl
QIAseq Beads	10 ml	26 ml	10 ml
UPX 3' Targeted Panel Pool	125 µl	500 µl	125 µl
Indented flat 12-cap strips	1 bag	1 bag	—
Optical adhesive film	—	—	1
384-EasyLoad Covers	—	—	1
Quick-Start Protocol*	2	2	2

\* See [www.qiagen.com/HB-2473](http://www.qiagen.com/HB-2473) (part 1) and [www.qiagen.com/HB-2474](http://www.qiagen.com/HB-2474) (part 2) to view this handbook's corresponding Quick-Start Protocol.

<b>QIAseq UPX 3' Targeted RNA 12 index</b>			<b>(48)</b>
<b>Catalog no.</b>			<b>333044</b>
<b>Number of reactions</b>	<b>Index i7*</b>	<b>Index i5*</b>	<b>48</b>
UPX 3' Tar I1	N702	S505	48 µl
UPX 3' Tar I2	N704	S506	48 µl
UPX 3' Tar I3	N705	S508	48 µl
UPX 3' Tar I4	N701	S502	48 µl
UPX 3' Tar I5	N703	S503	48 µl
UPX 3' Tar I6	N707	S510	48 µl
UPX 3' Tar I7	N710	S511	48 µl
UPX 3' Tar I8	N711	S513	48 µl
UPX 3' Tar I9	N712	S515	48 µl
UPX 3' Tar I10	N714	S516	48 µl
UPX 3' Tar I11	N715	S517	48 µl
UPX 3' Tar I12	N706	S507	48 µl
QIAseq A Read 1 Primer I (100 µM)	–	–	24 µl
QIAseq C Read 2 Primer I (100 µM)	–	–	24 µl

\*Sample Indices are adapted from Nextera V2.

<b>QIAseq UPX 3' Targeted RNA 96-Index A, B, C or D</b>		<b>(384)</b>
<b>(96 sample index for 384 samples on Illumina platform)</b>		
<b>Catalog no.</b>		<b>333051</b> <b>333052</b> <b>333053</b> <b>333054</b>
<b>Number of reactions</b>		<b>384</b>
<p>Box containing four tubes of QIAseq A Read 1 Primer I (100 µM, 24 µl), QIAseq C Read 2 Primer I (100 µM, 24 µl), Indented flat 12-cap strips (48) and arrays. Each array well contains one PCR primer pair for amplification and dual sample indexing – enough for a total of 384 samples (for indexing up to 96 samples per run) for targeted RNA sequencing on Illumina platforms. All four sets support indexing up to 384 samples per run.</p>		<b>4</b>

---

## Shipping and Storage

The QIAseq UPX 3' Targeted RNA Panel (cat. nos. 333041, 333042, 333043) is shipped in several boxes.

- Box 1 (QIAseq UPX 3' Targeted RNA Panel) is shipped on dry ice, and upon receipt, all components in Box 1 should be stored immediately at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.
- Box 2 (QIAseq Beads and Cell Index RT Plate) is shipped on blue ice. Upon receipt, all components in Box 2 should be stored at  $2$ – $8^{\circ}\text{C}$ .
- Do not freeze QIAseq Beads.

The QIAseq UPX 3' Targeted RNA 12 index (sold separately) (cat. no. 333044) is shipped on blue ice and should be stored at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

The QIAseq UPX 3' Targeted RNA 96-Index A, B, C and D (sold separately) (cat. nos. 333051, 333052, 333053, 333054, respectively) are shipped on dry ice and should be stored at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

## Intended Use

The QIAseq UPX 3' Targeted RNA Panel is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

---

## Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at [www.qiagen.com/safety](http://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 the QIAseq UPX 3' Targeted RNA Panel is tested against predetermined specifications to ensure consistent product quality.

---

# Introduction

Tissues are heterogeneous mixtures of different cell populations with each cell contributing a unique proteome and transcriptome. For example, normal- and disease-biology are both inherently heterogeneous, and cells can respond individually and in concert to internal and external stimuli. Furthermore, individual cells can differ due to epigenetics, circadian clock, cell cycle, microenvironments, cell-to-cell contacts, and intrinsic transcriptional “noise”. While bulk transcriptomic analysis of mRNA, lncRNA, and miRNA expression is critical for understanding biological systems, the consequential “cellular averages” mask intrinsic transcriptional variability across individual cell subpopulations. In fact, the contribution of rare cell subtypes may be completely obscured when cells are assessed in bulk. Single-cell expression analysis brings into focus the individual contribution of every cell providing a complete, granular understanding of a specific biological response.

QIAseq Ultraplex (UPX) 3' Targeted RNA Panels enables Sample to Insight, high-throughput next-generation sequencing (NGS) of polyadenylated RNAs from single cells on Illumina® NGS instruments. In addition, QIAseq UPX is intended for library construction and analysis of cell pellets (up to 100 cells) and purified RNA (10 pg to 1 ng). QIAseq UPX presents an innovative advantage in that, during reverse transcription, each cell is tagged with a unique ID (up to 384 different IDs) and each RNA molecule is tagged with a unique index. Following reverse transcription, all cDNAs are combined, which enables all subsequent library construction steps to be performed in a single tube. This prevents sample mixup, saves substantial time and dramatically reduces library prep costs. Target enrichment technology further enhances the power of QIAseq UPX by enabling users to sequence specific genes of interest, which effectively increases sequencing depth and sample throughput, while minimizing cost. Universal library amplification then assigns up to 384 different sample IDs. Together, the combination of Cell IDs and the Sample IDs enables up to 147,456 targeted libraries to be sequenced together. Ultimately, QIAseq UPX data analysis enables primary mapping, single-cell clustering analysis and differential expression analysis. Collectively, the

Sample to Insight workflow of QIAseq UPX defines a new generation of high-throughput NGS technologies (Figure 1).

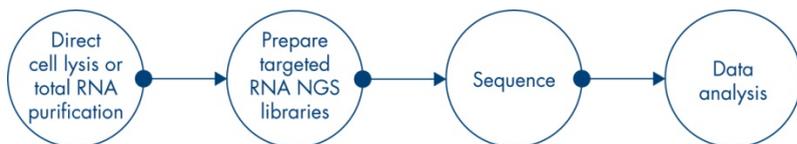


Figure 1. QIAGEN's Sample to Insight QIAseq UPX workflow.

## Principle and procedure

The QIAseq UPX 3' Targeted RNA Panel workflow (Figure 2 and Figure 3) is described below. A magnetic, bead-based cleanup is included between each step.

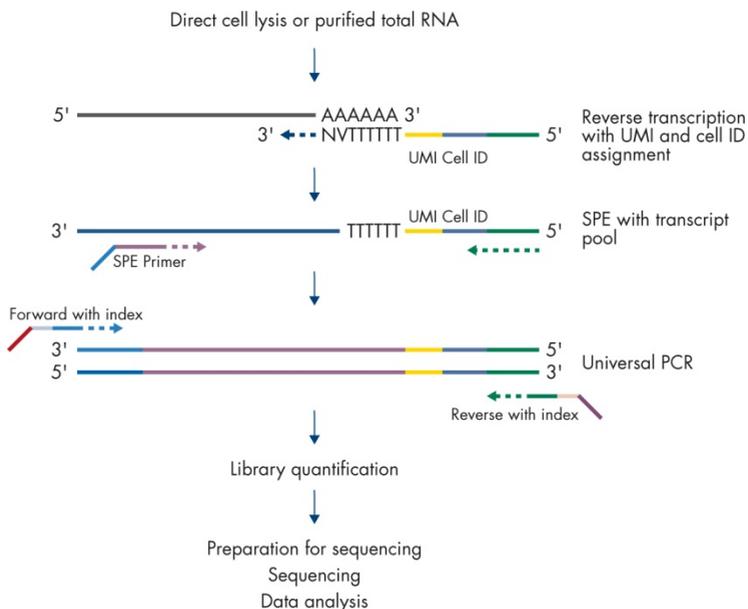
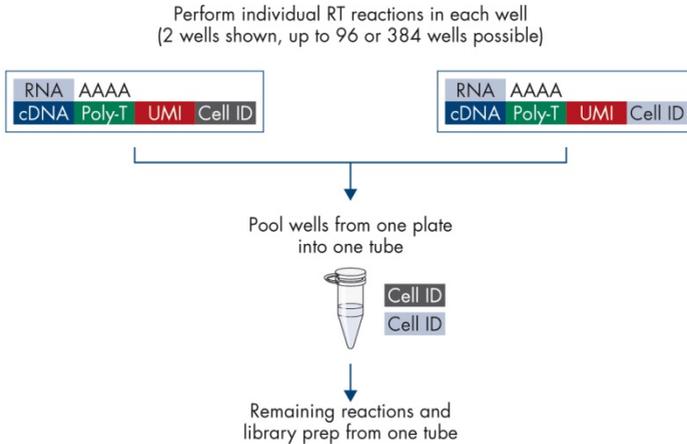


Figure 2. QIAseq UPX 3' Targeted RNA Panel workflow.

- **Direct cell lysis or purified total RNA:** Single cells and pellets of up to 100 cells can be lysed using the provided cell lysis buffer and RNase inhibitor. Alternatively, between 10 pg and 1 ng of purified total RNA can be added to the reaction.



**Figure 3. QIAseq UPX 3' Targeted RNA Panel handling.** Individual reverse transcription reactions are performed for each sample that tag the cDNA with a unique Cell ID and each molecule with a unique molecular index (UMI). Following reverse transcription, all wells for a plate, up to 96 or 384 respectively, can be pooled into a single tube. The remaining reactions and library prep associated with that plate are performed in a single tube.

- **Reverse transcription:** The LNA-enhanced anchored oligo-dT reverse transcription (RT) primer contains an integrated Cell ID and UMI (Figure 4A) and is provided in single-use or multi-use 96-well plates or single-use 384-well plates (Figure 4B and 4C). For the 96-well plates, 96 different RT primers are provided, each with a unique Cell ID. For the 384-well plates, 384 different RT primers are provided, each with a unique Cell ID. The UMI is a 12-base fully random sequence (i.e., the UMI). Statistically, this process provides  $4^{12}$  possible indices per RT primer, and each cDNA molecule in the sample receives a unique UMI sequence. Following reverse transcription, all cDNAs from a single plate, whether it is 96-well or 384-well, can be combined in a single tube due to the unique Cell IDs.

**A**

- NVTTTTT<sub>(n)</sub>-[UMI]-[Cell ID]-\_\_\_\_\_

**B**

	1	2	3	4	5	6	7	8	9	10	11	12
A	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
B	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
C	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34	C35	C36
D	C37	C38	C39	C40	C41	C42	C43	C44	C45	C46	C47	C48
E	C49	C50	C51	C52	C53	C54	C55	C56	C57	C58	C59	C60
F	C61	C62	C63	C64	C65	C66	C67	C68	C69	C70	C71	C72
G	C73	C74	C75	C76	C77	C78	C79	C80	C81	C82	C83	C84
H	C85	C86	C87	C88	C89	C90	C91	C92	C93	C94	C95	C96

**C**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
B	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34	C35	C36	C37	C38	C39	C40	C41	C42	C43	C44	C45	C46	C47	C48
C	C49	C50	C51	C52	C53	C54	C55	C56	C57	C58	C59	C60	C61	C62	C63	C64	C65	C66	C67	C68	C69	C70	C71	C72
D	C73	C74	C75	C76	C77	C78	C79	C80	C81	C82	C83	C84	C85	C86	C87	C88	C89	C90	C91	C92	C93	C94	C95	C96
E	C97	C98	C99	C100	C101	C102	C103	C104	C105	C106	C107	C108	C109	C110	C111	C112	C113	C114	C115	C116	C117	C118	C119	C120
F	C121	C122	C123	C124	C125	C126	C127	C128	C129	C130	C131	C132	C133	C134	C135	C136	C137	C138	C139	C140	C141	C142	C143	C144
G	C145	C146	C147	C148	C149	C150	C151	C152	C153	C154	C155	C156	C157	C158	C159	C160	C161	C162	C163	C164	C165	C166	C167	C168
H	C169	C170	C171	C172	C173	C174	C175	C176	C177	C178	C179	C180	C181	C182	C183	C184	C185	C186	C187	C188	C189	C190	C191	C192
I	C193	C194	C195	C196	C197	C198	C199	C200	C201	C202	C203	C204	C205	C206	C207	C208	C209	C210	C211	C212	C213	C214	C215	C216
J	C217	C218	C219	C220	C221	C222	C223	C224	C225	C226	C227	C228	C229	C230	C231	C232	C233	C234	C235	C236	C237	C238	C239	C240
K	C241	C242	C243	C244	C245	C246	C247	C248	C249	C250	C251	C252	C253	C254	C255	C256	C257	C258	C259	C260	C261	C262	C263	C264
L	C265	C266	C267	C268	C269	C270	C271	C272	C273	C274	C275	C276	C277	C278	C279	C280	C281	C282	C283	C284	C285	C286	C287	C288
M	C289	C290	C291	C292	C293	C294	C295	C296	C297	C298	C299	C300	C301	C302	C303	C304	C305	C306	C307	C308	C309	C310	C311	C312
N	C313	C314	C315	C316	C317	C318	C319	C320	C321	C322	C323	C324	C325	C326	C327	C328	C329	C330	C331	C332	C333	C334	C335	C336
O	C337	C338	C339	C340	C341	C342	C343	C344	C345	C346	C347	C348	C349	C350	C351	C352	C353	C354	C355	C356	C357	C358	C359	C360
P	C361	C362	C363	C364	C365	C366	C367	C368	C369	C370	C371	C372	C373	C374	C375	C376	C377	C378	C379	C380	C381	C382	C383	C384

**Figure 4. UPX anchor oligo-dT RT primer and Cell Index (ID) RT Plate.** (A) The LNA-enhanced anchored oligo-dT reverse transcription (RT) primer contains an integrated Cell ID and UMI. (B) Layout of 96-well single-use and multi-use Cell ID RT Plates. (C) Layout of 384-well single-use Cell ID RT Plate.

- Single primer extension (SPE): Targeted SPE enrichment is performed post-UMI assignment to ensure that cDNA molecules containing UMIs are sufficiently enriched in the sequenced library. For enrichment, the pooled cDNAs are subject to several cycles of targeted PCR using transcript-specific primers and one universal primer complementary to a universal sequence added during reverse transcription. Recommendations for the number of single cells that can be multiplexed are given, based on the number of multiplexed primers in Table 1. Recommendations for the number of 1 ng samples that can be multiplexed are given, based on the number of multiplexed primers in Table 2.

**Table 1. Single cell multiplexing recommendations, based on panel size and sequencing instrument (>15 reads per UMI)**

Instrument	Version	Capacity	250 primers	500 primers	1000 primers	2000 primers
MiSeq®	V2	15 M	922	461	230	115
MiSeq	V3	25 M	1536	768	384	192
NextSeq® 550	High output	400 M	24576	12288	6144	3072
NextSeq 550	Mid output	130 M	7987	3994	1997	998
HiSeq® 2500	Rapid run	150 M/lane	9216	4608	2304	1152
HiSeq 2500	High output	250 M/lane	15360	7680	3840	1920
HiSeq 3000/4000	–	312.5 M/lane	19200	9600	4800	2400

**Table 2. Multiplexing recommendations for 1 ng sample, based on panel size and sequencing instrument (>15 reads per UMI)**

Instrument	Version	Capacity	250 primers	500 primers	1000 primers	2000 primers
MiSeq	V2	15 M	230	115	58	29
MiSeq	V3	25 M	384	192	96	48
NextSeq 550	High output	400 M	6144	3072	1536	768
NextSeq 550	Mid output	130 M	1997	998	499	250
HiSeq 2500	Rapid run	150 M/lane	2304	1152	576	288
HiSeq 2500	High output	250 M/lane	3840	1920	960	480
HiSeq 3000/4000	–	312.5 M/lane	4800	2400	1200	600

- 
- Universal PCR: Library amplification introduces up to 384 sample indices using a dual indexing approach. This universal amplification approach also ensures that sufficient target is present for next-generation sequencing. For each sample index, up to 384 Cell IDs can be included from the reverse transcription reaction. As a result, up to 147,456 can be sequenced together at one time.
  - Library quantification: The library yield measurements of the Agilent® Bioanalyzer or Agilent TapeStation system using fluorescence dyes which intercalate into DNA or RNA cannot discriminate between cDNA with or without 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 or Assay Kit, which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library.
  - Next-generation sequencing: The QIAseq UPX 3' Targeted RNA Library Kit is compatible with Illumina NGS systems (MiniSeq®, MiSeq Personal Sequencer, NextSeq 500, HiSeq 1000, HiSeq 1500, HiSeq 2000, HiSeq 2500, HiSeq 3000/4000, and NovaSeq™). A custom sequencing primer for Read 1 (QIAseq A Read 1 Primer I) is required for all Illumina NGS systems. In addition, a custom sequencing primer for Read 2 (QIAseq C Read 2 Primer I) is required when using the NextSeq.
  - Data analysis: The QIAseq UPX 3' Targeted RNA Panel analysis pipeline is available at [www.qiagen.com/GeneGlobe](http://www.qiagen.com/GeneGlobe). The pipeline automatically performs all steps necessary to perform primary mapping, single cell cluster analysis, or differential expression.

---

# Equipment and Reagents to Be Supplied by User

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

- Nuclease-free pipette tips and tubes
- Microcentrifuge tubes (2 ml)
- PCR tubes (0.2 ml individual tubes or tubes strips) (VWR, cat. no. 20170-012 or 93001-118)
- Ice
- Microcentrifuge
- Optional spike-in for single cell application
- MagneSphere® Technology Magnetic Separation Stand ( Promega, cat. no. Z5342) or DynaMag™-2 Magnet (Thermo Fisher Scientific, cat. no. 12321D)
- Optional spike-in for single cell application: ERCC ExFold RNA Spike-In Mixes (Thermo Fisher Scientific, cat. no. 4456739)
- Library QC:
  - Agilent 2100 Bioanalyzer
  - High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626)
- Preferred Library Quantification Method: qPCR instrument and QIAseq Library Quant System: QIAseq Library Quant Array Kit (cat. no. 333304) or QIAseq Library Quant Assay Kit (cat. no. 333314)

# Important Notes

For direct cell lysis, single cells and pellets of up to 100 cells can be lysed using the provided cell lysis buffer and RNase inhibitor. Alternatively, between 10 pg and 1 ng of purified total RNA can be added to the reaction. QIAGEN provides a range of solutions for purification of total RNA (Table 3).

**Table 3. Recommended kits for purification of total RNA**

Kit	Starting material	Cat. no.
RNeasy® Micro Kit	Small amounts of cells and tissue	74004
RNeasy Mini Kit	Animal/human tissues and cells	74104, 74106
RNeasy 96 Kit	Animal/human tissues and cells	74181, 74182

- Ensure that total RNA samples are of high quality relative to their sample type. For additional information, see “Appendix A: General remarks on handling RNA”, page 50.
- RNA quantification: The concentration and purity of total RNA isolated from cells and fresh/frozen tissues should be determined by measuring the absorbance in a spectrophotometer. As the spectral properties of nucleic acids are highly dependent on pH we recommend preparing dilutions and measure absorbance in 10 mM Tris-Cl, pH 7.5 instead of RNase-free water. Pure RNA has an  $A_{260}/A_{280}$  ratio of 1.9–2.1 in 10 mM Tris-Cl, pH 7.5. It is not useful to assess the concentration and purity of total RNA derived from fluids and/or exosomes.
- RNA integrity: The integrity and size distribution of total RNA purified from cells and fresh/frozen tissue can be confirmed using an automated analysis system (such as the QIAxcel® Advanced System or the Agilent 2100 Bioanalyzer) that assess RNA integrity using a RNA integrity score (RIS) or RNA integrity number (RIN). Although the RNA Integrity Number (RIN) should ideally be  $\geq 8$ , successful library prep is still possible with samples whose RIN values are  $\leq 8$ .

- 
- Ensure reactions are thoroughly mixed, as well as 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 particular sections.

# Protocol: Cell Lysis

## Important points before starting

- The recommended starting amount is 1–100 cells.
- When working with single cells, a minimum of 24 cells must be multiplexed per sample index.
- Three options exist for cell collection (described in Figure 4, page 12):
  - CID-96S Plate: 96-well, single-use Cell ID RT Plate
  - CID-384 Plate: 384-well, single-use Cell ID RT Plate
  - Empty 96-well plates
- **Important:** When cells have been collected into empty 96-well plates, RT primers need to be dispensed from the CID-96M Plate, the multi-use 96-well Cell ID RT Plate described in Figure 4, during setup of the reverse transcription reactions.
- Ensure reactions are thoroughly mixed, prepared at recommended temperatures, and incubated at recommended temperatures.
- Do not vortex reactions or reagents unless instructed.

## Procedure

1. Thaw Cell Lysis Buffer and Nuclease-free Water at room temperature (15–25°C). Mix by flicking the tube. Centrifuge the tubes briefly to collect residual liquid from the sides of the tubes and keep at room temperature.

**Note:** RNase Inhibitor should be removed from the –20°C freezer just before preparation of the master mix, and placed on ice. RNase Inhibitor should be returned to the freezer immediately after use.

2. Prepare the Cell Lysis Premix on ice as described in Table 4. Briefly centrifuge, mix by pipetting up and down 12 times, and briefly centrifuge.

**Note:** The cell lysis buffer contains all components required for cell lysis (25% greater than that required for the total number of reactions).

**Table 4. Preparation of cell lysis premix**

<b>Component</b>	<b>Volume 8 wells</b>	<b>Volume 24 wells</b>	<b>Volume 96 wells</b>	<b>Volume 384 wells</b>
Cell Lysis Buffer	10 $\mu$ l	30 $\mu$ l	120 $\mu$ l	480 $\mu$ l
RNase Inhibitor	2.5 $\mu$ l	7.5 $\mu$ l	30 $\mu$ l	120 $\mu$ l
Nuclease-free Water	17.5 $\mu$ l	52.5 $\mu$ l	210 $\mu$ l	840 $\mu$ l
<b>Total volume</b>	<b>30 <math>\mu</math>l</b>	<b>90 <math>\mu</math>l</b>	<b>360 <math>\mu</math>l</b>	<b>1440 <math>\mu</math>l</b>

3. Aliquot 3  $\mu$ l of Cell Lysis Premix into the required wells of the single-use Cell ID RT Plate (CID-96S Plate or CID-384 Plate) or empty 96-/384-well plates.

**Note:** The CID-96S Plate is breakable, allowing cells to be processed in batches.

4. Capture cells into the plate containing the Cell Lysis Premix.

5. Incubate for 15 min on ice.

6. Freeze at  $-90$  to  $-60^{\circ}\text{C}$  or proceed immediately with "Protocol: Reverse Transcription of Lysed Cells" on page 18.

# Protocol: Reverse Transcription of Lysed Cells

## Important points before starting

- 3  $\mu$ l cell lysates from “Protocol: Cell Lysis” is the starting materials for the reverse-transcription reactions.
- Important: When cells have been collected into empty 96-well plates, RT primers must be dispensed from CID-96M Plate, the multi-use 96-well Cell ID RT Plate, described in Figure 4, during setup of the reverse transcription reactions. Each well contains a pre-dispensed, anchored oligo-dT primer containing a UMI and Cell ID. Prior to use, add 2.5  $\mu$ l Nuclease-free Water into each well, vortex the plate, centrifuge briefly, and incubate for 10 min at room temperature to fully dissolve the primer.
- Ensure reactions are thoroughly mixed, prepared at recommended temperatures, and incubated at recommended temperatures.
- Do not vortex reactions or reagents unless instructed.

## Procedure

1. Prepare reagents required for the reverse-transcription reactions. Thaw 3' RT Tar Buffer and Nuclease-free Water at room temperature (15–25°C). Mix by flicking the tube. Centrifuge the tubes briefly to collect residual liquid from the sides of the tubes and keep on ice.

**Note:** EZ Reverse Transcriptase should be removed from the  $-20^{\circ}\text{C}$  freezer just before preparation of the master mix, and placed on ice. Both enzymes should be returned to the freezer immediately after use.

2. Prepare the RT Premix on ice as described in Table 5 (when using single-use Cell ID RT Plates) or Table 6 (when aliquoting RT primers from a multi-use 96-well Cell ID RT Plate).

**Table 5. Preparation of RT premix for single-use Cell ID RT Plates (CID-96S Plate or CID-384 Plate)**

Component	Volume 8 wells	Volume 24 wells	Volume 96 wells	Volume 384 wells
3' Tar RT Buffer	10 $\mu$ l	30 $\mu$ l	120 $\mu$ l	480 $\mu$ l
EZ Reverse Transcriptase	2.5 $\mu$ l	7.5 $\mu$ l	30 $\mu$ l	120 $\mu$ l
Nuclease-free Water	5 $\mu$ l	15 $\mu$ l	60 $\mu$ l	240 $\mu$ l
ERCC diluted 1.25 x 10 <sup>6</sup> -fold* or Nuclease-free Water	2.5 $\mu$ l	7.5 $\mu$ l	30 $\mu$ l	120 $\mu$ l
<b>Total volume</b>	<b>20 <math>\mu</math>l</b>	<b>60 <math>\mu</math>l</b>	<b>240 <math>\mu</math>l</b>	<b>960 <math>\mu</math>l</b>

\* For single-cell analysis, ERCC can be added as a control. Prepare the ERCC by diluting the stock 1.25 x 10<sup>6</sup>-fold using 0.1X TE Buffer.

**Table 6. Preparation of RT premix when using multi-use Cell ID RT Plates (CID-96M Plate)**

Component	Volume 8 wells	Volume 24 wells	Volume 96 wells	Volume 384 wells
3' Tar RT Buffer	10 $\mu$ l	30 $\mu$ l	120 $\mu$ l	480 $\mu$ l
EZ Reverse Transcriptase	2.5 $\mu$ l	7.5 $\mu$ l	30 $\mu$ l	120 $\mu$ l
ERCC diluted 1.25 x 10 <sup>6</sup> -fold* or Nuclease-free Water	2.5 $\mu$ l	7.5 $\mu$ l	30 $\mu$ l	120 $\mu$ l
Cell ID RT Primer <sup>†</sup>	0.5 $\mu$ l added per well	0.5 $\mu$ l added per well	0.5 $\mu$ l added per well	0.5 $\mu$ l added per well
<b>Total volume</b>	<b>15 <math>\mu</math>l</b>	<b>45 <math>\mu</math>l</b>	<b>180 <math>\mu</math>l</b>	<b>720 <math>\mu</math>l</b>

\* For single-cell analysis, ERCC can be added as a control. Prepare the ERCC by diluting the stock 1.25x10<sup>6</sup>-fold using 0.1X TE Buffer.

<sup>†</sup> Added separately from a multi-use Cell ID RT Plate in step 3.

3. Aliquot the RT Premix and RT Primers from the multi-use Cell RT Plate (when applicable) as follows.

For single-use Cell ID RT Plates (CID-96S Plate or CID-384 Plate, aliquot 2  $\mu$ l of RT Premix from Table 5 into the wells of the plate.

When using the multi-use Cell ID RT Plate (CID-96M Plate), aliquot 1.5  $\mu$ l of reverse transcription premix from Table 6 into the plate wells containing 3  $\mu$ l of lysed cell, and subsequently aliquot 0.5  $\mu$ l of each Cell ID RT Primer from the multi-use Cell ID RT Plate into the appropriate well.

**Note:** In the same experiment, a different Cell ID RT Primer must be used per cell/sample.

4. Incubate as described in Table 7.

**Table 7. Reverse transcription incubation conditions**

Time	Temperature
5 min	25°C
1 h	42°C
5 min	95°C
$\infty$	4°C

5. Upon completion of reverse-transcription reactions, combine the synthesized cDNA from the different wells into one, 2 ml microcentrifuge tube. Up to 96 wells can be combined in one tube.

**Note:** The cDNA generated from each well of a Cell ID RT Plate contains a specific Cell ID that enables tracking of that particular sample.

6. Add 1.3X (volume) QIAseq Beads to the combined cDNA synthesis reactions as described in Table 8. Mix well by pipetting up and down 12 times.

**Table 8. Addition of QIAseq Beads for cDNA cleanup**

Number of wells combined	QIAseq Bead volume
8	52 $\mu$ l
24	156 $\mu$ l
96*	624 $\mu$ l

\* When working with 384 wells, perform the cleanup as 4 sets of 96 wells. Combine supernatants at step 14.

7. Incubate for 5 min at room temperature.
8. Place the tubes on a magnetic rack for 5–10 min. Higher volumes may take longer to clear. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.  
**Important:** Do not discard the beads as they contain the DNA of interest.
9. With the beads still on the magnetic stand, add 400  $\mu$ l of 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.
10. Repeat the ethanol wash.  
**Important:** Completely remove all traces of the ethanol wash after this second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200  $\mu$ l pipette, and then a 10  $\mu$ l pipette to remove any residual ethanol.
11. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 10 min.  
**Note:** Visually inspect that the pellet is completely dry.
12. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 52  $\mu$ l Nuclease-free Water. Mix well by pipetting.
13. Return the tubes to the magnetic rack until the solution has cleared.
14. Transfer 50  $\mu$ l of the supernatant to clean tubes.  
**Important:** With 384 wells, the mixture can be combined across all four sets to consolidate all 384 indices into one tube.
15. Add 1.3X volume QIAseq Beads to the supernatant. Mix well by pipetting up and down 12 times.  
**Note:** QIAseq Bead volume will be 65  $\mu$ l if supernatant is 50  $\mu$ l.
16. Incubate for 5 min at room temperature.
17. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

---

18. With the beads still on the magnetic stand, add 400  $\mu$ l 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.

19. Repeat the ethanol wash. Completely remove all traces of the ethanol wash.

**Important:** Completely remove all traces of the ethanol wash after this second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200  $\mu$ l pipette, and then a 10  $\mu$ l pipette to remove any residual ethanol.

20. With the tubes (caps opened) still on the magnetic stand, air dry for 10 min.

**Note:** Visually inspect that the pellet is completely dry. Ethanol carryover to the next step will affect reaction efficiency.

21. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 12  $\mu$ l Nuclease-free Water. Mix well by pipetting.

22. Return the tubes to the magnetic rack until the solution has cleared.

23. Transfer 10  $\mu$ l of the supernatant to clean tubes.

**Note:** From this point of the protocol, the procedures assume that all cDNA wells (8, 24, 96, or 384) have been combined into a single tube.

24. Proceed with "Protocol: Single Primer Extension" on page 28. Alternatively, the samples can be stored at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

---

# Protocol: Reverse Transcription of RNA

Fresh and frozen tissue can be used for RNA purification. The yield and quality of RNA obtained will depend on the tissue type, source, and storage conditions.

## Important points before starting

- This protocol can be used with low amounts of purified RNA (10 pg to 1 ng).
- When working with purified RNA, three options exist:
  - CID-96S Plate: 96-well single-use Cell ID RT Plate
  - CID-384 Plate: 384-well single-use Cell ID RT Plate
  - Empty 96-well plates
- **Important:** When using empty 96-well plates, RT primers must be dispensed from CID-96M Plate, the multi-use 96-well Cell ID RT Plate described in Figure 4, during setup of the reverse transcription reactions. Each well contains a pre-dispensed, anchored oligo-dT primer containing a UMI and Cell ID. Prior to use, add 2.5  $\mu$ l Nuclease-free Water into each well, vortex the plate, centrifuge briefly, and incubate for 10 min at room temperature to fully dissolve the primer.
- Ensure reactions are thoroughly mixed, prepared at recommended temperatures, and incubated at recommended temperatures.
- Do not vortex reactions or reagents unless instructed.

## Procedure

1. Prepare reagents required for the reverse-transcription reactions. Thaw Cell Lysis Buffer, 3' Tar RT Buffer, and Nuclease-free Water at room temperature (15–25°C). Mix by flicking the tube. Centrifuge the tubes briefly to collect residual liquid from the sides of the tubes and keep on ice.

**Note:** EZ Reverse Transcriptase and RNase Inhibitor should be removed from the –20°C freezer just before preparation of the master mix, and placed on ice. Both enzymes should be returned to the freezer immediately after use.

2. Prepare the RT Premix on ice as described in Table 9 (when using single-use Cell ID RT Plates) or Table 10 (when RT primers will be aliquoted from a multi-use 96-well Cell ID RT Plate).

**Table 9. Preparation of RT premix for single-use Cell ID RT Plates (CID-96S Plate or CID-384 Plate)**

Component	Volume 8 wells	Volume 24 wells	Volume 96 wells	Volume 384 wells
Cell Lysis Buffer	10 µl	30 µl	120 µl	480 µl
RNase Inhibitor	2.5 µl	7.5 µl	30 µl	120 µl
3' Tar RT Buffer	10 µl	30 µl	120 µl	480 µl
EZ Reverse Transcriptase	2.5 µl	7.5 µl	30 µl	120 µl
RNA*	2.5 µl added per well			
<b>Total volume</b>	<b>25 µl</b>	<b>75 µl</b>	<b>300 µl</b>	<b>1200 µl</b>

\* Added separately in step 3.

**Table 10. Preparation of RT premix when using multi-use Cell ID RT plates (CID-96M Plate)**

Component	Volume 8 wells	Volume 24 wells	Volume 96 wells	Volume 384 wells
Cell Lysis Buffer	10 µl	30 µl	120 µl	480 µl
RNase Inhibitor	2.5 µl	7.5 µl	30 µl	120 µl
3' Tar RT Buffer	10 µl	30 µl	120 µl	480 µl
EZ Reverse Transcriptase	2.5 µl	7.5 µl	30 µl	120 µl
RNA*	2 µl added per well			
Cell ID RT Primer†	0.5 µl added per well	0.5 µl added per well	2.5 µl added per well	2.5 µl added per well
<b>Total volume</b>	<b>25 µl</b>	<b>75 µl</b>	<b>300 µl</b>	<b>1200 µl</b>

\* Added separately in step 3.

† Added separately from a multi-use Cell ID RT Plate in step 3.

3. Aliquot the RT Premix, purified RNA, and RT Primers from the multi-use 3' Cell ID RT Plate (when applicable), as follows.

For single-use Cell ID RT plates (CID-96S Plate or CID-384 Plate), aliquot 2.5  $\mu$ l of RT Premix from Table 9 into the wells of the plate. Subsequently add 2.5  $\mu$ l of purified RNA.

When using the multi-use Cell ID RT Plate (CID-96M Plate), aliquot 2.5  $\mu$ l of RT Premix from Table 10 per well of **an empty** 96-/384-well plate. Subsequently, add 2  $\mu$ l of purified RNA and aliquot 0.5  $\mu$ l of each Cell ID RT primer from the multi-use Cell ID RT Plate into the appropriate well.

**Note:** A different Cell ID RT Primer must be used for every sample.

4. Incubate as described in Table 11.

**Table 11. Reverse transcription incubation**

Time	Temperature
5 min	25°C
1 h	42°C
5 min	95°C
$\infty$	4°C

5. Upon completion of reverse transcription reactions, combine the synthesized cDNA from the different wells into one, 2 ml microcentrifuge tube. Up to 96 wells can be combined in one tube.

**Note:** The cDNA generated from each well of a Cell ID RT Plate contains a specific Cell ID that enables tracking of that particular sample.

6. Add 1.3X (volume) QIAseq Beads to the combined cDNA synthesis reactions as described in Table 12. Mix well by pipetting up and down 12 times.

**Table 12. Addition of QIAseq Beads for cDNA cleanup**

Number of wells combined	QIAseq Bead volume
8	52 $\mu$ l
24	156 $\mu$ l
96*	624 $\mu$ l

\* When working with 384 wells, perform the cleanup as 4 sets of 96 wells. Combine supernatants at step 14.

7. Incubate for 5 min at room temperature.
8. Place the tubes on a magnetic rack for 5–10 min. Higher volumes may need longer to clear. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

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

9. With the beads still on the magnetic stand, add 400  $\mu$ l of 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.
10. Repeat the ethanol wash.

**Important:** Completely remove all traces of the ethanol wash after this second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200  $\mu$ l pipette, and then a 10  $\mu$ l pipette to remove any residual ethanol.

11. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 10 min.

**Note:** Visually inspect that the pellet is completely dry.

12. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 52  $\mu$ l Nuclease-free Water. Mix well by pipetting.
13. Return the tubes to the magnetic rack until the solution has cleared.
14. Transfer 50  $\mu$ l of the supernatant to clean tubes/plate.

**Important:** With 384 wells, the mixture can be combined across all four sets to consolidate all 384 indices into one tube.

---

15. Add 1.3X volume QIAseq Beads to the supernatant. Mix well by pipetting up and down 12 times.

**Note:** QIAseq Bead volume will be 65  $\mu$ l if supernatant is 50  $\mu$ l.

16. Incubate for 5 min at room temperature.

17. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

18. With the beads still on the magnetic stand, add 400  $\mu$ l 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.

19. Repeat the ethanol wash. Completely remove all traces of the ethanol wash.

**Important:** Completely remove all traces of the ethanol wash after this second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200  $\mu$ l pipette, and then a 10  $\mu$ l pipette to remove any residual ethanol.

20. With the tubes (caps opened) still on the magnetic stand, air dry for 10 min.

**Note:** Visually inspect that the pellet is completely dry. Ethanol carryover to the next step will affect reaction efficiency.

21. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 12  $\mu$ l Nuclease-free Water. Mix well by pipetting.

22. Return the tubes to the magnetic rack until the solution has cleared.

23. Transfer 10  $\mu$ l of the supernatant to clean tubes.

**Note:** From this point of the protocol, the procedures assume that all cDNA wells (8, 24, 96, or 384) have been combined into a single tube.

24. Proceed with "Protocol: Single Primer Extension" on page 28. Alternatively, the samples can be stored at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

---

# Protocol: Single Primer Extension

## Important points before starting

- 10  $\mu$ l of the product from the cDNA cleanup in “Protocol: Reverse Transcription of Lysed Cells” or “Protocol: Reverse Transcription of RNA” is the starting material for the single primer extension (SPE) reaction.
- From this point forward in the protocol, the procedures assume that all cDNA wells (8, 24, 96, or 384) have been combined into a single tube.

## Procedure

1. Prepare reagents required for the SPE reaction. Thaw UPX 3' SPE Buffer, UPX AMP Primer, and UPX 3' Targeted Panel Pool at room temperature (15–25°C). Mix by flicking the tube. Centrifuge the tube briefly to collect residual liquid from the sides of the tube and keep at room temperature.

**Note:** HotStarTaq DNA Polymerase should be removed from the –20°C freezer just before preparation of the master mix, and placed on ice. HotStarTaq DNA Polymerase should be returned to the freezer immediately after use.

2. Prepare the SPE reaction on ice as described in Table 13. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly again.

**Table 13. Setup of SPE reactions**

Component	Volume/reaction
Product from cDNA cleanup	10 $\mu$ l
SPE Buffer	8 $\mu$ l
HotStarTaq DNA Polymerase	1.6 $\mu$ l
UPX 3' Targeted Panel Pool	8 $\mu$ l
UPX AMP Primer	1.6 $\mu$ l
Nuclease-free Water	10.8 $\mu$ l
<b>Total volume</b>	<b>40 <math>\mu</math>l</b>

3. Incubate as described in Table 14.

**Table 14. SPE cycling protocol**

Step	Time	Temperature
Hold	15 min	95°C
<b>2-step cycling</b>		
Denaturation	15 s	95°C
Annealing/Extension	10 min	68°C
<b>10 cycles</b>		
Hold	5 min	72°C
Hold	$\infty$	4°C

4. Add 120  $\mu$ l Nuclease-free Water to bring each sample to 160  $\mu$ l.
5. Add 208  $\mu$ l QIAseq Beads. Mix well by pipetting up and down 12 times.
6. Incubate for 5 min at room temperature.
7. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

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

- 
8. With the beads still on the magnetic stand, add 400  $\mu$ l of 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.
  9. Repeat the ethanol wash.

**Important:** Completely remove all traces of the ethanol wash after this second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200  $\mu$ l pipette, and then a 10  $\mu$ l pipette to remove any residual ethanol.

10. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 10 min.

**Note:** Visually inspect that the pellet is completely dry. Ethanol carryover to the next step will affect reaction efficiency.

11. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 12  $\mu$ l Nuclease-free Water. Mix well by pipetting.
12. Return the tubes to the magnetic rack until the solution has cleared.
13. Transfer 10  $\mu$ l of the supernatant to clean tubes.
14. Proceed immediately with “Optional Protocol: Quantitative Universal PCR”, page 31. Alternatively, the samples can be stored at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

**Note:** If the number of cycles required for universal amplification has already been determined, proceed with “Protocol: Universal PCR”, page 35.

---

# Optional Protocol: Quantitative Universal PCR

## Important points before starting

- 1  $\mu$ l of the product from the cDNA cleanup in “Protocol: Single Primer Extension” is the starting material for the quantitative universal PCR reaction.
- This protocol is used to determine the number of cycles required in “Protocol: Universal PCR”. If the number of cycles required for universal amplification has already been determined, proceed with “Protocol: Universal PCR” on page 35.
- uQuant Buffer contains a fluorescent dye that binds double-stranded DNA molecules.
- Do not vortex the HotStarTaq DNA Polymerase or library amplification reactions.

## Procedure

1. Prepare reagents required for the quantitative universal PCR reaction. Thaw Tar uQuant Buffer and required index primer pair (options: QIAseq UPX 3' Targeted RNA 12 Index or QIAseq UPX 3' Targeted 96 Index Set A, B, C or D). Mix by flicking the tube. Centrifuge the tubes briefly to collect residual liquid from the sides of the tubes.

**Note:** Layout and use of QIAseq UPX 3' Targeted 96 Index Set A, B, C or D is described in Figure 5, next page.

**Note:** HotStarTaq DNA Polymerase should be removed from the  $-20^{\circ}\text{C}$  freezer just before preparation of the master mix and placed on ice. HotStarTaq DNA Polymerase should be returned to the freezer immediately after use.

### Set A

	1	2	3	4	5	6	7	8	9	10	11	12
A	N701- S502	N702- S502	N703- S502	N704- S502	N705- S502	N706- S502	N707- S502	N710- S502	N711- S502	N712- S502	N714- S502	N715- S502
B	N701- S503	N702- S503	N703- S503	N704- S503	N705- S503	N706- S503	N707- S503	N710- S503	N711- S503	N712- S503	N714- S503	N715- S503
C	N701- S505	N702- S505	N703- S505	N704- S505	N705- S505	N706- S505	N707- S505	N710- S505	N711- S505	N712- S505	N714- S505	N715- S505
D	N701- S506	N702- S506	N703- S506	N704- S506	N705- S506	N706- S506	N707- S506	N710- S506	N711- S506	N712- S506	N714- S506	N715- S506
E	N701- S507	N702- S507	N703- S507	N704- S507	N705- S507	N706- S507	N707- S507	N710- S507	N711- S507	N712- S507	N714- S507	N715- S507
F	N701- S508	N702- S508	N703- S508	N704- S508	N705- S508	N706- S508	N707- S508	N710- S508	N711- S508	N712- S508	N714- S508	N715- S508
G	N701- S510	N702- S510	N703- S510	N704- S510	N705- S510	N706- S510	N707- S510	N710- S510	N711- S510	N712- S510	N714- S510	N715- S510
H	N701- S511	N702- S511	N703- S511	N704- S511	N705- S511	N706- S511	N707- S511	N710- S511	N711- S511	N712- S511	N714- S511	N715- S511

### Set B

	1	2	3	4	5	6	7	8	9	10	11	12
A	N716- S502	N718- S502	N719- S502	N720- S502	N721- S502	N722- S502	N723- S502	N724- S502	N726- S502	N727- S502	N728- S502	N729- S502
B	N716- S503	N718- S503	N719- S503	N720- S503	N721- S503	N722- S503	N723- S503	N724- S503	N726- S503	N727- S503	N728- S503	N729- S503
C	N716- S505	N718- S505	N719- S505	N720- S505	N721- S505	N722- S505	N723- S505	N724- S505	N726- S505	N727- S505	N728- S505	N729- S505
D	N716- S506	N718- S506	N719- S506	N720- S506	N721- S506	N722- S506	N723- S506	N724- S506	N726- S506	N727- S506	N728- S506	N729- S506
E	N716- S507	N718- S507	N719- S507	N720- S507	N721- S507	N722- S507	N723- S507	N724- S507	N726- S507	N727- S507	N728- S507	N729- S507
F	N716- S508	N718- S508	N719- S508	N720- S508	N721- S508	N722- S508	N723- S508	N724- S508	N726- S508	N727- S508	N728- S508	N729- S508
G	N716- S510	N718- S510	N719- S510	N720- S510	N721- S510	N722- S510	N723- S510	N724- S510	N726- S510	N727- S510	N728- S510	N729- S510
H	N716- S511	N718- S511	N719- S511	N720- S511	N721- S511	N722- S511	N723- S511	N724- S511	N726- S511	N727- S511	N728- S511	N729- S511

### Set C

	1	2	3	4	5	6	7	8	9	10	11	12
A	N701- S513	N702- S513	N703- S513	N704- S513	N705- S513	N706- S513	N707- S513	N710- S513	N711- S513	N712- S513	N714- S513	N715- S513
B	N701- S515	N702- S515	N703- S515	N704- S515	N705- S515	N706- S515	N707- S515	N710- S515	N711- S515	N712- S515	N714- S515	N715- S515
C	N701- S516	N702- S516	N703- S516	N704- S516	N705- S516	N706- S516	N707- S516	N710- S516	N711- S516	N712- S516	N714- S516	N715- S516
D	N701- S517	N702- S517	N703- S517	N704- S517	N705- S517	N706- S517	N707- S517	N710- S517	N711- S517	N712- S517	N714- S517	N715- S517
E	N701- S518	N702- S518	N703- S518	N704- S518	N705- S518	N706- S518	N707- S518	N710- S518	N711- S518	N712- S518	N714- S518	N715- S518
F	N701- S520	N702- S520	N703- S520	N704- S520	N705- S520	N706- S520	N707- S520	N710- S520	N711- S520	N712- S520	N714- S520	N715- S520
G	N701- S521	N702- S521	N703- S521	N704- S521	N705- S521	N706- S521	N707- S521	N710- S521	N711- S521	N712- S521	N714- S521	N715- S521
H	N701- S522	N702- S522	N703- S522	N704- S522	N705- S522	N706- S522	N707- S522	N710- S522	N711- S522	N712- S522	N714- S522	N715- S522

### Set D

	1	2	3	4	5	6	7	8	9	10	11	12
A	N716- S513	N718- S513	N719- S513	N720- S513	N721- S513	N722- S513	N723- S513	N724- S513	N726- S513	N727- S513	N728- S513	N729- S513
B	N716- S515	N718- S515	N719- S515	N720- S515	N721- S515	N722- S515	N723- S515	N724- S515	N726- S515	N727- S515	N728- S515	N729- S515
C	N716- S516	N718- S516	N719- S516	N720- S516	N721- S516	N722- S516	N723- S516	N724- S516	N726- S516	N727- S516	N728- S516	N729- S516
D	N716- S517	N718- S517	N719- S517	N720- S517	N721- S517	N722- S517	N723- S517	N724- S517	N726- S517	N727- S517	N728- S517	N729- S517
E	N716- S518	N718- S518	N719- S518	N720- S518	N721- S518	N722- S518	N723- S518	N724- S518	N726- S518	N727- S518	N728- S518	N729- S518
F	N716- S520	N718- S520	N719- S520	N720- S520	N721- S520	N722- S520	N723- S520	N724- S520	N726- S520	N727- S520	N728- S520	N729- S520
G	N716- S521	N718- S521	N719- S521	N720- S521	N721- S521	N722- S521	N723- S521	N724- S521	N726- S521	N727- S521	N728- S521	N729- S521
H	N716- S522	N718- S522	N719- S522	N720- S522	N721- S522	N722- S522	N723- S522	N724- S522	N726- S522	N727- S522	N728- S522	N729- S522

**Figure 5. QIAseq UPX 3' Targeted 96 Index Set A, Set B, Set C, Set D.** Dual indices are pre-dried as single-use plates. During reaction setup, components are added directly to the plate. There is no need to reconstitute and transfer indices to a separate plate.

**Note:** The QIAseq UPX 3' Targeted 96 Index plates are breakable, allowing reactions to be processed in batches.

2. On ice, prepare the library amplification reaction according to Table 15. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly again.

**Table 15. Setup of quantitative universal PCR reactions**

Component	Volume/reaction index tubes	Volume/reaction index plates
Product from SPE cleanup	1 $\mu$ l	1 $\mu$ l
Tar uQuant Buffer	5 $\mu$ l	5 $\mu$ l
HotStarTaq DNA Polymerase	1 $\mu$ l	1 $\mu$ l
QIAseq UPX 3' Targeted Index*	5 $\mu$ l	0 $\mu$ l
Nuclease-free Water	13 $\mu$ l	18 $\mu$ l
<b>Total volume</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>

\* QIAseq UPX 3' Targeted RNA 12 Index (tubes) or QIAseq UPX 3' Targeted 96 Index Set A, Set B, Set C or Set D (plates). See Figure 5 (page 38) for Index Sets A, B, C and D.

- Transfer 20  $\mu$ l to a real-time PCR plate and incubate the reaction in a real-time PCR instrument as described in to Table 16.

**Table 16. Quantitative universal PCR cycling protocol**

Step	Time	Temperature
Hold	15 min	95°C
<b>2-step cycling</b>		
Denaturation	15 s	95°C
Annealing/Extension*	2 min	65°C
<b>40 cycles</b>		
Hold	$\infty$	4°C

\* Perform fluorescence data collection.

- When the run has finished, analyze the data to obtain raw  $C_T$  values.

**Baseline:** Define the baseline using auto baseline.

**Threshold:** Using the “Log View” of the amplification plot, place the threshold above the background signal but within the lower half of the log-linear range of the amplification plot.

- 
5. Determine the required number of universal PCR amplification cycles by adding two units to the  $C_T$  determined in Step 4. For example, if the  $C_T$  is 13, then 15 is the required number of universal PCR amplification cycles.
  6. Proceed with "Protocol: Universal PCR", next page. Alternatively, the completed library amplification product can be stored at  $-30$  to  $-15^\circ\text{C}$  in a constant-temperature freezer.

---

# Protocol: Universal PCR

## Important points before starting

- 9 µl of the product from the cDNA cleanup in “Protocol: Single Primer Extension” is the starting material for the universal PCR reaction.
- The number of cycles of universal PCR amplification should already have been determined using “Optional Protocol: Quantitative Universal PCR”.
- Do not vortex the HotStarTaq DNA Polymerase or library amplification reactions.

## Procedure

1. Prepare reagents required for the universal PCR reaction. Thaw UPX 3' uPCR Buffer and required index primer pair (options: QIAseq UPX 3' Targeted RNA 12 Index or QIAseq UPX 3' Targeted 96 Index Set A, B, C or D). Mix by flicking the tube. Centrifuge the tubes briefly to collect residual liquid from the sides of the tubes.

**Note:** Layout and use of QIAseq UPX 3' Targeted 96 Index Set A, B, C or D is described in Figure 5, page 38.

**Note:** HotStarTaq DNA Polymerase should be removed from the –20°C freezer just before preparation of the master mix and placed on ice. HotStarTaq DNA Polymerase should be returned to the freezer immediately after use.

2. On ice, prepare the library amplification reaction according to Table 17. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly again.

**Table 17. Setup of universal PCR reactions**

Component	Volume/reaction index tubes	Volume/reaction index plates
Product from SPE cleanup	9 $\mu$ l	9 $\mu$ l
UPX 3' uPCR Buffer	5 $\mu$ l	5 $\mu$ l
HotStarTaq DNA Polymerase	1 $\mu$ l	1 $\mu$ l
QIAseq UPX 3' Targeted Index*	5 $\mu$ l	0 $\mu$ l
Nuclease-free Water	5 $\mu$ l	10 $\mu$ l
<b>Total volume</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>

\* QIAseq UPX 3' Targeted RNA 12 Index (tubes) or QIAseq UPX 3' Targeted 96 Index Set A, Set B, Set C, Set D. See Figure 5 (page 38) for Sets A, B, C, and D (plates).

3. Incubate the reaction in a real-time PCR instrument as described in Table 18.

**Table 18. Universal PCR cycling protocol**

Step	Time	Temperature
Hold	15 min	95°C
<b>2-step cycling</b>		
Denaturation	15 s	95°C
Annealing/Extension	2 min	65°C
<b>Cycle number determined in "Protocol: Quantitative Universal PCR"</b>		
Hold	$\infty$	4°C

4. Add 75  $\mu$ l Nuclease-free Water to bring each sample to 100  $\mu$ l.
5. Add 130  $\mu$ l QIAseq Beads. Mix well by pipetting up and down 12 times.
6. Incubate for 5 min at room temperature.
7. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

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

- 
8. With the beads still on the magnetic stand, add 400  $\mu$ l of 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.
  9. Repeat the ethanol wash.

**Important:** Completely remove all traces of the ethanol wash after this second wash. To do this, briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200  $\mu$ l pipette, and then a 10  $\mu$ l pipette to remove any residual ethanol.

10. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 10 min.

**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  $\mu$ l Nuclease-free Water. Mix well by pipetting.
12. Return the tubes to the magnetic rack until the solution has cleared.
13. Transfer 20  $\mu$ l of the supernatant to clean tubes.
14. Proceed with "Protocol: Library QC and Quantification", next page. Alternatively, the completed library amplification product can be stored at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

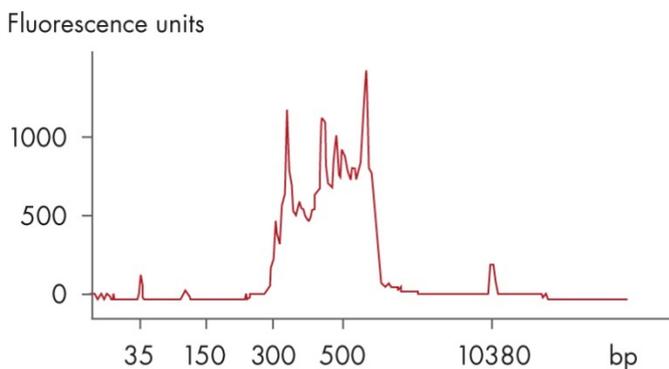
# Protocol: Library QC and Quantification

## Important points before starting

- A portion of the 20  $\mu$ l sequencing library is the starting material for the library QC and quantification. When not in use, the sequencing library should be stored on ice.
- Library QC involves use of an Agilent 2100 Bioanalyzer.
- Library quantification involves use of the QIAseq Library Quant System: QIAseq Library Quant Array Kit (cat. no. 333304) or QIAseq Library Quant Assay Kit (cat. no. 333314).

## Library QC (Agilent 2100 Bioanalyzer)

1. Analyze 1  $\mu$ l of the sequencing library on an Agilent Bioanalyzer using a High Sensitivity DNA chip according to the manufacturer's instructions. A typical library is shown in Figure 5.



**Figure 5.** Bioanalyzer trace of library prepared with the QIAseq UPX 3' Targeted RNA Panel.

2. Proceed with "Library quantification".

---

## Library quantification

1. The library yield measurements of the Bioanalyzer or TapeStation system use fluorescent dyes that intercalate into DNA or RNA and cannot discriminate between cDNA with or without 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 or Assay Kit, which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library.  
4 nM RNA-seq libraries should be used as input for the denaturation procedure to ultimately load 12 pM for the MiSeq (V3 kit) and 1.5 pM for the NextSeq.
2. Proceed with "Protocol: Sequencing Setup on Illumina MiSeq and NextSeq" in the next page.

# Protocol: Sequencing Setup on Illumina MiSeq and NextSeq

## Important points before starting

- **Important:** Recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System.
- **Important:** QIAseq A Read 1 Primer I (Custom Read 1 Sequencing Primer) **MUST** be used when performing sequencing on Illumina platform. QIAseq A Read 1 Primer I goes into the following specific reagent cartridge positions:

MiSeq Position #18

NextSeq Position #7

- **Important:** QIAseq C Read 2 Primer I (Custom Read 2 Sequencing Primer) **MUST** be used when performing sequencing on the NextSeq. QIAseq C Read 2 Primer I goes into the following specific reagent cartridge positions:  
NextSeq Position #8
- For complete instructions on how to denature sequencing libraries, prepare custom index primers and set up a sequencing run, please refer to the system-specific Illumina documents.
- Instrument-specific imagery is included to aid in sequencing preparations.

## Sequencing Preparations for MiSeq

1. **Sample sheet setup:** Set up a sample sheet with Custom Sequencing Read 1 primer using Illumina Experiment Manager v1.2, or later. Sample index of QIAseq UPX 3' Targeted RNA Panel is compatible with Illumina Nextera XT v2 adapter sample index system. Select and check the parameters as follows:

**Category:** Other

**Select Application:** FASTQ Only

**Sample Prep Kit:** Nextera XT v2

**Index Reads:** 2

**Read Type:** Select Paired End Read

**Cycles for both Read 1:** 95

**Cycles for both Read 2:** 51

**Important:** Check Custom Primer for Read 1

**Important:** Check Use Adapter Trimming

- Sample dilution and pooling:** Dilute libraries to 4 nM for MiSeq. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.
- Library preparation and loading:** Prepare and load library to load on a MiSeq according to the MiSeq System Denature and Dilute Libraries Guide. The final library concentration is 12 pM on MiSeq (V3 chemistry).
- Custom Sequencing Primer for Read 1 preparation and loading:** Use 597  $\mu$ l HT1 (Hybridization Buffer) to dilute 3  $\mu$ l of QIAseq A Read 1 Primer I (provided) to obtain a final concentration of 0.5  $\mu$ M. Load 600  $\mu$ l of the diluted QIAseq A Read 1 Primer I to Position 18 of the MiSeq reagent cartridge. For more details, please refer to Illumina's Protocol: [miseq\\_using\\_custom\\_primers\\_15041638\\_b.pdf](#) for the MiSeq.
- Upon completion of the sequencing run, proceed with " Protocol: Data Analysis," page 46.

## Sample Sheet Wizard - MiSeq Application Selection

Select Category

 Targeted Resequencing	 Small Genome Sequencing	 RNA Sequencing	 Other
--	--	---	--

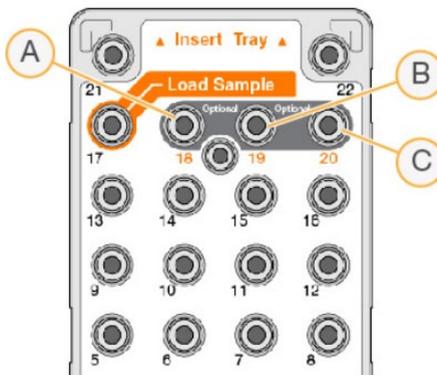
Select Application

 TruSight HLA	 Library QC	 FASTQ Only	 ChIP-Seq
---	---	---	---

## Sample Sheet Wizard - Workflow Parameters

FASTQ Only Run Settings	FASTQ Only Workflow-Specific Settings
Reagent Cartridge Barcode* <input type="text"/>	<input checked="" type="checkbox"/> Custom Primer for Read 1
Sample Prep Kit <input type="text" value="Nextera XT v2"/>	<input type="checkbox"/> Custom Primer for Index
Index Reads <input type="radio"/> 0 <input type="radio"/> 1 <input checked="" type="radio"/> 2	<input type="checkbox"/> Custom Primer for Read 2
Experiment Name <input type="text"/>	<input type="checkbox"/> Reverse Complement
Investigator Name <input type="text"/>	<input checked="" type="checkbox"/> Use Adapter Trimming
Description <input type="text"/>	
Date <input type="text" value="10/26/2017"/>	
Read Type <input checked="" type="radio"/> Paired End <input type="radio"/> Single Read	
Cycles Read 1 <input type="text" value="95"/>	
Cycles Read 2 <input type="text" value="51"/>	
* - required field	

Figure 6. Sample sheet using Illumina Experiment Manager.



A Position 18 for Read 1 Custom Primer

Figure 7. Loading the primers into positions on the MiSeq reagent cartridge.

## NextSeq: Setup custom library prep kit in BaseSpace Sequence Hub

The steps outlined here are intended for customers generating sequencing data on a NextSeq and using the BaseSpace Sequence Hub or a BaseSpace Onsite Sequence Hub system for data analysis, which requires the use of the Prep tab for setup. To ensure proper sample index demultiplexing, a custom library prep kit must be created and uploaded through the Prep tab. To add a custom library prep kit for the UPX 3' Targeted RNA Library, perform the following steps.

1. Download the following four templates from the “Resource” tab of [www.qiagen.com/shop/sequencing/QIAseq-UPX-3-Targeted-RNA-Panels](http://www.qiagen.com/shop/sequencing/QIAseq-UPX-3-Targeted-RNA-Panels):  
libraryprep\_template\_UPX\_setA.csv  
libraryprep\_template\_UPX\_setB.csv  
libraryprep\_template\_UPX\_setC.csv  
libraryprep\_template\_UPX\_setD.csv
2. Log in to BaseSpace or BaseSpace Onsite and go to the “Prep” tab screen.
3. Select “Biological Samples” from the “Prep” tab start page.
4. Choose the samples and click “Prep Libraries.”
5. Select “+Custom Library Prep Kit” from the “Library Prep Kit” drop-down menu.
6. In the screen that appears, name the custom kit “UPXsetA” and specify any other options, such as read types (Paired End), indexing strategies (Dual Index), and default read cycles (Read1 Cycles 95 and Read2 Cycles 27). Then, click “Choose .csv File” and select “libraryprep\_template\_UPX\_setA.csv”.
7. Click “Create New Kit” to generate library prep kit “UPXsetA.” This new kit now appears in the drop-down menu and is ready for any future runs.

**Custom Library Prep Kit**

Name of your new kit

Supported Read Types

Single Read

Paired End

Supported Indexing Strategy

None

Single Index

Dual Index

Default Read Cycles

Read 1 Cycles	<input type="text" value="95"/>
Read 2 Cycles	<input type="text" value="27"/>

Import the indexes following this [template](#).

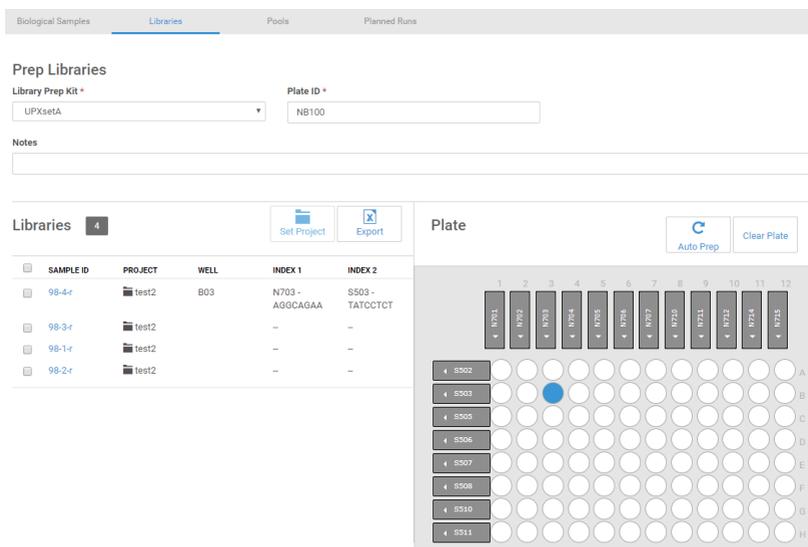
libraryprep\_template\_UPX\_setA.csv

**Figure 8. Custom library prep kit setup for the UPX 3' Targeted RNA Library.**

- Repeat steps 5 through 7 to create new library prep kits "UPXsetB," "UPXsetC," and "UPXsetD."

### NextSeq: Run planning and sequencing preparations

- From the drop-down menu on the "Libraries" tab, select library prep kit "UPXsetA," "UPXsetB," "UPXsetC," or "UPXsetD". Check the individual sample and drag it into corresponding well to assign INDEX 1 and INDEX 2.



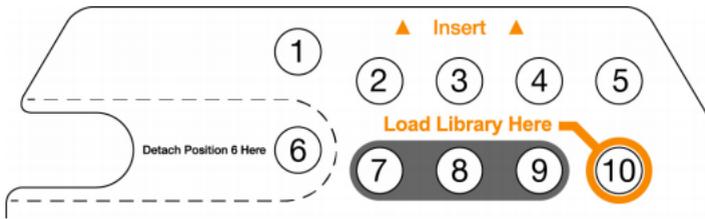
**Figure 9. Assigning sample indices in the “Libraries” tab.**

- Once indices are assigned, select pool on the “Pools” tab and then click “Plan Run.”
- Under “Plan Run”:
  - Select “NextSeq” from the “Instrument” drop-down menu.
  - Check both R1 and R2 for “Use Custom Primer.”
  - Check “Paired End” and verify 95 for “Read 1 Cycles” and 27 for “Read 2 Cycles.”
  - Check “Dual Index” and verify 8 for both “Index 1 Cycles” and “Index 2 Cycles.”
- Sample dilution and pooling:** Dilute libraries to 4 nM for NextSeq. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.
- Library preparation and loading:** Prepare and load library to load on a NextSeq according to the NextSeq System Denature and Dilute Libraries Guide. The final library concentration is 1.5 pM on NextSeq.
- Custom Sequencing Primer for Read 1 preparation and loading:** Use 1994  $\mu$ l HT1 (Hybridization Buffer) to dilute 6  $\mu$ l of QIAseq A Read 1 Primer I (provided) to obtain a

final concentration of 0.3  $\mu$ M. Load 2 ml of the diluted QIAseq A Read 1 Primer I to Position 7 of NextSeq reagent cartridge.

- Custom Sequencing Primer for Read 2 preparation and loading:** Use 1994  $\mu$ l HT1 (Hybridization Buffer) to dilute 6  $\mu$ l of QIAseq C Read 2 Primer I (provided) to obtain a final concentration of 0.3  $\mu$ M. Load 2 ml of the diluted QIAseq C Read 2 Primer I to Position 8 of NextSeq reagent cartridge.

**Note:** All other steps refer to run setup workflow as described in the NextSeq 500 SystemGuide (part # 15046563) or NextSeq 550 System Guide (part # 15069765-02). Upon completion of the sequencing run, proceed with " Protocol: Data Analysis," page 46.



Position #	Custom Primer
7	Custom Read 1 primer
8	Custom Read 2 primer

**Figure 10.** Loading the primers into positions on the NextSeq reagent cartridge. Protocol: Data Analysis

Important point before starting

- Data analysis is available at [www.qiagen.com/GeneGlobe](http://www.qiagen.com/GeneGlobe).

Primary data analysis procedure

- Click "Data Analysis Center" and log in.

- 
2. Under “Choose format,” select “QIAseq UPX Single Cell Analysis,” or “QIAseq UPX Primary Analysis.”
  3. Under “Choose Instrument,” select your instrument.
  4. Click “Start Analysis.”
  5. In the “BaseSpace Samples” tab, “.fastq,” or “.fast.gz,” files can be directly downloaded into the software from BaseSpace.
  6. In the “File Upload” tab, add “.fastq” or “.fastq.gz” files. Click “Start upload.”
  7. In the “File Management” tab, all files that have been uploaded to the portal are listed. Here, you can share and delete files.
  8. In the “UPX 3' Analysis Jobs” tab, select “Protocol,” “Catalog #,” “Species,” “Cell Index Set,” “Select Reads Files,” “File Lanes,” “Instrument,” and “Single Cell Analysis or “Low Input Primary Analysis.” Once the selections are made, click “Create Job.”
  9. When the job has been completed, the single-cell cluster analysis can be downloaded using the “Download Single Cell Report” link or the primary mapping report can be downloaded using the “Download Report” link. Alternatively, when using ultralow input samples, secondary differential expression analysis can be performed by using the “Secondary Analysis” link.

# Troubleshooting Guide

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

## Comments and suggestions

---

### Low library yield

- |  |   |
|--|---|
| a) Not enough cells multiplexed per sample index                                 | A minimum of 24 cells must be multiplexed per sample index.   |
| b) Using the multi-use Cell ID RT Plate, primers were not properly reconstituted | Prior to use, add 2.5 $\mu$ l 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   | Ensure reactions are thoroughly mixed (12 times), prepared, and incubated at recommended temperatures. Do not vortex.   |
| d) Inefficient SPE reaction  | Ensure that all components, including the UPX 3' Targeted Panel Pool and UPX AMP Primer, have been added to the SPE reaction. In addition, ensure that the SPE cycling program described in Table 14 has been followed.   |
| e) 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 $\mu$ l pipette, and then a 10 $\mu$ l pipette to remove any residual ethanol. In addition, allow beads to dry for the appropriate amount of time. |
| f) Insufficient universal PCR amplification cycles                               | Determine optimal universal PCR amplification cycles using "Optional Protocol: Quantitative Universal PCR," page 31.  |

### Primer-dimers observed

- |  |   |
|--|---|
| 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 $\mu$ l pipette, and then a 10 $\mu$ l pipette to remove any residual ethanol. In addition, allow beads to dry for the appropriate amount of time. |
|--|---|

## Comments and suggestions

---

### Sequencing issues

- |  |   |
|--|---|
| a) Too low or too high cluster density | Accurate library quantification is the key for optimal cluster density on any sequencing instrument. PCR-based quantification method is recommended. Other methods may lead to the incorrect quantification of the library especially when there is over-amplification.   |
| b) Very low clusters passing filter    | Make sure the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument. In addition, the QIAseq A Read 1 Primer I Custom Read 1 Sequencing primer MUST be used when sequencing on any Illumina platform. On a NextSeq, the QIAseq C Read 2 Primer I Custom Read 2 Sequencing primer MUST also be used. |

---

# Appendix A: General remarks on handling RNA

## Handling RNA

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

## General handling

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

---

To remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipettes and electrophoresis tanks), use of RNaseKiller (cat. no 2500080) from 5 PRIME ([www.5prime.com](http://www.5prime.com)) is recommended. RNase contamination can alternatively be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA\* followed by RNase-free water (see “Solutions”, page 52), or rinse with chloroform\* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent\* (e.g., 0.5% SDS), rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant) and allow to dry.

### Disposable plasticware

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

### Glassware

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

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

---

## Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. DEPC is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers\*. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO<sub>2</sub>. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA-RNA or RNA-RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

**Note:** QIAGEN solutions are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

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

# Ordering Information

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
QIAseq UPX 3' Targeted RNA Panel (96)	For 3' targeted RNA sequencing of 96 cells, cell pellets or ultralow input RNA samples	333041
QIAseq UPX 3' Targeted RNA Panel (96-M)	For 3' targeted RNA sequencing of 4 x 96 cells, cell pellets or ultralow input RNA samples	333042
QIAseq UPX 3' Targeted RNA Panel (384)	For 3' targeted RNA sequencing of 384 cells, cell pellets or ultralow input RNA samples	333043
QIAseq UPX 3' Targeted RNA 12 index (48)	Indexes and custom read primers compatible with Illumina platforms.	333044
QIAseq UPX 3' Targeted 96 index A (384)	High-throughput sample index plates and custom read primers compatible with Illumina platforms.	333051
QIAseq UPX 3' Targeted 96 index B (384)	High-throughput sample index plates and custom read primers compatible with Illumina platforms.	333052
QIAseq UPX 3' Targeted 96 index C (384)	High-throughput sample index plates and custom read primers compatible with Illumina platforms.	333053
QIAseq UPX 3' Targeted 96 index D (384)	High-throughput sample index plates and custom read primers compatible with Illumina platforms.	333054

Product	Contents	Cat. no.
<b>Related products</b>		
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
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

\* Larger kit sizes available; see [www.qiagen.com](http://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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

# Document Revision History

Date	Changes
02/2018	Initial release.
05/2018	Updated Tables 5 and 6 to include a recommendation for ERCC addition. Removed "Perform fluorescence data collection" footnote from Annealing/Extension in Table 18.
12/2021	Revised the Shipping and Storage section: corrected the shipping conditions of QIAseq Index kits. Editorial and layout changes

---

## Notes

### Limited License Agreement for QIAseq UPX 3' Targeted RNA Library Panel

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](http://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.

For updated license terms, see [www.qiagen.com](http://www.qiagen.com).

Trademarks: Trademarks: QIAGEN<sup>®</sup>, Sample-to-Insight<sup>®</sup>, QIAseq<sup>®</sup>, QIAxcel<sup>®</sup>, HotStarTaq<sup>®</sup>, miScript<sup>®</sup>, RNeasy<sup>®</sup> (QIAGEN Group); Agilent<sup>®</sup> (Agilent Technologies); Illumina<sup>®</sup>, HiSeq<sup>®</sup>, MiSeq<sup>®</sup>, MiniSeq<sup>®</sup>, NextSeq<sup>®</sup>, NovaSeq™ (Illumina, Inc.); Ion Torrent™, SYBR<sup>®</sup> (Thermo Fisher Scientific); MagneSphere<sup>®</sup> (Promega). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

12/2021 HB-2480-003 © 2021 QIAGEN, all rights reserved.

---

Ordering [www.qiagen.com/shop](http://www.qiagen.com/shop) | Technical Support [support.qiagen.com](http://support.qiagen.com) | Website [www.qiagen.com](http://www.qiagen.com)