May 2026

QIAseq® RNA Fusion XP Panel Handbook

For constructing molecularly barcoded libraries from RNA for gene fusion, gene expression, and RNA SNV/InDel analysis using QIAseq Unique Dual Index V2

For use with UDIs V2 references 331745, 331755, 331765, 331775

Sample to Insight

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Kit Contents

QIAseq RNA Fusion XP Panel	XP catalog panel (12)	XP catalog panel (96)	XP custom panel (96)	XP booster panel (96)*
Catalog number	**334602**	**334605**	**334625**	**334635**
Box 1 of 2				
QIAseq RNA Fusion XP Panel	48 µL	384 µL	384 µL	80 µL (3uM)
RP Primer II	12 µL	96 µL	96 µL	
EZ Reverse Transcriptase	15 µL	96 µL	96 µL	
BC3 Buffer, 5x	24 µL	192 µL	192 µL	
RNase Inhibitor	12 µL	96 µL	96 µL	
RH RNase	12 µL	96 µL	96 µL	
dNTP II	18 µL	144 µL	144 µL	
BLU Buffer, 10x	24 µL	192 µL	192 µL	
POL Enzyme	12 µL	96 µL	96 µL	
ERA Enzyme	120 µL	960 µL	960 µL	
ERA Buffer, 10x	60 µL	480 µL	480 µL	
DNA Ligase	120 µL	960 µL	960 µL	
Ligation Buffer, 5x	240 µL	2 × 960 µL	2 × 960 µL	
TEPCR Buffer, 5x	60 µL	400 µL	400 µL	
RNA Buffer II, 5x	48 µL	2 × 384µL	2 × 384 µL	
TaqIT Plus enzyme	15 µL	2 × 120 µL	2 × 120 µL	
HotStarTaq® DNA Polymerase	30 µL	240 µL	240 µL	

QIAseq RNA Fusion XP Panel	XP catalog panel (12)	XP catalog panel (96)	XP custom panel (96)	XP booster panel (96)*
Catalog number	**334602**	**334605**	**334625**	**334635**
Box 2 of 2				
QIAseq Beads	10 mL	55 mL	55 mL	

* Please contact QIAGEN technical support team for how to design and order this booster panel.

QIAseq Unique Dual Index V2 (96)	Set A	Set B	Set C	Set D
Catalog no.	**331745**	**331755**	**331765**	**331775**
AUDI-96AX orAUDI-96BX or AUDI-96CX or AUDI-96DX	(96)			
One plate, each containing 96 molecularly barcoded adapters, each well corresponding to 1 sample index; the kit is sufficient for 96 samples	One plate each (10 µL per adapter)			
XUDI-96AX or XUDI-96BX or XUDI-96CX or XUDI-96DX				
Containing one-index primer array. Each array well contains 1 unique index primer and IL-Universal PCR primer for PCR amplification and sample indexing; the kit is sufficient for 96 samples	One plate each (9 uL per index primer)			
Primers				
IL-Forward Primer	310 µL			
QIAseq A Read 1 Primer I (100 µM)	24 µL			

* 10 bp dual indices.

QIAseq RNA Fusion XP Catalog Panel Information

Catalog no.	Product Name	Total Number of Primers
JHS-001Z	Human Leukemia Panel	2010
JHS-002Z	Human Solid Tumor Panel	3161
JHS-003Z	Human Lung Cancer Panel	1210
JHS-004Z	Human Lymphoma Panel	1371
JHS-005Z	Human Sarcoma Panel	1238
JHS-3001Z	Human Oncology Research Panel	4366
JHS-3002Z	Human Pan Heme Panel	2393

Storage

The QIAseq RNA Fusion XP Panel Kit is shipped with dry ice (Box 1) and must be stored at –20°C upon arrival. Box 2 (QIAseq Beads) is shipped on cold packs and should be stored at 4°C. When stored under these conditions and handled correctly, the product can be kept based on the expiration date on each component without reduction in performance.

Intended Use

The QIAseq RNA Fusion XP Panel Kits are for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq RNA Fusion XP Panel Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

Misregulation of gene expression, both temporally and spatially, often leads to or results from disease. Additionally, alterations in DNA coding sequences, structural rearrangements, and post-transcriptional RNA processing can modify mRNA function, potentially causing or contributing to disease. Next-generation sequencing (NGS) of RNA (RNAseq) has been used to detect gene fusion, alternatively spliced transcripts, post-transcriptional modifications, mutations/single nucleotide variants (SNVs), and changes in gene expression. A fusion gene is a hybrid gene formed from two previously discrete genes. It can occur because of translocation, deletion, chromosomal inversion, or transcription-generated chimeras. The QIAseq RNA Fusion XP Panels use QIAGEN Enrichment Technology and unique molecular index (UMI) technologies in NGS to help identify and characterize fusion gene events, gene expression, and SNV/InDel at the RNA level with high efficiency, sensitivity, and flexibility.

Principle and workflow

The QIAseq RNA Fusion XP Panels rely on highly efficient RNA conversion, gene-specific target enrichment, and molecular barcoding for sensitive fusion, gene expression, and RNA SNV/InDel detection.

Unique molecular indices

The concept of UMIs or sometime called molecular barcoding is that, prior to any amplification, each original target molecule is "tagged by" a unique sequence "barcode". This is accomplished by the ligation of double-strand cDNA with a sample index adapter containing a 12-base random sequence. Statistically, this provides 4^{12} = 16,777,216 unique molecular tags for each adapter and each converted double-strand cDNA molecule in the sample receives a unique UMI sequence.

The barcoded cDNA molecules are then amplified using QIAseq Enrichment Technology and library amplification. Due to intrinsic noise and sequence-dependent bias, barcoded cDNA molecules may be amplified unevenly between different enriched targets. Therefore, target transcripts can be better evaluated by counting the number of unique molecular indices in the reads rather than counting the number of total reads for each transcript. Sequence reads having distinct UMIs represent different original molecules, while sequence reads having the same UMI are the results of PCR duplication from 1 original molecule and are counted together as 1 molecule.

Procedure

The QIAseq RNA Fusion XP Panels are provided as a single tube of primer mix, with up to 10,000 primers per tube (custom panel). The QIAseq RNA Fusion XP Panels can enrich selected transcripts using 10–250 ng of fresh total RNA or FFPE RNA. Although libraries can be constructed with as little as 1 ng fresh RNA, more RNA input will increase fusion detection sensitivity due to limited amount of original fusion RNA molecules present in low-input samples. Our general recommendation is to use 50–100 ng fresh total RNA or 100–200 ng FFPE RNA as the starting point if you have no prior experience with fusion analysis in your samples.

RNA samples are initially converted to first-strand cDNA. A separate, second-strand synthesis is used to generate double-stranded cDNA (ds-cDNA). This ds-cDNA is then end-repaired and A-tailed in a single-tube protocol. The prepared ds-cDNAs are then ligated at their 5′ ends to a sequencing platform-specific adapter containing UMI and sample index.

Adapter-ligated cDNA molecules are subject to limited target-barcode enrichment. This reaction ensures that intended targets are enriched sufficiently to be represented in the final library.

A universal PCR is then carried out with highly efficient, low error rate, fast processing Taq enzyme to amplify the library and add a second sample index (unique dual index, UDI, is recommended if available, platform specific) and other platform-specific required sequences.

The raw sequencing results should be analyzed using the QIAseq RNA Fusion XP Panel Analysis Software in GeneGlobe® at **geneglobe.qiagen.com**, which will automatically perform all steps necessary to generate a fusion, gene expression, and RNA SNV/InDel call report from your NGS data.

Data from QIAseq RNA Fusion XP Panels can also be analyzed using the QIAGEN CLC Genomics Workbench, which allows you to optimize analysis parameters for your specific panels. The parameters can then be locked for routine use. All detected fusions and variants can be further interpreted using QCI-I for QIAseq.

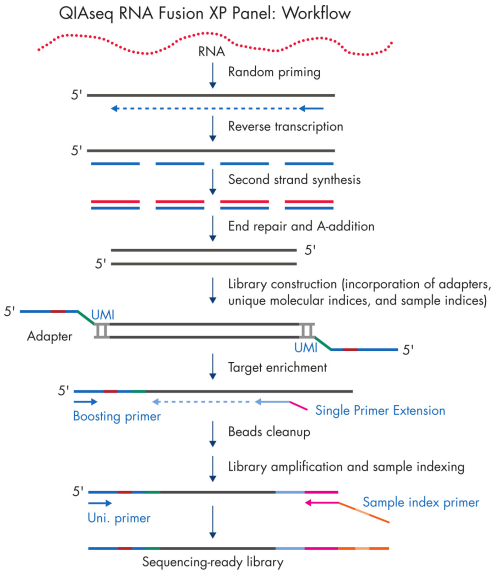


Figure 1. QIAseq RNA Fusion XP Panels workflow.

Description of protocols

This handbook contains a protocol for QIAseq RNA Fusion XP workflow for the Illumina platform with the QIAseq Unique Dual Index V2 kit. It needs to be sequenced with the QIAseq A Read 1 Primer I (100 µM) custom sequencing primer.

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.

In addition to the QIAseq RNA Fusion XP Panel Kits and QIAseq Unique Dual Index V2 (96), the following supplies are required:

- High-quality nuclease-free water
- Microcentrifuge
- 1.5 mL or 2 mL LoBind® tubes (Eppendorf® AG)
- 0.2 mL PCR tubes and 0.2 mL 96-well PCR reaction plates (BIOplastics, cat. no. AB17500 or equivalent) or PCR strip tubes and caps
- Eppendorf twin.tec® PCR plate 96 LoBind (Eppendorf AG, cat. no. 0030129504 or equivalent) for handling with 96-well plate format in beads wash and library storage.
- Thermal cycler
- Multichannel pipette
- Single-channel pipette
- Nuclease-free pipette tips and tubes
- QIAxcel Connect, Cat. No. / ID: 9003110; Agilent® 4200 TapeStation®
- QIAxcel® DNA High Resolution Kit (1200), Cat. No. / ID: 929002; Agilent High Sensitivity D1000 ScreenTape® kit (cat. no. 5067-5584)
- 80% ethanol

- Magnet rack for 1.5 mL or 2 mL tubes (DynaMag™-2 Magnet, Thermo Fisher Scientific cat. no. 12321D, or equivalent)
- Magnetic separation rack for 96-well plates (DynaMag-96 Side Magnet, Thermo Fisher cat. no. 12331D, or equivalent)
- QIAseq Library Quant System: QIAseq Library Quant Array Kit (cat. no. 333304), QIAseq Library Quant Assay Kit (cat. no. 333314), or QIAxpert® Instrument (cat. no. 9002340; **www.qiagen.com/qiaxpertsystem-orderinginfo**)
- NGS sequencing platform (Illumina system)
- Controls: Seraseq® Fusion RNA Mix v4 and Seraseq FFPE Tumor Fusion RNA v4 Reference Material from SeraCare Life Sciences; ALK RET ROS RNA fusion positive control from Horizon Discovery
- For 96-well format handling, Axygen Silicone 96 Round Well Compression Flat Mat for PCR Microplates, Axygen Sealing Film Roller, and AlumaSeal II sealing films are recommended.

Important Notes

Preparing RNA

High-quality RNA is essential for obtaining good sequencing results.

The most important prerequisite for RNA sequence analysis is consistent, high-quality RNA from every experimental sample. Accordingly, sample handling and RNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, or other contaminants may degrade RNA. Furthermore, contaminants can also decrease or completely block enzyme activities necessary for PCR performance.

Recommended RNA preparation method

QIAGEN's RNeasy® Mini Kit (cat. nos. 74104 and 74106), RNeasy Micro Kit (cat. no. 74004), AllPrep® DNA/RNA Mini Kit (cat. no. 80204), AllPrep DNA/RNA FFPE Kit (cat. no. 80234), and RNeasy FFPE Kit (cat. no. 73504) are recommended for the preparation of RNA samples from fresh tissues and FFPE tissue samples. If RNA samples need to be harvested from biological samples for which kits are not available, please contact Technical Support for suggestions. On-column DNase treatment is not recommended.

For best results, all RNA samples should be resuspended in RNase-free water. Do not use DEPC-treated water.

RNA quantification and quality control

For best results, all RNA samples should also demonstrate consistent quality according to the following:

The concentration and purity of RNA should be determined by measuring the absorbance in a spectrophotometer such as the QIAxpert.

Pure RNA has an A_{260}:A_{280} ratio of 1.9–2.1 in 10 mM Tris-HCl, pH 7.5.

Run an aliquot of each RNA sample on the Agilent 4200 TapeStation using RNA ScreenTape or the QIAxcel Connect using QIAxcel RNA QC Kit v2.0 (cat. no. 929104) (Figure 2). Verify that there are sharp bands/peaks present for both the 18S and 28S ribosomal RNAs (Figure 3). Any smearing of the RNA bands or shoulders on the RNA peaks indicates that degradation has occurred in the RNA sample.



Figure 2. QIAxcel Connect.

For best results, the ribosomal bands should appear as sharp peaks. Ideally, the RIN number for non-FFPE RNA from the Agilent Bioanalyzer/TapeStation should be higher than 5. For low-quality RNA samples (like FFPE sample), RNA quality needs to be evaluated by the percentage of RNA fragments >200 nucleotides. It is better to be >70% or at least to be above 30% though the QIAseq RNA Fusion XP may still generate library with fragmented samples when this number is as low as 20%.

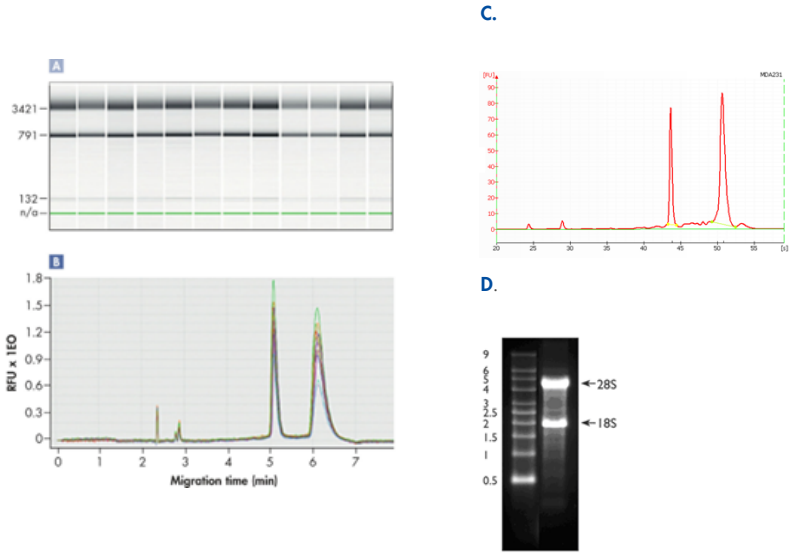


Figure 3. Ribosomal RNA integrity. A) Streamlined RNA analysis using the QIAxcel system. a gel image and **B)** a superimposed electropherogram view. **C)** Agilent Bioanalyzer electropherogram of high-quality total RNA showing sharp peaks for the 18S (left) and 28S (right) ribosomal RNA. Due to high quality of the RNA, peaks do not have shoulders (especially to the left of each peak). **D)** Agarose gel electrophoresis shows sharp bands (especially at the bottom of each band) for 28S and 18S ribosomal RNA.

Starting RNA amounts

The QIAseq RNA Fusion XP Panels provide results with as little as 10 ng or as much as 250 ng total RNA per cDNA synthesis reaction (input lower than 10 ng could be evaluated based on research needs; also, higher than 250 ng could be used under special situation with custom validation).

The optimal amount of starting material depends on the relative abundance of the transcripts of interest. Lower-abundance transcripts require more RNA; high-abundance transcripts require less RNA. Greater amounts of input total RNA will provide greater sensitivity for fusion gene call when enough read budget is allocated.

For successful results, we recommend that first-time users start with 50–100 ng total RNA or 100–200 ng FFPE RNA. We recommend using a consistent amount of total RNA for all reactions in a single experiment.

Sample index and sample plex

Using the QIAseq Unique Dual Index V2 to work with the RNA Fusion XP is recommended to overcome the Illumina platform-related index bleed/hopping issue as RNA Fusion XP has high sensitivity, especially for fusion detection. The pairing of i7 and i5 needs to be maintained based on the setting of the QIAseq Unique Dual Index V2 Kit for best performance.

The QIAseq RNA Fusion XP Panels are compatible with Illumina NGS platforms including MiSeq®, MiniSeq®, NextSeq® 500/550, NextSeq® 1000/2000, and NovaSeq® 6000, NovaSeq® X, NovaSeq® X Plus. The QIAseq RNA Fusion XP Panel cannot be used on Illumina's iSeq® 100 platform due to primer setting. The QIAseq RNA Fusion XP libraries generated with QIAseq Unique Dual Index V2 need QIAseq A Read 1 Primer I (100 µM) for sequencing; no custom Read 2 primer and custom index primer is needed. Sequencing cartridges with 300 cycles or higher are needed.

Read budget and sample plex level

Sample multiplexing capacity is defined by the size of the panel and sequencing platform read capacity. For using QIAseq Unique Dual Index V2 with Illumina platforms, sample indexing barcodes are available to multiplex up to 384 samples. The number of samples that can be sequenced together will depend on the size of your panel and the sequencing capacity of the intended instrument and kit. Fine tuning for your read budget will be possible after your first test run. Hereafter is a preliminary starting sample multiplex suggestion. Adjustments can be made as necessary.

Table 1. Estimated sequencing depth for catalog panels.

Catalog no.	Product Name	Reads (M)
JHS-001Z	Human Leukemia Panel	3.0–7.0
JHS-002Z	Human Solid Tumor Panel	4.0–7.0
JHS-003Z	Human Lung Cancer Panel	2.0–4.0
JHS-004Z	Human Lymphoma Panel	2.5–4.0
JHS-005Z	Human Sarcoma Panel	2.0–4.0
JHS-3001Z	Human Oncology Research Panel	5.5–10
JHS-3002Z	Human Pan Heme Panel	4.0–7.0

Note: The recommended reads number is for reference only; the best reads depth for each panel fully relies on the sample type, the input, and sensitivity requirement. User is recommended to optimize it with some control samples.

For the estimated sequencing depth and plex level under different instruments with different kits, please refer to *QIAseq RNA Fusion XP All-in-One Tool* (**www.qiagen.com/PROM-17069**). It is an estimation only as initial test setting. The best performance may need personally optimized setting based on instrument and kit's technical recommendations and user experience, as well as real sample validation.

Plate format handling

The QIAseq RNA Fusion XP Panels provide convenient workflow for handling 96 samples in 96-well plate format. Follow the special instructions to use 300 µL 96-well low-binding plates in the QIAseq Beads cleanup stage for 96-well plate format handling.

Automation processing needs to be optimized individually. Please contact QIAGEN's customer service for any special requirement and product customization needs.

Protocol: QIAseq RNA Fusion XP Panel for Illumina Instruments

First-strand cDNA synthesis

Primer priming

1. Pre-heat the PCR cycler to 65°C with a heated lid (at 103°C).
2. Put a PCR strip or a 96-well plate on ice.
3. Add 1–5 µL total RNA (10–250 ng total RNA) to the well, add 1 µL of RP Primer II to each tube, and then add water to reach the total volume of 6 µL if necessary.

Table 2. Primer priming

	1 reaction (µL)
RNA sample (x µL)	x (≤ 5)
RP Primer II	1
Nuclease-free water	5 – x
Total	**6**

Note: Please refer to "Appendix G: Blocking Unexpected rRNA and/or Globin Signal with FastSelect in RNA Fusion XP Workflow" on page 59 .

4. Mix by pipetting up and down 7 times and then spin down briefly. If using a 96-well plate, cover the plate with an aluminum foil seal, then briefly but gently vortex, and spin down briefly afterwards.
5. Transfer the strip/plate from ice to the cycler and incubate at 65°C for 5 min (silicone compression mat is recommended with plate).

Table 3. Cycler setting

Step	Incubation temperature	Incubation time (min)
1	65°C	5
2	Ice	≥2

6. Remove the strip/plate from the cycler and place on ice for at least 2 min.
7. Briefly centrifuge before next step.

Reverse transcription

8. Add each of the following reagents to the same tube/plate from the previous reaction. If handling more than 1 sample, prepare a first-strand synthesis mix according to Table 4.

Table 4. Reverse transcription

Step	Reaction volume (µL)
Random primed RNA from previous section	6
BC3 Buffer, 5x	2
RNase inhibitor	1
EZ Reverse Transcriptase	1
Total	**10**

9. Add 4 µL first-strand synthesis mix to each tube/well.
10. Mix by pipetting up and down 7 times and spin down briefly. If using a 96-well plate, cover the plate with an aluminum foil seal, then briefly but gently vortex, and spin down briefly afterwards.

11. Place the PCR strip/plate into a cycler (silicone compression mat is recommended with plate) with a heated lid (103°C) and incubate as indicated in Table 5.

Table 5. Cycler settings for reverse transcription

Step	Incubation temperature (°C)	Incubation time (min)
1	25	10
2	42	30
3	70	15
4	4	Hold

12. Remove the PCR strip/plate from the thermal cycler, briefly spin down, and place on ice.

    If reactions are to be stored after reverse transcription, transfer them to a –20°C freezer. Samples are stable overnight.

Second-strand synthesis

13. Add each of the following reagents to the same tube/well of the previous reaction. If handling more than 1 sample, prepare a second-strand synthesis mix based on Table 6.

Table 6. Second-strand synthesis

	1 reaction (µL)
cDNA from previous section	10
Nuclease-free water	5
BLU buffer	2
RH RNase	1
dNTP II	1
POL enzyme	1
Total	**20**

14. Add 10 µL second-strand synthesis mix to each tube/well.
15. Mix by pipetting up and down 7 times, and then spin down briefly. If using a 96-well plate, cover the plate with an aluminum foil seal, then briefly but gently vortex, and spin down briefly afterwards.
16. Place the PCR strip/plate into a cycler (silicone compression mat is recommended with plate) with a heated lid (103°C) and incubate as indicated below in Table 7.

Table 7. Cycler settings for second-strand synthesis

Step	Incubation temperature (°C)	Incubation time (min)
1	37	7
2	65	10
3	80	10
4	4	Hold

17. Remove the PCR strip/plate from the thermal cycler, briefly spin down, and place on ice.

    Note: It is okay for an overnight storage at –20°C if there is no time to process the following steps.

End repair/dA tailing

18. Enter the following program into a thermal cycler (Table 8).

    Note: If using cycler's temperature-controlled lid, be certain to set the instrument's heated lid to approximately 70°C if possible.

    Note: If there is no temperature-controlled lid, run with cycler lid open for step 2 and seal the strip or plate well. When the cycler reaches step 3, close the lid to avoid evaporation. Please spin down carefully after the run to remove any condensation.

Table 8. Cycler settings for end repair/dA tailing

Step	Incubation temperature (°C)	Incubation time (min)
1	4	1 (pause before add tube)
2	20	30
3	65	30
4	4	Hold

19. When the cycler block reaches 4°C, pause the program.
20. It is important to follow the procedure described below to achieve optimal results. The final total reaction volume is 50 µL.
21. Prepare a reaction mix in a new LoBind tube on ice by combining ERA Buffer/Enzyme and nuclease-free water as indicated in Table 9 (10% or more extra volume should be

added to compensate for the pipetting loss when preparing the master mix for multiple samples).

Table 9. End repair/dA tailing

	1 reaction (µL)
Second-strand product from previous section	20
ERA Buffer, 10x	5
Nuclease-free water	15
ERA Enzyme, 5x	10
Total	**50**

22. Add 30 µL reaction mix to each reaction and gently mix well by pipetting up and down 6–8 times. It is recommended to keep the PCR tube on ice for the whole-time during reaction setup.
23. Briefly spin down the sample tube/plate and immediately transfer to the pre-chilled thermal cycler (4°C; silicone compression mat is recommended with plate). Resume the cycling program.
24. When the program is complete and sample block has returned to 4°C, remove samples from block and place on ice.
25. Immediately proceed to the next step.

Adapter ligation

26. If working with more than 1 sample, prepare a ligation mix according to Table 10, next page.
27. Record each dual index and its corresponding sample as planned.

28. Transfer 5 µL of i7 adapter with molecular tags into the PCR tube/plate with 50 µL of A-tailed DNA from previous reaction. Mix gently by pipetting and keep cool on ice (see Table 11–Table 14 for index layout)

29. Prepare the following ligation reaction master mix in a separate tube on ice and mix well by pipetting. It can be scaled as needed for the desired number of samples (10% extra volume added to compensate for the pipetting loss when preparing the master mix for multiple samples).

Table 10. Ligation mix

	1 reaction (µL)
Ligation Buffer, 5x	20
DNA Ligase	10
Nuclease-free water	15
Total	**45**

Table 11. Adaptor plate of QIAseq Unique Dual Index Set A V2 (96): AUD-96AX

	1	2	3	4	5	6	7	8	9	10	11	12
A	AUD I001	AUD I009	AUD I017	AUD I025	AUD I033	AUD I041	AUD I049	AUD I057	AUD I065	AUD I073	AUD I081	AUD I089
B	AUD I002	AUD I010	AUD I018	AUD I026	AUD I034	AUD I042	AUD I050	AUD I058	AUD I066	AUD I074	AUD I082	AUD I090
C	AUD I003	AUD I011	AUD I019	AUD I027	AUD I035	AUD I043	AUD I051	AUD I059	AUD I067	AUD I075	AUD I083	AUD I091
D	AUD I004	AUD I012	AUD I020	AUD I028	AUD I036	AUD I044	AUD I052	AUD I060	AUD I068	AUD I076	AUD I084	AUD I092
E	AUD I005	AUD I013	AUD I021	AUD I029	AUD I037	AUD I045	AUD I053	AUD I061	AUD I069	AUD I077	AUD I085	AUD I093
F	AUD I006	AUD I014	AUD I022	AUD I030	AUD I038	AUD I046	AUD I054	AUD I062	AUD I070	AUD I078	AUD I086	AUD I094
G	AUD I007	AUD I015	AUD I023	AUD I031	AUD I039	AUD I047	AUD I055	AUD I063	AUD I071	AUD I079	AUD I087	AUD I095
H	AUD I008	AUD I016	AUD I024	AUD I032	AUD I040	AUD I048	AUD I056	AUD I064	AUD I072	AUD I080	AUD I088	AUD I096

Table 12. Adaptor plate of QIAseq Unique Dual Index Set B V2 (96): AUD-96BX

	1	2	3	4	5	6	7	8	9	10	11	12
A	AUD 097	AUD 105	AUD 113	AUD 121	AUD 129	AUD 137	AUD 145	AUD 153	AUD 161	AUD 169	AUD 177	AUD 185
B	AUD 098	AUD 106	AUD 114	AUD 122	AUD 130	AUD 138	AUD 146	AUD 154	AUD 162	AUD 170	AUD 178	AUD 186
C	AUD 099	AUD 107	AUD 115	AUD 123	AUD 131	AUD 139	AUD 147	AUD 155	AUD 163	AUD 171	AUD 179	AUD 187
D	AUD 100	AUD 108	AUD 116	AUD 124	AUD 132	AUD 140	AUD 148	AUD 156	AUD 164	AUD 172	AUD 180	AUD 188
E	AUD 101	AUD 109	AUD 117	AUD 125	AUD 133	AUD 141	AUD 149	AUD 157	AUD 165	AUD 173	AUD 181	AUD 189
F	AUD 102	AUD 110	AUD 118	AUD 126	AUD 134	AUD 142	AUD 150	AUD 158	AUD 166	AUD 174	AUD 182	AUD 190
G	AUD 103	AUD 111	AUD 119	AUD 127	AUD 135	AUD 143	AUD 151	AUD 159	AUD 167	AUD 175	AUD 183	AUD 191
H	AUD 104	AUD 112	AUD 120	AUD 128	AUD 136	AUD 144	AUD 152	AUD 160	AUD 168	AUD 176	AUD 184	AUD 192

Table 13. Adaptor plate of QIAseq Unique Dual Index Set C V2 (96): AUD-96CX

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

Table 14. Adaptor plate of QIAseq Unique Dual Index Set D V2 (96): AUD-96DX

	1	2	3	4	5	6	7	8	9	10	11	12
A	AUD 289	AUD 297	AUD 305	AUD 313	AUD 321	AUD 329	AUD 337	AUD 345	AUD 353	AUD 361	AUD 369	AUD 377
B	AUD 290	AUD 298	AUD 306	AUD 314	AUD 322	AUD 330	AUD 338	AUD 346	AUD 354	AUD 362	AUD 370	AUD 378
C	AUD 291	AUD 299	AUD 307	AUD 315	AUD 323	AUD 331	AUD 339	AUD 347	AUD 355	AUD 363	AUD 371	AUD 379
D	AUD 292	AUD 300	AUD 308	AUD 316	AUD 324	AUD 332	AUD 340	AUD 348	AUD 356	AUD 364	AUD 372	AUD 380
E	AUD 293	AUD 301	AUD 309	AUD 317	AUD 325	AUD 333	AUD 341	AUD 349	AUD 357	AUD 365	AUD 373	AUD 381
F	AUD 294	AUD 302	AUD 310	AUD 318	AUD 326	AUD 334	AUD 342	AUD 350	AUD 358	AUD 366	AUD 374	AUD 382
G	AUD 295	AUD 303	AUD 311	AUD 319	AUD 327	AUD 335	AUD 343	AUD 351	AUD 359	AUD 367	AUD 375	AUD 383
H	AUD 296	AUD 304	AUD 312	AUD 320	AUD 328	AUD 336	AUD 344	AUD 352	AUD 360	AUD 368	AUD 376	AUD 384

30. Add 45 µL of the ligation reaction mix to the sample from step 28 and mix well by pipetting.

31. Incubate the ligation reaction as in Table 15 on the facing page using a thermal cycler with the lid open.

    IMPORTANT: Do not use a heated lid. Pause the cycler during the first step before adding the sample tubes.

Table 15. Cycler settings for ligation

Step	Incubation temperature (°C)	Incubation time (min)
1	4	1 (hold till sample is ready)
2	20	15
3	4	Hold

32. Proceed immediately to adapter ligation cleanup.

    Note: Mix the QIAseq Beads well before usage.

Sample cleanup 1

33. Transfer the 100 µL reaction product into a 1.5 mL DNA LoBind tube or transfer the samples into a 300 µL 96-well low-binding plate for sample cleanup.
34. Add 80 µL QIAseq Beads to 100 µL reaction. Mix well by pipetting up and down at least 10 times.
35. Incubate for 5 min at room temperature.
36. Place the tube on a magnetic rack to separate the beads from supernatant. After the solution is clear (10 min usually), carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the cDNA target.
37. Completely remove residual supernatant (using 10 µL tip to aspirate the trace amount of residual supernatant after the first aspiration is recommended; spin down is helpful if any residual is on side wall).
38. Wash the beads with 220 µL 80% ethanol; rotate the tube 3 times. Wait 1 min with the tube on magnetic rack.
39. Remove the 80% ethanol completely.
40. Repeat the above wash step once. Carefully aspirate the trace amount of residual ethanol.

41. Dry beads by leaving the cap open for 10 min (no more than 15 min is needed).

    Note: Enough drying time is essential to ensure that all traces of ethanol are removed. Cracks will be observed in the bead pellet when drying is complete. Do not over dry the beads as this will significantly decrease elution efficiency, especially for larger fragments.

42. Elute ligated cDNA target from beads with 50 µL nuclease-free water. Mix well by pipetting. No bead separation is needed (this is for faster and easy handling; traditional method by adding 52 µL water to elute 50 µL supernatant is still fine).

43. Add 55 µL QIAseq Beads to 50 µL reaction. Mix well by pipetting up and down at least 10 times.

44. Incubate for 5 min at room temperature.

45. Place the tube or low-binding plate on the magnetic rack to separate the beads from supernatant. After the solution is clear (approx. 5–10 min), carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the ligated cDNA.

46. Add 220 µL freshly made 80% ethanol to the tube or well while it is on the magnetic rack. Rotate the tube once on the magnet to wash the beads, and then carefully remove and discard the supernatant.

47. Repeat the above wash step once more.

48. Completely remove residual ethanol and dry the beads for 5–10 min while the tube or plate is on the rack with the lid open.

    Note: Enough drying time is essential to ensure that all traces of ethanol are removed. Cracks will be observed in the bead pellet when drying is complete. Do not over dry the beads as this will significantly decrease elution efficiency, especially for larger fragments.

49. Elute the beads by adding 12.2 µL nuclease-free water. Mix well by pipetting. Place the tube or plate on the magnetic rack until solution is clear.

50. Transfer 10.4 µL supernatant to a clean PCR strip or 96-well PCR plate.

If the reactions are to be stored after bead-based cleanup, transfer them to a –20°C freezer. Samples are stable overnight.

Target enrichment with QIAseq Enrichment Technology

51. Transfer the 10.4 µL eluted sample into PCR strip or plate.
52. Prepare target enrichment reaction mix for each sample according to Table 16; add 9.6 µL mix in PCR strips or a 96-well PCR plate. Mix gently by pipetting up and down.

    Note: Extra volume of 10% or more should be added to compensate for the pipetting loss when preparing the master mix for multiple samples.

Table 16. Target enrichment reaction

	1 reaction (µL)
Purified sample from previous section	10.4
TEPCR Buffer, 5x	4
QIAseq RNA Fusion XP Panel	4
IL-Forward Primer	0.8
HotStarTaq DNA Polymerase	0.8
Total	**20**

53. Seal the wells with PCR tube caps. Place strips or plate in thermal cycler (silicone compression mat is recommended with plate) and set up reaction parameters according to Table 17, next page.

Table 17. Cycler setting for target enrichment

Step	Cycles	Incubation temperature (°C)	Incubation time
1	1	95	13 min
		98	2 min
2	8	98 68	15 s 10 min
3	1 1	72 4	2 min Hold

54. After the reaction is complete, place the reactions on ice and proceed to the next step.

    Optional: Target enrichment reaction may be run overnight and left in the thermal cycler at 4°C.

Sample cleanup 2

55. Add 30 µL nuclease-free water to 20 µL reaction to bring the volume to 50 µL and transfer into a 1.5 mL DNA LoBind tube or keep in the 96-well PCR plate for purification.
56. Mix the QIAseq Beads well before usage. Add 55 µL QIAseq Beads to 50 µL reaction. Mix well by pipetting up and down at least 10 times.
57. Incubate for 5 min at room temperature.
58. Place the tube on a magnetic rack to separate the beads from supernatant. After the solution is clear (approx. 5 min), carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the DNA target.
59. Completely remove residual supernatant.
60. Add 220 µL freshly made 80% ethanol to the tube or well while it is on the magnetic rack. Rotate the tube or move the plate side to side in the 2 positions of the magnet to wash the

beads. Wait 1 min with the tube on magnetic rack, and then carefully remove and discard the supernatant.

61. Repeat the above wash step once more.

62. Completely remove residual ethanol and dry the beads for 5–10 min while the tube or plate is on the rack with the lid open.

    Note: Enough drying time is essential to ensure that all traces of ethanol are removed. Cracks will be observed in the bead pellet when drying is complete. Do not over dry the beads as this will significantly decrease elution efficiency, especially for larger fragments.

63. Elute the beads into 14.2 µL sterile water. Mix well by pipetting. Place the tube or plate on the magnetic rack until the solution is clear.

64. Transfer 12.2 µL supernatant to a clean PCR strip or 96-well PCR plate.

Universal PCR amplification

65. Prepare universal PCR reaction mix for each sample according to Table 18 in PCR strips or a 96-well PCR plate. Mix gently by pipetting up and down.

    Note: Please refer to the next pages for index primer mix layout.

Table 18. Universal PCR mix using QIAseq Unique Dual Index V2 kit

	1 reaction (µL)
Purified sample	12.2
RNA Buffer II, 5x	4
Index primer mix from QIAseq Unique Dual Index V2 plate	3
TaqIt Plus enzyme	0.8
Total	**20**

Note: This step applies different QIAseq Unique Dual Index V2 i5 index to the other side of the target for each sample, please be careful to avoid cross contamination. Please thaw, then mix, then spin down the plate. Carefully pipette 3 µL to each sample.

Table 19. Index primer plate of QIAseq Unique Dual Index Set A V2 (96): XUDI-96AX

	1	2	3	4	5	6	7	8	9	10	11	12
A	XUD I001	XUD I009	XUD I017	XUD I025	XUD I033	XUD I041	XUD I049	XUD I057	XUD I065	XUD I073	XUD I081	XUD I089
B	XUD I002	XUD I010	XUD I018	XUD I026	XUD I034	XUD I042	XUD I050	XUD I058	XUD I066	XUD I074	XUD I082	XUD I090
C	XUD I003	XUD I011	XUD I019	XUD I027	XUD I035	XUD I043	XUD I051	XUD I059	XUD I067	XUD I075	XUD I083	XUD I091
D	XUD I004	XUD I012	XUD I020	XUD I028	XUD I036	XUD I044	XUD I052	XUD I060	XUD I068	XUD I076	XUD I084	XUD I092
E	XUD I005	XUD I013	XUD I021	XUD I029	XUD I037	XUD I045	XUD I053	XUD I061	XUD I069	XUD I077	XUD I085	XUD I093
F	XUD I006	XUD I014	XUD I022	XUD I030	XUD I038	XUD I046	XUD I054	XUD I062	XUD I070	XUD I078	XUD I086	XUD I094
G	XUD I007	XUD I015	XUD I023	XUD I031	XUD I039	XUD I047	XUD I055	XUD I063	XUD I071	XUD I079	XUD I087	XUD I095
H	XUD I008	XUD I016	XUD I024	XUD I032	XUD I040	XUD I048	XUD I056	XUD I064	XUD I072	XUD I080	XUD I088	XUD I096

Table 20. Index primer plate of QIAseq Unique Dual Index Set B V2 (96): XUDI-96BX

	1	2	3	4	5	6	7	8	9	10	11	12
A	XUD I097	XUD I105	XUD I113	XUD I121	XUD I129	XUD I137	XUD I145	XUD I153	XUD I161	XUD I169	XUD I177	XUD I185
B	XUD I098	XUD I106	XUD I114	XUD I122	XUD I130	XUD I138	XUD I146	XUD I154	XUD I162	XUD I170	XUD I178	XUD I186
C	XUD I099	XUD I107	XUD I115	XUD I123	XUD I131	XUD I139	XUD I147	XUD I155	XUD I163	XUD I171	XUD I179	XUD I187
D	XUD I100	XUD I108	XUD I116	XUD I124	XUD I132	XUD I140	XUD I148	XUD I156	XUD I164	XUD I172	XUD I180	XUD I188
E	XUD I101	XUD I109	XUD I117	XUD I125	XUD I133	XUD I141	XUD I149	XUD I157	XUD I165	XUD I173	XUD I181	XUD I189
F	XUD I102	XUD I110	XUD I118	XUD I126	XUD I134	XUD I142	XUD I150	XUD I158	XUD I166	XUD I174	XUD I182	XUD I190
G	XUD I103	XUD I111	XUD I119	XUD I127	XUD I135	XUD I143	XUD I151	XUD I159	XUD I167	XUD I175	XUD I183	XUD I191
H	XUD I104	XUD I112	XUD I120	XUD I128	XUD I136	XUD I144	XUD I152	XUD I160	XUD I168	XUD I176	XUD I184	XUD I192

Table 21. Index primer plate of QIAseq Unique Dual Index Set C V2 (96): XUDI-96CX

	1	2	3	4	5	6	7	8	9	10	11	12
A	XUD I193	XUD I201	XUD I209	XUD I217	XUD I225	XUD I233	XUD I241	XUD I249	XUD I257	XUD I265	XUD I273	XUD I281
B	XUD I194	XUD I202	XUD I210	XUD I218	XUD I226	XUD I234	XUD I242	XUD I250	XUD I258	XUD I266	XUD I274	XUD I282
C	XUD I195	XUD I203	XUD I211	XUD I219	XUD I227	XUD I235	XUD I243	XUD I251	XUD I259	XUD I267	XUD I275	XUD I283
D	XUD I196	XUD I204	XUD I212	XUD I220	XUD I228	XUD I236	XUD I244	XUD I252	XUD I260	XUD I268	XUD I276	XUD I284
E	XUD I197	XUD I205	XUD I213	XUD I221	XUD I229	XUD I237	XUD I245	XUD I253	XUD I261	XUD I269	XUD I277	XUD I285
F	XUD I198	XUD I206	XUD I214	XUD I222	XUD I230	XUD I238	XUD I246	XUD I254	XUD I262	XUD I270	XUD I278	XUD I286
G	XUD I199	XUD I207	XUD I215	XUD I223	XUD I231	XUD I239	XUD I247	XUD I255	XUD I263	XUD I271	XUD I279	XUD I287
H	XUD I200	XUD I208	XUD I216	XUD I224	XUD I232	XUD I240	XUD I248	XUD I256	XUD I264	XUD I272	XUD I280	XUD I288

Table 22. Index primer plate of QIAseq Unique Dual Index Set D V2 (96): XUDI-96DX

	1	2	3	4	5	6	7	8	9	10	11	12
A	XUD I289	XUD I297	XUD I305	XUD I313	XUD I321	XUD I329	XUD I337	XUD I345	XUD I353	XUD I361	XUD I369	XUD I377
B	XUD I290	XUD I298	XUD I306	XUD I314	XUD I322	XUD I330	XUD I338	XUD I346	XUD I354	XUD I362	XUD I370	XUD I378
C	XUD I291	XUD I299	XUD I307	XUD I315	XUD I323	XUD I331	XUD I339	XUD I347	XUD I355	XUD I363	XUD I371	XUD I379
D	XUD I292	XUD I300	XUD I308	XUD I316	XUD I324	XUD I332	XUD I340	XUD I348	XUD I356	XUD I364	XUD I372	XUD I380
E	XUD I293	XUD I301	XUD I309	XUD I317	XUD I325	XUD I333	XUD I341	XUD I349	XUD I357	XUD I365	XUD I373	XUD I381
F	XUD I294	XUD I302	XUD I310	XUD I318	XUD I326	XUD I334	XUD I342	XUD I350	XUD I358	XUD I366	XUD I374	XUD I382
G	XUD I295	XUD I303	XUD I311	XUD I319	XUD I327	XUD I335	XUD I343	XUD I351	XUD I359	XUD I367	XUD I375	XUD I383
H	XUD I296	XUD I304	XUD I312	XUD I320	XUD I328	XUD I336	XUD I344	XUD I352	XUD I360	XUD I368	XUD I376	XUD I384

Note: Each well contains a mixture of sample index primer and universal primer pair. Use 3 µL for each reaction. The QIAseq Unique Dual Index V2 adapter plates and wells used in ligation must be paired with the same set and well for the index primer plates in universal PCR step, respectively. Each sample index in QIAseq Unique Dual Index V2 is 10 nt in length.

66. Seal the wells with PCR tube caps or seal the 96-well PCR plate with sealing film. Place strips or plate (with compression pad) in thermal cycler and set up reaction parameters according to Table 23, next page.

Table 23. Cycler settings for universal PCR

Step	Cycles	Incubation temperature (°C)	Incubation time
1	1	95	2 min
		98	1 min
2	25*	95	15 s
		60	1 min
3	1	72	1 min
	1	4	hold

* **Note**: Cycle numbers can be adjusted based on library generation experience as the target expression level could vary significantly between different experiments. Library yield is also related with input and sample type, as well as panel primer number. It is recommended using 18–25 cycles for regular input, fresh high-quality RNA samples (low-plex panel could be 26) and using 28–30 cycles for low-input (≤20 ng) or FFPE samples.

67. After the reaction is complete, place the reactions on ice and proceed to the next step.

    If reactions are to be stored after bead-based clean up, transfer them to a –20°C freezer. Samples are stable overnight.

Sample cleanup 3

68. Add 30 µL nuclease-free water to 20 µL reaction to bring the volume to 50 µL.
69. Transfer 50 µL PCR reactions to a 1.5 mL LoBind tube or leave it in 96-well PCR plate for purification.
70. Mix the QIAseq Beads well before usage. Add 55 µL QIAseq Beads to 50 µL reaction. Mix well by pipetting up and down at least 10 times.
71. Incubate for 5 min at room temperature.
72. Place the tube on a magnetic rack to separate the beads from supernatant. After the solution is clear (approx. 5 min), carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the DNA target.

73. Add 220 µL freshly made 80% ethanol to the tube or plate while it is on the magnetic rack. Rotate the tube or move the plate side to side in the 2 positions of the magnet to wash the beads. Wait 1 min with the tube on magnetic rack, and then carefully remove and discard the supernatant.

74. Repeat the above wash step once more.

75. Place the tube or plate on the magnetic rack. Completely remove residual ethanol and dry the beads for 5–10 min while the tube or plate is on the rack with the lid open.

    Note: Enough drying time is essential to ensure that all traces of ethanol are removed. Cracks will be observed in the bead pellet when drying is complete. Do not over dry the bead as this will significantly decrease elution efficiency, especially for larger fragments.

76. Elute DNA target beads into 25 µL sterile water. Mix well by pipetting. Place the tube or plate on the rack until the solution is clear.

77. Transfer 21 µL supernatant to a clean PCR strip or 96-well PCR plate.

78. Proceed to library quantification. The concentration of the library can be determined using QIAGEN's QIAseq Library Quant Array for Illumina or QIAxpert (see "Appendix E: Library Quantification" on page 57). Library quality can be checked with TapeStation HSD1000.

    If libraries are to be stored after bead-based clean up, transfer them to a –20°C freezer. Individual stored samples are stable overnight or longer (re-do quantification and quality check after long time storage).

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 at 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**).

	Comments and suggestions
Low library yield	
Low quality sample	High quality RNA samples are always recommended for optimal performance of our well-optimized chemistry. DV200 ≥ 30% is needed while DV200>=70% is preferred.
Less efficient target enrichment and/or universal PCR	Target enrichment and universal PCR step is critical for library generation, especially for low input and/or low-quality samples. Correct volume of enzyme and buffer, enough cycles, and right temperature for PCR need to be carefully controlled.
Loss of sample during beads cleanup	The QIAseq Beads cleanup step is critical for high recovery efficiency. Carefully handle the bead without any beads lost. Ethanol carryover will affect elution efficiency and following reactions.
Wrong library size peak	
Large size peak and/or flat peak in large size region	RNA Fusion XP-enriched molecule size varied in nature, and during universal PCR, the overamplification may happen that generate single-stranded amplicons. They can be partially bound and can mimic large size molecules. Overamplified libraries will not affect the sequencing.
Small size peak (<200 bp)	The primer and/or primer dimmer need to be removed with each bead's cleanup step. Low input and/or low-quality samples may challenge the cleanup and adjust the sample, and/or cleanup step may help for that.

	Comments and suggestions
Poor sequencing	
High cluster density and/or low passing filter rate	Library quantification is important; underestimation of library concentration or wrong dilution calculation will have higher than expected density and low passing filter rate.
Low cluster density	Library quantification error could be the reason; overestimation of library concentration or wrong dilution calculation will be the reason; high NaOH will also make the cluster density lower.
Detection issue	
Fusion detection	Sample quality and tumor/normal cell ratio are important factors for the fusion detection sensitivity. Input level and/or sequencing depth will also affect it.
RNA SNV/InDel	Expression level and allele express frequency and tumor/normal cell ratio as well as sequencing depth all contribute to the detection sensitivity and accuracy.
Gene expression	Over high or over low expression level and rare transcript may challenge the gene expression detection, optimization of target list and/or design, as well as deep sequencing may help.

Appendix A: QIAseq Unique Dual Index V2 Run Setting with Illumina Platform

Prepare sample sheet with index sequence for QIAseq Unique Dual Index V2

For most of the Illumina sequencing platforms such as MiSeq, NextSeq500/550 and MiniSeq Sequencing Systems, Illumina offers the sample sheet loading function to set up the run with the index information input. Please find the corresponding index information from the All-in-One tool (Excel file). Please use the sample sheet from a successful run with QIAseq UDI kit before and modify the index information or use a standard temple for the instrument then modify the reads and custom primer setting for QIAseq Unique Dual Index V2.

QIAseq Unique Dual Index V2 Set A (96)

QIAseq Unique Dual Index V2 Set B (96)

QIAseq Unique Dual Index V2 Set C (96)

QIAseq Unique Dual Index V2 Set D (96)

The QIAseq Unique Dual Index V2 uses the optimized QIAGEN 10 nt index design. Please download the All-in-One Tool Excel file from QIAGEN.com (please note it is different than the QIAseq 96-UDI that was previously used for RNA Fusion XP.

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Local Run Manager

LRM software is an integrated solution designed to create sequencing runs, monitor run status, analyze sequencing data, and view results. LRM software integrates with the instrument control software and can be directly accessed on the instrument through a web browser. An off-instrument version of the same easy-to-use LRM software is available and compatible with the MiniSeq System, the MiSeq Series, and the NextSeq Series.

Please be aware that the format for LRM sample sheet may not be the same as sample sheet from IEM. The best way to generate the right LRM usable sample sheet is opening a sample sheet that had been be used successfully to copy all the text except the index information to a new file in Notepad; modify the run name, user name, run date, and setting of read 1 and 2 length (standard setting is 231/71 or 229/69 for some instrument with QIAseq Unique Dual Index V2 ; run with QIAseq DNA library needs 151/151 or 149/149 for some instruments with QIAseq Unique Dual Index V2 and please be aware that lower fusion detection sensitivity is expected; copy the right QIAseq Unique Dual Index V2 information to the index information part; then save as csv file so LRM can load the sample sheet for run setting.

LRM also provides an option to run the sequencing without sample sheet option. Just input the right cycles for the run, for example, run MiSeq with QIAseq Unique Dual Index V2 and set as Read1: 231 cycles, Index 1: 10 cycles, Index 2: 10 cycles, Read 2: 71 cycles. After running, the data can be analyzed with LRM to do demultiplexing with sample sheet later, or directly use LRM to do run setting before sequencing.

Appendix B: Sequencing Setup on Illumina MiSeq and NextSeq

Prepare library for sequencing

After library quantification, dilute library to 4 nM for MiSeq or NextSeq 1000/2000 and 0.5 nM for NextSeq500/550 then combine libraries with different sample indices in equimolar amounts if similar sequencing depth is needed for each library. If combining libraries with same number of primers, pool equal volume of individual library at 4 nM (or 0.5 nM for NextSeq) together.

Prepare library to load on the MiSeq or NextSeq according to Illumina's protocol. If using the QIAseq Library Quant Array to determine concentration, the final total library concentration is 8–10 pM on MiSeq and 0.8–1.0 pM on NextSeq 500/550, using 750 pM for NextSeq 1000/2000 without denature (in process denature). If using a QIAxpert and 3.5 converting factor to determine concentration, use 10–12 pM on MiSeq and 1.2–1.5 pM on NextSeq 500/550; using 700 pM for NextSeq 1000/2000 without denature (in process denature).

When setting up sequencing run. QIAseq Pro 96-UDI is a 10 nt index UDI system designed by QIAGEN. Please ask our tech support for the index sequence that needs to be used in sample sheet.

Reads setting:

- Cycles for Read 1:231 and Read 2:71: this setting is for high sensitivity fusion detection, especially for libraries generated with high quality RNA samples.
- Alternative setting when running RNA Fusion XP with QIAseq DNAseq is Read 1:151 and Read 2:151 or for low quality FFPE sample-generated libraries that the advantage of

asymmetry reads setting for fusion detection is limited when using standard protocol. For using our low quality FFPE protocol, a standard asymmetry setting is still recommended.

Sequencing setup on NextSeq 500/550

Please refer to Illumina protocol for NextSeq run with more details.

Run setting selection: Paired end read.

Cycles for Read 1:229 and cycles for Read 2:69

Sequencing setup on NextSeq 1000/2000

For more details, please refer to Illumina protocol for NextSeq1000/2000 run:

Run setting selection: Paired end read.

Loading suggestion: 750 pM

Cycles for Read 1:231 and cycles for Read 2:71 for 300 cycles kit.

Cycles for Read 1:301 and cycles for Read 2:301 for 600 cycles kit.

Custom Read 1 primer Loading:

MiSeq: Loading custom Read 1 primer (3 µL Custom Read 1 Primer mixed with 597 µL HT1) 600 µL to Well 18

MiniSeq: Loading custom Read 1 primer (3 µL Custom Read 1 Primer mixed with 997 µL HT1) 1000 µL to Well 15

NextSeq 500/550: Loading custom Read 1 primer (6 µL Custom Read 1 Primer mixed with 1997 µL HT1) 2000 µL to Well 7

NextSeq 1000/2000: Loading custom Read 1 primer (3 µL Custom Read 1 Primer mixed with 997 µL HT1) 550 µL to Custom 1 Well

NovaSeq 6000: Loading custom Read 1 primer (12 µL Custom Read 1 Primer mixed with 3988 µL HT1) 3500 µL (S4) or 2000uL (S1, S2, SP) to Well 5

NovaSeq X/X plus: Loading custom Read 1 primer (15 µL Custom Read 1 Primer mixed with 4985 µL HT1) 5000 µL (for 10B and 25B Flow Cell) or 3000 µL (for 1.5B Flow Cell) to Well CP1

Appendix C: FFPE RNA Quality and Quantity

Total RNA present in FFPE archives is usually damaged and fragmented to an uncertain extent. Commonly used RNA quantification methods including spectrometers or fluorometers do not differentiate between amplifiable and non-amplifiable RNA. Therefore, they cannot reliably measure the amplifiable amounts of RNA that are able to participate in the targeted enrichment step in the NGS workflow such as QIAseq RNA Fusion XP Panel.

The performance of the QIAGEN QIAseq RNA Fusion XP Panels is optimized for high-quality FFPE RNA samples. We recommend using the QIAxcel Advanced or an Agilent Bioanalyzer/TapeStation to check the RNA quality first.

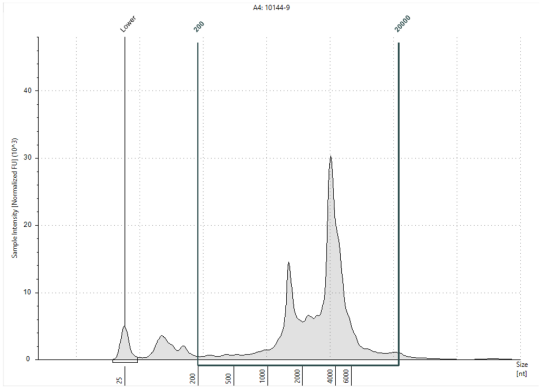
In TapeStation Analysis Software, select **Region** view, then go to **Region** settings to define the region from 200 to 20,000, then **apply** the change to the data file. Then in the Region table, it will have % of Total shown. This number can be used to judge the FFPE sample quality. It is better to use the sample with DV200 >30% (Samples with DV200 between 20% and 30% could be tested as it still has the opportunity to generate library with highly efficient and highly sensitive QIAseq RNA Fusion XP system).

Low quality FFPE RNA will have (1) low sensitivity for fusion call and poor RNA SNV/Indel call, (2) low yield or even failed on library generation, and (3) higher background (higher noise level fusion call). (4) Internal QC will report warning for gDNA signal level and low RNA reference UMI level.

4. gDNA Control Primers ⚠ Found 106 tags on average for DNA contamination control primers. Should be below 50

Target Location	Fragments	Tags	Sizes
chr18:66284615 (+)	2,649	113	33
chr18:66284626 (-)	3,437	99	33
Average control primer counts	3,043.0	106.0	33.0

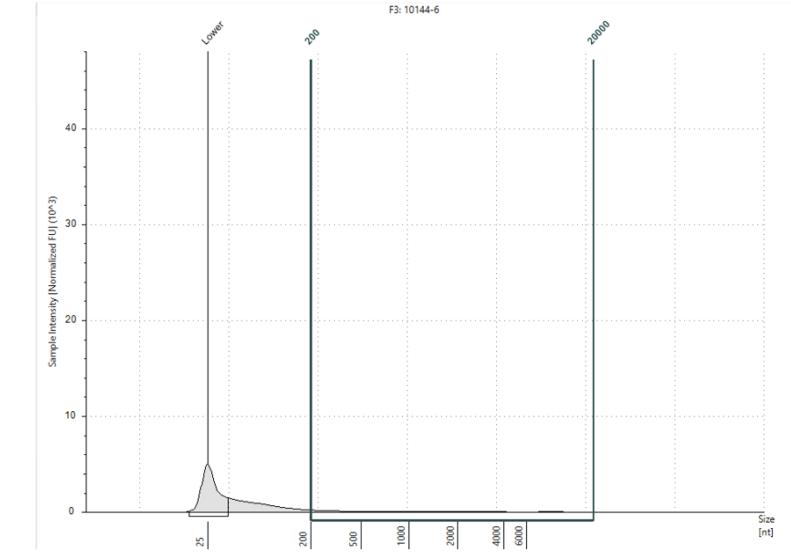
Figure 4. A result showing low quality RNA sample.



Region Table | Sample Table ⚠

From [nt]	To [nt]	Average Size [nt]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total
200	20000	5719	428	220	89.18

Figure 5. Good quality of FFPE RNA, DV200 > 70%. Low quality FFPE samples may generate higher gDNA signal and a warning will be shown. It does not affect the strong fusion call but provides information for troubleshooting of false negative or high background noise (multiple fusion calls with weak signal).



Region Table | Sample Table

From [nt]	To [nt]	Average Size [nt]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total
200	20000	6262	3.41	1.60	18.34

Figure 6. Bad quality FFPE RNA, DV200 < 30%.

Appendix D: Fast Target Enrichment

The RNA Fusion XP workflow has an option for fast target enrichment reaction if the short library generation time is critical. Please be aware that the efficiency could be lower with the fast protocol; please verify protocol based on user's sample type, sample quality and sample input, as well as sensitivity and specificity requirement.

Continued from the standard protocol after first beads cleanup and elution

79. Transfer the 10.4 µL eluted sample into PCR strip or plate.

80. Prepare target enrichment reaction mix for each sample according to Table 24 in PCR strips or a 96-well PCR plate. Mix gently by pipetting up and down.

Table 24. Target enrichment reaction mix

	1 reaction (µL)
Purified sample	10.4
TEPCR Buffer, 5x	4
QIAseq RNA Fusion XP Panel	4
IL-Forward primer	0.8
TaqIT Plus	0.8
Total	**20**

82. Seal the wells with PCR tube caps. Place strips or plate in thermal cycler and set up reaction parameters according to Table 25.

Table 25. Cycler settings for target enrichment

Step	Cycles	Incubation temperature (°C)	Incubation time
1	1	95	2 min
		98	1 min
2	8	95	15 s
		68	1 min
3	1	72	2 min
	1	4	Hold

84. After the reaction is complete, place on ice and proceed to next step.

Appendix E: Library Quantification

Library concentration of the QIAseq RNA Fusion XP Panels can be determined by using QIAGEN's QIAseq Library Quant System. With this system, the correct dilution of the library can be determined for sequencing. Please refer to the QIAseq Library Quant user manual for library quantification.

Please note that the concentration measured with QIAseq Library Quant System with standard settings could be 1.5–2 times less than its actual concentration, so if using the measured concentration directly, use 6–8 pM for MiSeq and 0.8–1.0 pM for NextSeq.

Library concentration of the QIAseq RNA Fusion XP Panels also can be determined by using the QIAGEN's QIAxpert system. With this system, the concentration can be determined as ng/µL; it can be converted to nM by using 3.5 as converting factor. For example, 80ng/µL × 3.5 = 280 nM, then it can be diluted to 4 nM or 2 nM for further Illumina sequencing processing. The final input will be 10–12 pM for MiSeq and NovaSeq and 1.0-1.5pM for NextSeq/MiniSeq. Best loading concentration may need to be optimized based on the real sample loading test.

Appendix F: Combining an Existing Panel with a Booster Panel

Catalog panel or customer panel may not be perfect to meet the user requirement. If additional target site/region and/or primer coverage is needed, QIAseq Fusion XP provides an option to design and order a QIAseq RNA Fusion XP Booster Panel. Please contact the technical support for details for designing/ordering this type of panel. It is a high concentration primer mix (3 µM for each primer) that can be added to an existing catalog/custom panel to enhance the coverage. The maximum primer number in Booster Panel is 100. Please calculate the required booster panel volume that needs to be added to the desired volume of original panel as below.

Goal for pool primer concentration:

- Primer number <2000, pool concentration =100 nM each
- Primer number 2000–4999, pool concentration=75 nM each
- Primer number >=5000, pool concentration=50 nM each.

Booster panel volume calculation:

$$y = v \times \frac{c}{3000}$$

- y-the volume of booster panel needed to be added (µL).
- v-the volume (µL) the original panel that plan to use.
- c-the goal for pool primer concentration.
- The booster panel primer concentration is fixed as 3000 nM.

Appendix G: Blocking Unexpected rRNA and/or Globin Signal with FastSelect in RNA Fusion XP Workflow

The RNA Fusion XP catalog panel and custom panels are designed with high specificity for the primer pool, so most of the time there is very limited non-specific signal from rRNA and/or globin molecules (depending on the sample type). Due to the sample variation and or specific design request challenge, occasionally a large portion of reads may go with rRNA and/or globin under very limited number of primers. It is recommended to optimizing the final primer pool by removing those primers and/or primer re-design. For a quick improvement on initial test or for a quick solution for the unexpected non-specific signal, a modified step of primer priming is provided as below, Table 26.

It is recommended to dilute the FastSelect with water first. Dilution of 1:10– 20 is recommended as initial test. It may be adjusted based on input and rRNA signal strength. Mix the diluted FastSelect as below with RNA sample and RP Primer II, then heat at 65°C for 5 min and cool down with ice for 2 min.

Table 26. Alternative primer priming

	1 reaction (µL)
RNA sample (x µL)	x (≤4)
Diluted FastSelect	1
RP Primer II	1
Nuclease-free water	4 – x
Total	**6**

It is fine to adjust the FastSelect concentration with less volume so more RNA can be used or if 5 µL RNA is needed to maintain the enough input, mixing 30 µL RNA with 3 µL FastSelect first, then take 5 µL for primer reaction.

Go with the standard first-strand synthesis after the primer step.

Appendix H: Data Analysis using QIAGEN's QIAseq RNA Fusion XP Data Analysis Software

After sequencing, results can be analyzed using QIAGEN's Cloud-Based QIAseq RNA Fusion XP Panel Data Analysis Software. It can be approached within QIAGEN.com webpage under GeneGlobe® Data Analysis Center. Our data analysis software will perform read trimming (removing adapter sequences), mapping, UMI counting, and fusion identification and classification. Please refer to the corresponding document for data analysis.

It also can be analyzed by QIAGEN's Genomic Workbench (**www.digitalinsights.qiagen.com**). Please contact QIAGEN's Technical Support team for more details.

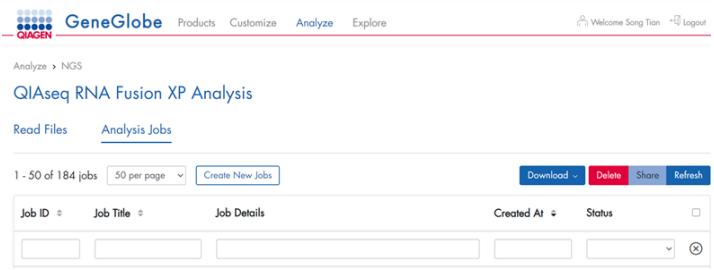
Currently, GeneGlobe data analysis tool acceptable file extensions are "**.fastq**" or "**.fastq.gz**" for Illumina reads and "**.basecaller.bam**" for Ion reads. Please submit only unaligned base-caller BAM files generated by Torrent Server 3.4.1 or higher. Please do not submit aligned BAM files.

IMPORTANT: Please do not refresh the browser or navigate to other pages while uploading files.

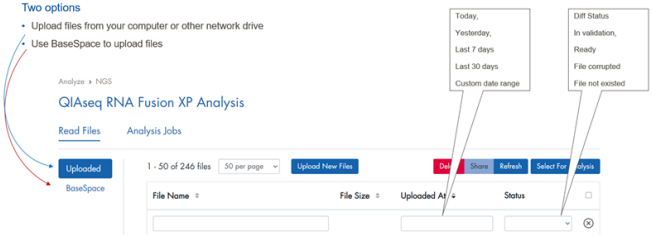
Procedure

1. Please go to **www.qiagen.com/GeneGlobe**, then go to the **Analyze Data**. Please select the **NGS** (log in if you already registered; otherwise please register at QIAGEN.com first).
2. Find the QIAseq RNA Fusion XP Analysis to access the free online analysis tool. In Read Files tab, the customer can sort the reads by uploaded date or file name. Files can be selected as Preselect Files for analysis or as Share Files for data sharing. Currently the

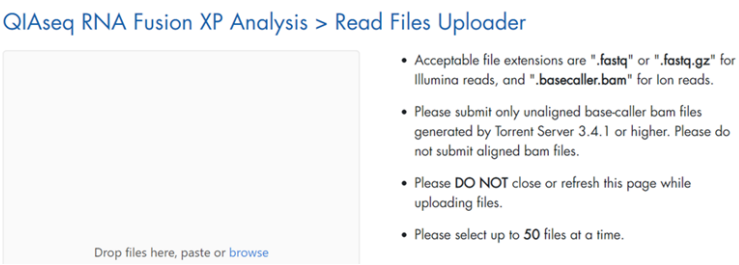
database will keep the reads file for at least 3 months after uploading is finished. Please save your raw reads in a safe drive for any needs of additional analysis.



3. Select **direct upload FASTQ files** (will be kept for 3 months) or link your BaseSpace account files with the analysis tool.

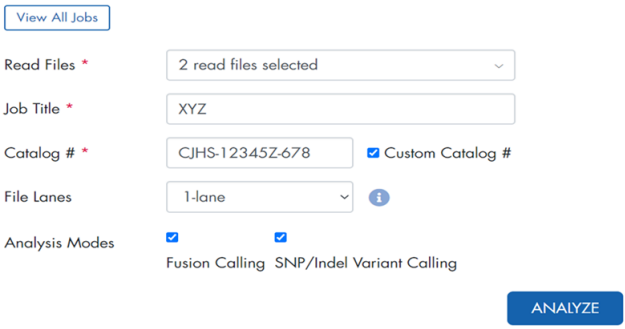


- **Direct upload FASTQ files:** Drop files to the uploader or browser file for upload.

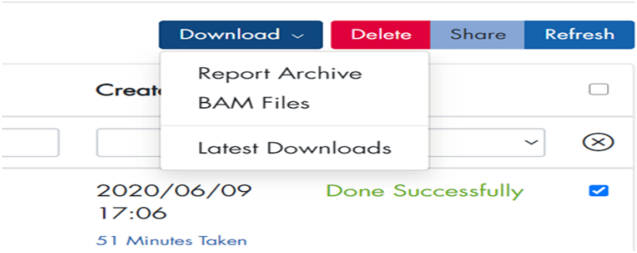


- **BaseSpace files link:** Log in your BaseSpace account, and find the files from Runs/Projects, grant download and then select the files for analysis.

4. After selecting the files for analysis, input the job title. For catalog panel, select from the dropdown list; if using custom panel, highlight the Custom Catalog # on the right, then input the number. Select lane number and select the analysis you want. Fusion analysis is selected by default; if you want SNV/InDel and gene expression analysis, please also select the next option (we currently cannot do transcript variants calling), then click **ANALYZE**.



5. Getting result: Select the finished jobs. Click **Download**, then select **Report Archive** for regular analysis report; if more details need to be checked, BAM files can be downloaded by selecting BAM Files. The report generation will need some time; it will be shown as "queued" first; it can be checked by clicking the Latest Downloads (may need refresh page). It will have a green "ready" sign when it is done. The top 1 will be latest combined analysis report. Please download the result in time by clicking the Download under Status, as it will be removed automatically after 7 days. The result will be downloaded as a zip file. Please unzip it first. The jobs will be retained for 7 days from their created date.



Result folder 1: Summary

~Combined.enrichment-metrics: The summary of the run, like reads number, trimmed reads number, on-target percentage, etc.

~Combined.fusion.xlsx: The same one as RNAscan summary file. It has fusion summary and more details.

Combined.gene-expression: It reports the average UMI for all the genes that are targeted as GEX (gene expression); if no GEX targets, then it will only report the reference assay result.

~Combined.gene-expression-extra: It will report other genes that are not defined as GEX targets (it may be from fusion and SNV/InDel primers).

~Combined.snv-indel: It reports RNA SNV/InDel similar as DNA SNP/InDel.

Result folder 2: Tables

- ~Fusion: HTML report as RNAscan and report other details in per_primer file, filtered fusion, passed fusion, and VCF file for fusion report.
- ~Gene-expression: More reports by using different ways for calculation like min max, for reads or UMI count.
- ~Snv-indel: More details for SNV/InDel call.

Ordering Information

Product	Contents	Cat. no.
QIAseq RNA Fusion XP Panel (12) *	Kit containing ALL reagents (except indices) sufficient to process 12 samples for RNA sequencing analysis of gene fusions, gene expression and RNA SNP/InDel	334602
QIAseq RNA Fusion XP Panel (96) *	Kit containing ALL reagents (except indices) sufficient to process 96 samples for RNA sequencing analysis of gene fusions, gene expression and RNA SNP/InDel	334605
QIAseq RNA Fusion XP Custom Panel*	Kit containing ALL reagents (except indices) sufficient to process 96 samples for RNA sequencing analysis of gene fusions, gene expression and RNA SNP/InDel	334625
QIAseq RNA Fusion XP Booster Panel	Pool of primers used in combination with either catalogued or custom panels	334635
QIAseq 96-Unique Dual Index Set A V2 (96) *	Box containing unique dual-indexed adapters, for indexing up to 96 samples for QIAseq Targeted Panel sequencing on Illumina platforms; enough to process a total of 96 samples; four sets are required for multiplexing up to 384 samples	331745
QIAseq 96-Unique Dual Index Set B V2 (96) *	Box containing unique dual-indexed adapters, for indexing up to 96 samples for QIAseq Targeted Panel sequencing on Illumina platforms; enough to process a total of 96 samples; four sets are required for multiplexing up to 384 samples	331755
QIAseq 96-Unique Dual Index Set C V2 (96) *	Box containing unique dual-indexed adapters, for indexing up to 96 samples for QIAseq Targeted Panel sequencing on Illumina platforms; enough to process a total of 96 samples; four sets are required for multiplexing up to 384 samples	331765
QIAseq 96-Unique Dual Index Set D V2 (96) *	Box containing unique dual-indexed adapters, for indexing up to 96 samples for QIAseq Targeted Panel sequencing on Illumina platforms; enough to process a total of 96 samples; four sets are required for multiplexing up to 384 samples	331775

Product	Contents	Cat. no.
Human XpressRef Universal Total RNA	2 tubes each containing 100 µg human RNA at 1mg/mL	338112
RNeasy Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 mL and 2 mL), RNase-free reagents, and buffers	74104
RNeasy FFPE Kit (50)	50 RNeasy MinElute® Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-free buffers, and Nuclease-Free Water	73504
PAXgene® Blood RNA Kit (50)	50 PAXgene Spin Columns, 50 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-free reagents, and buffers; to be used in conjunction with PAXgene Blood RNA Tubes	762174
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
QIAamp® RNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 mL and 2 mL), RNase-free reagents, and buffers	52304
QIAseq FastSelect – rRNA HMR Kit (24)	Includes 3 tubes of QIAseq FastSelect reagent for rRNA removal: sufficient for 24 reactions from human, mouse, and rat samples	334386
QIAseq FastSelect – rRNA/Globin Kit (24)	Includes 1 tube of QIAseq FastSelect reagent for rRNA removal and 1 tube of QIAseq FastSelect reagent for globin mRNA removal	335376

* Visit www.qiagen.com/GeneGlobe to search for and order these products.

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Document Revision History

Date	Changes
12/2024	Initial release.
05/2026	Updated volume of step 50 in the Protocol: QIAseq RNA Fusion XP Panel for Illumina Instruments

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