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QIAseq® Targeted RNA Panel TCR Library Kit Handbook

Sample to Insight® solution for T-cell receptor sequencing from RNA

Sample to Insight

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Kit Contents

Box 1 of 2

QIAseq® Targeted RNA Panel TCR (Human or Mouse)	**12**	**96**
Catalog no.	**334651/334671**	**334665/334685**
Number of samples.	**12**	**96**
Human/Mouse TCR Panel Alpha IMLH-001A pool / IMLM-001A pool	12 µL	96 µL
Human/Mouse TCR Panel Beta IMLH-001B pool / IMLM-001B pool	12 µL	96 µL
Human/Mouse TCR Panel Delta IMLH-001D pool / IMLM-001D pool	12 µL	96 µL
Human/Mouse TCR Panel Gamma IMLH-001G pool / IMLM-001G pool	12 µL	96 µL
Human/ Mouse TCR RT Primers	12 µL	96 µL
Zip Buffer, 5x	24 µL	192 µL
RNase Inhibitor	12 µL	96 µL
RH RNase	12 µL	96 µL
dNTP II	18 µL	144 µL
StableScript	12 µL	96 µL
BLU Buffer	24 µL	192 µL
POL Enzyme	12 µL	96 µL
ERA Enzyme	120 µL	960 µL
ERA Buffer, 10x	60 µL	480 µL
DNA Ligase	120 µL	960 µL
Ligation Buffer, 5x	240 µL	2 x 1250 µL
TaqIT Plus	15 µL	120 µL
RNA Buffer II	2 x 48 µL	768 µL
Ligation solution	2 x 125 µL	2 x 970 µL
TUDI universal F primer	9.6 µL	76.8 µL
HotStarTaq® DNA Polymerase	30 µL	240 µL

TUDI-Phased Adapter	30 µL	240 µL

Box 2 of 2

QIAseq Targeted RNA Panel TCR	12	96
QIAseq Beads	10 mL	38.4 mL
Nuclease Free water	2 x 1.5 mL	3 x 1.5 mL

QIAseq TCR Unique Dual Indices (TUDIs)

The QIAseq TCR UDI kit (334792 / 334805) is required (sold separately) to complete the workflow. This kit is necessary to complete the library preparation protocol by adding a 10 base pair unique sample index and Illumina specific sequences for flow cell attachment. Each well contains one pair of a sample index primer and a universal primer for sample amplification and indexing using a PCR reaction.
Each kit can process 24 or 96 samples.

Product Name	QIAseq 24-Index TCR UDI (24)	QIAseq 96-Index TCR UDI Set A (96)
Catalog no.	334792	334805
Number of samples	24	96
TUDI-24K	1	N/A
TUDI-96AK	N/A	1
8-cap strips (24/bag)	1 bag	1 bag

Shipping and Storage

The QIAseq Targeted RNA Panel TCR Library Kit (334651 / 334665 / 334671 / 334685) is shipped in 2 boxes:

- Box 1 is shipped on dry ice and must be stored at –30°C to –15°C in a constant temperature freezer upon arrival.
- Box 2 is shipped on cold packs.

  Important: Open immediately and store the QIAseq Beads at the 4°C.

When stored under these conditions and handled correctly, the product can be kept based on the expiration date on each box without a reduction in performance.

QIAseq Index TCR UDI Kits (334792/334805) (ordered separately) are shipped at ambient temperature and can be stored at 4 or –20°C in a constant-temperature fridge or freezer upon arrival. The product can be kept until the expiration date on the box.

Intended Use

The QIAseq Targeted RNA Panel TCR Library Kit 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**, 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 Targeted RNA Panel TCR Library Kit is tested against predetermined specifications, to ensure consistent product quality.

Introduction

The QIAseq Targeted RNA Panel TCR Library Kit enables targeted next-generation sequencing (NGS) of the human or mouse expressed T-cell receptor (TCR), which includes 4 distinct genes: TCR-alpha, TCR-beta, TCR-gamma and TCR-delta. This highly optimized solution incorporates unique molecular indices (UMIs) to facilitate ultrasensitive and accurate characterization of the immune repertoire in cells and tissues starting from 200 pg to 1000 ng of total RNA.

The adaptive immune system is composed of T and B lymphocytes that bind antigens via highly specific T cell receptors (TCRs) and B cell receptors (BCRs) on their cell surfaces. To recognize a nearly infinite number of potential antigens, extensive sequence diversity of TCRs and BCRs is generated by somatic V(D)J recombination of the TCR and BCR loci, and by subsequent somatic hypermutation and class-switching recombination upon antigen stimulation. Accurate characterization of the TCR and BCR repertoires is key to understanding adaptive immune responses and has many applications across different fields, including vaccine development, autoimmunity, monitoring treatment response in lymphoid malignancies, and immunotherapy.

Compared to traditional methods, NGS provides an unprecedented, high-resolution picture of the immune repertoire. However, many available immune repertoire sequencing methods involve multiplex PCR with primers targeting different V or J regions, which can introduce substantial amplification bias and unintended PCR artifacts.

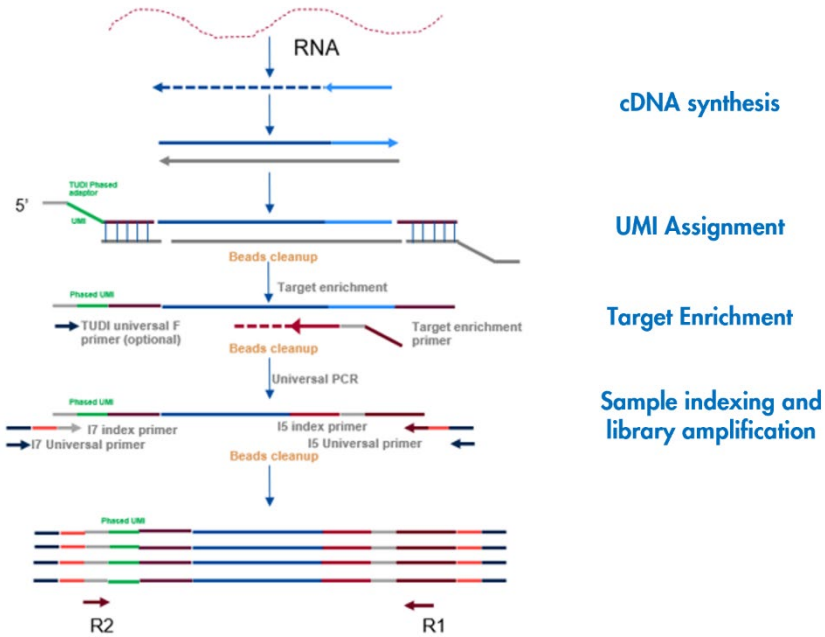
The QIAseq Targeted RNA Panel TCR Library Kit utilizes unique molecular index (UMIs) with QIAseq enrichment technology to robustly create targeted RNA-seq libraries for NGS instruments. This library construction approach greatly improves amplification uniformity compared to multiplexed PCR-based V and J primer pools. The incorporation of UMIs before the library amplification step further reduces amplification bias and allows for accurate and sensitive TCR clonotype and repertoire diversity assessment.

The QIAseq Targeted RNA Panel TCR Library Kit is a complete sample-to-insight solution for precise characterization of the TCR immune repertoire using NGS. The purchase of the TCR Panel includes access to GeneGlobe Analyze (**https://geneglobe.qiagen.com/analyze**) where you can simply upload your FASTQ files. For customized analysis with desktop and server support, QIAGEN Genomics Workbench (**https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-clc-genomics-workbench/**) can be utilized. Both offerings contain highly optimized pipelines with all the necessary steps of NGS data processing, read mapping and clonotype identification.

Principle and procedure

The QIAseq Targeted RNA Panel TCR Library Kit relies on a highly efficient, TCR-specific cDNA synthesis, TCR gene-specific QIAseq enrichment technology and unique molecular indexing (UMI) for accurate and sensitive TCR clonotype and diversity assessment. TCR reverse transcriptase and enrichment panel primers are provided, together with all the necessary library construction reagents. The QIAseq Targeted RNA Panel TCR Library Kit is designed to enrich TCR α, β, γ and δ subunits (individually or combined) in a single reaction using 200 pg to 1000 ng of RNA (Figure 1, next page).

Figure 1. QIAseq Target RNA Panel TCR workflow



cDNA synthesis

RNA samples are first reverse transcribed into cDNA with RT specific primers. Subsequently, second-strand synthesis occurs, which generates double-stranded cDNA (ds-cDNA). This ds-cDNA is then end-repaired with an A-addition in a single-tube protocol.

Unique Molecular Index (UMI) assignment

Prior to target enrichment and library amplification, each original cDNA molecule is assigned a unique molecular index (UMI) by ligating an adapter containing as many as 18 base unique molecular index (UMI) to the ds-cDNA. Statistically, this process provides >268 million possible indices per adapter, with each DNA -molecule in the sample receiving a unique UMI sequence.

Target enrichment and final library construction

Following UMI assignment, QIAGEN target enrichment is performed to capture TCR cDNA molecules with UMIs into the NGS library. For enrichment, ligated cDNA molecules are subjected to a TCR specific enrichment using a TCR targeted panel. After enrichment, a universal PCR is performed to amplify the library and introduce unique dual indices.

NGS adapter and unique dual index (UDI) technologies

The QIAseq Targeted RNA Panel TCR Library Kits require the QIAseq TCR UDI kit (334792 / 334805) which contains the TUDI adapters and dual index primers. The UDI design significantly reduces the risk of index bleeding issues ("index hopping") associated with Illumina sequencing instruments that utilize patterned flow cells. With unique dual indexing (UDI), each sample will be assigned two unique sample indices to overcome the error introduced by image analysis, sequencing error, and demultiplexing, and to remove mis-assignment of sequencing data to the wrong samples.

Next-generation sequencing on Illumina NGS systems

The QIAseq Targeted RNA Panel TCR Library Kit is compatible with Illumina NGS systems (MiSeq®, NextSeq 1000/2000, and NovaSeq™ 6000).

Data analysis

The raw NGS data produced by the QIAseq Targeted RNA Panel TCR Library Kit can be analysed using cloud, desktop, and server software packages, using highly optimized pipelines to automatically perform all steps necessary to generate TCR diversity and clonotype report. For cloud-based analysis, you can utilize the QIAseq section of GeneGlobe Analyze (**https://geneglobe.qiagen.com/analyze**). For desktop and server support, QIAGEN Genomics Workbench (**https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysisand-visualization/qiagen-clc-genomics-workbench/**) can be utilized.

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.

- 80% ethanol (made fresh daily)
- Nuclease-Free Water
- Nuclease-free pipette tips and tubes
- 1.5 mL LoBind® tubes (Eppendorf, cat. no. 022431021)
- 0.2 mL PCR tubes, 96-well PCR plates or PCR strips and caps
- Ice
- Microcentrifuge
- Thermal cycler
- Multichannel and single-channel pipettes
- Library QC: QIAxcel Advanced System (QIAGEN, Cat.No. 9002123); Agilent® 2100 Bioanalyzer® (Agilent, cat. no. G2939BA) or Agilent TapeStation® (Agilent, cat. no. G2991AA) and Agilent High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626)
- Magnetic separation stands for 1.5 mL or 2 mL tubes (DynaMag™-2; Thermo Fisher Scientific, 12321D or equivalent)
- Magnetic separation stands for 96-well plates (DynaMag-96 Side Magnet; Thermo Fisher Scientific, cat. no. 12331D or equivalent)
- QIAseq Library Quant system: QIAseq Library Quant Array Kit (cat. no. 333304) or
- QIAseq Library Quant Assay Kit (cat. no. 333314)

Important Notes

RNA quality

The most important prerequisite for RNA sequence analysis is consistent, high-quality RNA from every experimental sample. Therefore, sample handling and RNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts or other contaminants may either degrade the RNA or decrease the efficiency of, if not block completely, the enzymatic activity necessary for optimal targeted enrichment. RNA concentrations and sample purity can be checked using a fluorescence based assay or a spectrophotometer depending on the amount of RNA present. Due to the large intronic region between the TCR J and constant regions, it is recommended that RNA should be free of DNA to avoid wasting sequencing reads on intron region.

The QIAGEN kits listed in Table 1 are recommended for the preparation of RNA samples from cells, tissues, and serum/plasma samples. For best results, all RNA samples should be resuspended in RNase-free water.

Important: Do not use DEPC-treated water.

Note: If RNA samples must be harvested from biological samples for which kits are not available, please contact Technical Support representatives for suggestions.

Table 1. Recommended kits for purification of total RNA

Kit	Starting material	Cat. no.
RNeasy® Mini Kit	Cells and tissue	74104, 74106
RNeasy Micro Kit	Small amount tissues and cells	74004
QIAamp RNA Blood Mini Kit (50)	Whole blood, tissue	52304
AllPrep® DNA/RNA Mini Kit (50)	Animal/human tissues and cells	80204
PAXgene® Blood RNA Kit (50)	Whole blood	762164
RNeasy FFPE Kit	FFPE	73504

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

RNA quantification

The concentration and purity 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 measuring absorbance in 10 mM Tris·Cl, pH 7.5. The A_{260}/A_{280} ratio is better to be >1.8.

Ribosomal RNA band integrity

Run an aliquot of each RNA sample on the Agilent Bioanalyzer using an RNA 6000 Nano LabChip®. Verify that there are sharp bands/peaks present for both the 18S and 28S ribosomal RNAs (Figure 2). Any smearing of the RNA bands or shoulders on the RNA peaks indicates that degradation has occurred in the RNA sample. For best results, the ribosomal bands should appear as sharp peaks.

Sometimes research needs to use FFPE RNA though it is not the best sample type for QIAseq Target RNA panel TCR kit. For checking the RNA quality, DV600 (percentage of RNA size >=600nt in total RNA) can be used as quality value. It is better to be >=70%, if DV600 is between 30% to 70%, it would be fine for a test run, though the useful information may not be enough. It is not recommended using DV600 <30% samples.

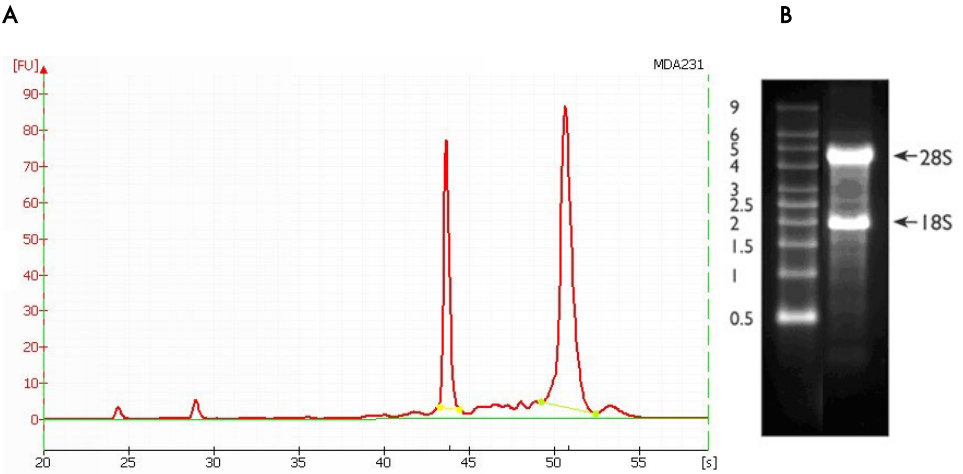


Figure 2. Ribosomal RNA integrity. **A** Agilent Bioanalyzer electropherogram of high-quality total RNA showing sharp peaks for the 18S (left) and 28S (right) ribosomal RNA. Due to high quality of the RNA, peaks do not have shoulders (especially to the left of each peak) **B** Agarose gel electrophoresis shows sharp bands (especially at the bottom of each band) for 28S and 18S ribosomal RNA.

RNA input amount

QIAseq Targeted RNA Panel TCR Library Kit provides results with as little as 200 pg or as much as 1000 ng total RNA per cDNA synthesis reaction. Libraries from total RNA (0.2 ng to 1 µg from peripheral blood leukocytes, 0.5 ng to 200 ng of total RNA from whole blood, or 0.2 ng to 100 ng T-cells, 100ng to 1ug from tumor tissues) or purified T cells (10 to 10,000 cells) can be used to generate data for TCR profiling.

The optimal amount of starting material depends on the relative TCR immune repertoire diversity and the abundance of the TCR clonotype. Detection of lower-abundance clonotypes requires more RNA; high-abundance clonotypes require less RNA. Greater amounts of input total RNA will provide better sensitivity for specific clonotype detection when sufficient instrument read budget is allocated.

For successful results, we recommend that first-time users start with 10ng total RNA. We recommend using a consistent amount of total RNA for all reactions in a single experiment.

Sequencing capacity and sample multiplex level

QIAseq Targeted RNA Panel TCR Library Kits must use the (334792) QIAseq 24-index TCR UDI kit or (334805) QIAseq 96-Index TCR UDI sets to complete the library construction. Other QIAGEN index kits are not compatible with this library kit.

Sample multiplexing is one of the most important NGS tools for increasing throughput and reducing costs. It works by combining multiple samples to be processed together in a single sequencing run; therefore, sequencing reads need to be demultiplexed by reassigning each single read to its original source library. This is facilitated by the integration of index sequences into the individual adapter molecules.

Sample multiplexing capacity is defined by the immune repertoire diversity and sequencing platform read capacity. General guidelines are provided for the number of samples that can be multiplexed in different sequencing platforms, based on 10ng PBMC RNA (Table 2). Fine-tuning the read depth and read budget will be possible after the first test run. Adjustments should be made for different sample type, input, and specific applications, as necessary.

Table 2. Estimated number of multiplexed samples based on sequencing need*

Instrument	Version	Capacity (paired-ends reads)	Shallow	Deep
MiSeq	V2 Reagents	30 M	6	1
MiSeq	V3 Reagents	50 M	10	2
NextSeq 1000/2000	P1/P2/P3	200/800/2400M	40/160/480	8/32/96
NoveSeq 6000		1.6/3.2/8.2/20B	300/600/4000	50/120/800

* Based on 2 x 249 bp paired-end reads for TUDIs for complete variable regions.

Next-generation sequencing read-length recommendations

The QIAseq Targeted RNA Panel TCR libraries can be sequenced two ways depending on whether the complete variable region or just the CDR3 region want to be sequenced.

Variable region characterization (Preferred method)

Illumina NGS Instruments Supported: miSeq (500 cycles and 600 cycles), NextSeq 1000/2000 (P1 600 cycles, P2 600 cycles), NovaSeq 6000 (SP 500 cycles) To sequence the complete variable regions, the QIAseq Targeted RNA Panel TCR libraries require at least 249 bps paired-end reads and 10 bp indices. We recommend utilizing the QIAseq NextSeq 2000 with 600 cycle kits for the best performance.

CDR3 Region only

Illumina NGS instruments supported: MiSeq (300 cycles), NextSeq 1000/2000 (P1 300 cycles, P2 300 cycles, P3 300 cycles), NovaSeq 6000 (SP 300 cycles, S1 300 cycles, S2 300 cycles and S4 300 cycles)

QIAseq Targeted RNA Panel TCR libraries require asymmetrical 259 bp for Read 1 and 39 bp for Read 2 paired-end reads and dual 10 bp indices. It is possible there will be V region ambiguity due to the difficulty to differentiate two very similar V regions with the limited length of reading in Read 2.

Protocol: QIAseq Targeted RNA TCR Kit for Illumina Instruments

Important points before starting

- This protocol covers all procedures required for the preparation of libraries for Illumina sequencers from "standard RNA" (i.e., cells or tissues).
- Before setting up the reaction, it is critical to accurately determine the amount of input RNA (200pg–1000 ng). We recommend using 10 ng total RNA (such as PBMC) as starting input. Lower input amounts may reduce detection of low abundance clonotypes due to the sampling effect.
- Set up reactions on ice.
- Mix well for any reagents or reactions but avoid vigorous mixing for enzymes.

Protocol: RT primer hybridization

Procedure: RT primer hybridization

1. Pre-heat a thermal cycler to 65°C with a heated lid (set at 103°C).
2. Thaw the RNA samples on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and then return to ice.
3. Prepare the reagents required for RT primer hybridization.
   3a. Thaw TCR RT primer if required at room temperature.
   3b. Mix by flicking the tube, and centrifuge briefly.
4. On ice, prepare the RT primer hybridization reactions as described in Table 3. Briefly centrifuge, mix by pipetting up and down 8 times (do not vortex), and briefly centrifuge again.

Table 3. Preparation of RT primer hybridization reactions

Component	1 reaction
RNA sample	Variable
TCR RT Primer (Human/ Mouse TCR RT Primers (IMHS -001Z / IMMM -001Z))	1 µL
Nuclease-free Water	Variable
Total	**6 µL**

5. Transfer the tube from ice to the pre-heated thermal cycler and incubate for 5 min at 65°C followed by incubating on ice for 2 minutes.
6. Upon completion, proceed immediately with "Protocol: Reverse transcription".

Protocol: Reverse transcription

Important points before starting

- The 6 µL product from "Protocol: RT primer hybridization" above, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure: Reverse Transcription

7. Prepare the reagents required for Reverse Transcription.

   7a. Thaw Zip Buffer, 5x, at room temperature.

   7b. Mix by flicking the tube, and centrifuge briefly.

   Note: The RNase Inhibitor and StableScript Reverse Transcriptase should be removed from the freezer just before use and placed on ice. After use, immediately return the enzymes to the freezer.

8. On ice, prepare the reverse transcription reactions as described in Table 4. Briefly centrifuge, mix by pipetting up and down 8 times (do not vortex), and briefly centrifuge again.

Table 4. Preparation of reverse transcription reactions

Component	1 reaction (µL)
RT primer hybridization reaction (already in tube)	6
Zip buffer, 5x	2
RNase Inhibitor	1
StableScript	1
Total	**10**

9. Incubate the tube in a thermal cycler with a heated lid (103°C) according to Table 5. Please note for best specificity, we recommend using a "hot start". Pre-heat the cycler to

55°C first, then add the sample to pre-heated cycler to start the 45 min 55°C incubation as show below.

Table 5. Thermal cycler settings for reverse transcription reactions

Step	Temperature (°C)	Time
0	55	1 minute, then pause
1	55	45 minutes
2	70	15 minutes
3	4	Hold

10. Upon completion, proceed with "Protocol: Second-strand synthesis". Alternatively, the samples can be stored at –30°C to –15°C in a constant temperature freezer.

Protocol: Second-strand synthesis

Important points before starting

- The 10 µL product from "Protocol: Reverse transcription" above, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure: Second-strand synthesis

11. Prepare the reagents required for second-strand synthesis.

    11a. Thaw BLU Buffer and dNTP II at room temperature.

    11b. Mix by flicking the tube, and then centrifuge briefly.

12. On ice, prepare the second-strand reactions as described in Table 6. Briefly centrifuge, mix by pipetting up and down 8 times (do not vortex), and briefly centrifuge again.

Table 6. Preparation of second-strand synthesis reactions

Component	1 reaction (µL)
Reverse-transcription reaction (already in tube)	10
BLU buffer	2
RH RNase	1
dNTP II	1
POL enzyme	1
Nuclease-free Water	5
Total	**20**

13. Incubate the tube in a thermal cycler with a heated lid (103°C) according to Table 7 (next page).

Table 7. Thermal cycler settings for second-strand synthesis

Step	Temperature (°C)	Time
1	37	7 min
2	65	10 min
3	80	10 min
4	4	Hold

14. Upon completion, proceed with “Protocol: End-repair and A-addition”.

Protocol: End-repair and A-addition

Important points before starting

- The entire 20 µL product from "Protocol: Second-strand synthesis" above, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure: End-repair and A-addition

15. Prepare the reagents required for end-repair and A-addition.

    15a. Thaw ERA Buffer, 10x, at room temperature.

    15b. Mix by flicking the tube, and then centrifuge briefly.

16. On ice, prepare the end-repair and A-addition reactions as described in Table 8. Briefly centrifuge, mix by pipetting up and down 8 times (do not vortex), and briefly centrifuge again.

Table 8. Preparation of end-repair and A-addition reactions

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

Important: Keep the reactions on ice during the entire reaction setup.

17. Program a thermal cycler according to Table 9. Set the heated lid to 70°C.

Note: If using a non-temperature–controlled lid, run with cycler lid open for step 4 and seal the strip or plate well. When the cycler reaches step 5, close the lid to avoid evaporation. Centrifuge after the run to remove any condensation.

18. Prior to adding the tubes/plate to the thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.

    Important: The thermal cycler must be pre-chilled and paused at 4°C.

19. Transfer the tubes/plate prepared in step 17 to the pre-chilled thermal cycler and resume the cycling program.

Table 9. Thermal cycler settings for end-repair and A-addition

Step	Temperature (°C)	Time
1	4	1 min
2	20	30 min
3	65	30 min
4	4	Hold

20. Upon completion, immediately proceed with "Protocol: Adapter ligation".

Protocol: Adapter ligation

Important points before starting

- The 50 µL product from "Protocol: End-repair and A-addition" above, is the starting material for this protocol.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure: Adapter Ligation

21. Prepare the reagents required for the Adapter Ligation.

    21a. Thaw DNA Ligation Adapter; Ligation Buffer, 5x; and Ligation Solution at room temperature.

    21b. Mix by flicking the tube, and then centrifuge briefly.

22. On ice, prepare the adapter ligation reactions as described in Table 10. Briefly centrifuge, mix by pipetting up and down at least 10 times (do not vortex) after adding all reagents, and briefly centrifuge again.

    Note: If setting up more than one reaction, prepare a volume of master mix 20% greater than what is required for the total number of reactions.

Table 10. Preparation of adapter ligation reactions

Component	1 reaction (µL)
End-repair and A-addition reaction (already in the tube)	50
Ligation Buffer, 5x	20
TUDI Phased adapter*	2.5
Ligation solution†	15
DNA Ligase	10
Nuclease-free Water	2.5
Total	**100**

* This TUDI phased adaptors is the universal one that can apply to 12 or 96 samples for later TUDI index assignment.
† Ligation solution is very viscous. It should be added into each reaction individually and not pre-mixed with other components for a master mix. Do not coat the outside of the pipette tip with ligation solution or excess volume may be added.

23. Incubate the reactions in a thermal cycler, with the lid open, according to Table 11.

Important: Do not use a heated lid.

Table 11. Incubation conditions for DNA ligation

Step	Temperature (°C)	Time (min)
1	4	1
2	20	15
3	4	30

24. Upon completion, place the reactions on ice and proceed with "Protocol: Cleanup of Adapter-Ligated cDNA". Alternatively, the samples can be stored at –30°C to –15°C in a constant temperature freezer for up to 3 days.

Protocol: Cleanup of adapter-ligated cDNA

Important points before starting

- The entire 100 µL adapter ligated cDNA from "Procedure: Adapter Ligation" is the starting material for the cleanup of adapter-ligated cDNA.
- Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color. If a delay in the protocol occurs, simply vortex the beads (No needs to warm up the beads, it is better to work on ice especially when sample number is high).

Important: Prepare fresh 80% ethanol daily.

Procedure: Cleanup of adapter-ligated DNA

25. Transfer the 100 µL adapter ligation product into a 1.5 mL DNA LoBind tube or a 300 µL 96-well LoBind PCR plate.

    Note: Keep the ligation product on ice especially if you have more samples or need more time before you can process to the next step.

26. Add 65 µL QIAseq beads. Mix well by pipetting up and down 10 times.

27. Incubate for 5 min

    Note: Keep the incubation on ice.

28. Place the tubes/plate on a magnetic rack for 10 min (for 1.5 mL LoBind tubes) or ~15 min (for 300 µL plates) to separate the beads from the supernatant. Once 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.

29. With the beads still on the magnetic stand, completely remove residual supernatant.

30. With the beads still on the magnetic stand, add 200 µL 80% ethanol. Carefully remove and discard the wash.

31. Repeat the beads wash with 200µL of 80% ethanol.

    Important: Completely remove all traces of the ethanol wash after this second wash. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol with a 200 µL pipette first, and then use a 10 µL pipette to remove any residual ethanol.

32. With the beads still on the magnetic stand, air-dry at room temperature for 10 min.

    Note: Visually inspect that the pellet is completely dry, and that all residual ethanol is evaporated.

33. Remove the tube from the magnetic stand and elute the DNA from the beads by adding 100 µL Nuclease-free Water. Mix well by pipetting.

34. Add 70 µL QIAseq Beads to the 100 µL supernatant. Mix well by pipetting up and down 10 times or vortexing.

35. Incubate for 5 min at room temperature.

36. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates) to separate the beads from the supernatant. Once 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.

37. With the beads still on the magnetic stand, add 200 µL 80% ethanol. Carefully remove and discard the wash.

38. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol wash after this second wash. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol with a 200 µL pipette first, and then use a 10 µL pipette to remove any residual ethanol.

39. With the beads still on the magnetic stand, air-dry at room temperature for 10 min.

Note: Visually inspect that the pellet is completely dry, and that all residual ethanol is evaporated. Ethanol carryover to the target enrichment PCR step will affect PCR efficiency.

40. Remove the beads from the magnetic stand and elute cDNA from beads by adding 12.4 µL Nuclease-free Water. Mix well by pipetting.
41. Return the tubes/plate to the magnetic rack until the solution has cleared.
42. Transfer 10.4 µL of the supernatant to clean PCR tubes or plate.
43. Proceed with "Protocol: Target enrichment". Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer for up to 3 days.

Protocol: Target enrichment

Important points before starting

- TCR panel for target enrichment
- It is important to prepare the TCR enrichment panel correctly before the reaction as each sub-gene panel is in high concentration and can be mixed for different targeting options. See Table 12 below for mixing sub-panel together.

Table 12. Preparation of QIAseq TCR panel for target enrichment (1 reaction as example, unit=uL, total 4uL mix per reaction)

Primer Pool	TR-alpha only	TR-beta only	TR-delta only	TR-gamma only	Alpha+ Beta	delta+ gamma	All receptors (α β γ δ)
Human IMLH-001A / Mouse IMLM-001A	1	0	0	0	1	0	1
Human IMLH-001B / Mouse IMLM-001B	0	1	0	0	1	0	1
Human IMLH-001D / Mouse IMLM-001D	0	0	1	0	0	1	1
Human IMLH-001G / Mouse IMLM-001G	0	0	0	1	0	1	1
Nuclease-free water	3	3	3	3	2	2	0

- The entire 10.4 µL product from "Protocol: Cleanup of adapter-ligated DNA" in previous reaction is the starting material for this protocol.
- Set up reactions on ice.
- Mixing by inversion 8 times for each component.

Procedure: Target enrichment

44. Prepare the reagents required for the target enrichment.

    44a. Thaw QIAseq RNA Buffer II, 5x; QIAseq TCR Panel; and Universal F Primer at room temperature.

    44b. Mix by flicking the tube, and then centrifuge briefly.

    Note: HotStarTaq DNA Polymerase should be removed from the freezer just before use and placed on ice. After use, immediately return the enzyme to the freezer.

45. Prepare the target enrichment reactions as described in Table 13, on ice. Briefly centrifuge, mix gently by pipetting up and down 8 times, and briefly centrifuge again.

Table 13. Preparation of target enrichment reactions

Component Input	1 reaction (>100-1000ng- high input)	1 reaction (200pg-100ng-regular input)
Adapter-ligated DNA (from "Protocol: Cleanup of adapter-ligated DNA")	10.4 µL	10.4 µL
QIAseq RNA Buffer II, 5x	4 µL	4 µL
QIAseq TCR panel	4 µL	4 µL
TUDI-Universal F primer	0 µL	0.8 µL
Nuclease-free water	0.8 µL	0 µL
HotStarTaq DNA Polymerase	0.8 µL	0.8 µl
Total	**20 µL**	**20 µL**

46. Program a thermal cycler using the cycling conditions in Table 14.

Table 14. Cycling conditions for target enrichment procedure

Step	Cycles	Temperature	Time
1	1	95°C	15 min
2	High input 16 Regular input 8	95°C 68°C	15 s 5 min (high input) 10min (regular input
3	1 1	72°C 4°C	5 min Hold

47. Place the target enrichment reaction in the thermal cycler and start the run.
48. After the reaction is complete, place the reactions on ice and proceed with "Protocol: Cleanup of target enrichment". Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer for up to 3 days.

Protocol: Cleanup of target enrichment

Important points before starting

- The entire 20 µL Target Enriched DNA reaction from the "Procedure: Target Enrichment" is the starting material for the cleanup of target enrichment.
- Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color. If a delay in the protocol occurs, simply vortex the beads.

Important: Prepare fresh 80% ethanol daily.

Procedure: Cleanup of target enrichment

49. Transfer the completed target enrichment reaction into a 1.5 mL DNA LoBind tube or a 96well LoBind PCR plate.
50. Add 80 µL Nuclease-free Water to bring each sample to 100 µL.
51. Add 75 µL QIAseq beads. Mix well by pipetting up and down at 10 times or vortexing.
52. Incubate for 5 min at room temperature.
53. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates) to separate the beads from the supernatant. Once 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.
54. With the beads still on the magnetic stand, add 200 µL 80% ethanol. Carefully remove and discard the wash.
55. Repeat the ethanol wash with 200 µL of 80% ethanol.

    Important: Completely remove all traces of the ethanol wash after this second wash. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol with a 200 µL pipette first, and then use a 10 µL pipette to remove any residual ethanol.

56. With the beads still on the magnetic stand, air-dry at room temperature for 10 min.

    Note: Visually inspect that the pellet is completely dry, and that all residual ethanol is evaporated. Ethanol carryover to the next step will affect PCR efficiency.

57. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 17.5 µL Nuclease-free Water. Mix well by pipetting.
58. Return the tubes/plate to the magnetic rack until the solution has cleared.
59. Transfer 15.2 µL of the supernatant to the TCR UDI index plate (or break the plate to use the column as 8-well strip as needed).
60. Proceed with "Protocol: Universal PCR". Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer for up to 3 days.

Protocol: Dual Index assignment and Universal PCR

Important points before starting

- The 15.2 µL product from "Protocol: Cleanup of target enrichment" above, is the starting material for this protocol.
- It is better to prepare the reaction mix on ice all the time before PCR runs, especially for FFPE samples or low input samples.

Procedure: Universal PCR

61. Prepare the reagents required for the universal PCR.
    61a. Thaw QIAseq RNA Buffer II, 5x, at room temperature and bring the appropriate index plates to room temperature.
    61b. Mix by flicking the tube, and then centrifuge briefly.
    Note: TaqIT plus enzyme should be removed from the freezer just before use and placed on ice. After use, immediately return the enzyme to the freezer.
62. Prepare the Universal PCR according to Table 15, depending on index set. Briefly centrifuge, mix by pipetting up and down 8 times, and briefly centrifuge again.

Table 15. Setup of Universal PCR using QIAseq TDUI-24K or QIAseq TUDI-96AK Set A

Component	1 reaction
Purified sample	15.2 µL
QIAseq RNA Buffer II, 5x	4 µL
Taq IT plus enzyme	0.8 µL
Total	**20 µL**

QIAseq TUDI-24K QIAseq TUDI-96AK

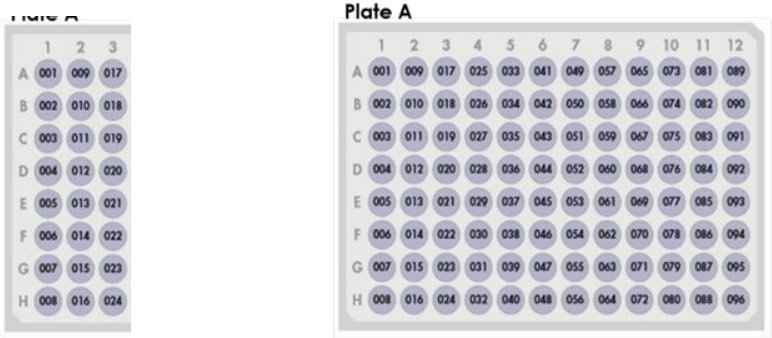


Figure 3. Layout of the QIAseq TUDI-24K or TUDI-96AK (Set A)

63. Program a thermal cycler using the cycling conditions in Table 16.

Table 16. Cycling conditions for universal PCR

Step	Cycles	Temperature	Time
1	1	95°C	3 min
2	18–30*	95°C 62°C	15 s 1 min
3	1 1	72°C 4°C	3 min Hold

* Cycle numbers can be adjusted based on sample type and user experience. Library yield is related to input amount and sample type. For normal PBMC RNA, it is recommended to use 25-30 cycles for 10–1000 pg, 21–25 cycles for 1–50 ng and 18-21 cycles for 50–1000 ng.

64. After the reaction is complete, place the reactions on ice and proceed with "Protocol: Cleanup of Universal PCR". Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer for up to 3 days.

Protocol: Cleanup of Universal PCR

Important points before starting

- The entire 20 µL Universal PCR reaction from the "Procedure: Universal PCR" is the starting material for the cleanup of Universal PCR.
- Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color. If a delay in the protocol occurs, simply vortex the beads.

Important: Prepare fresh 80% ethanol daily.

Procedure: Cleanup of Universal PCR

65. Transfer the completed Universal PCR reaction into a 1.5 ml DNA LoBind tube or a 96-well LoBind PCR plate.
66. Add 80 µL Nuclease-free Water to bring each sample to 100 µl.
67. Add 70 µL QIAseq beads. Mix well by pipetting up and down at 10 times or vortexing.
68. Incubate for 5 min at room temperature.
69. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates) to separate the beads from the supernatant. Once 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.
70. With the beads still on the magnetic stand, add 200 00B5l 80% ethanol. Carefully remove and discard the wash.
71. Repeat the ethanol wash with 200 µL of 80% ethanol.

Important: Completely remove all traces of the ethanol wash after this second wash. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove the ethanol with a 200 µL pipette first, and then use a 10 µL pipette to remove any residual ethanol.

72. With the beads still on the magnetic stand, air-dry at room temperature for 10 min.

    Note: Visually inspect that the pellet is completely dry, and that all residual ethanol is evaporated, but be careful not to over dry the beads as this will significantly decrease elution efficiency.

73. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 30 µL Nuclease-free Water. Mix well by pipetting.

74. Return the tubes/plate to the magnetic rack until the solution has cleared.

75. Transfer 23 µL supernatant to clean PCR tubes or plate.

76. Proceed with "Appendix A: Library Quantification Using the QIAseq Library Quant System", page 44. Alternatively, the library can be stored in a –30 to –15°C in a constant temperature freezer for up to 3 months prior to quantification using the QIAseq Library Quant System. Once quantification is performed, proceed with the "Protocol: Sequencing Setup on Illumina MiSeq and NextSeq", next page.

    Note: The Agilent Bioanalyzer can be used to check the fragment size and concentration with the High Sensitivity DNA Kit. For details, please refer to Appendix C: Library QC.

    Note: For very low input sample libraries, if some small size primer dimmer is still there after beads wash and library yield is limit, a pooled library can be further cleaned up before sequencing.

    Note: For very low input sample libraries, it is recommended to spik in PhiX Control v3 Library (catalog number FC-110-3001, Illumina) for sequencing. For MiSeq, 10% is recommended, for NovaSeq, 10% is recommended. For NextSeq 1000/2000 300 cycles kit, we recommend to spike in >=10%, and for NextSeq 1000/2000 P1 and P2 600 cycle kits, we recommend to spike in 40% to account for potential variation in final % read alignment metric.

Protocol: Sequencing Setup on Illumina MiSeq and NextSeq

Important notes before starting

- Accurate library quantification is essential for accurate flow cell clustering and instrument loading. Libraries quantitated by qPCR is the most accurate. We highly recommend using the QIAseq Library Quant System (cat. no. 333304 or 333314).
- Due to the library size, the extension time should be 4 min at 72°C instead of 2 min indicated in the QIAseq Library Quant System protocol.

Recommendations for library dilution concentrations and library loading concentrations are based on the QIAseq Library Quant System (see Appendix A: Library Quantification Using the QIAseq Library Quant System, page 44).

- Download Illumina compatible sample sheets for different sequencing instruments at www.qiagen.com, from the "Resources" tab of the QIAseq Targeted RNA Panel TCR.
- Paired-end sequencing is required for the QIAseq Targeted RNA Panel TCR on Illumina platforms.

When using 10 bp Indexing kits such as QIAseq 24-index TUDI (24) 334792, QIAseq 96-Index TUDI Set A (96) 334805,

  - Index 1 and Index 2 are 10 bp cycles each
  - 249 bp or 299bp paired-end sequencing is recommended for complete variable regions sequencing (500 cycles or 600 cycles kits)
  - 259/39 bp asymmetric paired-end sequencing is recommended for CDR3 region sequencing (300 cycles kit)

For complete instructions on how to denature sequencing libraries, set up a sequencing run, please refer to the system specific Illumina documentation.

Instrument-specific imagery is included to aid in sequencing preparations.

1. **Sample dilution and pooling:** Dilute libraries to 2 or 4 nM for MiSeq. Then, combine libraries with different sample indexes in equimolar amounts if similar sequencing depth is needed for each library.

Note: Recommendations for library dilution concentrations are based on QIAseq Library Quant System.

2. **Library preparation and loading:** Prepare and load the library onto a MiSeq according to the MiSeq System Denature and Dilute Libraries Guide. The final library concentration is 8–10 pM on the MiSeq. For loading to NextSeq 2000, the initial concentration would be 650pM

Note: Recommendations for library loading concentrations are based on QIAseq Library Quant System.

3. **Data analysis:** Upon completion of the sequencing run, proceed to "Appendix B: Data Analysis Using QIAGEN's QIAseq Targeted RNA Panel TCR Library Kit Data".

Sequencing setting

When working with the QIAseq Targeted RNA Panel TCR libraries and QIAseq 96-Index TUDIs, use Local Run Manager (LRM) on the instrument to upload a sample sheet (see the "Index" tab of the QIAseq Targeted RNA Panel TCR and download the appropriate template) and proceed with sequencing: Read 1 is 259, Read 2 is 39, and each Index Read is 10 for 300 cycles kit. Read 1 is 249 and Read 2 is 249 and each index read is 10 for 500 cycles kit. Read 1 is 301 and Read 2 is 301 and each index read is 10 for 600 cycles kit.

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** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low library yield	
a) Suboptimal reaction conditions due to low RNA quality	Make sure to use high-quality RNA to ensure optimal activity of the library enzymes.
b) Inefficient PCR	QIAseq Beads need to be completely dried before elution. Ethanol carryover to target enrichment and universal PCR will affect reaction efficiency.
c) Insufficient universal PCR cycles	Increase universal PCR cycle numbers to achieve sufficient library amount for sequencing.
Unexpected signal breaks	
a) Larger library fragments after universal PCR	After the universal PCR, library fragments are larger than the intended peak and can be a PCR artifact due to over-amplification of the library. Overamplification of the library won't affect the QIAseq Targeted RNA Panel TCR Library Kit sequencing performance. Decreasing the universal PCR cycle numbers can reduce over-amplification.
b) Small fragments in final library	Make sure the correct bead amount is used. PCR tube or plate must be sealed well to prevent evaporation during PCR reaction.
Sequencing issues	
a) Too low or too high cluster density	Accurate library quantification is the key for optimal cluster density on any sequencing instrument. The QIAseq Library Quant System method, which involves PCR-based quantification, is recommended. Other methods may lead to the incorrect quantification of the library especially when there is overamplification or the library concentration is very high.
b) Very low clusters passing filter	Make sure the library is quantified accurately and that the correct amount is
Clonotype detection issues	
a) Known clonotype not detected	Clonotype and immune repertoire diversity detection sensitivity is directly related to the input RNA and read depth. See Table 2 for suggested sequencing capacity needed.

Appendix A: Library Quantification Using the QIAseq Library Quant System

The library yield measurements can be done with, Qubit, Bioanalyzer or TapeStation, though they may not fully reflect the quantity of complete QIAseq Targeted RNA Panel TCR libraries with full adapter sequences. The conversion of quantification to molar concentration could be different and could be adjusted based on experience. For example, the measurement in ng/uL concentration can be x 1.25 to get nM concentration and loading at 8pM.

For accurate quantification of library, we still recommend quantifying the libraries using QIAGEN's QIAseq Library Quant Array Kit (cat. no. 333304) or QIAseq Library Quant Assay Kit (cat. no. 333314), which contains laboratory-verified forward and reverse primers, together with a DNA standard. With this system, the correct dilution of the library can be determined for sequencing. Please refer to the relevant handbook(s) (available at **www.qiagen.com**) for library quantification.

Important: The extension time should be 4 minutes at 72°C instead of the 2 minutes indicated in the standard protocol of the QIAseq Library Quant Systeml.

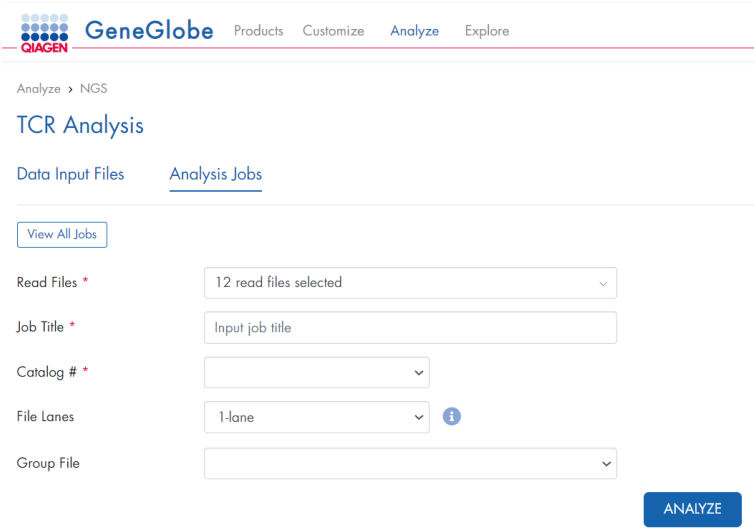
Appendix B: Data Analysis Using QIAGEN's QIAseq Targeted RNA Panel TCR Library Kit Data

Analysis Portal

After sequencing, the results can be analyzed using QIAGEN's QIAseq Targeted RNA Panel TCR Library Kit data analysis portal. Our data analysis software will perform mapping to the reference sequence, UMI counting, read trimming (removing primer sequences) as well as immune repertoire diversity assessment and clonotype classification. For the analysis, use Google Chrome™ or Mozilla® Firefox®, as the portal is not compatible with Internet Explorer™ (IE). Log in or create a QIAGEN account at **www.qiagen.com**. From there, log in to the GeneGlobe Data Analysis Center at: **www.qiagen.com/shop/genes-and-pathways/dataanalysis-center-overview-page/**.

To start the analysis, select:

1. Next-generation sequencing
2. mRNA/lncRNA
3. QIAseq Targeted RNA Panel TCR Library Kit



Select the reads by go through the BaseSpace or upload the fastq files then select the files for analysis.

Name your Job with preferred job title.

Select the catalog number (human or mouse)

Option: If you want to do grouped analysis (such as control and test group), please upload a group file first then select from the list (download TCR_group_template.txt).

Run analysis by clicking the ANALYZE.

Appendix C: Library QC

NGS Library QC

After the library is constructed and purified, QC can be performed with the QIAxcel, Agilent's Bioanalyzer or TapeStation can be used to check for the correct fragment size distribution of library fragments and for the absence of adapters or adapter-dimers (~<200 bp) and concentration with the corresponding kit.

For using QIAxcel Advanced System for NGS library QC, please refer the "NGS sample quality control using the QIAxcel Advanced System" in QIAGEN website.

- Refer to the Application Guide for Medium-Concentration Libraries. To access guides and system files for Library QC, contact QIAGEN Technical Services.
- QIAxcel Cartridge: QIAxcel DNA High Resolution Kit (cat. no. 929002)
- QX Alignment Marker: 15 bp/10 kb (cat. no. 929523)
- QX DNA Size Marker: 100bp – 2.5kb (cat. no. 929559)

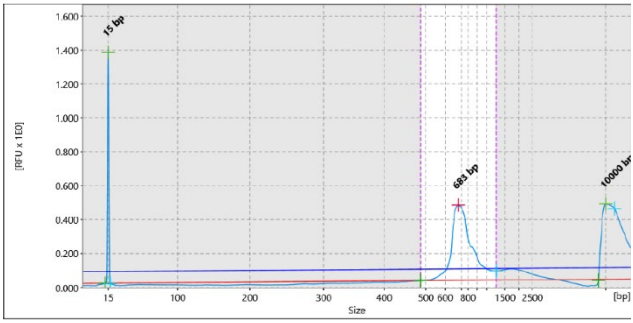


Figure 4. Sample QIAxcel image of QIAseq Targeted RNA TCR panel libraries for Illumina instruments. The library assessed using QIAxcel illustrates the size of the majority of the library fragments are between 500 and 1500 bp.

For using TapeStation (such as 4200) for library QC, please refer Agilent user manual for more details. Agilent High Sensitivity D1000 ScreenTape Libraries prepared with QIAseq

Targeted RNA Panel TCR Library Kits demonstrate a size distribution between 500–1500 bp (Figure 4). Amounts of DNA under the appropriate peaks can be used to quantify the libraries. However, due to the superior sensitivity of qPCR, we recommend quantifying the libraries using the QIAseq Library Quant System (see “Appendix A: Library Quantification Using the QIAseq Library Quant System”, page 44).

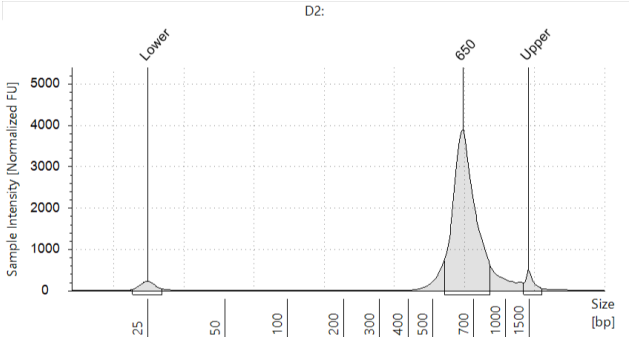


Figure 5. TapeStation image of QIAseq Targeted RNA Panel TCR Kit library for Illumina instruments. The size of most of the library fragments are between 600–1500 bp. Library was prepared using the QIAseq Targeted RNA panel TCR Kit and assessed using an Agilent T

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
QIAseq Targeted RNA Panel Human TCR kit (12)*	All reagents (except indexes) for human TCR immune repertoire sequencing; fixed panel for 12 samples	334651
QIAseq Targeted RNA panel Human TCR kit (96)*	All reagents (except indexes) for human TCR immune repertoire sequencing; fixed panel for 96 samples	334665
QIAseq Targeted RNA panel Mouse TCR kit (12)*	All reagents (except indexes) for mouse TCR immune repertoire sequencing; fixed panel for 12 samples	334671
QIAseq Unique Dual Indices		
QIAseq TUDI-96AK Set A (96)	Box containing unique molecularly indexed primers, enough for a total of 96 reactions – for indexing up to 96 samples uniquely for targeted panel sequencing on Illumina platforms;	334805
QIAseq TUDI-24K (24)	Box containing molecularly-indexed primers, enough for a total of 24 reactions – for indexing up to 24 samples for targeted panel sequencing on Illumina platforms	334792

Related Products

QIAseq Library Quant System	PCR arrays with accessory components for sample library quantification prior to next-generation sequencing	333304
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms, assay format	333314
RNeasy Mini Kit (50)	Reagents for purification of high-quality RNA from cells, tissues, and yeast, 100 µg RNA capacity	74104
RNeasy FFPE Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water	73504
RNeasy Micro Kit (50)	Reagents for purification of high-quality RNA from cells, tissues, and yeast, 45 µg RNA capacity	74004
AllPrep DNA / RNA Mini Kit (50)	Reagents for simultaneous purification of genomic DNA and total RNA from the same biological sample	80204
PAXgene Blood RNA Kit (50)	Reagents for isolation and purification of intracellular RNA from blood stabilized in PAXgene Blood RNA Tubes	762164
QIAamp® RNA Blood Mini Kit (50)	Reagents for purification of cellular RNA from fresh whole blood	52304

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

Document Revision History

Revision	Description
09/2023	Initial release

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Limited License Agreement for QIAseq Targeted RNA panel TCR Kit

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