

Standard Operating Procedure for Bulk RNA TCR Sequencing (Bulk rhTCRseq<sup>1</sup>)


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**Reagents**

- Total RNA samples, at least 20  $\mu\text{L}$  at a concentration of 25 ng/ $\mu\text{L}$
- ProNex Size-Selective Purification System (125 mL, Promega, cat. no. NG2002)
- Water, PCR certified (Teknova, cat. no. W3331)
- TE Buffer (10 mM Tris, pH 8.0; 1 mM EDTA, Teknova, cat. no. T0224)
- DNA Suspension Buffer (10 mM Tris, pH 8.0; 0.1 mM EDTA, Teknova, cat. no. T0221)
- P5.IDTxxx.Rd1x.x1 (Table 2) and P7.IDTyyy.Rd2x.x1 (Table 3) oligonucleotides (Integrated DNA Technologies, custom product). All oligonucleotides are listed in the 5' to 3' direction.
- RNase H2 Enzyme Kit (includes RNase H2 Enzyme at 2 units/ $\mu\text{L}$  and RNase H2 Dilution Buffer, Integrated DNA Technologies, cat. no. 11-02-12-01)
- 1 M Tris-HCl, pH 8.4 (Teknova, cat. no. T1084)
- 1 M KCl (Teknova, cat. no. P0325)
- 1 M  $\text{MgCl}_2$  (Teknova, cat. no. M0302)
- dNTP Mix, 25 mM each (Thermo Fisher, cat. no. FERR1121)
- Rd1.AVxx.x1 and Rd1.BVxx.x1 oligonucleotides at 500  $\mu\text{M}$  each (Table 4, Integrated DNA Technologies, custom product)
- Hot Start Taq DNA Polymerase (New England BioLabs, cat. no. M0495L)
- AMPure XP beads (Beckman Coulter, cat. no. A63880)
- 200 Proof Ethanol (Decon Labs, cat. no. 2716) ! CAUTION Ethanol is flammable, keep away from open flame.
- Beads Buffer: 20% polyethylene glycol (PEG 8000), 2.5M sodium chloride (Teknova, cat. no. P4137)
- P5 primer, AATGATACGGCGACCACCGAGATCTACAC at 10  $\mu\text{M}$  (Integrated DNA Technologies, custom product)
- P7 primer, CAAGCAGAAGACGGCATACTGAGAT at 10  $\mu\text{M}$  (Integrated DNA Technologies, custom product)
- Q5 Hot Start HiFi PCR Master Mix (New England BioLabs, cat. no. M0543S)
- 1 N NaOH (MilliporeSigma, cat. no. 109137) ! CAUTION Causes severe skin burns and eye damage.
- PhiX spike-in library (Control v3, Illumina, cat. no. FC-110-3001)
- MiSeq 300-cycle Reagent Kit v2 (Illumina, cat. no. MS-102-2002)
- 10% NP-40 (Thermo Fisher, cat. no. 28324)
- Rd2.UM7.AC primer, gtgactggagttcagacgtgtgctcttccgatctNHNHNHVTCAGCTGGTACACGGCA at 10  $\mu\text{M}$  (Integrated DNA Technologies, custom product)

- Rd2.UM7.BC primer, gtgactggagttcagacgtgtgctcttccgatctNHNNNHVTCTCTGCTTCTGATGGCTCAA at 10  $\mu$ M (Integrated DNA Technologies, custom product)
- 50% glycerol (Teknova, cat. no. G1796)
- 1 M dithiothreitol (Teknova, cat. no. D9750) ! **CAUTION** Dithiothreitol is harmful if swallowed. It causes skin irritation and serious eye irritation.
- RNaseOUT (40 units/ $\mu$ L, Thermo Fisher, cat. no. 10777019)
- SuperScript II Reverse Transcriptase (200 units/ $\mu$ L, Thermo Fisher, cat. no. 18064014)
- Exonuclease I (20 units/ $\mu$ L, New England BioLabs, cat. no. M0293S)
- RNase H2 Dilution Buffer (Integrated DNA Technologies, cat. no. 11-01-02-12)

### Equipment

- Twin.tec PCR plate (96 wells, LoBind, skirted, PCR clean, colorless, Eppendorf, cat. no. 0030129512)
- 0.2-mL PCR 8-tube FLEX-FREE strip (attached clear flat caps, natural, USA Scientific, cat. no. 1402-4700)
- 20- $\mu$ L 8-channel pipette (Pipet-Lite Multi Pipette L8-20XLS+, Rainin, cat. no. 17013803)
- 200- $\mu$ L 8-channel pipette (Pipet-Lite Multi Pipette L8-200XLS+, Rainin, cat. no. 17013805)
- 2- $\mu$ L pipette (Pipet-Lite LTS Pipette L-2XLS+, Rainin, cat. no. 17014393)
- 20- $\mu$ L pipette (Pipet-Lite LTS Pipette L-20XLS+, Rainin, cat. no. 17014392)
- 200- $\mu$ L pipette (Pipet-Lite LTS Pipette L-200XLS+, Rainin, cat. no. 17014393)
- 1000- $\mu$ L pipette (Pipet-Lite LTS Pipette L-1000XLS+, Rainin, cat. no. 17014382)
- 20- $\mu$ L pipette tips (RT-LTS-A-10 $\mu$ L-/F/L-960/10, Rainin, cat. no. 30389226)
- 200- $\mu$ L pipette tips (RT-LTS-A-200 $\mu$ L-/F/L-960/10, Rainin, cat. no. 30389240)
- 1000- $\mu$ L pipette tips (RT-LTS-A-1000 $\mu$ L-/F/L-768/8, Rainin, cat. no. 30389213)
- 10x magnetic separation stand for 8-tube strip (10x Genomics, cat. no. 230003)
- DynaMag-96 side skirted magnetic separation stand (Thermo Fisher, cat. no. 12027)
- MagneSphere magnetic separation stand (12-hole, 1.5 mL vial, Promega, cat. no. Z5342)
- Cooling block for 96-well PCR plate (Eppendorf, cat. no. 022510509) for keeping plate cool on the deck of the Mantis liquid dispenser. When not in use, block is stored at -20°C.
- DNA LoBind microcentrifuge tubes (1.5 mL, PCR clean, colorless, Eppendorf, cat. no. 022431021)
- LSE vortex mixer (Corning, cat. no. 6775)
- 3-inch head for vortex mixer (Corning, cat. no. 480100)
- Mantis liquid dispenser (Formulatrix, cat. no. MANTV3.2)
- 1250- $\mu$ L pipette tips (Sterile Non-Filtered Extra Long Pipet Tip, Thomas Scientific, cat. no. 1158U40). For use as reservoir on Mantis liquid dispenser.
- 1000- $\mu$ L pipette (PIPETMAN Classic P1000, Gilson, cat. no. F123602). Required for using the 1250- $\mu$ L pipette tips.

- C1000 Touch Thermal Cycler (with 96 Deep Well Reaction Module, Bio-Rad, cat. no. 1851197)
- PX1 PCR Plate Sealer (Bio-Rad, cat. no. 1814000)
- Peelable foil heat seal (Bio-Rad, cat. no. 1814045)
- 5430 microcentrifuge (with 2-place microplate swing bucket rotor, Eppendorf, cat. no. 022620572)
- 2100 Bioanalyzer (Agilent, cat. no. G2939BA)
- High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626)
- MiSeq system (Illumina, cat. no. SY-410-1003)

### Software

- BLAST<sup>2</sup> version NCBI-BLAST-2.2.30+  
(<ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/2.2.30/>)
- MiXCR<sup>3</sup> version 2.1.5 (<https://github.com/milaboratory/mixcr/releases/tag/v2.1.5>)
- Java JDK version 8  
(<https://www.oracle.com/technetwork/java/javase/downloads/jdk8-downloads-2133151.html>)
- GNU parallel version 20180722 (<http://git.savannah.gnu.org/cgit/parallel.git/>)  
[Tange, O. *GNU Parallel 2018* at  
<[https://zenodo.org/record/1146014#.W\\_1SJhNKjmV](https://zenodo.org/record/1146014#.W_1SJhNKjmV)>]
- Scripts and reference files from the Github repository at  
<https://github.com/julietforman/rhTCRseq>

### Reagent setup

#### **P5.IDTxxx.Rd1x.x1 and P7.IDTyyy.Rd2x.x1 index primer plates**

There are 96 P5.IDTxxx.Rd1x.x1 index primers in Plate P5.2 (sequences in Table 2). There are 96 P7.IDTyyy.Rd2x.x1 index primers in Plate P7.3 (sequences in Table 3). These were obtained with each primer at a concentration of 200  $\mu$ M and are stored at -80°C. Tables 2 and 3 include the well position of each primer and its corresponding index, with the indexes designated IDT097-IDT192 for Plate P5.2 and IDT193-IDT288 for Plate P7.3. The 8-nucleotide index is underlined in each of the primer sequences. Prepare Barcode Primer Plate at concentration 6  $\mu$ M each primer by mixing 3  $\mu$ L 200  $\mu$ M P5 barcode primer, 3  $\mu$ L 200  $\mu$ M P7 barcode primer, and 94  $\mu$ L TE Buffer for each well in the 96-well plate.

#### **Rd1.AV.x1 / Rd1.BV.x1 primer mix**

Combine 69 rhPCR primers at a concentration of 5  $\mu$ M each by mixing 5  $\mu$ L 500  $\mu$ M each primer with 155  $\mu$ L TE Buffer. These primers are specific for the V segments of the human alpha and beta TCR genes and are designed to amplify all productive alpha and beta alleles. The sequences are in Table 4. Store at -20°C for up to one year.

#### **Ethanol, 80% (vol/vol)**

Prepare 80% (vol/vol) ethanol just before use by mixing 4 mL 200 proof ethanol with 1 mL water.

#### **5× RT Buffer (75 mM Tris-HCl, pH 8.4; 375 mM KCl; 50 mM MgCl<sub>2</sub>; 25% glycerol)**

Mix 1.5 mL 1 M Tris-HCl, pH 8.4, 7.5 mL 1 M KCl, 1 mL 1 M MgCl<sub>2</sub>, 10 mL 50% glycerol. Store at room temperature for up to one year.

### NaOH, 0.2 N

Prepare just before use by mixing 4  $\mu$ L 1 N NaOH with 16  $\mu$ L water.

## Procedure

For bulk RNA rhTCRseq, use 25 ng total RNA per reaction. RNA samples should be diluted to the same concentration 25 ng/ $\mu$ L. RNA can be isolated by any of the standard methods for purifying total RNA. Four replicates are run for each sample. The protocol is written for analyzing 22 samples. Sample 23 is a Positive Control consisting of an RNA sample that has been successfully analyzed previously. Sample 24 is a Negative Control where water, rather than RNA, is added to each of the four replicates.

### cDNA synthesis • Timing 3 h

- 1 Prepare the following mix and keep on ice:

Component	Volume per well ( $\mu$ L)	Volume for 24 wells with average ( $\mu$ L)	Final concentration in RT reaction
NP-40 (10%)	0.5	15	0.25%
dNTP Mix (25 mM each)	0.4	12	0.5 mM
Rd2.UM7.AC primer (10 $\mu$ M)	0.42	12.6	210 nM
Rd2.UM7.BC primer (10 $\mu$ M)	0.14	4.2	70 nM
Water	3.54	106.2	-
<b>Total volume</b>	<b>5</b>	<b>150</b>	

- 2 Dispense 5  $\mu$ L of this mix to each of 24 wells in 96-well plate.
- 3 Add 5  $\mu$ L 25 ng/ $\mu$ L RNA (or water for the Negative Control) to each well.
- 4 From each well, transfer 2  $\mu$ L to each of 4 wells in a 96-well PCR plate.
- 5 Transfer plate to thermal cycler and run the protocol: 65°C for 5 min, hold at 4°C.
- 6 Prepare the following RT mix and keep on ice:

Component	Volume per reaction ( $\mu$ L)	Volume for 96 reactions with average ( $\mu$ L)	Final concentration in RT reaction
RT Buffer (5 $\times$ )	0.8	96	1 $\times$
Dithiothreitol (1 M)	0.04	4.8	10 mM
RNaseOUT (40 units/ $\mu$ L)	0.08	9.6	0.8 units/ $\mu$ L
SuperScript II Reverse Transcriptase (200 units/ $\mu$ L)	0.02	2.4	1 units/ $\mu$ L
Water	1.06	127.2	-
<b>Total volume</b>	<b>2</b>	<b>240</b>	

- 7 With a 96-well sample plate on the cooling block on the deck of the Mantis, use Mantis to dispense 2  $\mu$ L RT mix to each of the 96 wells.
- 8 Cover the plate with foil and heat seal using plate sealer at 168°C for 3 s.
- 9 Transfer plate to thermal cycler and run the protocol: 42°C for 90 min, 85°C for 5 min, hold at 4°C.
- 10 Prepare diluted exonuclease as follows and keep on ice:

Component	Volume per reaction ( $\mu$ L)	Volume for 96 reactions with overage ( $\mu$ L)	Final concentration in exonuclease reaction
Exonuclease I (20 units/ $\mu$ L)	0.025	3	0.083 units/ $\mu$ L
RNase H2 Dilution Buffer	1.975	237	-
<b>Total volume</b>	<b>2</b>	<b>240</b>	

- 11 With the 96-well sample plate on the cooling block on the deck of the Mantis, use Mantis to dispense 2  $\mu$ L diluted exonuclease to each of the 96 wells.
- 12 Cover the plate with foil and heat seal using plate sealer at 168°C for 3 s.
- 13 Transfer the plate to the thermal cycler and run the protocol: 37°C for 15 min, 85°C for 15 min, hold at 4°C.

**TCR-specific amplification • Timing 3 h**

- 14 From the Barcode Primer Plate, use an 8-channel pipette to add 4  $\mu$ L 6  $\mu$ M each P5.IDTxxx.Rd1x.x1 / P7.IDTyyy.Rd2x.x1 to each of the 96 wells.
- 15 Dilute RNase H2 to 20 mU/ $\mu$ L by mixing 1  $\mu$ L 2 units/ $\mu$ L RNase H2 Enzyme with 99  $\mu$ L RNase H2 Dilution Buffer and keep on ice.
- 16 Prepare the following PCR mix (PCR1) and keep on ice:

Component	Volume per reaction ( $\mu$ L)	Volume for 96 reactions with overage ( $\mu$ L)	Final concentration in PCR reaction
Tris-HCl, pH 8.4 (1 M)	0.18	21.6	15 mM
KCl (1 M)	0.3	36	25 mM
MgCl <sub>2</sub> (1 M)	0.048	5.76	4 mM
dNTP Mix (25 mM each)	0.192	23.04	0.4 mM each
Rd1.AV.x1 / Rd1.BV.x1 primer mix (5 $\mu$ M each)	0.12	14.4	50 nM each
RNase H2 (20 mU/ $\mu$ L)	0.3	36	0.5 mU/ $\mu$ L
Hot Start Taq DNA Polymerase (5 units/ $\mu$ L)	0.48	57.6	0.2 units/ $\mu$ L
Water	0.38	45.6	-
<b>Total volume</b>	<b>2</b>	<b>240</b>	

- 17 With the 96-well sample plate on the cooling block on the deck of the Mantis, use Mantis to dispense 2  $\mu$ L PCR mix to each of the 96 wells.
- 18 Cover the plate with foil and heat seal using plate sealer at 168°C for 3 s.
- 19 Transfer plate to thermal cycler and run the protocol:

Cycle number	Denature	Anneal	Extend
1	95°C, 5 min		
2-21	96°C, 20 s	60°C, 6 min	

**Pooling, purification, and final PCR.** • Timing 3.5 h

20 Pool all the reactions by mixing 2 µL of each sample. Pooling is done by using an 8-channel pipette to transfer samples from all wells of each column repeatedly into the same wells of one column of a new 96-well PCR plate. This requires 12 transfers for a 96-well plate. The eight pooled samples are then combined by transferring to a 1.5-mL microcentrifuge tube to generate a 192-µL pooled sample for a 96-well plate. Store remainder of unpooled samples at -20°C for up to six months.

21 Warm AMPure XP beads to room temperature.

22 To the pooled sample, add:

Component	Volume for pool from 96-well plate (µL)
AMPure XP beads	19.2
Beads Buffer	96

23 Pipet up and down, then incubate at room temperature for 5 min.

24 Place the tube on the magnetic stand and wait 5 min or until solution clears for beads to collect.

25 Transfer supernatant to fresh tube and add:

Component	Volume for pool from 96-well plate (µL)
AMPure XP beads	6.4
Beads Buffer	32

26 Pipet up and down, then incubate at room temperature for 5 min.

27 Place the tube on the magnetic stand, wait until solution clears (approximately 5 min for beads to collect), and discard supernatant.

28 With tube remaining on the magnetic stand, wash the beads by adding 1 mL freshly prepared 80% ethanol, waiting 30 s, and discarding the supernatant.

29 Repeat Step 28 one more time. Remove as much residual 80% ethanol as possible.

30 After air drying the beads for 5 min, add 21 µL DNA Suspension Buffer.

31 Pipet up and down, place the tube on the magnetic stand, wait 2 min for beads to collect, then transfer 20 µL supernatant to one of the unused wells in the 96-well PCR plate used to pool samples (Step 20).

32 Add 16 µL AMPure XP beads.

33 Repeat Steps 26-31 using the 96-well magnetic stand and 100 µL 80% ethanol for Steps 28 and 29, then transferring 20 µL supernatant to another unused well in the 96-well PCR plate.

34 In an adjacent unused well in the 96-well PCR plate, prepare 1:10 dilution by mixing 2 µL purified pooled sample and 18 µL DNA Suspension Buffer.

■PAUSE POINT Samples can be stored for up to one week at -20°C.

35 In 8-tube PCR strip, prepare two PCR reactions (PCR2) on ice. Each reaction contains:

Component	Volume per sample (µL)	Final concentration in PCR reaction
Purified pooled sample from Step 33 (original) or Step 34 (1:10 dilution)	5	-
P5 primer (10 µM)	2.5	0.5 µM
P7 primer (10 µM)	2.5	0.5 µM
Q5 Hot Start HiFi PCR Master Mix (2×)	25	1×
Water	15	-
<b>Total volume</b>	<b>50</b>	

36 Transfer strip to thermal cycler and run the protocol:

Cycle number	Denature	Anneal	Extend
1	98°C, 30 s		
2-18	98°C, 10 s	62°C, 2 min	
19			75°C, 2 min

37 Warm ProNex beads to room temperature.

38 To each of the two PCR samples from Step 36, add 55 µL ProNex beads.

39 Pipet up and down, then incubate at room temperature for 10 min.

40 Place the strip on the 10x magnetic stand in the High orientation, wait until solution clears (approximately 2 min for beads to collect), then transfer supernatants to two unused tubes in the strip.

41 Add 15 µL ProNex beads to each, pipet up and down, and incubate at room temperature for 10 min.

42 Place the strip on the 10x magnetic stand in the High orientation, wait until solution clears (approximately 2 min for beads to collect), then discard supernatants.

43 With strip remaining in the magnetic stand, wash the beads by adding 100 µL Promega Wash Buffer per well, waiting 30 s, and discarding the supernatants.

44 Repeat wash step 43 one time. Remove as much residual Wash Buffer as possible.

45 After air drying the beads for 10 min, add 20 µL Promega Elution Buffer to each sample.

46 Pipet up and down, place in the 10x magnetic stand in the Low orientation, wait until solution clears (approximately 2 min for beads to collect), then transfer the two supernatants to unused wells in the 96-well plate used to store the samples after purification of the pooled TCR-specific PCR (Step 34).

47 Use 1 µL of each of the two final PCR products from Step 46 to measure the fragment size distribution and estimate concentration using the 2100 BioAnalyzer and High Sensitivity DNA Kit. Fragment size should be a set of peaks predominantly in the range 260-400 bp. Concentration is determined setting the

size range to 200-1000 bp. Of the two libraries, the one with the flattest baseline is used for sequencing.

- PAUSE POINT Amplified libraries can be stored for at least one year at -20°C.

### Sequencing • Timing 28 h

Perform sequencing using the 300-cycle Reagent Kit v2 on the Illumina MiSeq according to the manufacturer's protocol. As part of the set-up, upload a sample sheet like the one found in Table 5 to the instrument. This sample sheet specifies 248 nt read 1, 48 nt read 2, 8 nt index 1, and 8 nt index 2. We have found that the sequencing depth using the MiSeq has been adequate for analyzing 96 bulk RNA reactions at 25 ng RNA per reaction.

- 48 Dilute the selected (see Step 47) final library from Step 46 to 4 nM using water.
- 49 Mix 4.5 µL 4 nM library, 0.5 µL PhiX spike-in, and 5 µL freshly prepared 0.2 N NaOH, then incubate at room temperature for 5 min.
- 50 Add 990 µL Hyb Buffer (from MiSeq kit), mix by inversion, and place on ice. The library concentration is now 20 pM.
- 51 To prepare final loading library at 8 pM, mix 400 µL 20 pM library with 600 µL Hyb Buffer.
- 52 Load 600 µL 8 pM library into MiSeq cartridge then follow the Illumina instructions to run the sequencer.

### Data analysis • Timing 8 h

The required input for the rhTCRseq pipeline includes the sequencing reads in fastq.gz files separated by well, with four files per well. Each filename for each well should begin with the fastq\_basename prefix for that well, and the four filenames should end in \_L001\_R1\_001, \_L001\_R2\_001, \_L001\_I1\_001, and \_L001\_I2\_001. Also required is the SampleSheet.csv file for the run.

- 53 Install MiXCR-2.1.5, NCBI Blast, Java, and GNU Parallel.
- 54 Set up directory structure as follows:
  - choose a `ROOT_DIR` in which all requirements for the pipeline will go
  - create a directory `<ROOT_DIR>/scripts` (add to this folder all of the files from the Github repository folder `rhTCRseq/scripts`)
  - create a directory `<ROOT_DIR>/data`
  - create a directory `<ROOT_DIR>/out`
  - create a directory `<ROOT_DIR>/blast_database` (add to this folder all of the blast database files from the Github repository folder `rhTCRseq/blast_database`)
- 55 Prepare to analyze a particular run:
  - open the config file `<ROOT_DIR>/scripts/config.py` and edit the variables in the file to match your run
  - create a directory in `<ROOT_DIR>/data/` with the same name as `RUN_NAME` in `config.py` (place the fastq.gz files from the sequencing run in this folder)



create a directory in <ROOT\_DIR>/out/ with the same name as RUN\_NAME  
in config.py (place SampleSheet.csv for the run into this folder)

After these steps are complete, the directory structure should look like this:

```

ROOT_DIR
├── blast_database
│   ├── TRV_primer.fasta
│   ├── TRV_primer.fasta.nhr
│   ├── TRV_primer.fasta.nin
│   ├── TRV_primer.fasta.nog
│   ├── TRV_primer.fasta.nsd
│   ├── TRV_primer.fasta.nsi
│   ├── TRV_primer.fasta.nsq
│   ├── TRC_primer.fasta
│   ├── TRC_primer.fasta.nhr
│   ├── TRC_primer.fasta.nin
│   ├── TRC_primer.fasta.nog
│   ├── TRC_primer.fasta.nsd
│   ├── TRC_primer.fasta.nsi
│   ├── TRC_primer.fasta.nsq
│   ├── target_gene_primer_forward.fasta
│   ├── target_gene_primer_forward.fasta.nhr
│   ├── target_gene_primer_forward.fasta.nin
│   ├── target_gene_primer_forward.fasta.nog
│   ├── target_gene_primer_forward.fasta.nsd
│   ├── target_gene_primer_forward.fasta.nsi
│   ├── target_gene_primer_forward.fasta.nsq
│   ├── target_gene_primer_reverse.fasta
│   ├── target_gene_primer_reverse.fasta.nhr
│   ├── target_gene_primer_reverse.fasta.nin
│   ├── target_gene_primer_reverse.fasta.nog
│   ├── target_gene_primer_reverse.fasta.nsd
│   ├── target_gene_primer_reverse.fasta.nsi
│   ├── target_gene_primer_reverse.fasta.nsq
│   ├── target_gene_reverse.fasta
│   ├── target_gene_reverse.fasta.nhr
│   ├── target_gene_reverse.fasta.nin
│   ├── target_gene_reverse.fasta.nog
│   ├── target_gene_reverse.fasta.nsd
│   ├── target_gene_reverse.fasta.nsi
│   ├── target_gene_reverse.fasta.nsq
│   ├── target_gene_forward.fasta
│   ├── target_gene_forward.fasta.nhr
│   ├── target_gene_forward.fasta.nin
│   ├── target_gene_forward.fasta.nog
│   ├── target_gene_forward.fasta.nsd
│   ├── target_gene_forward.fasta.nsi
│   └── target_gene_forward.fasta.nsq

```

```

data
  <RUN_NAME>
    <fastq.gz files>
out
  <RUN_NAME>
    SampleSheet.csv
scripts
  blast.sh
  count_umi.py
  separate_fastq.py
  collapse_rules.txt
  parse_blast_results.py
  analyze_tcr.py
  compare_clonotype.py
  merge_TRBV.py
  mixcr.sh
  get_parallel_range.py
  print_description.py
  config.py
  run_pipeline.sh
  make_index_list.py

```

#### 56 Run the pipeline:

```

cd <ROOT_DIR>/scripts
./run_pipeline.sh

```

The output files are shown in Table 1. There are separate [sample]\_clones\_umi\_count.csv files for alpha and beta. These files combine the UMI counts for the four replicates and are the files that are used to calculate the clonotype frequencies for each sample. Because there are four replicates of 25 ng each, the final results for each sample are for 100 ng total RNA.

#### References

1. Li, S. *et al.* RNase H-dependent PCR-enabled T-cell receptor sequencing for highly specific and efficient targeted sequencing of T-cell receptor mRNA for single-cell and repertoire analysis. *Nat. Protoc.* **14**, 2571–2594 (2019).
2. Camacho, C. *et al.* BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421 (2009).
3. Bolotin, D. A. *et al.* MiXCR: software for comprehensive adaptive immunity profiling. *Nat. Methods* **12**, 380–381 (2015).

Table 1 | Data analysis output files for bulk RNA protocol

Results folder	Content
[well]_clonotype_count_TRA.csv	For each well, reports all unique TRA clonotypes with V identity, J identity, CDR3 nucleic acid sequence, CDR3 amino acid sequence, read count, and UMI count
[well]_clonotype_count_TRB.csv	For each well, reports all unique TRB clonotypes with V identity, J identity, CDR3 nucleic acid sequence, CDR3 amino acid sequence, read count, and UMI count
[sample]_clones_count.csv	Read counts for combined replicates
[sample]_clones_umi_count.csv	UMI counts for combined replicates
[sample]_observation_across_replicates.csv	Number of replicates in which each clonotype appears
[sample]_clonotype_count.csv	Number of clonotypes with each V gene for combined replicates
[sample]_clonotype_count.png	Bar plot of clonotypes per V gene for combined replicates

Table 2. | Plate P5.2

Well Position	Name	Sequence
A01	P5.IDT097.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GCCTATCA</u> AACTCTTTCCCTACrACGACa/3SpC3/
B01	P5.IDT098.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTTGGATG</u> AACTCTTTCCCTACrACGACa/3SpC3/
C01	P5.IDT099.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGTCTCAC</u> AACTCTTTCCCTACrACGACa/3SpC3/
D01	P5.IDT100.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTCATCAG</u> AACTCTTTCCCTACrACGACa/3SpC3/
E01	P5.IDT101.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TGTACCGT</u> AACTCTTTCCCTACrACGACa/3SpC3/
F01	P5.IDT102.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACA <u>AGTCGAG</u> AACTCTTTCCCTACrACGACa/3SpC3/
G01	P5.IDT103.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CCACGTTGT</u> AACTCTTTCCCTACrACGACa/3SpC3/
H01	P5.IDT104.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTCACAGCA</u> AACTCTTTCCCTACrACGACa/3SpC3/
A02	P5.IDT105.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTACTTGG</u> AACTCTTTCCCTACrACGACa/3SpC3/
B02	P5.IDT106.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CCCTCAGTT</u> AACTCTTTCCCTACrACGACa/3SpC3/
C02	P5.IDT107.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TCCCTACCT</u> AACTCTTTCCCTACrACGACa/3SpC3/
D02	P5.IDT108.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ATGGCGAA</u> AACTCTTTCCCTACrACGACa/3SpC3/
E02	P5.IDT109.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTTACCTG</u> AACTCTTTCCCTACrACGACa/3SpC3/
F02	P5.IDT110.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTCGATA</u> CACTCTTTCCCTACrACGACa/3SpC3/
G02	P5.IDT111.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TCCGTGAA</u> AACTCTTTCCCTACrACGACa/3SpC3/
H02	P5.IDT112.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TAGAGCTC</u> AACTCTTTCCCTACrACGACa/3SpC3/
A03	P5.IDT113.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TGACTGAC</u> AACTCTTTCCCTACrACGACa/3SpC3/
B03	P5.IDT114.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TAGACGTG</u> AACTCTTTCCCTACrACGACa/3SpC3/
C03	P5.IDT115.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CCCGGAATT</u> AACTCTTTCCCTACrACGACa/3SpC3/
D03	P5.IDT116.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTCCTAGA</u> AACTCTTTCCCTACrACGACa/3SpC3/
E03	P5.IDT117.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CAACGGAT</u> AACTCTTTCCCTACrACGACa/3SpC3/
F03	P5.IDT118.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TGGCTATC</u> AACTCTTTCCCTACrACGACa/3SpC3/
G03	P5.IDT119.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGGTCATA</u> AACTCTTTCCCTACrACGACa/3SpC3/
H03	P5.IDT120.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TCCAATCG</u> AACTCTTTCCCTACrACGACa/3SpC3/

A04	P5.IDT121.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GAGCTTGT</u> ACTCTTTCCCTACrACGACa/3SpC3/
B04	P5.IDT122.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GAAGGTT</u> CACACTCTTTCCCTACrACGACa/3SpC3/
C04	P5.IDT123.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ATCTCGCT</u> ACTCTTTCCCTACrACGACa/3SpC3/
D04	P5.IDT124.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGTTACGG</u> ACACTCTTTCCCTACrACGACa/3SpC3/
E04	P5.IDT125.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTGTCTGA</u> ACTCTTTCCCTACrACGACa/3SpC3/
F04	P5.IDT126.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>GACTTCG</u> ACACTCTTTCCCTACrACGACa/3SpC3/
G04	P5.IDT127.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>TGGATCAC</u> ACTCTTTCCCTACrACGACa/3SpC3/
H04	P5.IDT128.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ACACCAGT</u> ACTCTTTCCCTACrACGACa/3SpC3/
A05	P5.IDT129.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CAGGTTAG</u> ACTCTTTCCCTACrACGACa/3SpC3/
B05	P5.IDT130.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGTTGGCT</u> ACTCTTTCCCTACrACGACa/3SpC3/
C05	P5.IDT131.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>CAACTGG</u> ACTCTTTCCCTACrACGACa/3SpC3/
D05	P5.IDT132.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTGC</u> ACTTACTCTTTCCCTACrACGACa/3SpC3/
E05	P5.IDT133.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ACACGGT</u> TACTCTTTCCCTACrACGACa/3SpC3/
F05	P5.IDT134.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AATACGCG</u> ACTCTTTCCCTACrACGACa/3SpC3/
G05	P5.IDT135.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>TGCG</u> AACTACTCTTTCCCTACrACGACa/3SpC3/
H05	P5.IDT136.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGCTG</u> ACTAACTCTTTCCCTACrACGACa/3SpC3/
A06	P5.IDT137.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTGGTGT</u> TACTCTTTCCCTACrACGACa/3SpC3/
B06	P5.IDT138.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTGCTT</u> ACTACTCTTTCCCTACrACGACa/3SpC3/
C06	P5.IDT139.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>CAAGG</u> ACACTCTTTCCCTACrACGACa/3SpC3/
D06	P5.IDT140.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>GAACTG</u> ACTCTTTCCCTACrACGACa/3SpC3/
E06	P5.IDT141.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGTGTGG</u> ACTCTTTCCCTACrACGACa/3SpC3/
F06	P5.IDT142.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTA</u> CTCTCACACTCTTTCCCTACrACGACa/3SpC3/
G06	P5.IDT143.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CCGTAT</u> CTACTCTTTCCCTACrACGACa/3SpC3/
H06	P5.IDT144.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGAAGA</u> ACACTCTTTCCCTACrACGACa/3SpC3/
A07	P5.IDT145.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGCGGA</u> ATACTCTTTCCCTACrACGACa/3SpC3/
B07	P5.IDT146.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTGAGCT</u> TACTCTTTCCCTACrACGACa/3SpC3/
C07	P5.IDT147.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGTGAT</u> CAACTCTTTCCCTACrACGACa/3SpC3/

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D07	P5.IDT148.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>TCGCATTG</u> ACTCTTTCCCTACrACGACa/3SpC3/
E07	P5.IDT149.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>TGACGCAT</u> ACTCTTTCCCTACrACGACa/3SpC3/
F07	P5.IDT150.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CCGATGTA</u> ACTCTTTCCCTACrACGACa/3SpC3/
G07	P5.IDT151.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>TTGCGAGT</u> ACTCTTTCCCTACrACGACa/3SpC3/
H07	P5.IDT152.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ACGACAGA</u> ACTCTTTCCCTACrACGACa/3SpC3/
A08	P5.IDT153.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGCTTG</u> AGACTCTTTCCCTACrACGACa/3SpC3/
B08	P5.IDT154.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GAGTGGT</u> TACTCTTTCCCTACrACGACa/3SpC3/
C08	P5.IDT155.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GCTGTA</u> AGACTCTTTCCCTACrACGACa/3SpC3/
D08	P5.IDT156.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CCAAGACT</u> TACTCTTTCCCTACrACGACa/3SpC3/
E08	P5.IDT157.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ATTGCGT</u> GACTCTTTCCCTACrACGACa/3SpC3/
F08	P5.IDT158.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTGAAGCT</u> TACTCTTTCCCTACrACGACa/3SpC3/
G08	P5.IDT159.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>AA</u> CGAGGACTCTTTCCCTACrACGACa/3SpC3/
H08	P5.IDT160.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>TCGTCTCA</u> ACTCTTTCCCTACrACGACa/3SpC3/
A09	P5.IDT161.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>TCCTGT</u> GACTCTTTCCCTACrACGACa/3SpC3/
B09	P5.IDT162.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGTTGAGT</u> TACTCTTTCCCTACrACGACa/3SpC3/
C09	P5.IDT163.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGTCGCTT</u> TACTCTTTCCCTACrACGACa/3SpC3/
D09	P5.IDT164.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>AGGTAGG</u> ACTCTTTCCCTACrACGACa/3SpC3/
E09	P5.IDT165.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CCAGGAGAT</u> ACTCTTTCCCTACrACGACa/3SpC3/
F09	P5.IDT166.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CATCGTGA</u> ACTCTTTCCCTACrACGACa/3SpC3/
G09	P5.IDT167.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>GTTGTG</u> GACTCTTTCCCTACrACGACa/3SpC3/
H09	P5.IDT168.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ACAGACCT</u> TACTCTTTCCCTACrACGACa/3SpC3/
A10	P5.IDT169.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGTCCTTCT</u> TACTCTTTCCCTACrACGACa/3SpC3/
B10	P5.IDT170.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACACT <u>GTATACGC</u> ACTCTTTCCCTACrACGACa/3SpC3/
C10	P5.IDT171.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTGTGTTG</u> ACTCTTTCCCTACrACGACa/3SpC3/
D10	P5.IDT172.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AACGTGGA</u> ACTCTTTCCCTACrACGACa/3SpC3/
E10	P5.IDT173.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTTGCGAT</u> ACTCTTTCCCTACrACGACa/3SpC3/
F10	P5.IDT174.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AACGACGT</u> TACTCTTTCCCTACrACGACa/3SpC3/

G10	P5.IDT175.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGTATTCG</u> ACTCTTTCCCTACrACGACa/3SpC3/
H10	P5.IDT176.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGCAAGCA</u> AACTCTTTCCCTACrACGACa/3SpC3/
A11	P5.IDT177.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TGTTTCGAG</u> ACTCTTTCCCTACrACGACa/3SpC3/
B11	P5.IDT178.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTCCATGT</u> ACTCTTTCCCTACrACGACa/3SpC3/
C11	P5.IDT179.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGTCTTGT</u> ACTCTTTCCCTACrACGACa/3SpC3/
D11	P5.IDT180.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ATAAGGCG</u> ACTCTTTCCCTACrACGACa/3SpC3/
E11	P5.IDT181.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>TGTCTGCT</u> ACTCTTTCCCTACrACGACa/3SpC3/
F11	P5.IDT182.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CGCTTAAC</u> AACTCTTTCCCTACrACGACa/3SpC3/
G11	P5.IDT183.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GATCCATG</u> ACTCTTTCCCTACrACGACa/3SpC3/
H11	P5.IDT184.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ACCTCTGT</u> ACTCTTTCCCTACrACGACa/3SpC3/
A12	P5.IDT185.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GCCACTTA</u> AACTCTTTCCCTACrACGACa/3SpC3/
B12	P5.IDT186.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ACCTGACT</u> ACTCTTTCCCTACrACGACa/3SpC3/
C12	P5.IDT187.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTTAAGGC</u> ACTCTTTCCCTACrACGACa/3SpC3/
D12	P5.IDT188.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ATGCCAAC</u> AACTCTTTCCCTACrACGACa/3SpC3/
E12	P5.IDT189.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>AGAGGTTG</u> ACTCTTTCCCTACrACGACa/3SpC3/
F12	P5.IDT190.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>ACCATCCA</u> AACTCTTTCCCTACrACGACa/3SpC3/
G12	P5.IDT191.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>GTGGATAG</u> ACTCTTTCCCTACrACGACa/3SpC3/
H12	P5.IDT192.Rd1x.x1	AATGATACGGCGACCACCGAGATCTACAC <u>CTGAGATC</u> AACTCTTTCCCTACrACGACa/3SpC3/

Table 3. | Plate P7.3

Well Position	Name	Sequence
A01	P7.IDT193.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CCTTCGTT</u> CGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B01	P7.IDT194.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GTCTAGGT</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
C01	P7.IDT195.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>ACGTCGT</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D01	P7.IDT196.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GAGCTCA</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
E01	P7.IDT197.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CGTGTACT</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
F01	P7.IDT198.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CAC</u> TGACAGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G01	P7.IDT199.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCGTAGT</u> CGTGACTGGAGTTCAGArCGTGTa/3SpC3/
H01	P7.IDT200.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GCACG</u> TAAAGTGACTGGAGTTCAGArCGTGTa/3SpC3/
A02	P7.IDT201.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CAAGCAG</u> TGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B02	P7.IDT202.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>ACATAGG</u> CGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C02	P7.IDT203.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TGTGGT</u> ACGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D02	P7.IDT204.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CACCACT</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
E02	P7.IDT205.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CTGCGT</u> ATGTGACTGGAGTTCAGArCGTGTa/3SpC3/
F02	P7.IDT206.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>ACGGTCT</u> TGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G02	P7.IDT207.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GATTGG</u> AGGTGACTGGAGTTCAGArCGTGTa/3SpC3/
H02	P7.IDT208.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TGTCCAG</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
A03	P7.IDT209.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CCAGTGT</u> TGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B03	P7.IDT210.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TGCACCA</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C03	P7.IDT211.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TTTACAG</u> GGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D03	P7.IDT212.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>AGGCAT</u> AGGTGACTGGAGTTCAGArCGTGTa/3SpC3/
E03	P7.IDT213.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TAGCCGA</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
F03	P7.IDT214.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TTTGT</u> CGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G03	P7.IDT215.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CATCTAC</u> GGTGACTGGAGTTCAGArCGTGTa/3SpC3/
H03	P7.IDT216.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GCATA</u> CAGGTGACTGGAGTTCAGArCGTGTa/3SpC3/



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A04	P7.IDT217.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATACAGCAACGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B04	P7.IDT218.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCTGGTTCTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C04	P7.IDT219.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATTTCGACATCGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D04	P7.IDT220.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATAAACCCTCCTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
E04	P7.IDT221.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCAGCGATTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
F04	P7.IDT222.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATAGGTCACCTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G04	P7.IDT223.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATGCAATTCGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
H04	P7.IDT224.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATGCTTCTTGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
A05	P7.IDT225.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATAACTGGTGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B05	P7.IDT226.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCGGAATACGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C05	P7.IDT227.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATGCTTCGAAAGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D05	P7.IDT228.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCAAGGTCTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
E05	P7.IDT229.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATAACCTTGGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
F05	P7.IDT230.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCCATACGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G05	P7.IDT231.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATTGGTCTTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
H05	P7.IDT232.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATACCGCATAGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
A06	P7.IDT233.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCCTTCCTTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B06	P7.IDT234.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATTACACGCTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C06	P7.IDT235.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATTGCGTAGAGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D06	P7.IDT236.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATAAGAGCCAGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
E06	P7.IDT237.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATATGGAAGGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
F06	P7.IDT238.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATGCCAGTATGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G06	P7.IDT239.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCGTAGGTTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
H06	P7.IDT240.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCGAGTATGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
A07	P7.IDT241.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCAAGTGCAGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B07	P7.IDT242.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATTCGAGTGAGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C07	P7.IDT243.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATCTACAGTGGTGTGACTGGAGTTCAGArCGTGTa/3SpC3/

D07	P7.IDT244.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GATCGTAC</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
E07	P7.IDT245.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CTTACCAG</u> TGACTGGAGTTCAGArCGTGTa/3SpC3/
F07	P7.IDT246.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CTCAGCTAG</u> TGACTGGAGTTCAGArCGTGTa/3SpC3/
G07	P7.IDT247.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCTGCTCT</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
H07	P7.IDT248.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATA <u>AACCGAAG</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
A08	P7.IDT249.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GCCTGTTGT</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
B08	P7.IDT250.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TTTACGGCT</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
C08	P7.IDT251.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GACAAGAG</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
D08	P7.IDT252.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>AGGATCTGG</u> TGACTGGAGTTCAGArCGTGTa/3SpC3/
E08	P7.IDT253.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GTAGCATC</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
F08	P7.IDT254.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GTGTTCC</u> TGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G08	P7.IDT255.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>AGGATGGT</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
H08	P7.IDT256.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCACGTT</u> CGTGACTGGAGTTCAGArCGTGTa/3SpC3/
A09	P7.IDT257.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GCGTTCT</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B09	P7.IDT258.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CTCTGGT</u> TGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C09	P7.IDT259.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TTTAGGT</u> CGGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D09	P7.IDT260.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCTGAGAG</u> GTGACTGGAGTTCAGArCGTGTa/3SpC3/
E09	P7.IDT261.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TTTCAGC</u> CTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
F09	P7.IDT262.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCTCCG</u> ATGTGACTGGAGTTCAGArCGTGTa/3SpC3/
G09	P7.IDT263.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CAGGTAT</u> CGTGACTGGAGTTCAGArCGTGTa/3SpC3/
H09	P7.IDT264.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>AGTCAGG</u> AGTGACTGGAGTTCAGArCGTGTa/3SpC3/
A10	P7.IDT265.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATA <u>AAGGCT</u> GAGTGACTGGAGTTCAGArCGTGTa/3SpC3/
B10	P7.IDT266.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CGATGC</u> TTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
C10	P7.IDT267.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GTATTGG</u> CGTGACTGGAGTTCAGArCGTGTa/3SpC3/
D10	P7.IDT268.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATA <u>ACTGTG</u> TCGTGACTGGAGTTCAGArCGTGTa/3SpC3/
E10	P7.IDT269.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TGCC</u> TCTTGTGACTGGAGTTCAGArCGTGTa/3SpC3/
F10	P7.IDT270.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CAGTCT</u> TCGTGACTGGAGTTCAGArCGTGTa/3SpC3/

G10	P7.IDT271.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CATAACGGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
H10	P7.IDT272.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATA <u>CTGCTAGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
A11	P7.IDT273.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATAT <u>TCTGGCGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
B11	P7.IDT274.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TTCTCTCGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
C11	P7.IDT275.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCCGAGTTGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
D11	P7.IDT276.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CGAACTGTGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
E11	P7.IDT277.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATA <u>AACGGTCAGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
F11	P7.IDT278.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>AGCAGATGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
G11	P7.IDT279.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TATCAGCGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
H11	P7.IDT280.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCAGACGAGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
A12	P7.IDT281.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATA <u>CCATGTGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
B12	P7.IDT282.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CTAACTCGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
C12	P7.IDT283.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GCTTAGCTGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
D12	P7.IDT284.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>CATGGAACGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
E12	P7.IDT285.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TAGGATGCGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
F12	P7.IDT286.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>GTTTCATGGGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
G12	P7.IDT287.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGAT <u>TCGTGGATGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/
H12	P7.IDT288.Rd2x.x1	CAAGCAGAAGACGGCATAACGAGATA <u>ACCTTCTCGTGACTGGAGTTCAGArCGTGTa</u> /3SpC3/

**Table 4. | TCR-Specific Primers**

Name	Sequence	Notes
Rd1.AV01.x1	ctctttccctacacgacgctctttccgatctAACTGCACGTACCAGACATCTrGGGTa/3SpC3/	Amplifies TRAV1-1, TRAV1-2
Rd1.AV02.x1	ctctttccctacacgacgctctttccgatctTCATCGCTGCTCATCTCCrAGGTGa/3SpC3/	Amplifies TRAV2
Rd1.AV03.x1	ctctttccctacacgacgctctttccgatctCCTGGTTAAAGGCAGCTATGGrCTTTGc/3SpC3/	Amplifies TRAV3
Rd1.AV04.x1	ctctttccctacacgacgctctttccgatctGCCGACAGAAAGTCCAGCrACTCTa/3SpC3/	Amplifies TRAV4
Rd1.AV05.x1	ctctttccctacacgacgctctttccgatctTCTGCGCATTGCAGACACrCCAGAA/3SpC3/	Amplifies TRAV5
Rd1.AV06.x1	ctctttccctacacgacgctctttccgatctTGAAGGTACCTTTGATACCACCrCTTAAC/3SpC3/	Amplifies TRAV6
Rd1.AV07.x1	ctctttccctacacgacgctctttccgatctCCGTGCAGCCTGAAGATTCrAGCCAa/3SpC3/	Amplifies TRAV7
Rd1.AV08-1.x1	ctctttccctacacgacgctctttccgatctTGGTCAACACCTTCAGCTTCTrCCTCAc/3SpC3/	Amplifies TRAV8-1
Rd1.AV08-2/4/6.x1	ctctttccctacacgacgctctttccgatctAAGGACTCCAGCTTCTCTGrAAGTAG/3SpC3/	Amplifies TRAV8-2, TRAV8-4, TRAV8-6
Rd1.AV08-3.x1	ctctttccctacacgacgctctttccgatctGGAACCCCTCTGTGCATTGGrAGTGAc/3SpC3/	Amplifies TRAV8-3
Rd1.AV9.x1	ctctttccctacacgacgctctttccgatctGAAACCACTTCTTTCCACTTGGArGAAAGc/3SpC3/	Amplifies TRAV9-1, TRAV9-2
Rd1.AV10.x1	ctctttccctacacgacgctctttccgatctCACAAAGCAAAGCTCTCTGCArCATCAa/3SpC3/	Amplifies TRAV10
Rd1.AV12.x1	ctctttccctacacgacgctctttccgatctCAGTGATTCAGCCACCTACCTrCTGTGa/3SpC3/	Amplifies TRAV12-1, TRAV12-2, TRAV12-3
Rd1.AV13-1.x1	ctctttccctacacgacgctctttccgatctACAAGACAGCCAAACATTTCTCCrCTGCAa/3SpC3/	Amplifies TRAV13-1
Rd1.AV13-2.x1	ctctttccctacacgacgctctttccgatctTGCAGTACTCAACCTGGArGACTCc/3SpC3/	Amplifies TRAV13-2
Rd1.AV14.x1	ctctttccctacacgacgctctttccgatctACCTTGTCATCTCCGCTTCArCAACTa/3SpC3/	Amplifies TRAV14/DV4
Rd1.AV16.x1	ctctttccctacacgacgctctttccgatctGGCGAGACATCTTTCCACCTrGAAGAc/3SpC3/	Amplifies TRAV16
Rd1.AV17.x1	ctctttccctacacgacgctctttccgatctAGTCACGCTTGACACTTCCArAGAAAc/3SpC3/	Amplifies TRAV17
Rd1.AV18.x2	ctctttccctacacgacgctctttccgatctCAGTTCCTTCCACCTGGAGArAGCCCa/3SpC3/	Amplifies TRAV18
Rd1.AV19.x1	ctctttccctacacgacgctctttccgatctCACAGCCTCACAAAGTCGTGrGACTCc/3SpC3/	Amplifies TRAV19
Rd1.AV20.x1	ctctttccctacacgacgctctttccgatctCTGCACATCACAGCCCTArAACCTa/3SpC3/	Amplifies TRAV20
Rd1.AV21.x1	ctctttccctacacgacgctctttccgatctACATTGCAGCTTCTCAGCCTrGGTGAA/3SpC3/	Amplifies TRAV21
Rd1.AV22.x1	ctctttccctacacgacgctctttccgatctTCCTCTTCCCAGACCAGArCTCAGA/3SpC3/	Amplifies TRAV22
Rd1.AV23.x1	ctctttccctacacgacgctctttccgatctGATTCGCCAGCCTGGAGACTCrAGCCAa/3SpC3/	Amplifies TRAV23/DV6

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Rd1.AV24.x1	ctctttccctacacgacgctcttccgatctGTACATCAAAGGATCCCAGCCTrGAAGAA/3SpC3/	Amplifies TRAV24
Rd1.AV25.x1	ctctttccctacacgacgctcttccgatctGCCACCCAGACTACAGATGTrAGGAAA/3SpC3/	Amplifies TRAV25
Rd1.AV26-1.x1	ctctttccctacacgacgctcttccgatctCGCTACGCTGAGAGACACTrGCTGTa/3SpC3/	Amplifies TRAV26-1
Rd1.AV26-2.x1	ctctttccctacacgacgctcttccgatctTGGCAATCGCTGAAGACAGArAAGTca/3SpC3/	Amplifies TRAV26-2
Rd1.AV27.x1	ctctttccctacacgacgctcttccgatctTGCAAGAAAGGACAGTTCTCTCCrACATCc/3SpC3/	Amplifies TRAV27
Rd1.AV29.x1	ctctttccctacacgacgctcttccgatctTGGAGACTCTGCAGTGTACTTCTrGTGCAa/3SpC3/	Amplifies TRAV29/DV5
Rd1.AV30.x1	ctctttccctacacgacgctcttccgatctGCAAAGCTCCCTGTACCTTACGrGCCTCa/3SpC3/	Amplifies TRAV30
Rd1.AV34.x1	ctctttccctacacgacgctcttccgatctCCAGCCATGCAGGCATCTArCCTCTa/3SpC3/	Amplifies TRAV34
Rd1.AV35.x1	ctctttccctacacgacgctcttccgatctGCATCCATACCTAGTGATGTAGGCrATCTAa/3SpC3/	Amplifies TRAV35
Rd1.AV36.x1	ctctttccctacacgacgctcttccgatctAGCATCCTGAACATCACAGCCrACCCAA/3SpC3/	Amplifies TRAV36/DV7
Rd1.AV38.x1	ctctttccctacacgacgctcttccgatctGCAGCCAAATCCTTCAGTCTCArAGATCc/3SpC3/	Amplifies TRAV38-1,TRAV38-2
Rd1.AV39.x1	ctctttccctacacgacgctcttccgatctTGCATGACCTCTCTGCCArCTACTc/3SpC3/	Amplifies TRAV39
Rd1.AV40.x2	ctctttccctacacgacgctcttccgatctCCCCATTGTGAAATATTTCAGTCCrAGGTAc/3SpC3/	Amplifies TRAV40
Rd1.AV41.x1	ctctttccctacacgacgctcttccgatctCCCATCCAGAGACTCTGCrCGTCTc/3SpC3/	Amplifies TRAV41
Rd1.BV02.x1	ctctttccctacacgacgctcttccgatctTCTGAAGATCCGGTCCACAAAGrCTGGAA/3SpC3/	Amplifies TRBV2
Rd1.BV03.x1	ctctttccctacacgacgctcttccgatctCAATTCCTGGAGCTTGGTGArCTCTGa/3SpC3/	Amplifies TRBV3-1,TRBV3-2
Rd1.BV04.x1	ctctttccctacacgacgctcttccgatctTCTCACCTGAATGCCCAACrAGCTCc/3SpC3/	Amplifies TRBV4-1,TRBV4-2,TRBV4-3
Rd1.BV05-1.x1	ctctttccctacacgacgctcttccgatctCGCTCTGAGATGAATGTGAGCArCCTTGa/3SpC3/	Amplifies TRBV5-1
Rd1.BV05-4/5/6/8.x1	ctctttccctacacgacgctcttccgatctCTCTGAGCTGAATGTGAACGrCTTGGc/3SpC3/	Amplifies TRBV5-4,TRBV5-5,TRBV5-6,TRBV5-7,TRBV5-8
Rd1.BV06-1to6.x1	ctctttccctacacgacgctcttccgatctACATCTGTGACTTCTGTGCCAGrCAGTGC/3SpC3/	Amplifies TRBV6-1,TRBV6-2,TRBV6-3,TRBV6-4,TRBV6-5,TRBV6-6
Rd1.BV06-8/9.x1	ctctttccctacacgacgctcttccgatctCCTGGTATCGACAAGACCCAGrGCATGa/3SpC3/	Amplifies TRBV6-8,TRBV6-9
Rd1.BV07-2/6.x1	ctctttccctacacgacgctcttccgatctCTCCACTCTGACGATCCAGCrGCACAA/3SpC3/	Amplifies TRBV7-2,TRBV7-6
Rd1.BV07-3.x1	ctctttccctacacgacgctcttccgatctCTACTCTGAAGATCCAGCGCArCAGAGa/3SpC3/	Amplifies TRBV7-3
Rd1.BV07-4/6/7.x1	ctctttccctacacgacgctcttccgatctCGGTTCTCTGCAGAGAGGrCTGAGt/3SpC3/	Amplifies TRBV7-4,TRBV7-6,TRBV7-7
Rd1.BV07-8.x1	ctctttccctacacgacgctcttccgatctGGATCCGTCTCCACTCTGAAGrATCCAA/3SpC3/	Amplifies TRBV7-8
Rd1.BV07-9.x1	ctctttccctacacgacgctcttccgatctTCCACCTTGGAGATCCAGCrGCACAA/3SpC3/	Amplifies TRBV7-9
Rd1.BV09.x1	ctctttccctacacgacgctcttccgatctACGATTCTCCGCACAACAGTTrCCCTGc/3SpC3/	Amplifies TRBV9

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Rd1.BV10-1.x1	ctctttccctacacgacgctcttccgatctCCTCACTCTGGAGTCTGCTGrCCTCCa/3SpC3/	Amplifies TRBV10-1
Rd1.BV10-2.x1	ctctttccctacacgacgctcttccgatctCCCCTCACTCTGGAGTCAGrCTACCa/3SpC3/	Amplifies TRBV10-2
Rd1.BV10-3.x1	ctctttccctacacgacgctcttccgatctGCTACCAGCTCCCAGACATrCTGTGc/3SpC3/	Amplifies TRBV10-3
Rd1.BV11.x1	ctctttccctacacgacgctcttccgatctAGGCTCAAAGGAGTAGACTCCArCTCTCc/3SpC3/	Amplifies TRBV11-1,TRBV11-2,TRBV11-3
Rd1.BV12.x1	ctctttccctacacgacgctcttccgatctATCCAGCCCTCAGAACCCrAGGGAA/3SpC3/	Amplifies TRBV12-3,TRBV12-4,TRBV12-5
Rd1.BV13.x1	ctctttccctacacgacgctcttccgatctGAACTGAACATGAGCTCCTTGGArGCTGGA/3SpC3/	Amplifies TRBV13
Rd1.BV14.x1	ctctttccctacacgacgctcttccgatctCTACTCTGAAGGTGCAGCCrGCAGAc/3SpC3/	Amplifies TRBV14
Rd1.BV15.x1	ctctttccctacacgacgctcttccgatctCAGGAGGCCGAACACTTCTTrCTGTc/3SpC3/	Amplifies TRBV15
Rd1.BV16.x1	ctctttccctacacgacgctcttccgatctACGAAGCTTGAGGATTCAGCArGTGTAc/3SpC3/	Amplifies TRBV16
Rd1.BV18.x1	ctctttccctacacgacgctcttccgatctGCATCCTGAGGATCCAGCArGGTAGc/3SpC3/	Amplifies TRBV18
Rd1.BV19.x1	ctctttccctacacgacgctcttccgatctCCAAAAGAACCCGACAGCTTCTrATCTCc/3SpC3/	Amplifies TRBV19
Rd1.BV20.x1	ctctttccctacacgacgctcttccgatctCAGTGCCCATCTGAAGACArGCAGCc/3SpC3/	Amplifies TRBV20-1
Rd1.BV24.x1	ctctttccctacacgacgctcttccgatctTCTCCCTGTCCCTAGAGTCTGrCCATCa/3SpC3/	Amplifies TRBV24-1
Rd1.BV25.x1	ctctttccctacacgacgctcttccgatctACAGTCTCAGAAATAAGGACGGArGCATTc/3SpC3/	Amplifies TRBV25-1
Rd1.BV27.x1	ctctttccctacacgacgctcttccgatctCCCCAACAGACCTCTCTGTArCTTCTa/3SpC3/	Amplifies TRBV27
Rd1.BV28.x1	ctctttccctacacgacgctcttccgatctCCAGCACCAACCAGACATCTrATGTAA/3SpC3/	Amplifies TRBV28
Rd1.BV29.x1	ctctttccctacacgacgctcttccgatctGAGCAACATGAGCCCTGAAGArCAGCAa/3SpC3/	Amplifies TRBV29-1
Rd1.BV30.x1	ctctttccctacacgacgctcttccgatctCTCTCAGCCTCCAGACCCrCAGGAa/3SpC3/	Amplifies TRBV30

**Table 5. | MiSeq Sample Sheet**

[Header]

```

IEMFileVersion          4
Investigator
Name                   Shuqiang
Project Name           Shuqiang TCR
Experiment Name        16097-101
Date                   xx/xx/xxxx
Workflow                GenerateFASTQ
Application             FASTQ Only
Assay                   amplicon
Description
Chemistry               Amplicon
    
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[Reads]

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248
48
    
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[Settings]

[Data]

Sample_ID	Sample_Name	Sample_Plate	Sample_Well	Sample_Project	index	I7_Index_ID	index2	I5_Index_ID	GenomeFolder
A01	Sample 01				GAACGAAG	IDT193	GCCTATCA	IDT097	
A02	Sample 01				ACTGCTTG	IDT201	CTACTTGG	IDT105	
A03	Sample 01				AACACTGG	IDT209	TGACTGAC	IDT113	
A04	Sample 01				GTTGCTGT	IDT217	GAGCTTGT	IDT121	
A05	Sample 02				CACCAGTT	IDT225	CAGGTTAG	IDT129	
A06	Sample 02				AAGGAAGG	IDT233	GTGGTGTT	IDT137	
A07	Sample 02				TGCACTTG	IDT241	AGCGGAAT	IDT145	

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A08	Sample 02	ACAACAGC	IDT249	AGCTTGAG	IDT153
A09	Sample 03	TAGAACGC	IDT257	TTCTGTG	IDT161
A10	Sample 03	TCAGCCTT	IDT265	GTCTTCT	IDT169
A11	Sample 03	GCCAGAAT	IDT273	TGTTCGAG	IDT177
A12	Sample 03	CACATGGT	IDT281	GCCACTTA	IDT185
B01	Sample 04	ACCTAGAC	IDT194	CTTGATG	IDT098
B02	Sample 04	GCCTATGT	IDT202	CCTCAGTT	IDT106
B03	Sample 04	TTGGTGCA	IDT210	TAGACGTG	IDT114
B04	Sample 04	AGAACCAG	IDT218	GAAGGTT	IDT122
B05	Sample 05	GTATTCCG	IDT226	AGTTGGCT	IDT130
B06	Sample 05	AGCGTGTA	IDT234	GTGCTTAC	IDT138
B07	Sample 05	TCCTCGA	IDT242	GTGAGCTT	IDT146
B08	Sample 05	AGCCGTAA	IDT250	GAGTGGTT	IDT154
B09	Sample 06	AACCAGAG	IDT258	CGTTGAGT	IDT162
B10	Sample 06	AAGCATCG	IDT266	TGATACGC	IDT170
B11	Sample 06	CGAGAGAA	IDT274	CTCCATGT	IDT178
B12	Sample 06	CGAGTTAG	IDT282	ACCTGACT	IDT186
C01	Sample 07	TACGACGT	IDT195	AGTCTCAC	IDT099
C02	Sample 07	GTACCACA	IDT203	TCCTACCT	IDT107
C03	Sample 07	CCTGTCAA	IDT211	CCGGAATT	IDT115
C04	Sample 07	GATGTCGA	IDT219	ATCTCGCT	IDT123
C05	Sample 08	TTCGAAGC	IDT227	TCAACTGG	IDT131
C06	Sample 08	TCTACGCA	IDT235	TCAAGGAC	IDT139
C07	Sample 08	CACTGTAG	IDT243	CGTGATCA	IDT147
C08	Sample 08	CTCTTGTC	IDT251	GCTGTAAG	IDT155
C09	Sample 09	CGACCTAA	IDT259	AGTCGCTT	IDT163
C10	Sample 09	GCCAATAC	IDT267	CTGTGTTG	IDT171
C11	Sample 09	AACTCGGA	IDT275	CGTCTTGT	IDT179
C12	Sample 09	AGCTAAGC	IDT283	GTTAAGGC	IDT187



BULK RNA TCR SEQUENCING SOP V1

D01	Sample 10	TTGAGCTC	IDT196	CTCATCAG	IDT100
D02	Sample 10	TAGTGGTG	IDT204	ATGGCGAA	IDT108
D03	Sample 10	CTATGCCT	IDT212	CTCCTAGA	IDT116
D04	Sample 10	AGGAGGTT	IDT220	AGTTACGG	IDT124
D05	Sample 11	AGACCTTG	IDT228	CTGCACTT	IDT132
D06	Sample 11	TGGCTCTT	IDT236	TGAACCTG	IDT140
D07	Sample 11	GTACGATC	IDT244	TCGCATTG	IDT148
D08	Sample 11	CAGATCCT	IDT252	CCAAGACT	IDT156
D09	Sample 12	CTCTCAGA	IDT260	TAGGTAGG	IDT164
D10	Sample 12	GACACAGT	IDT268	AACGTGGA	IDT172
D11	Sample 12	ACAGTTCG	IDT276	ATAAGGCG	IDT180
D12	Sample 12	GTTCCATG	IDT284	ATGCCAAC	IDT188
E01	Sample 13	AGTACACG	IDT197	TGTACCGT	IDT101
E02	Sample 13	ATACGCAG	IDT205	CTTACCTG	IDT109
E03	Sample 13	TTCGGCTA	IDT213	CAACGGAT	IDT117
E04	Sample 13	AATCGCTG	IDT221	GTGTCTGA	IDT125
E05	Sample 14	CCAAGGTT	IDT229	ACACGGTT	IDT133
E06	Sample 14	CCTTCCAT	IDT237	AGTGTGG	IDT141
E07	Sample 14	TGGTGAAG	IDT245	TGACGCAT	IDT149
E08	Sample 14	GATGCTAC	IDT253	ATTGCGTG	IDT157
E09	Sample 15	AGGCTGAA	IDT261	CAGGAGAT	IDT165
E10	Sample 15	AAGAGGCA	IDT269	GTTGCGAT	IDT173
E11	Sample 15	TGACCGTT	IDT277	TGTCTGCT	IDT181
E12	Sample 15	GCATCCTA	IDT285	AGAGGTTG	IDT189
F01	Sample 16	TGTCAGTG	IDT198	AAGTCGAG	IDT102
F02	Sample 16	AAGACCGT	IDT206	CTCGATAC	IDT110
F03	Sample 16	ACCGACAA	IDT214	TGGCTATC	IDT118
F04	Sample 16	AGTGACCT	IDT222	TGACTTCG	IDT126
F05	Sample 17	ACGTATGG	IDT230	AATACGCG	IDT134

BULK RNA TCR SEQUENCING SOP V1

F06	Sample 17	ATACTGGC	IDT238	GTA CTCTC	IDT142
F07	Sample 17	TAGCTGAG	IDT246	CCGATGTA	IDT150
F08	Sample 17	AGGAACAC	IDT254	CTGAAGCT	IDT158
F09	Sample 18	ATCGGAGA	IDT262	CATCGTGA	IDT166
F10	Sample 18	GAAGACTG	IDT270	AACGACGT	IDT174
F11	Sample 18	CATCTGCT	IDT278	CGCTTAAC	IDT182
F12	Sample 18	CCATGAAC	IDT286	ACCATCCA	IDT190
G01	Sample 19	GACTACGA	IDT199	CACGTTGT	IDT103
G02	Sample 19	CTCCAATC	IDT207	TCCGTGAA	IDT111
G03	Sample 19	CGTAGATG	IDT215	CGGTCATA	IDT119
G04	Sample 19	CGAATTGC	IDT223	TGGATCAC	IDT127
G05	Sample 20	AAGGACCA	IDT231	TGCGAACT	IDT135
G06	Sample 20	AACCTACG	IDT239	CCGTATCT	IDT143
G07	Sample 20	AGAGCAGA	IDT247	TTCGCAGT	IDT151
G08	Sample 20	ACCATCCT	IDT255	TAACGAGG	IDT159
G09	Sample 21	GATACCTG	IDT263	TGTTGTGG	IDT167
G10	Sample 21	CCGTTATG	IDT271	CGTATTCG	IDT175
G11	Sample 21	CGCTGATA	IDT279	GATCCATG	IDT183
G12	Sample 21	ATCCACGA	IDT287	GTGGATAG	IDT191
H01	Sample 22	TTACGTGC	IDT200	TCACAGCA	IDT104
H02	Sample 22	TCTGGACA	IDT208	TAGAGCTC	IDT112
H03	Sample 22	CTGTATGC	IDT216	TCCAATCG	IDT120
H04	Sample 22	CAAGAAGC	IDT224	ACACCAGT	IDT128
H05	Positive control	TATGCGGT	IDT232	GCTGACTA	IDT136
H06	Positive control	CATACTCG	IDT240	CGAAGAAC	IDT144
H07	Positive control	CTTCGGTT	IDT248	ACGACAGA	IDT152
H08	Positive control	GAACGTGA	IDT256	TCGTCTCA	IDT160
H09	Negative control	TCCTGACT	IDT264	ACAGACCT	IDT168
H10	Negative control	CTAGCAGT	IDT272	AGCAAGCA	IDT176

## BULK RNA TCR SEQUENCING SOP V1

H11	Negative control	TCGTCTGA	IDT280	ACCTCTGT	IDT184
H12	Negative control	GAGAAGGT	IDT288	CTGAGATC	IDT192