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**Protocol status:** Working
We use this protocol and it's working

# 🌐 Slide-TCR-Seq v3 (IVT) V.1

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ABSTRACT

T cells mediate antigen-specific immune responses to disease through the specificity and diversity of their clonotypic T cell receptors (TCRs). Determining the spatial distributions of T cell clonotypes in tissues is essential to understanding T cell behavior, but spatial sequencing methods remain unable to profile the TCR repertoire.

We developed Slide-TCR-seq, a 10-μm-resolution method, to sequence whole transcriptomes and TCRs within intact tissues. Our method yields insights into the spatial relationships between clonality, neighboring cell types, and gene expression that drive T cell responses.

The most recent version of our protocol uses in vitro transcription in lieu of rhPCR amplfiication, which overcomes the barcode switching introduced by the rhPCR and results in higher mapping rates.

MATERIALS

**LIBRARY PREPARATION**

- 1.5 mL Eppendorf LoBind Tubes (Eppendorf, 0030122275)
- 0.2 mL TempAssure PCR Flex-Free 8-Tube Strips, Attached Individual Optical Caps (USA Scientific, 1402-4700)
- UltraPure Distilled Water (Invitrogen, 10977015)
- NxGen RNase Inhibitor (Lucigen, F83923-1)
- Maxima H minus Reverse Transcriptase + Maxima 5X RT Buffer (Thermo Scientific, EP0752)
- Deoxynucleotide (dNTP) solution mix (New England BioLabs, N0447L)
- AmPure XP (SPRI beads) (Beckman Coulter, A63881)
- SPRIselect (SPRI beads) (Beckman Coulter, B23319)

- Qubit dsDNA HS Assay Kit (Thermofisher, Q32851)
- Bioanalyzer High Sensitive DNA kit (Agilent, 5067-4626)
- HiScribe™ T7 Quick High Yield RNA Synthesis Kit (New England BioLabs, E2050S)
- 2x KAPA Hifi Hotstart Readymix (Roche, KK2602)

## OLIGONUCLEOTIDE SEQUENCES

| A | B |
|---|---|
| Truseq-P5 Hybrid | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT |
| T7 PCR primer | TCTAGATAATACGACTCACTATAGGG |
| Human T7-TCRV primer mix | See table 2 |
| Mouse T7-TCRV primer mix | See table 3 |
| | |

Table 1: Oligonucleotide sequences

| A | B |
|---|---|
| Name | Sequence |
| TRAV1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGTCGTTTTTCTTCATTCCTTAGTC |
| TRAV2 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGATACAACATGACCTATGAACGG |
| TRAV3.1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTTGAAGCTGAATTTAACAAGAGCC |
| TRAV4.1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCCTGTTTATCCCTGCCGAC |
| TRAV5.1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAACAAGACCAAAGACTCACTGTTC |
| TRAV6 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGACTGAAGGTCACCTTTGATACC |
| TRAV7 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTAAATGCTACATTACTGAAGAATGG |
| TRAV8 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCATCAACGGTTTTGAGGCTGAATTTAA |
| TRAV9 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAACCACTTCTTTCCACTTGGAGAA |
| TRAV10 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAGCAACTCTGGATGCAGACAC |
| TRAV12 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAGATGGAAGGTTTACAGCACA |
| TRAV13.1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACATTCGTTCAAATGTGGGCGAA |

| | A | B |
|---|---|---|
| | TRAV13.2 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCAAGGCCAAAGAGTCACCGT |
| | TRAV14 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCAGAAGGCAAGAAAATCCGCCA |
| | TRAV16 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGACCTTAACAAAGGCGAGACA |
| | TRAV17 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTAAGAGTCACGCTTGACACTTCCA |
| | TRAV18 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGAGGTTTTCAGGCCAGTCCT |
| | TRAV19 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCACCAGTTCCTTCAACTTCACC |
| | TRAV20 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCACATTAACAAAGAAGGAAAGCT |
| | TRAV21 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTCGCTGGATAAATCATCAGGA |
| | TRAV22 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGACTGTCGCTACGGAACGCTA |
| | TRAV23 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACAATCTCCTTCAATAAAAGTGCCA |
| | TRAV24 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGAATAAGTGCCACTCTTAATACCA |
| | TRAV25 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTTGGAGAAGCAAAAAAGAACAGCT |
| | TRAV26.1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGAAGACAGAAAGTCCAGCACCT |
| | TRAV26.2 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCGCTGAAGACAGAAAGTCCAGT |
| | TRAV27 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTAACCTTTCAGTTTGGTGATGCAA |
| | TRAV29 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTAAACAAAAGTGCCAAGCACCTC |
| | TRAV30 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATATCTGCTTCATTTAATGAAAAAAAGC |
| | TRAV34 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAAGTTGGATGAGAAAAAGCAGCA |
| | TRAV35 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCAGTTTGGTATAACCAGAAAGGA |
| | TRAV36 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAGACTAAGTAGCATATTAGATAAG |
| | TRAV38 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGTGAACTTCCAGAAAGCAGCCA |
| | TRAV39 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCACTTGATACCAAAGCCCGT |
| | TRAV40 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGCGGAAATATTAAAGACAAAAACTC |
| | TRAV41 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATTAATTGCCACAATAAACATACAGG |

| | A | B |
|---|---|---|
| | TRBV2 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGCCTGATGGATCAAATTTCACTCTG |
| | TRBV3-1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTCTCACCTAAATCTCCAGACAAAGCT |
| | TRBV4 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCCTGAATGCCCCAACAGCTCTC |
| | TRBVS-48 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCTCTGAGCTGAATGTGAACGCCT |
| | TRBVS-1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCGATTCTCAGGGCGCCAGTTCTCT |
| | TRBV6-1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTGGCTACAATGTCTCCAGATTAAACAA |
| | TRBV6-23 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCCCTGATGGCTACAATGTCTCCAGA |
| | TRBV6-4 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGTGTCTCCAGAGCAAACACAGATGATT |
| | TRBV6-56 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGTCTCCAGATCAACCACAGAGGAT |
| | TRBV6-8 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGTCTCTAGATTAAACACAGAGGATTTC |
| | TRBV6-9 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGGCTACAATGTATCCAGATCAAACA |
| | TRBV7-2 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTCGCTTCTCTGCAGAGAGGACTGG |
| | TRBV7-3 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCGGTTCTTTGCAGTCAGGCCTGA |
| | TRBV7-8 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCCAGTGATCGCTTCTTTGCAGAAA |
| | TRBV?-46 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTCTCCACTCTGAMGATCCAGCGCA |
| | TRBV7-7 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGCAGAGAGGCCTGAGGGATCCAT |
| | TRBV7-9 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCTGCAGAGAGGCCTAAGGGATCT |
| | TRBV9 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCTCCGCACAACAGTTCCCTGACTT |
| | TRBV10-13 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCAGATGGCTAYAGTGTCTCTAGATCAAA |
| | TRBV10-2 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGTTGTCTCCAGATCCAAGACAGAGAA |
| | TRBV11 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGCAGAGAGGCTCAAAGGAGTAGACT |
| | TRBV12-34 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGCTAAGATGCCTAATGCATCATTCTC |
| | TRBV12-5 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCTCAGCAGAGATGCCTGATGCAACT |
| | TRBV13 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTCTCAGCTCAACAGTTCAGTGACTA |

| A | B |
|---|---|
| TRBV14 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGAAAGGACTGGAGGGACGTAT |
| TRBV15 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATAACTTCCAATCCAGGAGGCCG |
| TRBV16 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTAAGTGCCTCCCAAATTCACCC |
| TRBV18 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAACGATTTTCTGCTGAATTTCCCA |
| TRBV19 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTACAGCGTCTCTCGGGAGAAGA |
| TRBV20-1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACAAGTTTCTCATCAACCATGCAA |
| TRBV24-1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGATACAGTGTCTCTCGACAGGC |
| TRBV25-1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAACAGTCTCCAGAATAAGGACGGA |
| TRBV27-1 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAAAGTCTCTCGAAAAGAGAAGAGGA |
| TRBV28 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGGGGTACAGTGTCTCTAGAGAGA |
| TRBV29 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTTCCCATCAGCCGCCCAAACCTA |
| TRBV30 | TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGACCCCAGGACCGGCAGTTCAT |

Table 2: Human T7-UPS2-TCRV oligo sequences

| A | B |
|---|---|
| mAV01 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCGCTCGAATGGGTACAGTTACCTGA |
| mAV02 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGGAAGCTCAGCACTCTGAACCTGA |
| mAV03 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACTCTCTGAACCTCACAGCTGCCCAA |
| mAV041 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGCTACAGCACCCTGCACATCAC |
| mAV042 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGGAGAGCTACAGCACCCTGCAA |
| mAV043 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGGAGAGCTACAGCACCCCGCAA |
| mAV044 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGGAGCTCTACAGCACCCTGCAA |
| mAV051 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTACAGCCACTCAGCCTGGAGACTA |
| mAV052 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCACAGACACCCAGCCTGGAGACA |

| | A | B |
|---|---|---|
| | mAV061 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCCTTCCACTTGCAGAAAGCCTCAGTA |
| | mAV062 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTCCTTCCACTTACAGAAAGCCTCAGTGCT |
| | mAV063 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGGAAGCAGCAGAGGTTTTGAAGCTACAC |
| | mAV071 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCAGCTCACCTCAATAAGGCCAGCCTGA |
| | mAV072 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGAGACTCCCAGCCCAGTGACTCA |
| | mAV073 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCAGAGAGTCGCAACCCAGTGACTCA |
| | mAV074 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTCAATAGAGCCAGCCTGCATGTTTCA |
| | mAV075 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGTGTCCATCTTCTCTGATGGTGAAAAGGT |
| | mAV081 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACAGCCACCCTTGACACCTCCAGCCT |
| | mAV082 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCAGTGGAAGACTCAGAGCCACCCTTA |
| | mAV091 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGAAAGCCTCCGTGCACTGGAGCGT |
| | mAV092 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGCTTCGAGGCTGAGTTCAGCAAGAC |
| | mAV093 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACAGTAACTCTTCCTTCCACCTGCGGAAAT |
| | mAV10 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACATCACAGCCACACAGCCTGAAGAC |
| | mAV11 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCACAGCACGCTGCACATCACAGA |
| | mAV12 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCAGCTCCTTCCATCTGCAGAAGTCCA |
| | mAV131 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGCTCTTTGCACATTTCCTCCTCCCAGAA |
| | mAV132 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGCTCTTTGACTATATCCTCCTCCCAGACCT |
| | mAV141 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACAGACTCTCAGCCTGGAGACTCAGCCT |
| | mAV142 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACAGGAAGATGGACGATTCACAATCTTCTTCAC |
| | mAV151 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCCGCTATTCTGTAGTCTTCCAGAAATCACA |
| | mAV152 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCCATCAGCCTTGTCATTTCAGCCTCACT |
| | mAV16 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACAGCCAAAAAGTTCCATCGGACTCATCAC |
| | mAV17 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCTTTCAACCTGAAGAAATCCCCAGCCCATA |

| | A | B |
|---|---|---|
| | mAV19 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTTCTCACTGCACATCACAGCCTCCCT |
| | mAV21 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGGCTATTGCCTCTGACAGAAAGTCAC |
| | mBV01 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTGATACGGAGCTGAGGCTGCAAGA |
| | mBV02 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCAGATCACAGCTCTAAAGCCTGATGACC |
| | mBV03 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCAACCCACAGCACTGGAGGACA |
| | mBV04 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGCTTCTCACCTCAGTCTTCAGATAAC |
| | mBV05 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGCCCAGACAGCTCCAAGCTACA |
| | mBV12 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCCAGCAGATTCTCAGTCCAACAGTC |
| | mBV131 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCACCAGAACAACGCAAGAAGC |
| | mBV132 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACAAGGCCTCCAGACCAAGCCAAT |
| | mBV14 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCTAAAGGAACTAACTCCACTCTCAAGAC |
| | mBV15 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGAAGATTCAACCTACAGAACCCAAGGACA |
| | mBV16 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGAAGATCCAGAGCACGCAACCCCT |
| | mBV17 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCTCTACATTGGCTCTGCAGGCCTAGT |
| | mBV19 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCACTGTGACATCTGCCCAGAAGAT |
| | mBV20 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCCCATCAGTCATCCCAACTTATCCTA |
| | mBV23 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCTGCAGCCTGGGAATCAGAACGA |
| | mBV24 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCATCCTGGAAATCCTATCCTCTGAAGAC |
| | mBV25 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCCAATCTCATCCTTCATCTTGGAAATGCT |
| | mBV26 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGCAGCCTAGAAATTCAGTCCTCTGAGA |
| | mBV29 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGGGAGCATTTCTCCCTGATTCTGGATTA |
| | mBV30 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCAAACCTAACATTCTCAACGTTGACAGA |
| | mBV31 | AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACGGAGAAGCTGCTTCTCAGCCACA |

Table 3: Mouse T7-UPS2-TCRV oligo sequences

## PCR to add T7 to cDNA libraries

1. This protocol amplifies TCRs from unfragmented, full-length cDNA from Slide-Seq.
   Prepare two 10-nanogram* dilutions of all samples into 12.25 µL of ultrapure water for amplifying TCR alpha and beta sequences in separate reactions.
   *We have successfully tested down to 2 ng for low-concentration samples.

2. Prepare the primer extension PCR master mix using KAPA Hifi Hotstart Readymix 2X and T7-TCRV primer pool. Gently mix by pipetting and run the PCR program below.

   **Note: TRAV and TRBV primers are pooled separately and are treated as individual reactions for each sample.**

   *Primer extension PCR mix per sample:*

   | A | B | C |
   |---|---|---|
   | Volume (µL) | Reagent | Final concentration |
   | 12.5 | KAPA Hifi Hotstart Readymix 2X | 1 X |
   | 0.25 | 100 µM T7-TCRV primer pool | 1 µM |
   | 4-10 ng | cDNA | |
   | up to 25 µL | Ultrapure water | |

   *Final volume 25 µL*

   *Primer extension PCR protocol:*

   | A | B | C |
   |---|---|---|
   | Cycles | Temp | Time |
   | 5 | 95 °C | 5 minutes |
   | | 65 °C for human primer pool/70 °C for mouse primer pool | 30 seconds |
   | | 72 °C | 3 minutes |

| A | B | C |
|---|---|---|
| 1 | 4 °C | hold |

**Safe stopping point, store at 4 °C**

3.  Add 25 µL of water to bring the reaction to 50 µL. Perform a PCR clean-up following the manufacturer's instructions using SPRIselect or AMPure XP beads at 0.6X (30 µL of SPRI beads to 50 µLPCR reaction volume). Elute in 9 µL of water.

4.  Prepare the T7/Truseq PCR master mix with KAPA Hifi Hotstart Readymix 2X. Add 16 µL of master mix to 9 µL of the sample. Gently mix by pipetting and run the PCR program below.

*T7/Truseq PCR mix per sample:*

| A | B | C |
|---|---|---|
| Volume (uL) | Reagent | Final concentration |
| 12.5 | KAPA Hifi Hotstart Readymix 2X | 1 X |
| 0.5 | 100uM Truseq-P5 Hybrid primer | 2 µM |
| 0.5 | 100uM T7 PCR primer | 2 µM |
| 9 | Sample | |
| 2.5 | Ultrapure water (up to 25) | |

*Final volume 25 µL*

*T7/Truseq PCR protocol:*

| A | B | C |
|---|---|---|
| Cycles | Temp | Time |
| 1 | 98 °C | 2 minutes |
| 10 | 98 °C | 1 minute |
| | 60 °C | 30 seconds |
| | 72 °C | 3 minutes |
| 1 | 72 °C | 5 minutes |
| | 4 °C | hold |

**Safe stopping point, store at 4 °C**

5.  Add 25 µL of water to bring the reaction to 50 µL. Perform a PCR clean-up following the

manufacturer's instructions using SPRIselect or AMPure XP beads at 0.6X (30 µL of SPRI beads to 50 µLPCR reaction volume). Elute in 8 µL of water.

## IVT amplification

6   Follow the manufacturer's instructions on the HiScribe RNA synthesis kit using 8 µL of the sample eluted in the previous step. Incubate reaction for 2 hours at 37 °C.

*HiScribe RNA Synthesis mix per sample:*

| A | B | C |
|---|---|---|
| Volume (uL) | Reagent | Final concentration |
| 10 | NTP Buffer Mix | 10 mM each NTP |
| 2 | T7 RNA Polymerase Mix | |
| 8 | Sample | |

*Final volume 20 µL*

7   Use RNAse away to clean all surfaces and pipettors.
Add 30 µL of water to bring the reaction to 50 µL. Perform a PCR clean-up using SPRIselect or AMPure XP beads at 0.6X (30 µL of SPRI beads to 50 µL PCR reaction volume), following the steps below:

*For a 50 µL reaction, add 30 uL of SPRI beads.*
*Incubate for 5 minutes at RT.*
*Incubate for 2 minutes on a magnet until the solution turns clear.*
*Discard supernatant.*
*Wash on a magnet for 30 sec with 200 µL of freshly made 80% EtOH.*
*Repeat wash.*
*Discard supernatant.*
*Spin down briefly on a table spinner.*
*Remove all EtOH with a 20 µL pipette.*
*Elute with 20 µL of H2O.*

8   Use a NanoDrop on the RNA setting to measure RNA concentration.

## RT

**9** Add 180 μL of the following RT mix to 20 μL of the RNA sample. Incubate reaction for 2 hours at 42 °C.

*Reverse Transcription Mix per sample:*

| A | B | C |
|---|---|---|
| Volume (uL) | Reagent | Final concentration |
| 40 | Maxima 5X RT buffer | 1 X |
| 20 | 10 mM dNTPs | 1 mM |
| 5 | RNAse inhibitor | |
| 2 | 100 μM Truseq-P5 Hybrid primer | 1 μM |
| 10 | Maxima H-RTase | |
| 20 | Template RNA (sample) | 1 pg - 5 ug |
| 103 | Ultrapure water (up to 200) | |

*Final volume 200 μL*

**Safe stopping point, store at 4 °C**

**10** Perform a PCR clean-up following the manufacturer's instructions using SPRIselect or AMPure XP beads at 0.6X (120 μL of SPRI beads to 200 μL RT reaction volume). Elute in 20 μL of water. Record concentrations using a NanoDrop on the ssDNA setting and save all samples.

## Index PCR

**11** Prepare the PCR master mix with KAPA Hifi Hotstart Readymix 2X, P5-Truseq PCR primer, and Nextera PCR primer index. Gently mix by pipetting and divide the total volume of each sample into 4 PCR tubes each containing 50 μL (25%) of the total.

Note: Each sample must use a different i7 index if you intend to pool samples for multiplexed sequencing. We do not recommend dual-indexing of samples.

*Index PCR mix per sample:*

| A | B | C |
|---|---|---|
| Volume (uL) | Reagent | Final Concentration |
| 100 | KAPA Hifi Hotstart Readymix 2X | 1 X |

| A | B | C |
|---|---|---|
| 4 | 100 µM Truseq-P5 Hybrid PCR primer | 2 µM |
| 4 | 100 µM Nextera PCR primer (i7) | 2 µM |
| 100 ng | Sample | |
| up to 200 | ultrapure water | |

*Final volume 200 µL*

*Index PCR protocol:*

| A | B | C |
|---|---|---|
| Cycles | Temp | Time |
| 1 | 98 °C | 2 minutes |
| 10 | 98 °C | 1 minute |
| | 67 °C | 20 seconds |
| | 72 °C | 3 minutes |
| 1 | 72 °C | 5 minutes |
| | 4 °C | hold |

**Safe stopping point, store at 4 °C**

**12**  Recombine the samples that were split into 4 parts in the previous step and perform PCR clean-up following the manufacturer's instructions using SPRIselect or AMPure XP beads at 0.6X (120 µL of SPRI beads to 200 µL PCR reaction volume). Elute in 10 µL of water.

To quantify the TCR libraries, use the Qubit dsDNA high-sensitivity kit and BioAnalyzer High-SensitivityThe expected DNA kit following the manufacturer protocols.
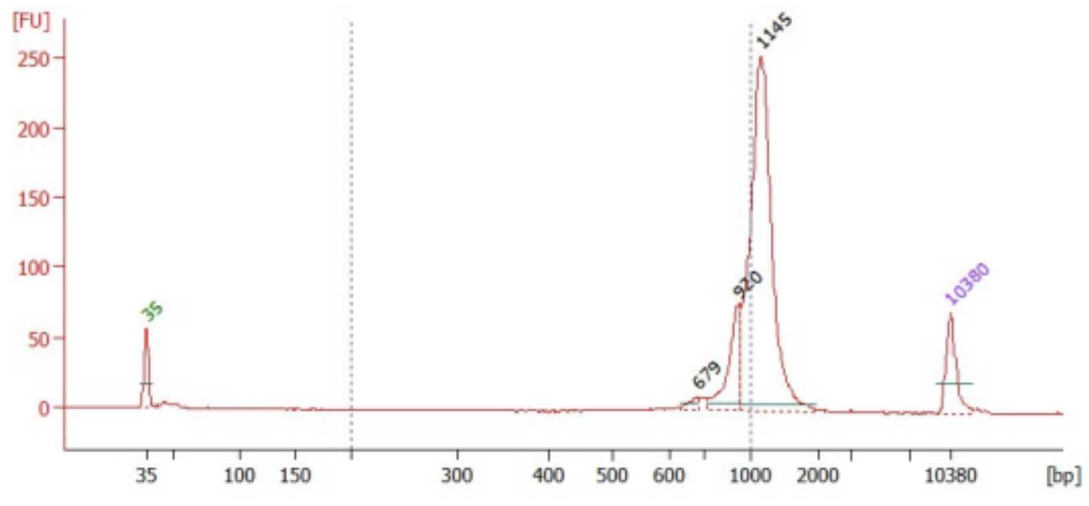
BioAnalyzer trace of a TRB library. The expected library length is around 1100bp.
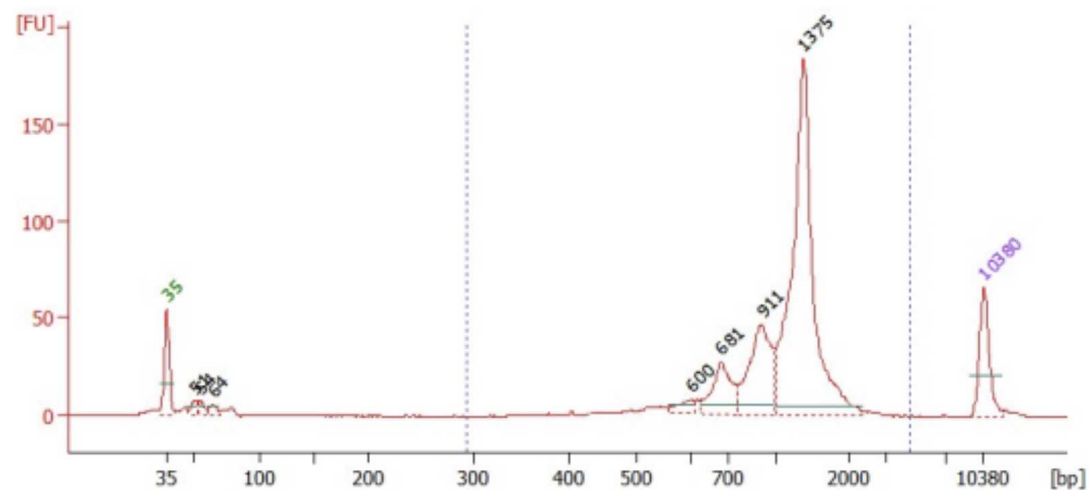


BioAnalyzer trace of a TRA library. The expected library length is around 1300bp.

## Sequencing

**13**  TRA libraries are best sequenced on a Nanopore. TRB libraries can be sequenced on a Nanopore or MiSeq.
For best results, it's generally advised to sequence each sample to a depth of 1-2 million reads.

MiSeq read structure is as follows:
Read 1: 42 bp
Index 1: 8 bp

Read 2: 270 bp
Index 2: 0 bp