VERSION 2

MAR 16, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldp6w8l5b/v2

Protocol Citation: Sophia Liu, Ruth Raichur, Fei Chen 2023. Slide-TCR-Seq v3 (IVT). **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldp6w8l5b/v2Version created by Ruth Raichur

MANUSCRIPT CITATION:
Liu, Sophia, et al. "Spatial Maps of T Cell Receptors and Transcriptomes Reveal Distinct Immune Niches and Interactions in the Adaptive Immune Response." *Immunity*, vol. 55, no. 10, 2022, https://doi.org/10.1016/j.immuni.2022.09.002.

Slide-TCR-Seq v3 (IVT) V.2

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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 amplification, 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)

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

Created: Mar 16, 2023

Last Modified: Mar 16, 2023

PROTOCOL integer ID: 78915

Keywords: T cell receptor, spatial transcriptomics, tertiary lymphoid structures, cancer niches, Slide-TCR-seq, IVT, in vitro transcription

- 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	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGCTGAAAGGACTGGAGGGACGTAT
	TRBV15	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGATAACTTCCAATCCAGGAGGCCG
	TRBV16	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGCTAAGTGCCTCCCAAATTCACCC
	TRBV18	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGGAACGATTTTCTGCTGAATTTCCCA
	TRBV19	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGGTACAGCGTCTCTCGGGAGAAGA
	TRBV20-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGGACAAGTTTCTCATCAACCATGCAA
	TRBV24-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTGGATACAGTGTCTCTCGACAGGC
	TRBV25-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCAACAGTCTCCAGAATAAGGACGGA
	TRBV27-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTACAAAGTCTCTCGAAAAGAGAAGAGGA
	TRBV28	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGGGGTACAGTGTCTCTAGAGAGA
	TRBV29	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGTTTCCCATCAGCCGCCCAAACCTA
	TRBV30	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCAGACCCCAGGACCGGCAGTTCAT

Table 2: Human T7-UPS2-TCRV oligo sequences

	A	B
	mAV01	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCCGCTCGAATGGGTACAGTTACCTGA
	mAV02	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCGGAAGCTCAGCACTCTGAACCTGA
	mAV03	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACACTCTCTCTGAACCTCACAGCTGCCCAA
	mAV041	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCGCTACAGCACCCTGCACATCAC
	mAV042	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTTCTAAGGAGAGCTACAGCACCCTGCAA
	mAV043	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTTCTAAGGAGAGCTACAGCACCCCGCAA
	mAV044	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTTCTAAGGAGCTCTACAGCACCCTGCAA
	mAV051	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTTACAGCCACTCAGCCTGGAGACTA
	mAV052	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCACAGACACCCAGCCTGGAGACA

	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	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCTTCTCACTGCACATCACAGCCTCCCT
	mAV21	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTGGCTATTGCCTCTGACAGAAAGTCAC
	mBV01	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCTGATACGGAGCTGAGGCTGCAAGA
	mBV02	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTCAGATCACAGCTCTAAAGCCTGATGACC
	mBV03	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCCAACCCACAGCACTGGAGGACA
	mBV04	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCGCTTCTCACCTCAGTCTTCAGATAAC
	mBV05	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTGCCCAGACAGCTCCAAGCTACA
	mBV12	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCCCAGCAGATTCTCAGTCCAACAGTC
	mBV131	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGCCACCAGAACAACGCAAGAAGC
	mBV132	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACACAAGGCCTCCAGACCAAGCCAAT
	mBV14	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGCCTAAAGGAACTAACTCCACTCTCAAGAC
	mBV15	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTGAAGATTCAACCTACAGAACCCAAGGACA
	mBV16	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTGAAGATCCAGAGCACGCAACCCCT
	mBV17	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTCTCTACATTGGCTCTGCAGGCCTAGT
	mBV19	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTCACTGTGACATCTGCCCAGAAGAT
	mBV20	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTTCCCATCAGTCATCCCAACTTATCCTA
	mBV23	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTCTGCAGCCTGGGAATCAGAACGA
	mBV24	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGCATCCTGGAAATCCTATCCTCTGAAGAC
	mBV25	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACCCCAATCTCATCCTTCATCTTGGAAATGCT
	mBV26	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACTGCAGCCTAGAAATTCAGTCCTCTGAGA
	mBV29	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGGGAGCATTTCTCCCTGATTCTGGATTA
	mBV30	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACGCCAAACCTAACATTCTCAACGTTGACAGA
	mBV31	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGT GTATAAGAGACACGGAGAAGCTGCTTCTCAGCCACA

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

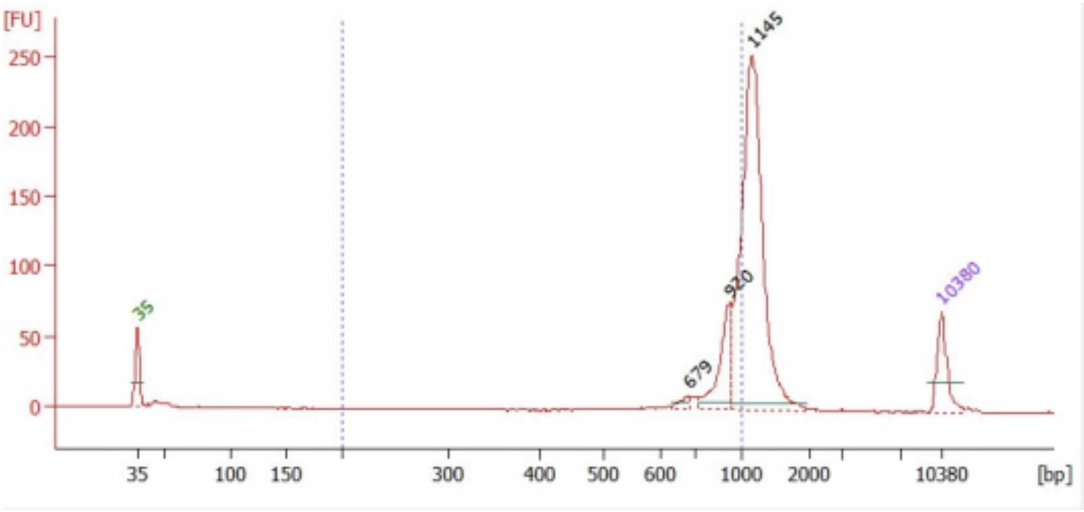
Index PCR protocol:

	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

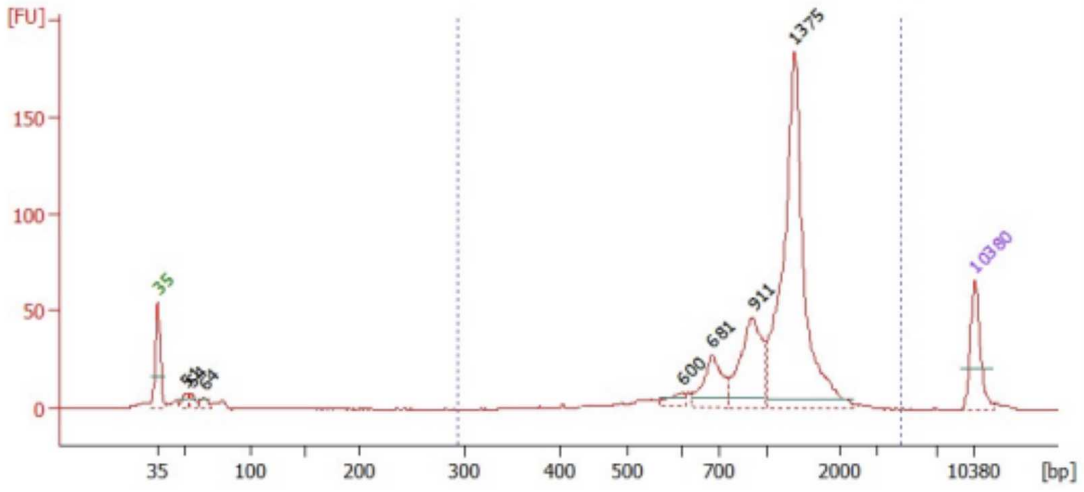
Safe stopping point, store at 4 °C

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