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Slide-TCR-Seq v3 (IVT) V.1

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We use this protocol and it's working

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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

	A	B
	TRAV3.1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTTGAAGC TGAATTTAACAAGAGCC
	TRAV4.1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCCTGTTT ATCCCTGCCGAC
	TRAV5.1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAACAAGAC CAAAGACTCACTGTTC
	TRAV6	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGACTGAA GGTCACCTTTGATACC
	TRAV7	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTAAATGCT ACATTACTGAAGAATGG
	TRAV8	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCATCAACG GTTTTGAGGCTGAATTTAA
	TRAV9	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAACCACT TCTTTCCACTTGGAGAA
	TRAV10	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAGCAACT CTGGATGCAGACAC
	TRAV12	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAGATGGA AGGTTTACAGCACA
	TRAV13.1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACATTCGTT CAAATGTGGGCGAA
	TRAV13.2	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCAAGGCC AAAGAGTCACCGT
	TRAV14	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCAGAAGG CAAGAAAATCCGCCA
	TRAV16	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGACCTT AACAAAGGCGAGACA
	TRAV17	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTAAGAGTCA CGCTTGACACTTCCA
	TRAV18	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGAGGTT TTCAGGCCAGTCCT
	TRAV19	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCACCAGTT CCTTCAACTTCACC
	TRAV20	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCACATTAA CAAAGAAGGAAAGCT
	TRAV21	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTCGCTG GATAAATCATCAGGA
	TRAV22	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGACTGTC GCTACGGAACGCTA

	A	B
	TRAV23	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACAATCTCC TTCAATAAAAGTGCCA
	TRAV24	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGAATAAGT GCCACTCTTAATACCA
	TRAV25	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTTGGAGA AGCAAAAAAGAACAGCT
	TRAV26.1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGAAGACA GAAAGTCCAGCACCT
	TRAV26.2	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCGCTGAA GACAGAAAGTCCAGT
	TRAV27	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTAACCTTT CAGTTTGGTGATGCAA
	TRAV29	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTAAACAA AAGTGCCAAGCACCTC
	TRAV30	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATATCTGCT TCATTTAATGAAAAAAAGC
	TRAV34	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAAGTTGG ATGAGAAAAAGCAGCA
	TRAV35	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCAGTTTG GTATAACCAGAAAGGA
	TRAV36	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAGACTA AGTAGCATATTAGATAAG
	TRAV38	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGTGAACTT CCAGAAAGCAGCCA
	TRAV39	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCACTTG ATACCAAAGCCCGT
	TRAV40	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGCGGAAA TATTAAAGACAAAAACTC
	TRAV41	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATTAATTGC CACAATAAACATACAGG
	TRBV2	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTGATGG ATCAAATTTCACTCTG
	TRBV3-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCACCTAA ATCTCCAGACAAAGCT
	TRBV4	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTGAATGC CCCAACAGCTCTC
	TRBVS-48	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCTGAGCT GAATGTGAACGCCT

	A	B
	TRBVS-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGATTCTCAGGGCGCCAGTTCTCT
	TRBV6-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGCTACAATGTCTCCAGATTAAACAA
	TRBV6-23	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCTGATGGCTACAATGTCTCCAGA
	TRBV6-4	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGTCTCCAGAGCAAACACAGATGATT
	TRBV6-56	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCCAGATCAACCACAGAGGAT
	TRBV6-8	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCTAGATTAAACACAGAGGATTTC
	TRBV6-9	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCTACAATGTATCCAGATCAAACA
	TRBV7-2	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCGCTTCTCTGCAGAGAGGACTGG
	TRBV7-3	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGGTTCTTTGCAGTCAGGCCTGA
	TRBV7-8	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAGTGATCGCTTCTTTGCAGAAA
	TRBV?-46	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCCACTCTGAMGATCCAGCGCA
	TRBV7-7	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGAGAGGCCTGAGGGATCCAT
	TRBV7-9	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGCAGAGAGGCCTAAGGGATCT
	TRBV9	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCGCACAACAGTTCCCTGACTT
	TRBV10-13	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGATGGCTAYAGTGTCTCTAGATCAAA
	TRBV10-2	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTGTCTCCAGATCCAAGACAGAGAA
	TRBV11	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGAGAGGCTCAAAGGAGTAGACT
	TRBV12-34	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTAAGATGCCTAATGCATCATTCTC
	TRBV12-5	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCAGCAGAGATGCCTGATGCAACT

	A	B
	TRBV13	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCAGCTCAACAGTTCAGTGACTA
	TRBV14	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGAAAGGACTGGAGGGACGTAT
	TRBV15	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATAACTTCCAATCCAGGAGGCCG
	TRBV16	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTAAGTGCCTCCCAAATTCACCC
	TRBV18	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAACGATTTTCTGCTGAATTTCCCA
	TRBV19	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTACAGCGTCTCTCGGGAGAAGA
	TRBV20-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACAAGTTTCTCATCAACCATGCAA
	TRBV24-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGATACAGTGTCTCTCGACAGGC
	TRBV25-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAACAGTCTCCAGAATAAGGACGGA
	TRBV27-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAAAGTCTCTCGAAAAGAGAAGAGGA
	TRBV28	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGGGTACAGTGTCTCTAGAGAGA
	TRBV29	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTTCCCATCAGCCGCCCAAACCTA
	TRBV30	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGACCCCAGGACCGGCAGTTCAT

Table 2: Human T7-UPS2-TCRV oligo sequences

	A	B
	mAV01	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCGCTCGAATGGGTACAGTTACCTGA
	mAV02	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGGAAGCTCAGCACTCTGAACCTGA
	mAV03	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACTCTCTCTGAACCTCACAGCTGCCCAA

	A	B
	mAV041	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGCTACAGCACCCTGCACATCAC
	mAV042	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGGAGAGCTACAGCACCCTGCAA
	mAV043	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGGAGAGCTACAGCACCCCGCAA
	mAV044	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGGAGCTCTACAGCACCCTGCAA
	mAV051	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTACAGCCACTCAGCCTGGAGACTA
	mAV052	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCACAGACACCCAGCCTGGAGACA
	mAV061	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCTTCCACTTGCAGAAAGCCTCAGTA
	mAV062	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCCTTCCACTTACAGAAAGCCTCAGTGCT
	mAV063	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGGAAGCAGCAGAGGTTTTGAAGCTACAC
	mAV071	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGCTCACCTCAATAAGGCCAGCCTGA
	mAV072	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGAGACTCCCAGCCCAGTGACTCA
	mAV073	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGAGAGTCGCAACCCAGTGACTCA
	mAV074	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCAATAGAGCCAGCCTGCATGTTTCA
	mAV075	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGTGTCCATCTTCTCTGATGGTGAAAAGGT
	mAV081	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCACCCTTGACACCTCCAGCCT
	mAV082	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGTGGAAGACTCAGAGCCACCCTTA
	mAV091	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGAAAGCCTCCGTGCACTGGAGCGT
	mAV092	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCTTCGAGGCTGAGTTCAGCAAGAC
	mAV093	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAACTCTTCCTTCCACCTGCGGAAAT

	A	B
	mAV10	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACATCACAGCCACACAGCCTGAAGAC
	mAV11	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCACAGCACGCTGCACATCACAGA
	mAV12	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGCTCCTTCCATCTGCAGAAGTCCA
	mAV131	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCTCTTTGCACATTTCCTCCTCCCAGAA
	mAV132	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCTCTTTGACTATATCCTCCTCCCAGACCT
	mAV141	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTCTCAGCCTGGAGACTCAGCCT
	mAV142	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAGATGGACGATTCACAATCTTCTTCAC
	mAV151	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCGCTATTCTGTAGTCTTCCAGAAATCACA
	mAV152	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCATCAGCCTTGTCATTTCAGCCTCACT
	mAV16	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAAAAAGTTCCATCGGACTCATCAC
	mAV17	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTTTCAACCTGAAGAAATCCCCAGCCCATA
	mAV19	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTTCTCACTGCACATCACAGCCTCCCT
	mAV21	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGGCTATTGCCTCTGACAGAAAGTCAC
	mBV01	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTGATACGGAGCTGAGGCTGCAAGA
	mBV02	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCAGATCACAGCTCTAAAGCCTGATGACC
	mBV03	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCAACCCACAGCACTGGAGGACA
	mBV04	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGCTTCTCACCTCAGTCTTCAGATAAC
	mBV05	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGCCCAGACAGCTCCAAGCTACA
	mBV12	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCCAGCAGATTCTCAGTCCAACAGTC

	A	B
	mBV131	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCACCA GAACAACGCAAGAAGC
	mBV132	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACAAGGC CTCCAGACCAAGCCAAT
	mBV14	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCTAAAG GAACTAACTCCACTCTCAAGAC
	mBV15	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGAAGATT CAACCTACAGAACCCAAGGACA
	mBV16	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGAAGATC CAGAGCACGCAACCCCT
	mBV17	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCTCTACA TTGGCTCTGCAGGCCTAGT
	mBV19	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCACTGTG ACATCTGCCCAGAAGAT
	mBV20	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCCCATC AGTCATCCCAACTTATCCTA
	mBV23	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCTGCAGC CTGGGAATCAGAACGA
	mBV24	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCATCCTG GAAATCCTATCCTCTGAAGAC
	mBV25	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCCAATCT CATCCTTCATCTTGGAAATGCT
	mBV26	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGCAGCCT AGAAATTCAGTCCTCTGAGA
	mBV29	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGGGAGCAT TTCTCCCTGATTCTGGATTA
	mBV30	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCAAAC CTAACATTCTCAACGTTGACAGA
	mBV31	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACGGAGAA GCTGCTTCTCAGCCACA

Table 3: Mouse T7-UPS2-TCRV oligo sequences

Troubleshooting

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
	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
	4	100 µM Truseq-P5 Hybrid PCR primer	2 µM

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

Final volume 200 µL

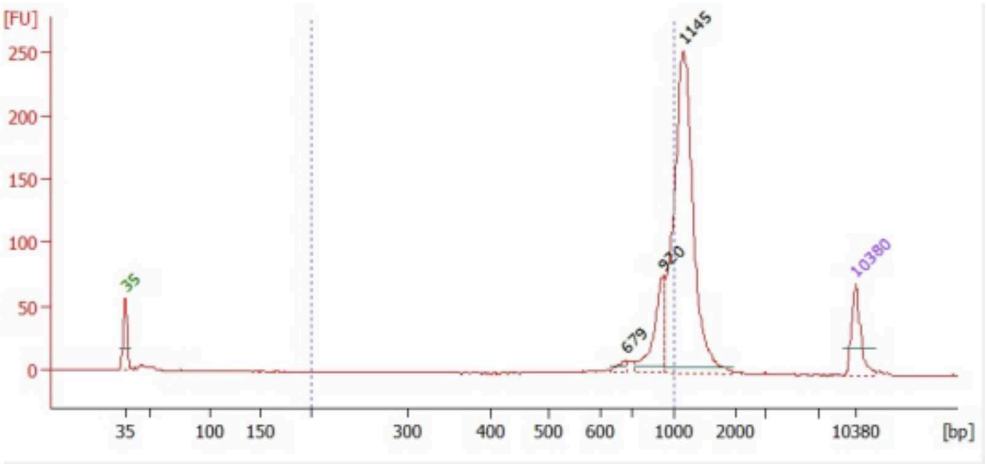
Index PCR protocol:

	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

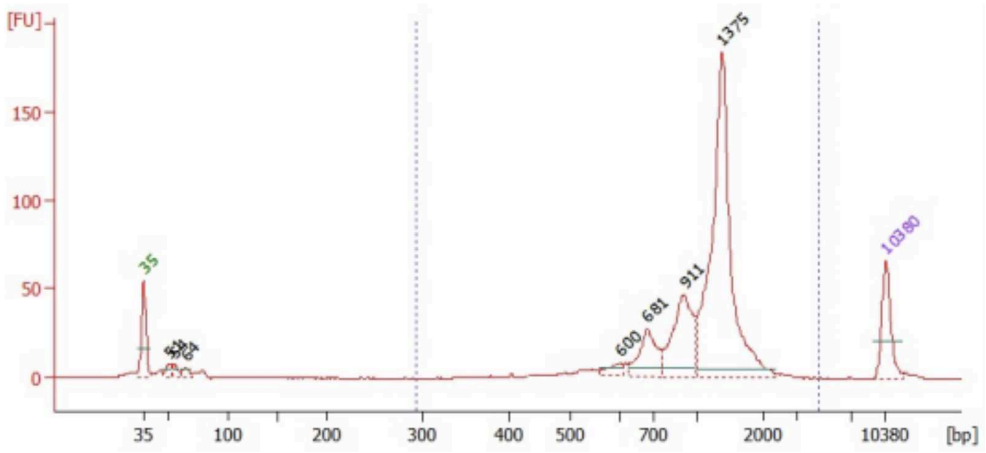
Safe stopping point, store at 4 °C

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