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Version 2

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

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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- μ m-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)
- 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	AATGATACGGCGACCAACGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
	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	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGTCGTTTTCTTCATTCCTTAGTC
	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 TCTTCCACTTGGAGAA
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	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCACCACTT 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 TCATTTAATGAAAAAAGC
TRAV34	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAAGTTGG ATGAGAAAAAGCAGCA
TRAV35	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCAGTTTG GTATAACCAGAAAGGA
TRAV36	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAGACTA AGTAGCATATTAGATAAG
TRAV38	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGTGAACCT CCAGAAAGCAGCCA
TRAV39	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCACTTG ATACCAAAGCCCGT
TRAV40	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGCGGAAA TATTAAAGACAAAACTC
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	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGTCTCCAAGACAAACACAGATGATT
TRBV6-56	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCCAGATCAACCACAGAGGAT
TRBV6-8	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCTAGATTAAACACAGAGGATTTC
TRBV6-9	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCTACAATGTATCCAGATCAAACA
TRBV7-2	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCGCTTCTCTGCAGAGAGGACTGG
TRBV7-3	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGGTTCTTTGCAGTCAGGCCTGA
TRBV7-8	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAGTGATCGTTCTTTGCAGAAA
TRBV?-46	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCCACTCTGAMGATCCAGCGCA
TRBV7-7	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGAGAGGCCTGAGGGATCCAT
TRBV7-9	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGCAGAGAGGCCTAAGGGATCT
TRBV9	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCGCACAACAGTTCCCTGACTT
TRBV10-13	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGATGGCTAYAGTGTCTCTAGATCAAA
TRBV10-2	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTGTCTCCAGATCCAAGACAGAGAA
TRBV11	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGAGAGGCTCAAAGGAGTAGACT
TRBV12-34	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTAAGATGCTAATGCATCATTCTC
TRBV12-5	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCAGCAGAGATGCCTGATGCAACT



A	B
TRBV13	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCAGCTCA ACAGTTCAGTGA
TRBV14	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGAAAGG ACTGGAGGGACGTAT
TRBV15	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATAACTTCC AATCCAGGAGGCCG
TRBV16	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTAAGTGC CTCCCAAATTCACCC
TRBV18	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAACGATT TTCTGCTGAATTTCCCA
TRBV19	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTACAGCG TCTCTCGGGAGAAGA
TRBV20-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACAAGTT TCTCATCAACCATGCAA
TRBV24-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGATACAGT GTCTCTCGACAGGC
TRBV25-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAACAGTCT CCAGAATAAGGACGGA
TRBV27-1	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAAAGTCT CTCGAAAAGAGAAGAGGA
TRBV28	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGGGTACAG TGTCTCTAGAGAGA
TRBV29	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTTCCCATC AGCCGCCCAAACCTA
TRBV30	TCTAGATAATACGACTCACTATAGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGACCCCA GGACCGGCAGTTCAT

Table 2: Human T7-UPS2-TCRV oligo sequences

A	B
mAV01	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCGCTCG AATGGGTACAGTTACCTGA
mAV02	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGGAAGC TCAGCACTCTGAACCTGA
mAV03	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACTCTCTC TGAACCTCACAGCTGCCCAA



A	B
mAV041	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGCTACAG CACCCTGCACATCAC
mAV042	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGG AGAGCTACAGCACCCCTGCAA
mAV043	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGG AGAGCTACAGCACCCCGCAA
mAV044	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCTAAGG AGCTCTACAGCACCCCTGCAA
mAV051	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTACAGCC ACTCAGCCTGGAGACTA
mAV052	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCACAGACA CCCAGCCTGGAGACA
mAV061	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCTTCCAC TTGCAGAAAGCCTCAGTA
mAV062	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCCTTCCA CTTACAGAAAGCCTCAGTGCT
mAV063	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGGAAGCA GCAGAGGTTTTGAAGCTACAC
mAV071	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGCTCAC CTCAATAAGGCCAGCCTGA
mAV072	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGAGACTCC CAGCCCAGTGACTCA
mAV073	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGAGAGT CGCAACCCAGTGACTCA
mAV074	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCAATAGA GCCAGCCTGCATGTTTCA
mAV075	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGTGTCCAT CTTCTCTGATGGTGAAAAGGT
mAV081	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCACC CTTGACACCTCCAGCCT
mAV082	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGTGGA GACTCAGAGCCACCCTTA
mAV091	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGAAAGCC TCCGTGCACTGGAGCGT
mAV092	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCTTCGAG GCTGAGTTCAGCAAGAC
mAV093	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAACTC TTCTTCCACCTGCGGAAAT



A	B
mAV10	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACATCACAGC CACACAGCCTGAAGAC
mAV11	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCACAGCAC GCTGCACATCACAGA
mAV12	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCAGCTCCT TCCATCTGCAGAAGTCCA
mAV131	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCTCTTTG CACATTTCTCCTCCCAGAA
mAV132	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCTCTTTG ACTATATCCTCCTCCCAGACCT
mAV141	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTCTC AGCCTGGAGACTCAGCCT
mAV142	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAGAT GGACGATTCAATCTTCTTAC
mAV151	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCGCTATT CTGTAGTCTTCCAGAAATCACA
mAV152	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCATCAGC CTTGTCATTTAGCCTCACT
mAV16	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAAA AAGTTCCATCGGACTCATCAC
mAV17	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTTTCAAC CTGAAGAAATCCCCAGCCCATA
mAV19	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTTCTCAC TGCACATCACAGCCTCCCT
mAV21	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGGCTATT GCCTCTGACAGAAAGTCAC
mBV01	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCTGATACG GAGCTGAGGCTGCAAGA
mBV02	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCAGATCA CAGCTCTAAAGCCTGATGACC
mBV03	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCAACCC ACAGCACTGGAGGACA
mBV04	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCGCTTCTC ACCTCAGTCTTCAGATAAC
mBV05	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGCCCAGA CAGCTCCAAGCTACA
mBV12	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCCAGCA GATTCTCAGTCCAACAGTC

A	B
mBV131	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCACCA GAACAACGCAAGAAGC
mBV132	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACACAAGGC CTCCAGACCAAGCCAAT
mBV14	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCCTAAAG GAACTAACTCCACTCTCAAGAC
mBV15	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGAAGATT CAACCTACAGAACCCAAGGACA
mBV16	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTGAAGATC CAGAGCACGCAACCCCT
mBV17	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCTCTACA TTGGCTCTGCAGGCCTAGT
mBV19	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCACTGTG ACATCTGCCCAAGAGAT
mBV20	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTTCCCATC AGTCATCCCAACTTATCCTA
mBV23	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACTCTGCAGC CTGGGAATCAGAACGA
mBV24	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACGCATCCTG GAAATCCTATCCTCTGAAGAC
mBV25	AGATAATACGACTCACTATAGGGTCGTGGGTCGTGGGCTCGGAGATGTGTATAAGAGACCCCAATCT CATCCTTCATCTTGGAATGCT
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 $^{\circ}\text{C}$	5 minutes
		65 $^{\circ}\text{C}$ for human primer pool/70 $^{\circ}\text{C}$ for mouse primer pool	30 seconds
		72 $^{\circ}\text{C}$	3 minutes
	1	4 $^{\circ}\text{C}$	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 µL PCR 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 (µL)	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 µL PCR 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 (μL)	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 μL 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 H_2O .

- 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 μ M Nextera PCR primer (i7)	2 μ M
	100 ng	Sample	
	up to 200	ultrapure water	

Final volume 200 μ L

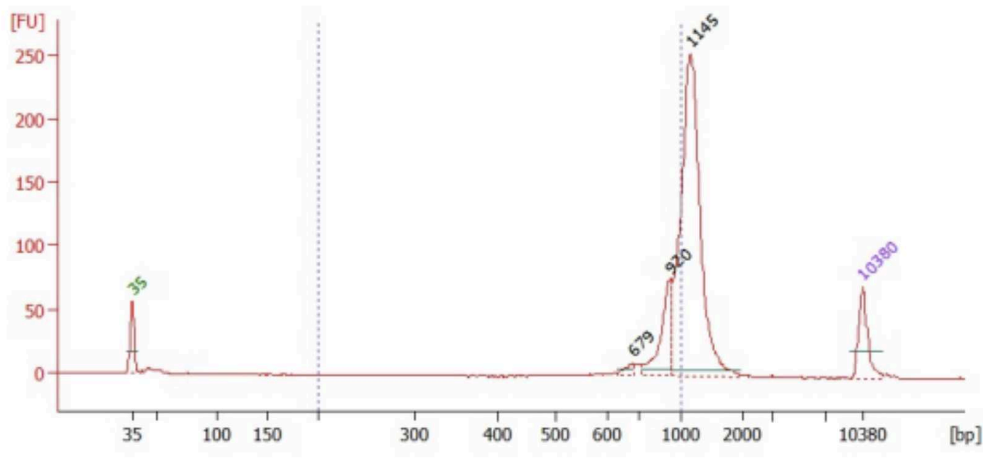
Index PCR protocol:

	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

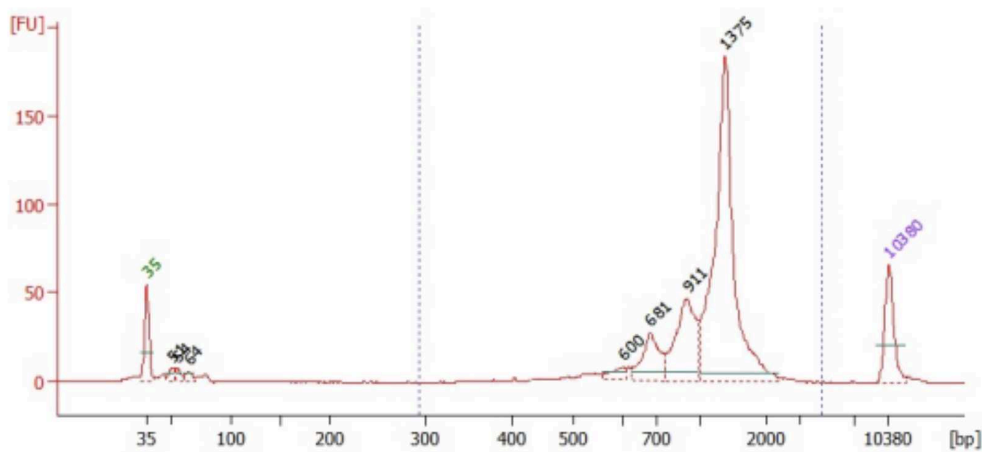
Safe stopping point, store at 4 °C

- 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 μ L of SPRI beads to 200 μ L PCR reaction volume). Elute in 10 μ L of water.

To quantify the TCR libraries, use the Qubit dsDNA high-sensitivity kit and BioAnalyzer High-Sensitivity. The 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