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***Method1: single cell multiomics analysis using the BD Rhapsody platform***

Cells were collected into a staining buffer at 200-800 cell/ul in ~500ul for each sample. Samples were stained with oligonucleotide-conjugated Sample Tags from the BD Human Single-Cell Multiplexing Kit in BD stain buffer following the manufacturer protocol. Barcoded samples were then washed and spun down at 350xg for 10 minutes and pooled. Pooled sample was then stained concurrently with oligonucleotide-conjugated antibody cocktail (custom table). Stain was in BD stain buffer for 30 minutes on ice, and samples were then spun down at 350xg for 10 minutes and washed three times. Pellet was resuspended in Rhapsody buffer for capture. Cell capture and library preparation were completed using the BD Rhapsody Targeted mRNA and AbSeq Reagent Kit. Briefly, cells were captured with beads in microwell plate, followed by cell lysis, bead retrieval, cDNA synthesis, template switching and Klenow extension, and library preparation in the Stanford Human Immune Monitoring Center following the BD Rhapsody protocol. Libraries were prepared for T cell receptor, sample tags, targeted mRNA using the human or mouse Immune Response or T cell panel with additional custom primer sets for genes (panel ID xxxxx), and AbSeq. Sequencing was completed on NovaSeq (Illumina, San Diego, CA) in (the sequencing facility). Rhapsody data were processed using the Seven Bridges Genomics online platform (San Francisco, CA) and BD Rhapsody Targeted Analysis Pipeline with V(D)J processing incorporated. After processing, data were imported into SeqGeq version 1.6.0 (BD, Ashland, OR). The import included a CSV file of all the data, and CSV files identifying the Sample Tag and V(D)J calls. Then the plug-in Lex BDSMK was run to separate out the Sample Tags, then the VDJ Explorer to identify clones. Unbiased clustering in SeqGeq was performed.

***Method2:*** ***Germline TRBV TCRseq using the ImmunoSEQ***

Sample gDNA were extracted using QIAGEN kit and quantified using fluorospectrometric measurement. Multiplexed PCR and barcoding amplification were performed per the manufacturer’s protocol. Briefly, Pipette 32 μL of Working Mix into the appropriate wells of a new 96-well PCR plate then Add 18 μL of each DNA sample to a well of the 1st PCR plate. Load sealed plate into thermal cycler and amplify samples using the following program: 95 °C 5 min; 94 °C 0.30 min, 62 °C 1.15 min, 72 °C 1.30 min × 21 cycles; 72 °C 10 min; 4 °C. Pipette 17 μL of Working Mix to the wells of a new 96-well PCR plate. Transfer 4 μL of primers from each appropriate well of the Barcode plate (included) to the same well locations in the 2nd PCR Plate. Transfer 4 μL of each diluted 1st PCR product to the same well locations in the 2nd PCR Plate. Load sealed plate into thermal cycler and amplify samples using the following program: 95 °C 15 min; 94 °C 0.30 min, 68 °C 0.40 min, 72 °C 1.00 min × 7 cycles; 72 °C 10 min; 12 °C. finally combine equal volumes (e.g., 10 μL) of each library replicate that will be sequenced together for further QCs and sequencing on MiSeq platform. Seq data was uploaded to ImmunoSEQ Analyzer using ImmunoSEQ Assistant software for further dada analysis. gDNA The ImmunoSEQ TRBV profiling kit (Adaptive Biotechnologies) was used for germline TCR profiling based on the principle of gDNA proportionally associated with number of T cells to calculate the proportion of antigen specificity. Each cell has only one V(D)J gene that has been successfully rearranged, thus gDNA can quantitatively better reflect the number of cells.

***Method3: human HLA typing using the AlloSeq Tx17***

Sample gDNA were extracted using QIAGEN kit and quantified using fluorospectrometric measurement. HLA typing was performed by using the AlloSeq Tx17 per the manufacturer’s protocol. Briefly, ~50 ng genomic DNA was bound, fragmented and tagged by the bead bound transponsons. Wash the fragmented DNA to remove any unbound DNA. Index PCR amplification in each well then pool all the sample Libraries into a single tube for cleanup & size selection. Hybridize the DNA library to the HLA probes to capture target fragments. Final enriched HLA library pool will be sequenced on MiSeq platform and resulting Fastq files were to be analyzed using the AlloSeq Assign software. Procedures for use of the AlloSeq Assign software can be found in the IFU082\_AlloSeq Assign IFU RUO. AlloSeq Tx leverages Nextera Flex for Enrichment hybrid capture technology from Illumina Inc. to enrich the genes of interest from a whole genome library preparation. The use of hybrid capture technology, as opposed to traditional long-range PCR techniques, has workflow benefits and enables the flexibility of variable gene/sequence content without the need for workflow changes.

***Method4: human or mouse whole transcriptome mRNAseq using the SMARTER kit***

Cells were sorted directly to 1000ul (or 500 ul if cell number<10,000) TRIzol and immediately stored at -80C for further processing. Total RNA was extracted using TRIzol’s method and QIAGEN RNeasy mini column clean up. First-strand cDNA synthesis directly from cells was primed by the 3’ SMART-Seq CDS Primer II A and uses the SMART-Seq v4 Oligonucleotide for template switching at the 5’ end of the transcript. Full length of cDNA was synthesized and amplified by LD PCR for 12 cycles using a SMARTseq v4 Ultra Low Input RNA kit (Cat. #634888) followed by the Illumina Nextera XT library preparation kit per the manufacturer’s protocol. An equal molar amount of tagmented cDNA library from each sample was pooled for sequencing on the Illumina HiSeq 4000 or NovaSeq 6000 platform at (the sequencing facility). FASTQ files were generated using the bcl2fastq2 Conversion v2.19 tool. Each set of samples seq data was aligned and normalized using STAR2 aligner or DRAGEN RNA pipeline to assemble the mapped reads into human transcriptome Homo Sapiens hg19 (Refseq) or mouse transcriptome Mus Musculus/MM10 (Refseq) and DEseq 2 was used for differential expression analysis. The R packages (or other tools to be specified) were used for secondary analysis and visualization (heatmap and clustering algorithm).

***Method5: Single cell TCR repertoire profiling using custom method (Davis Lab)***

Using single FACS-sorted T cells in 96-well plates, TCR sequence and gene expression analysis were obtained by a series of three nested PCR reactions. Cells were sorted directly into RT-PCR buffer. For the first reaction, reverse transcription and preamplification were performed with a One-Step RT-PCR kit (Qiagen) using multiplex PCR with multiple Vα and Vβ region primers, Cα and Cβ region primers, and primers for selected T cell phenotyping genes, in a 20-μl reaction. A 25-cycle first RT-PCR reaction was done per manufacturer's instructions using the following cycling conditions: 50 °C 30 min; 95 °C 15 min; 94 °C 30 s, 62 °C 1 min, 72 °C 1 min × 25 cycles; 72 °C 5 min; 4 °C. Next, a 1-μl aliquot of the first reaction was used as a template for a second 20-μl PCR using HotStarTaq DNA polymerase (Qiagen) for either TCR sequencing or phenotyping. The cycling conditions were: 95 °C 15 min; 94 °C 30 s, 64 °C 1 min, 72 °C 1 min × 25 cycles (TCR) or 35 cycles (phenotyping); 72 °C 5 min; 4 °C. A 1-μl aliquot of the second PCR product was used as a template for the third 20-μl PCR reaction, which incorporated barcodes and enabled sequencing on the Illumina MiSeq platform. The PCR products were combined at equal proportion by volume, run on a 1.2% agarose gel, and a band around 350 to 380 bp was excised and gel purified using a Qiaquick gel extraction kit (Qiagen). This purified product was then sequenced on Miseq for a pair-ended 2x250 run. The fastq files were used to run a customized TCRseq informatics pipeline, which assigned reads to wells, totaled phenotyping gene reads per well, assigned TCR Va, Ja, Vb, and Jb usage, along with alternate Va/Ja where applicable, and reported CDR3a and CDR3b sequences. This assay was performed by the Human Immune Monitoring Center at Stanford University. This method was based on Han et al., Nature Biotechnology 32, 684-692 (2014).

***Method6: Human or mouse Bulk TCR or BCR repertoire profiling using SMARTER kit***

Cells were collected into each Eppendorf tube preloaded with 1000 ul of TRIzol solution (thermoFisher Scientific, Cat. 10296010), mix well and keep at room temperature for 10 min then immediately stored at -80C for further processing. Total RNA was extracted using phenol/chloroform separation and isopropanol precipitation then using the RNeasy MinElute RNA cleanup kit (QIAGEN, Cat. 74204) per the manufacturer’s protocol. 10-50 ng total RNA was used for bulk TCRseq library prep. T-cell receptor profiling using The SMARTer Human TCR a/b Profiling Kit (Takara Bio, Cat. Nos. 635014) was performed and followed by the manufacturer’s protocol. Briefly, Following reverse transcription, two rounds of PCR are performed in succession to amplify cDNA sequences corresponding to variable regions of TRA and/or TRB transcripts. The first PCR uses the first-strand cDNA as a template and includes a forward primer with complementarity to the hTCR PCR1 Universal Forward and a reverse primer with complementarity to the constant region of TRA and/or TRB gene (hTCRa/b PCR1 Reverse). The second PCR takes the product from the first PCR as a template. It uses semi-nested primers (SMARTer RNA unique dual indexes and hTCRa/b PCR2 reverse primers) to amplify the entire variable region and a portion of the constant region of the TCRa and/or TCRb cDNA. The forward and reverse primers include adapter and UDI sequences which are compatible with Illumina sequencing platforms and allow for multiplexing. Following post-PCR purification, size selection, and quality analysis, the library is ready for sequencing. An equal amount of cDNA library from each sample was pooled for sequencing on the Illumina MiSeq platform. FASTQ files were automatically generated and demultiplexed using the real-time bcl2fastq2 Conversion v2.19 tool. The Immune Profiler (Takara bio) or the MIXCR (MI laboratories) was launched to call Human TRA/TRB, assemble clonotypes, and correct for PCR and sequencing errors. The output files include clonotype table and related details of germline assignments, Variable and Joining segment usage, mutations, CDR3 sequences, and clonotype abundances/sample clonality (Nature methods 12.5 (2015): 380-381).

***Method7: Human or Mouse cell-direct whole transcriptome mRNAseq using the SMARTER kit***

Cells (up to ~5000) were sorted directly to 10.5 ul of the 1x lysis buffer and immediately stored at -80C for further processing. first-strand cDNA synthesis directly from cells was primed by the 3’ SMART-Seq CDS Primer II A and uses the SMART-Seq v4 Oligonucleotide for template switching at the 5’ end of the transcript. Full length of cDNA was synthesized and amplified by LD PCR for 14 cycles using a SMARTseq v4 Ultra Low Input RNA kit (Cat. #634888) followed by the Illumina Nextera XT library preparation kit per the manufacturer’s protocol. An equal molar amount of tagmented cDNA library from each sample was pooled for sequencing on the Illumina NovaSeq 6000, Hiseq 4000, or NextSeq 550 platform at (the sequencing facility). FASTQ files were generated using the bcl2fastq2 Conversion v2.19 tool. Each set of samples seq data was aligned and normalized using STAR aligner or DRAGEN RNA pipeline to assemble the mapped reads into human transcriptome Homo Sapiens hg19 (Refseq) or mouse transcriptome Mus Musculus/MM10 (Refseq)and DEseq 2 was used for differential expression analysis. The R packages (or other tools to be specified) were used for secondary analysis and visualization (heatmap and clustering algorithm).

***Method8: human or mouse whole transcriptome mRNAseq using Kapa kit***

Cells were sorted into 1.5 ml Eppendorf tube preloaded with 1000 ul of the TRIzol LS Reagent (thermoFisher Scientific, Cat.#10296028). RNA was extracted using TRIzol method with chloroform phase separation and isopropanol precipitation then applied to the QIAGEN miRNeasy mini (QIAGEN, Cat. #217004) column for RNA purification. All RNAs were checked on a NANODRP1000 and Agilent bioanalyzer 2100 PICO chip for RNA purity and integrity. The KAPA mRNA HyperPrep Kits (KK8580) with the  IDT for Illumina Dual Index Adapter kit (Cat. #20021454) per the manufacturer’s protocol. Briefly, mRNA was captured using magnetic oligo-dT beads. Fragmentation was performed using heat and magnesium. 1st strand cDNA synthesis was completed using random priming. Combined 2nd strand synthesis and A-tailing, adapter ligation, library amplification, and KAPA Pure Beads clean-ups were performed for library preparation. The strand marked with dUTP is not amplified, allowing strand-specific sequencing. All final libraries were checked on Agilent’s bioanalyzer 2100 High Sensitivity DNA Chip. An equal amount of cDNA library from each sample was pooled for sequencing on the Illumina NovaSeq 6000 platform S4 flowcell. FASTQ files were generated using the bcl2fastq2 Conversion v2.19 tool. Each set of samples seq data was aligned and normalized using STAR aligner or DRAGEN RNA pipeline to assemble the mapped reads into human transcriptome Homo Sapiens hg19 (Refseq) or mouse transcriptome Mus Musculus/MM10 (Refseq)and DEseq 2 was used for differential expression analysis. The R packages (or other tools to be specified) were used for secondary analysis and visualization (heatmap and clustering algorithm).

***Method9: gDNA extraction from PMBC and Granulocytes***

Genomic DNA from PBMC (thawed routinely from liquid nitrogen, washed with cold PBS twice, and resuspend pellet in 200 ul cold PBS) and Granulocytes (resuspended in 200 ul cold PBS) was extracted using a commercially available kit (QIAamp DNA Mini kit, cat.# 51304, Qiagen, Valencia, CA, USA) following the manufacturer’s instructions. Optimized buffers and enzymes in the QIAamp DNA Mini Kit lyse samples, stabilize nucleic acids and enhance selective DNA adsorption to the QIAamp membrane. Alcohol is added and lysates loaded onto the QIAamp spin column. Wash buffers are used to remove impurities and pure, ready-to-use DNA is then eluted in resuspension buffer. All steps (lyse, bind, wash, and elute) in the purification procedure are fully automated for 12 samples per run. Yields of DNA were measured using spectrophotometer OD260nm reading and/or qubit fluorometer. DNA purified using the QIAamp DNA Mini Kit can be used directly in a wide range of downstream applications, including PCR and quantitative real-time PCR, Southern blotting, SNP and STR genotyping and pharmacogenomic research.

***Method10: gDNA extraction from the PAXgene DNA blood***

Blood collected into PAXgene Blood DNA Tubes, which contain a proprietary blend of reagents that both prevents blood coagulation and stabilizes white blood cells, were processed for gDNA extraction using the PAXgene Blood DNA Kit ( cat.# 761133, Qiagen, Valencia, CA, USA) following the manufacturer’s instructions. The PAXgene DNA solution was mixed to lyse red and white blood cells. Cell nuclei and mitochondria were pelleted by centrifugation, washed, and resuspended in digestion buffer. Protein contaminants were removed by incubation with a protease. DNA was precipitated in isopropanol, washed in 70% ethanol, dried, and resuspended in resuspension buffer. Yields of DNA were measured using spectrophotometer OD260nm reading and/or qubit fluorometer. DNA purified using the QIAamp DNA Mini Kit can be used directly in a wide range of downstream applications, including PCR and quantitative real-time PCR, Southern blotting, SNP and STR genotyping and pharmacogenomic research.

***Method11: total RNA extraction from PAXgene RNA blood***

whole blood (2-3 ml) samples were collected into the BD PreAnalytiX PAXgene blood RNA tubes then stored at -80c. The PAXgene blood samples can be stable at room temperature or at 4c degree for a few days and stored at -20c or -80c for a long term (12 mo). After spinning down PAXgene whole blood for 10 minutes at 3000–5000 x g using a swing-out rotor, the pellet was suspended in 350 µl lysis Buffer BR1 then loaded to QIAcube for automation. It took less than two hours to complete the whole RNA extraction. The yields were about 40-80 ng/ul from 2-3 ml human whole blood. Total RNA was pure with A260/A280 values between 1.8 and 2.2. The RIN (RNA Integrity Number), which was determined by Agilent’s bioanalyzer NANO RNA analysis, were >9 in ≥95% of all samples . No inhibitions have been observed in downstream assays, such as qPCR and mRNAseq etc.

***Method12: single cell DNA and surface protains multiomics using the Tapestri platform***

Cells were prepared (PBMC, Bone marrow, flow cytometry sorted, etc.) and provided using customer’s protocols. Cells were blocked with Blocking buffer then stained with resuspended Hemo panel oligo-tagged antibodies following the instruction in the Tapestri Single-Cell DNA + Protein Sequencing User Guide V2 (Tapestri User Guide V2). Briefly, 1M cells were stained with TotalSeq-D Heme Oncology Cocktail (BioLegend; Cat. num. 399906) reconstituted in 59 μl of Cell Staining buffer (BioLegend; and adding 1 μl of each TotalSeq-custom antibody) for 30 minutes on ice. A total of 120,000-140,000 cells were loaded into a Tapestri microfluidics cartridge. Upon encapsulation, cells were lysed. The DNA digestion and targeted PCR steps were performed in a SimpleAmp Thermal Cycler (thermoFisher Scientific). All other sample processing steps were performed following the Tapestri User Guide V2. All Tapestri related reagents were obtained using Tapestri Single-Cell DNA Kits and Cartridge (Mission Bio, Inc, Cat. num. MB02-0001 and MB03-0034). The Tapestri User Guide V2 was followed to prepare the libraries. Briefly, PCR products were retrieved from individual droplets, and purified with 0.7X Ampure XP beads (Beckman Coulter; Cat. num. A63881), to split the scDNA-seq library bound to the beads from the surface proteins library in the supernatant. Illumina i5/i7 sequencing indexes were added to the scDNA-seq PCR products, followed by two steps of purification, using 0.6X and 0.65X Ampure XP beads (these are different from the ratios indicated in the Tapestri protocol). PCR products from the surface proteins library were incubated with Tapestri Biotin Oligo at 96 °C for 5 minutes, followed by incubation on ice for 5 minutes, and purification using Tapestri Streptavidin Beads. Afterwards, the beads were used as PCR templates for the incorporation of i5/i7 Illumina indices, followed by purification using 0.9X Ampure XP beads. The quality of all scDNA-seq and surface proteins libraries were assessed by Bioanalyzer. Libraries were pooled and sequenced on the Illumina NovaSeq6000 platform at (the Sequencing facility), at a sequencing depth of 260 M reads/library in case of surface proteins libraries, and 420 M reads/library for scDNA-seq libraries.

Raw sequencing data was processed using the Mission Bio Tapestri Pipeline v2 (https://support.missionbio.com/hc/en-us/sections/360006255314-TapestriPipeline). Briefly, cell barcodes were extracted from the raw FASTQ data files and sequencing adapters trimmed using cutadapt v2.5. Trimmed reads were aligned to the reference genome version ‘hg19’ using bwa-mem v0.7.12. Subsequently, read pair information was verified using PicardTools (v1.126, https://github.com/broadinstitute/picard), and quantified with samtools (v1.9), and the cell barcode distribution was computed using the python scripts provided by Mission Bio.

***Major publications using HIMC genomics services:***

1. miR Profile of Chronic Right Ventricular Pacing: a Pilot Study in Children with Congenital Complete Atrioventricular Block. **Journal of Cardiovascular Translational Research**. Received: 16 June 2022 / Accepted: 1 September 2022 (Agilent micrroRNA microarray)
2. KIR+ CD8+ T cells suppress pathogenic T cells and are active in autoimmune diseases and COVID-19. ***Science***, 10.1126/science.abi9591, First release: 8 March 2022 (mRNAseq & TCRseq)
3. Early non-neutralizing, afucosylated antibody responses are associated with COVID-19 severity. ***Sci. Transl. Med***., 10.1126/scitranslmed.abm7853 (2022) (mRNAseq)
4. Allogeneic CAR Invariant Natural Killer T Cells Exert Potent Antitumor Effects through Host CD8 T-Cell Cross-Priming. ***CLINICAL CANCER RESEARCH***, Published OnlineFirst August 10, 2021; DOI: 10.1158/1078-0432.CCR-21-1329 (mRNAseq & TCRseq)
5. Alloantigen-specific type 1 regulatory T cells suppress through CTLA-4 and PD-1 pathways and persist long-term in patients. ***Science Translational Medicine***, 26 October, 2021 (bulk TCRseq)
6. Association of Premature Immune Aging and Cytomegalovirus After Solid Organ Transplant. ***Frontiers in Immunology***, 27 May, 2021 (Rhapsody single mRNA/TCR/AbSeq)
7. Development of immunosuppressive myeloid cells to induce tolerance in solid organ and hematopoietic cell transplant recipients. ***Blood Advances***, August 30, 2021 (mRNA & TCRseq)
8. High-Parametric Evaluation of Human Invariant Natural Killer T Cell to Delineate Heterogeneity in Allo- and Autoimmunity. ***Blood***. January 2020 (Rhapsody single mRNA/TCR/AbSeq)
9. Opposing T cell responses in experimental autoimmune encephalomyelitis.

***Nature***. 2019 Aug;572(7770):481-487.(scTCRseq)

1. Autoimmunity to hypocretin and molecular mimicry to flu in type 1 narcolepsy. ***Proc Natl Acad Sci*** U S A PNAS | vol. 115 | no. 52 | E12323–E12332.(scTCRseq)
2. mTORC1 underlies age-related muscle fiber damage and loss by inducing oxidative stress and catabolism. [***Aging Cell***.](https://www.ncbi.nlm.nih.gov/pubmed/30924297) 2019 Jun;18(3) (Gene expression microarray)
3. A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. ***Nature Medicine***, volume 25, pages487–495 (2019).(Gene expression microarray)
4. Whole-genome sequencing of Atacama skeleton shows novel mutations linked with dysplasia. [***Genome Res.***](https://www.ncbi.nlm.nih.gov/pubmed/29567674) 2018 Apr;28(4):423-431 (whole genome DNAseq)
5. Identifying specificity groups in the T cell receptor repertoire. [***Nature***.](https://www.ncbi.nlm.nih.gov/pubmed/28636589) 2017 Jul 6;547(7661):94-98. (scTCRseq)
6. Peripheral blood cell microRNA quantification during the first trimester predicts preeclampsia: Proof of concept. [***PLoS One***.](https://www.ncbi.nlm.nih.gov/pubmed/29293682) 2018 Jan 2; (Fluidigm qPCR microRNA dynamic arrays)
7. [Early first trimester peripheral blood cell microRNA predicts risk of preterm delivery in pregnant women: Proof of concept.](https://www.ncbi.nlm.nih.gov/pubmed/28692679) [***PLoS One***.](https://www.ncbi.nlm.nih.gov/pubmed/28692679) 2017 Jul 10;12(7)
8. First-trimester maternal cell microRNA is a superior pregnancy marker to immunological testing for predicting adverse pregnancy outcome. [***J Reprod Immunol*.**](https://www.ncbi.nlm.nih.gov/pubmed/25965838) 2015 Aug;110:22-35. (Fluidigm qPCR microRNA dynamic arrays)
9. First trimester PBMC microRNA predicts adverse pregnancy outcome. [***Am J Reprod Immunol*.**](https://www.ncbi.nlm.nih.gov/pubmed/24974972) 2014 Nov;72(5):515-26. (microRNA microarray & Fluidigm qPCR microRNA dynamic arrays)
10. Successful immunotherapy induces previously unidentified allergen-specific CD4+ T-cell subsets. [***Proc Natl Acad Sci*** U S A.](https://www.ncbi.nlm.nih.gov/pubmed/26811452) 2016 Mar 1;113(9). (scTCRseq)