Single Cell TCR and BCR

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Outline

1. Introduction of immunity
2. Single cell TCR and BCR
3. Analysis of scTCR and scBCR data 1：from 10X Genomics raw data (.fastq format) to contig files by cellranger vdj
4. Analysis of scTCR and scBCR data 2：scRepertoire

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Immunity is very important, and a hotspot in life science research

In 2024, it is the 50th anniversary of the founding of *Cell*. To commemorate this milestone, *Cell* plans to launch a year-long series of anniversary events, including a special issue on *Immunology*. On April 25th, *Cell* published an editorial article, emphasizing that immunology is a field for everyone, playing a crucial role in multiple areas such as tumor immunology, neuroimmunology, immunometabolism, and reproductive immunology. On the same day, *Cell* published three heavyweight reviews, covering adaptive immunity, innate immunity, and discussions on the latest perspectives in immunology, providing a comprehensive interpretation of human immunology.


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Cell Leading Edge

Review

Principles and therapeutic applications of adaptive immunity

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https://doi.org/10.1016/j.cell.2024.03.037


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Cell Leading Edge

Review

From periphery to center stage: 50 years of advancements in innate immunity

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https://doi.org/10.1016/j.cell.2024.03.036


Cell Leading Edge

CellPress

Perspective

Exploring new perspectives in immunology

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[3]Center for Infection and Immunity, Yale School of Medicine, New Haven, CT, USA
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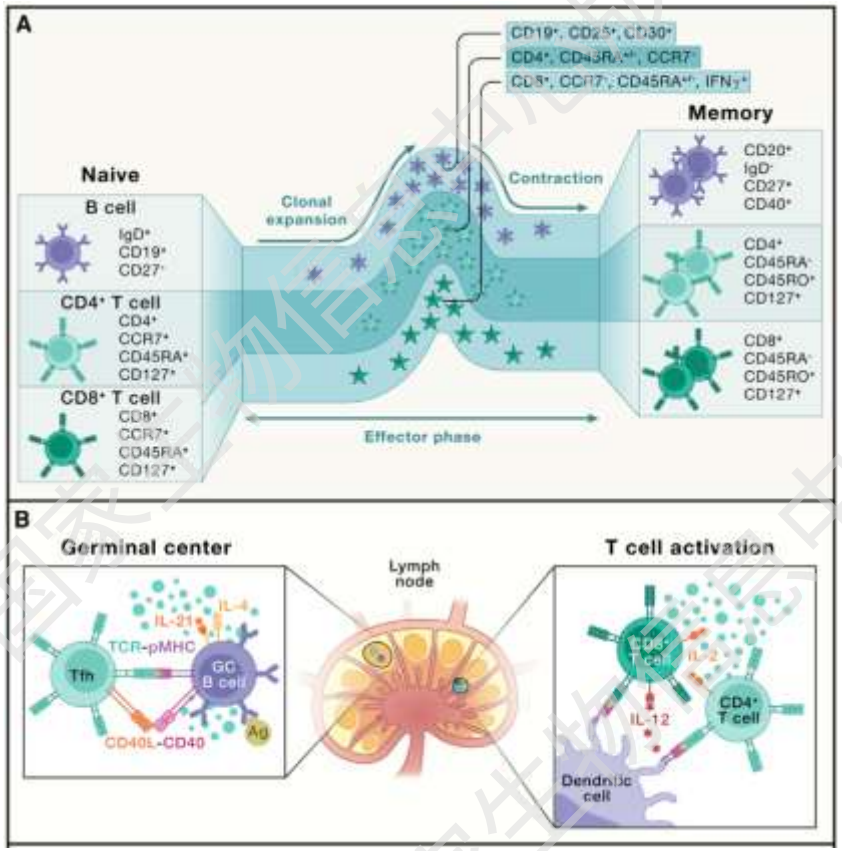
https://doi.org/10.1016/j.cell.2024.03.036

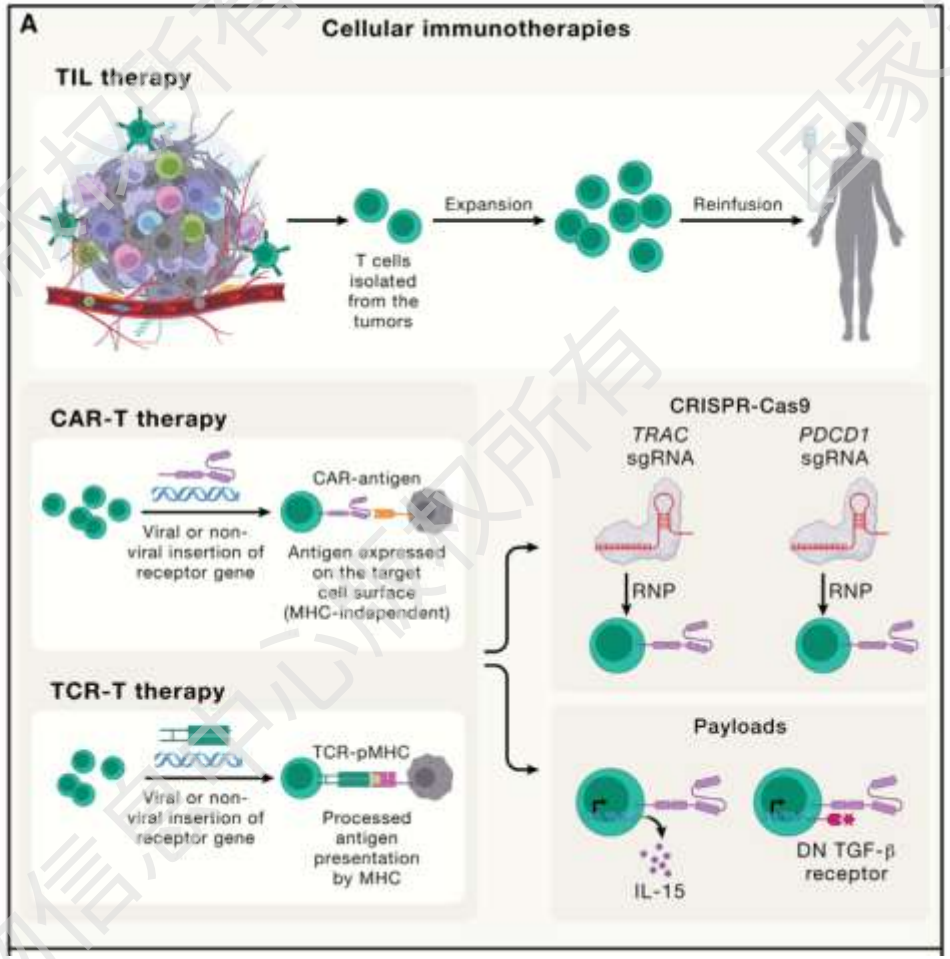
Adaptive immune cell states and the primary response

B cells, CD4+ T cells, and CD8+ T cells acquire multiple differentiation fates during a normal immune response. Naive T cells and B cells are quiescent cells. Upon activation, these cells undergo clonal expansion and acquire effector function (effector phase). Following pathogen control, most effector cells undergo apoptosis (contraction), leaving long-lived memory cells to provide continued immune surveillance (memory).



Engineering adaptive immune responses for cell- and antibody-based therapies

TIL, CAR-T, and TCR-T are three prominent cellular immunotherapies for cancer. For TIL therapy, T cells are isolated from the tumors for expansion and reinfusion into tumor patients; for CAR-T cells, CARs are engineered to recognize surface antigen independently of MHC-mediated antigen presentation; for TCR-T, a specific TCR gene recognizing the tumor antigen is ectopically expressed in T cells to expand tumor-specific T cells for tumor killing, which requires peptide-MHC (pMHC) expression on tumor cells. For CAR-T or TCR-T therapies, new strategies have been developed to modify intracellular signaling pathways in these tumor-specific T cells to enhance tumor killing, such as CRISPR-mediated genome editing of the *TRAC* or *PDCD1* locus or introduction of payloads (e.g., cytokines like IL-15 and dominant-negative [DN] TGF-β receptor) expressed on T cells. These strategies increase the antitumor function in these cellular therapies.



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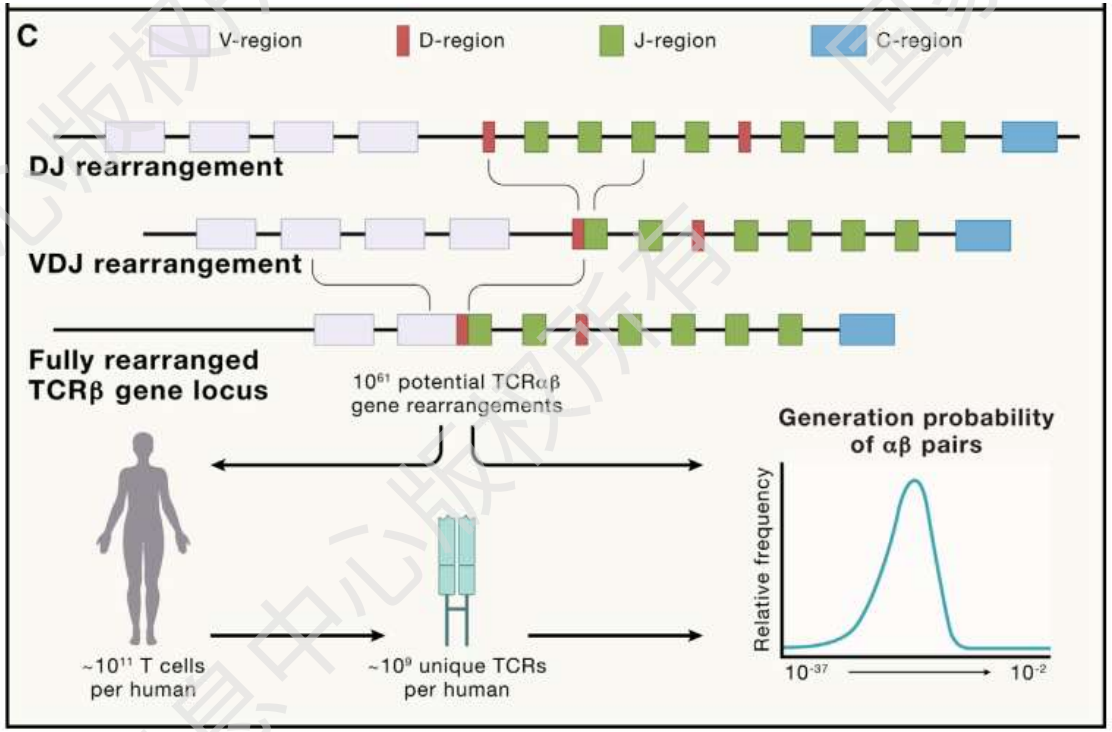
The generation of antigen receptor (TCRs and BCR) diversity are generated by the process of V(D)J recombination

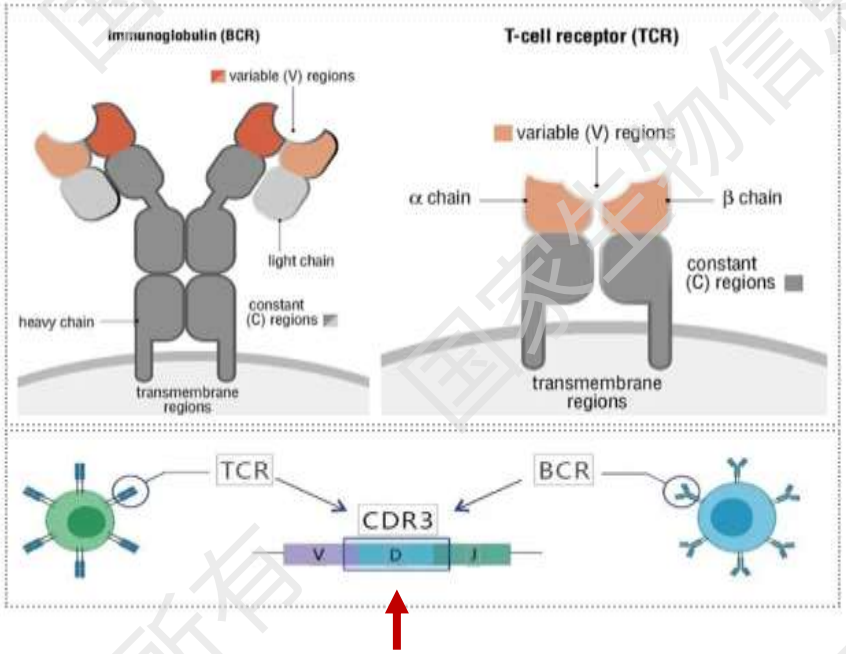
The generation of antigen receptor diversity. TCRs and BCRs are generated by the process of V(D)J recombination, which involves the fusion of separate V, D (on TCRβ and heavy chains), and J segments. Depicted is a TCRβ-chain recombination. Subsequent mRNA splicing brings the constant region together with the V(D)J fusion. This process can result in a vast potential diversity, which is only minimally represented in any individual organism. The distribution of probabilities for an individual TCR to recombine in an individual varies over 20 orders of magnitude.



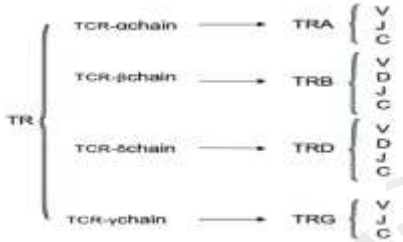
The main detection objects of scTCR (single-cell TCR sequencing) and scBCR (single-cell BCR sequencing)

TCR and BCR are two distinct receptors, differing in both structure and function.

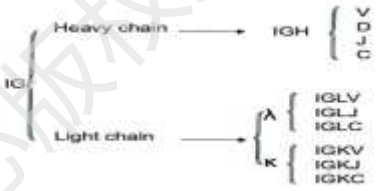
- TCR V(D)J recombination: The majority of TCRs are composed of α and β peptide chains (90-95%), while a minority of T cells have TCRs composed of γ and δ peptide chains. Each peptide chain can be divided into several parts. The most important part of them is the variable region. The variable region of the α chain is encoded by V and J gene segments, while the variable region of the β chain is encoded by V, D, and J gene segments.
- BCR V(D)J recombination: BCR contain two heavy chains (H) and two light chains (κ or λ). The heavy chain is divided into the variable region and other contact regions. The variable region of the light chain is encoded by V and J gene segments, while the variable region of the heavy chain is encoded by V, D, and J gene segments.
- CDR3: The BCR/TCR has a region known as the CDR, which plays a crucial role in antigen recognition. The CDR consists of CDR1, CDR2, and CDR3. Among them, CDR3 is formed by the encoding of V, D, and J genes, which has a higher degree of diversity and directly determines the antigen-binding specificity of TCR/BCR.



TCR



BCR



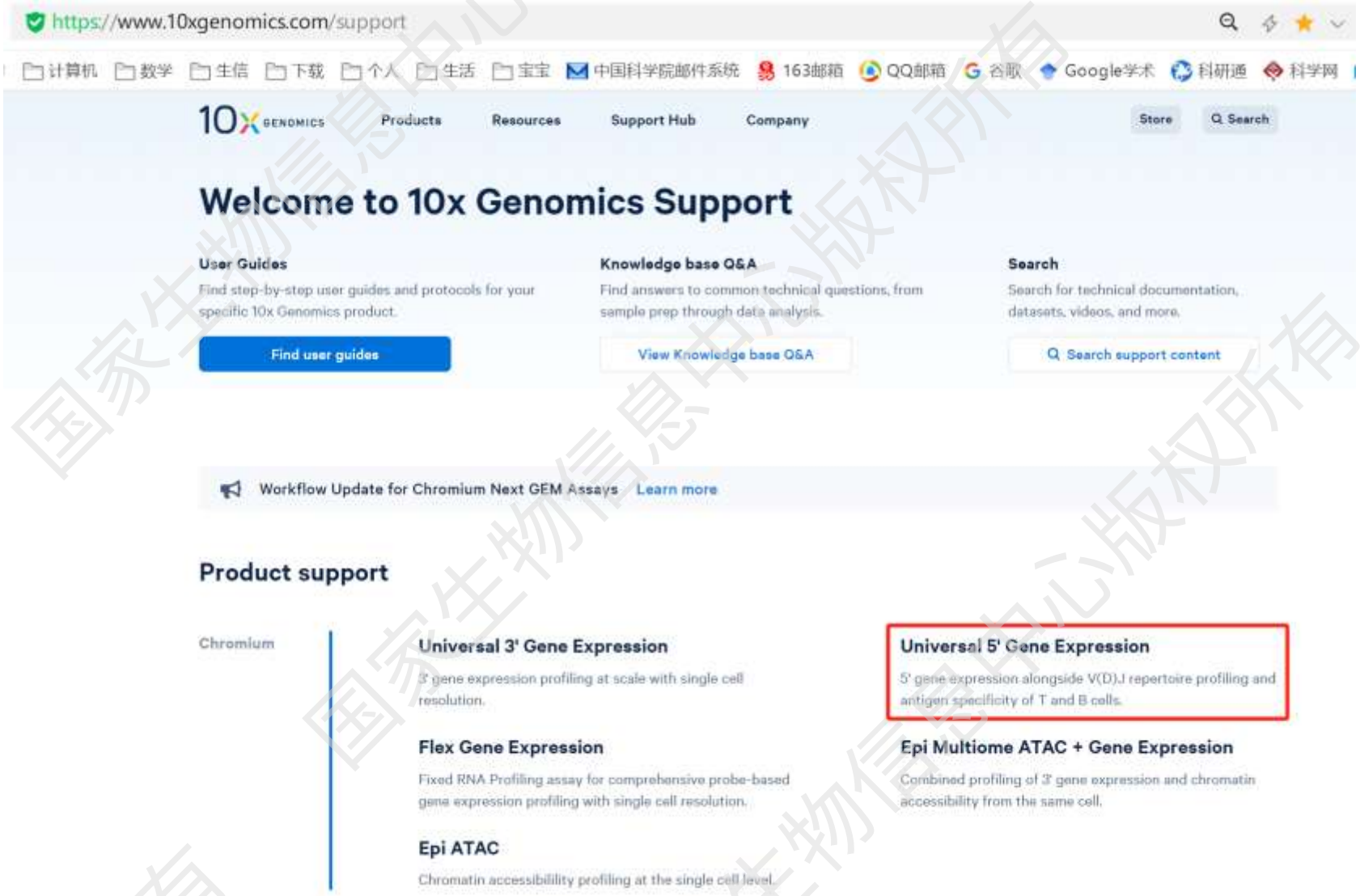
Locus	Major loci					
	Chromosomal localization	V	D	J	C	Total number of genes [2]
TRA	14q11.2	54*	0	61	1	116*
TRB	7q34	64-67	2	14	2	82-85
TRG	7q14	12-15	0	5	2	19-22
TRD	14q11.2	3 (8*)	3	4	1	11 (16*)
Total number of genes		133-139	5	84	6	228-234

TRA,TRB,TRD,TRG这4类的V,D,J,C基因数量

Locus	Major loci					
	Chromosomal localization	V	D	J	C	Total number of genes [1]
IGH	14q32.33	123-128	27	6	11*	170-176*
IGK	2p11.2	[40* or] 76	0	5	1	[46* or] 82
IGL	22q11.2	73-74	0	7-11	7-11	87-96
Total number of genes		236-278	27	21-25	15-23	303-354

IG分成IGH,IGK,IGL这3类的V,D,J,C基因数量

10X Genomics library preparation of 5’ scRNA and scTCR/scBCR

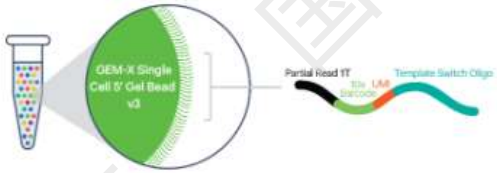


10X Genomics library preparation of 5’ scRNA and scTCR/scBCR

Chromium Single Cell

Universal 5' Gene Expression

This assay requires Chromium X/iX instrument Firmware v2.0 or newer. Refer to the Chromium X Series Firmware Update Procedure for details

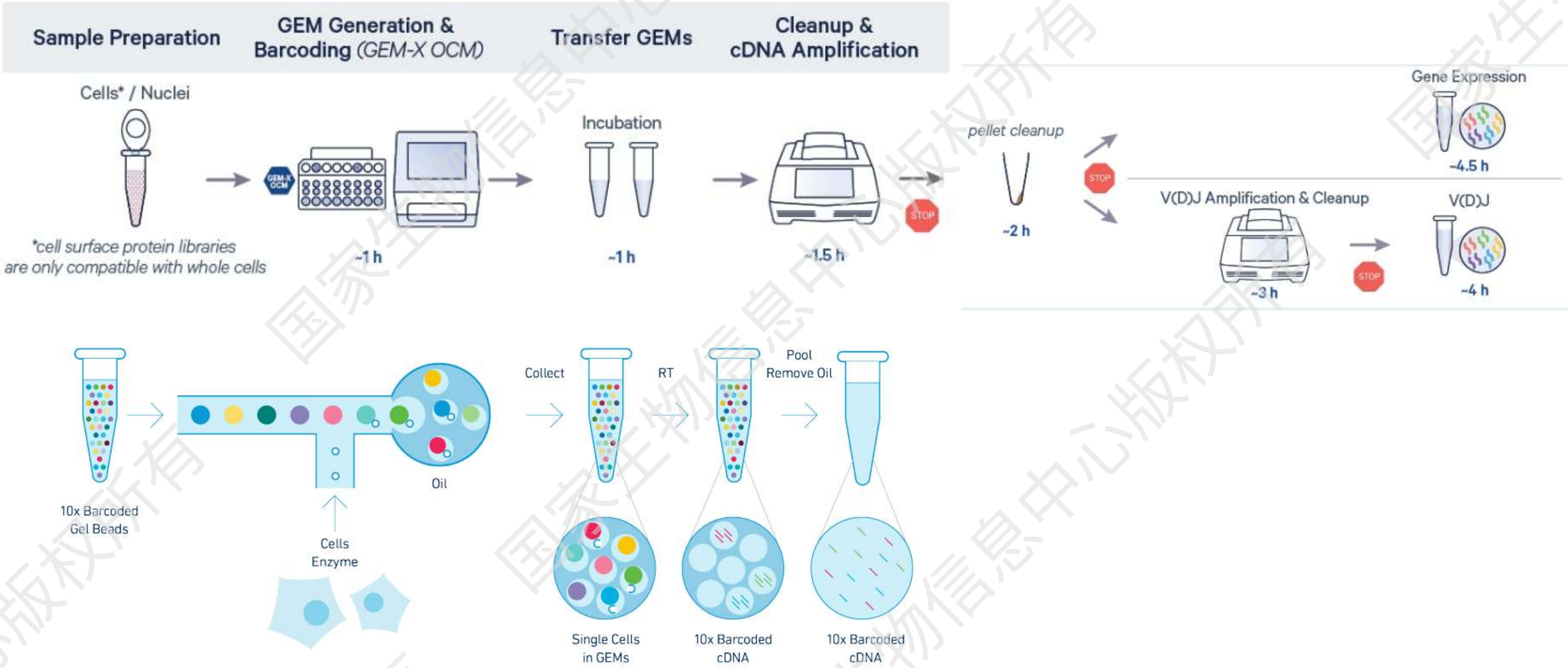


What is Universal 5' Gene Expression?

Chromium Universal 5' Gene Expression allows analysis of full-length, paired B-cell or T-cell receptor (BCR/TCR) sequences, surface protein expression, and 5’ gene expression, all from a single cell.

Find user guides Get started

10X Genomics library preparation of 5’ scRNA and scTCR/scBCR



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10X Genomics scRNA+scTCR/scBCR rawdata in .fastq format

Path to rawdata/scRNA:

```
Sub01_S1_L001_R1_001.fastq.gz
Sub01_S1_L001_R2_001.fastq.gz
Sub01_S1_L002_R1_001.fastq.gz
Sub01_S1_L002_R2_001.fastq.gz
Sub01_S1_L003_R1_001.fastq.gz
Sub01_S1_L003_R2_001.fastq.gz
Sub02_S1_L001_R1_001.fastq.gz
Sub02_S1_L001_R2_001.fastq.gz
Sub02_S1_L002_R1_001.fastq.gz
Sub02_S1_L002_R2_001.fastq.gz
Sub02_S1_L003_R1_001.fastq.gz
Sub02_S1_L003_R2_001.fastq.gz
```

Path to rawdata/scTCR:

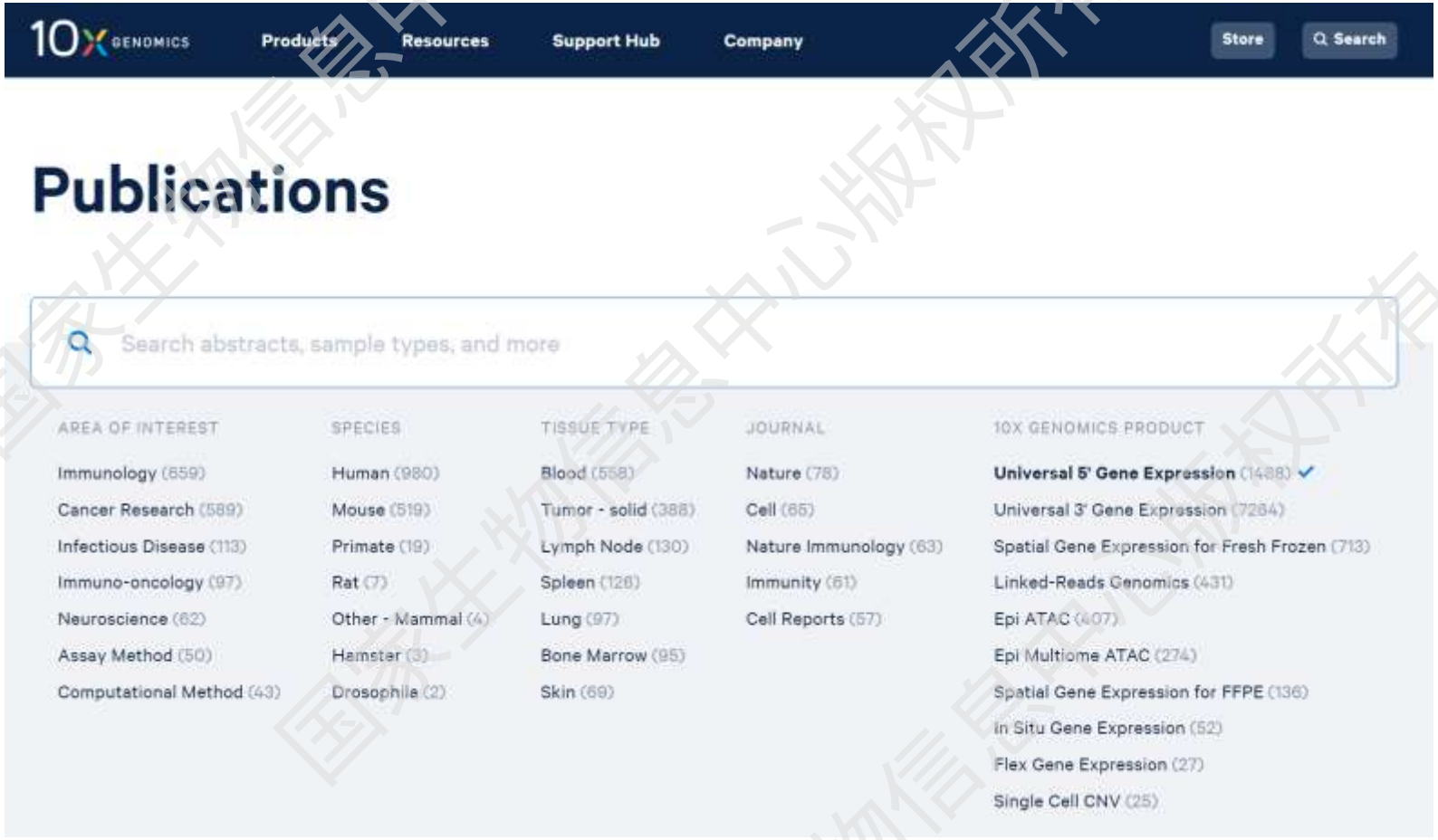
```
Sub01_S1_L001_R1_001.fastq.gz
Sub01_S1_L001_R2_001.fastq.gz
Sub01_S1_L002_R1_001.fastq.gz
Sub01_S1_L002_R2_001.fastq.gz
Sub01_S1_L003_R1_001.fastq.gz
Sub01_S1_L003_R2_001.fastq.gz
Sub02_S1_L001_R1_001.fastq.gz
Sub02_S1_L001_R2_001.fastq.gz
Sub02_S1_L002_R1_001.fastq.gz
Sub02_S1_L002_R2_001.fastq.gz
Sub02_S1_L003_R1_001.fastq.gz
Sub02_S1_L003_R2_001.fastq.gz
```

Path to rawdata/scBCR:

```
Sub01_S1_L001_R1_001.fastq.gz
Sub01_S1_L001_R2_001.fastq.gz
Sub01_S1_L002_R1_001.fastq.gz
Sub01_S1_L002_R2_001.fastq.gz
Sub01_S1_L003_R1_001.fastq.gz
Sub01_S1_L003_R2_001.fastq.gz
Sub02_S1_L001_R1_001.fastq.gz
Sub02_S1_L001_R2_001.fastq.gz
Sub02_S1_L002_R1_001.fastq.gz
Sub02_S1_L002_R2_001.fastq.gz
Sub02_S1_L003_R1_001.fastq.gz
Sub02_S1_L003_R2_001.fastq.gz
```

Same filename in different directory, just the same filename, not the same file!

10X Genomics scRNA+scTCR/scBCR rawdata in .fastq format



Cellranger vdj: rawdata → contig files

Example cmd in shell:

```
/xtdisk/jiangl_group/yuchw/software/cellranger-8.0.0/bin/cellranger vdj \
    --id=Sub01 \
    --sample=Sub01 \
    --reference=/xtdisk/jiangl_group/yuchw/reference/10X_ref/GRCh38/refdata-cellranger-vdj-GRCh38-alts-ensembl-7.1.0 \
    --fastqs=/p300s/ligchA_jianglgroup/ligchA/301_LiPing/batch5_20250223/data/UDA_scTCR \
    --localmem=200 \
    --localcores=10
```

Example output:

```
[ligchA@loginb2:/p300s/ligchA_jianglgroup/ligchA/301_LiPing/batch5_20250223_20250317/analysis/UDA_scTCR/1_cellranger]
$ ls Sub01/outs/
airr_rearrangement.tsv  all_contig_annotations.bed  concat_ref.bam.bai     consensus.fasta.fai          metrics_summary.csv
all_contig.bam          all_contig_annotations.csv  concat_ref.fasta       consensus_annotations.csv    vdj_contig_info.pb
all_contig.bam.bai      all_contig_annotations.json concat_ref.fasta.fai  donor_regions.fa             vdj_reference
all_contig.fasta        cell_barcodes.json          consensus.bam          filtered_contig.fasta        web_summary.html
all_contig.fasta.fai    clonotypes.csv              consensus.bam.bai      filtered_contig.fastq
all_contig.fastq        concat_ref.bam              consensus.fasta        filtered_contig_annotations.csv
```

https://www.10xgenomics.com/support/software/cell-ranger/latest

Content of contig files

less Sub01/outs/all_contig_annotations.csv:

```
barcode,is_cell,contig_id,high_confidence,length,chain,v_gene,d_gene,j_gene,c_gene,full_length,productive,fwr1,fwr1_nt,cdr1,cdr1_nt,fwr2,fwr2_nt,cdr2,cdr
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```

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scRepertoire in action

scRepertoire 2.3.4 | Getting Started | Install | Vignettes | News/Changelog | Reference | FAQ

scRepertoire

Introduction

Single-cell sequencing is an emerging technology in the field of immunology and oncology that allows researchers to couple RNA quantification and other modalities, like immune cell receptor profiling at the level of an individual cell. A number of workflows and software packages have been created to process and analyze single-cell transcriptomic data. These packages allow users to take the vast dimensionality of the data generated in single-cell-based experiments and distill the data into novel insights. Unlike the transcriptomic field, there is a lack of options for software that allow for single-cell immune receptor profiling. Enabling users to easily combine RNA and immune profiling, the scRepertoire framework supports use of 10x, AIRR, BD, MiXCR, Omniscope, TRUST4, and WAT3R single-cell clonal formats and interaction with popular R-based single-cell data pipelines.

https://www.borch.dev/uploads/screpertoire/

scRepertoire in action

scRepertoire 2.3.4 | Getting Started | Install | Vignettes | News/Changelog | Reference | FAQ

- Loading Data
- Combining Contigs into Clones
- Additional Processing
- Basic Clonal Analysis
- Visualizing Clonal Dynamics
- Summarizing Repertoires
- Clonal Diversity, Rarefaction, and Overlap
- Clustering Sequences by Edit Distance
- Combining Clones and Single-Cell Objects
- Visualizations for Single-Cell Objects
- Quantifying Clonal Bias
- Making Deep Learning Models with immApex
- Combining Deep Learning and TCRs with Trex
- Combining Deep Learning and BCRs with Ibex
- Single-cell Gene Set Enrichment Analysis

scRepertoire

Introduction

Single-cell sequencing is an emerging technology in the fie
researchers to couple RNA quantification and other modal
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data. These packages allow users to take the vast dimens
data into novel insights. Unlike the transcriptomic field, the
profiling. Enabling users to easily combine RNA and immu
MiXCR, Omniscope, TRUST4, and WAT3R single-cell clo

gle-cell transcriptomic
eriments and distill the
-cell immune receptor
of 10x, AIRR, BD,
e-cell data pipelines.

Applying Deep Learning to VDJ da

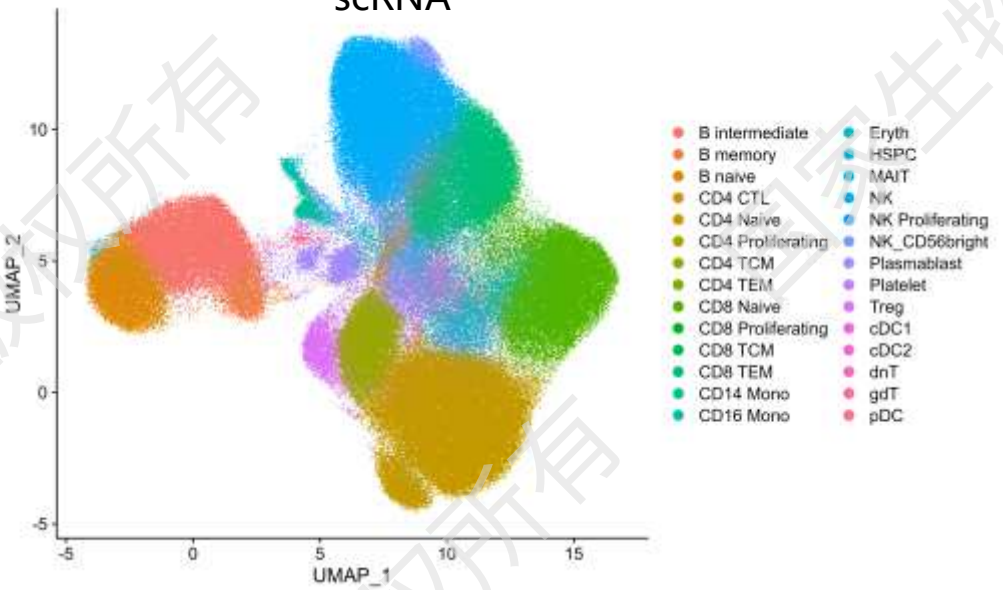
scRepertoire is compatible and integrated with the R packa
receptor and Ibex for the B cell receptor. If you are interest
see immApex.

oencoding of the T cell
mune receptors, please

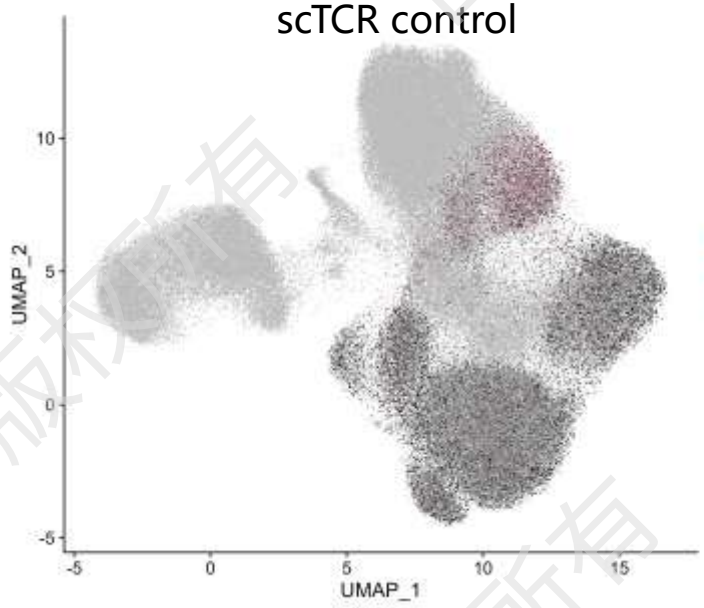
Integration of scTCR/scBCR and scRNA data

Calculate the distribution of clonotypes with different abundances across various cell types (function: DimPlot): It was found that in healthy individuals, mild patients, and severe patients, the clonotypes with the highest frequency in each group were mainly located in CD8+ effector T cells (CD8 TEM). Moreover, as the severity of the disease increased, the abundance of clonotypes in all types of T cells generally rose gradually.

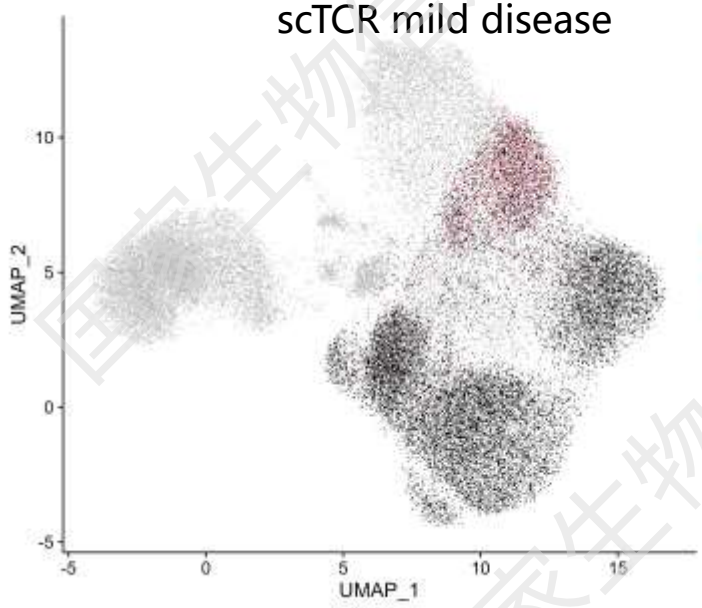
scRNA



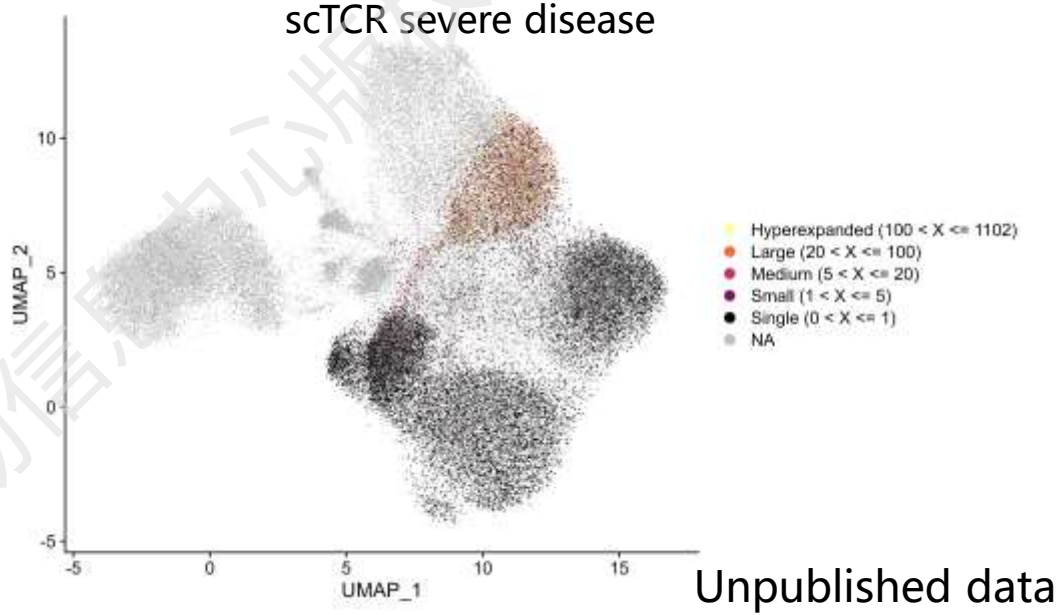
scTCR control



scTCR mild disease



scTCR severe disease



Unpublished data

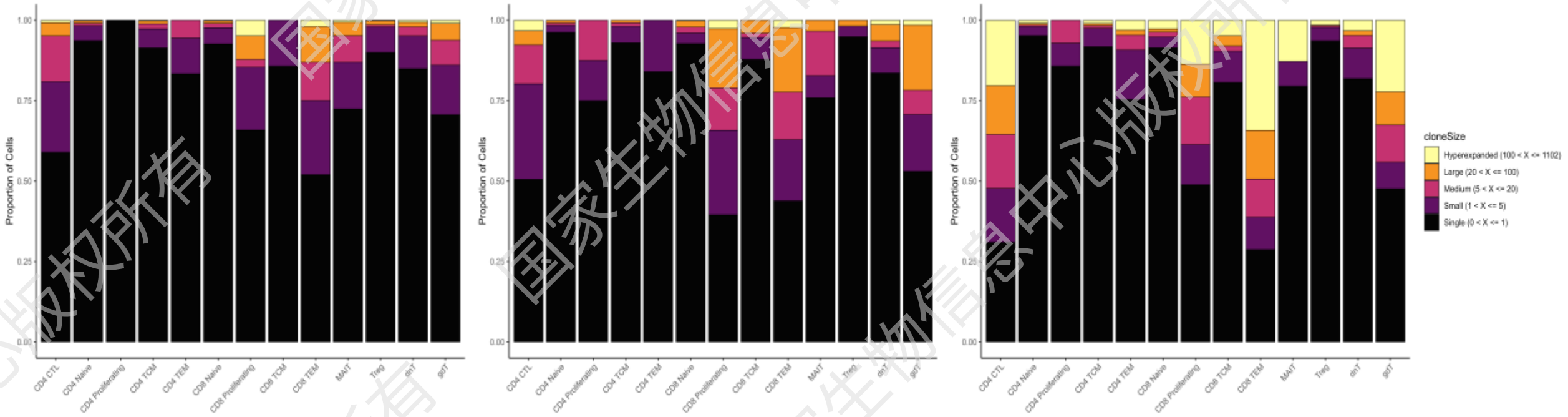
Integration of scTCR/scBCR and scRNA data

Use a stacked bar chart to calculate the proportion of clonotypes with different abundances in each cell type (function: clonalOccupy): As the disease severity increases, the abundance of clonotypes in all types of T cells generally rises gradually. The most significant changes are observed in CD4+ cytotoxic T cells (CD4 CTL), CD8+ proliferating T cells (CD8 Proliferating), CD8+ effector T cells (CD8 TEM), mucosal-associated invariant T cells (MAIT), and γδ T cells (gdT).

scTCR control

scTCR mild disease

scTCR severe disease



Unpublished data

Thank you!

http://jianglab.group