Immune receptor repertoires in SC technologies

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TRYNKA GROUP

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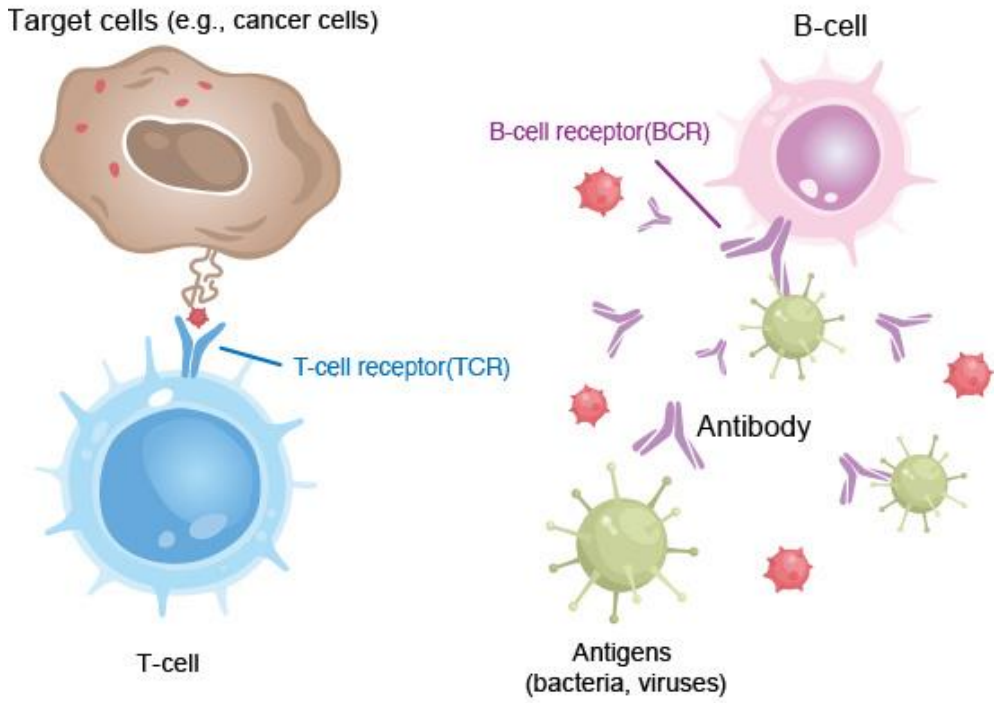
TCRs and BCRs (AIRR): keystones of adaptive immunity

TCR



BCR

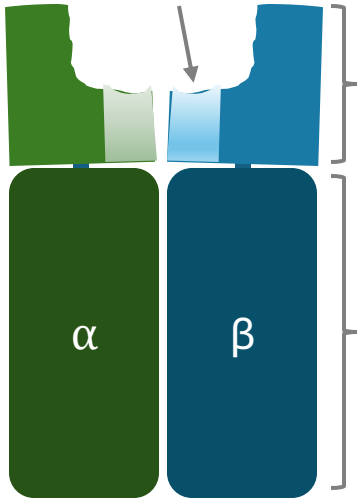
- Present on all T cells
- Reacts to a molecule (usually a short peptide) in HLA (MHC) context
- Results in the cell activation
- Depending on the type of the T cell: ↑ or ↓ immune response

- Present on all B cells
- React to a molecule
- When secreted – antibody
- Isotypes
- Many immune cells react to the external ‘handle’ of the antibody

picture: www.repertoire.co.jp

TCRs and BCRs (AIRR): keystones of adaptive immunity

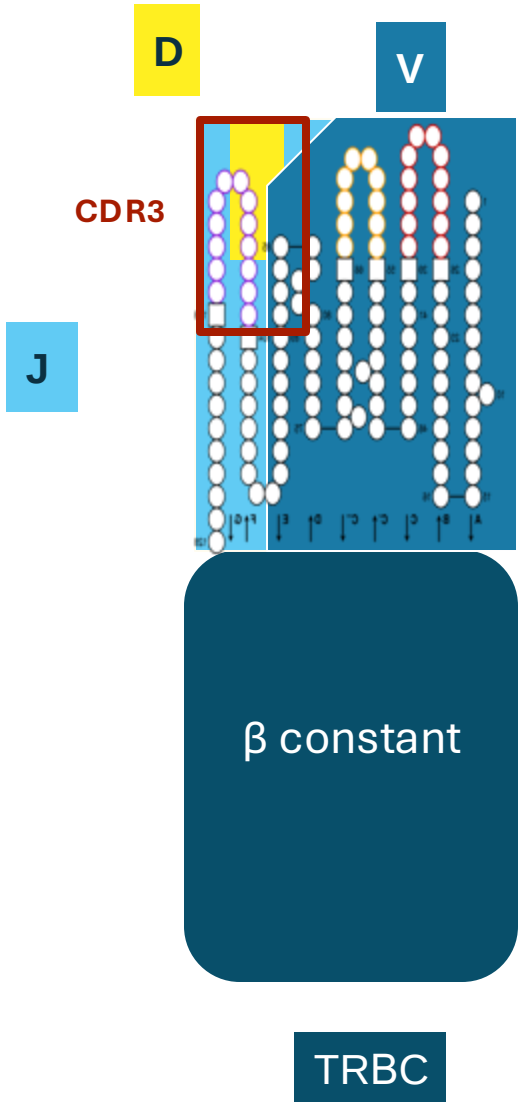
Complementarity
Determining
Region
3



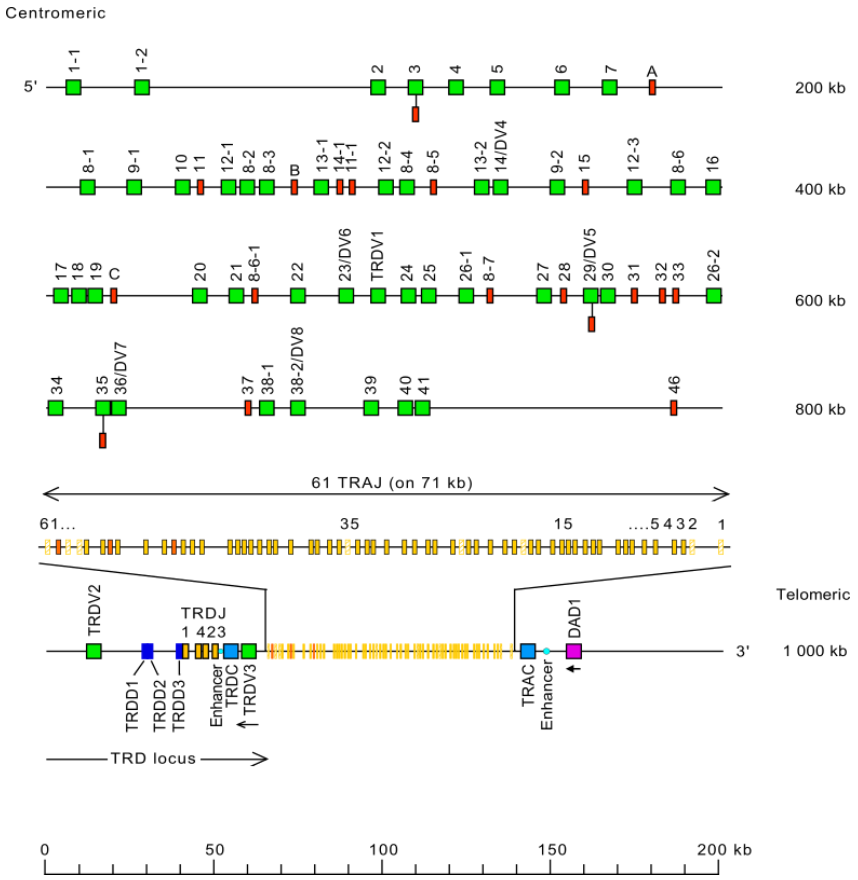
variable region

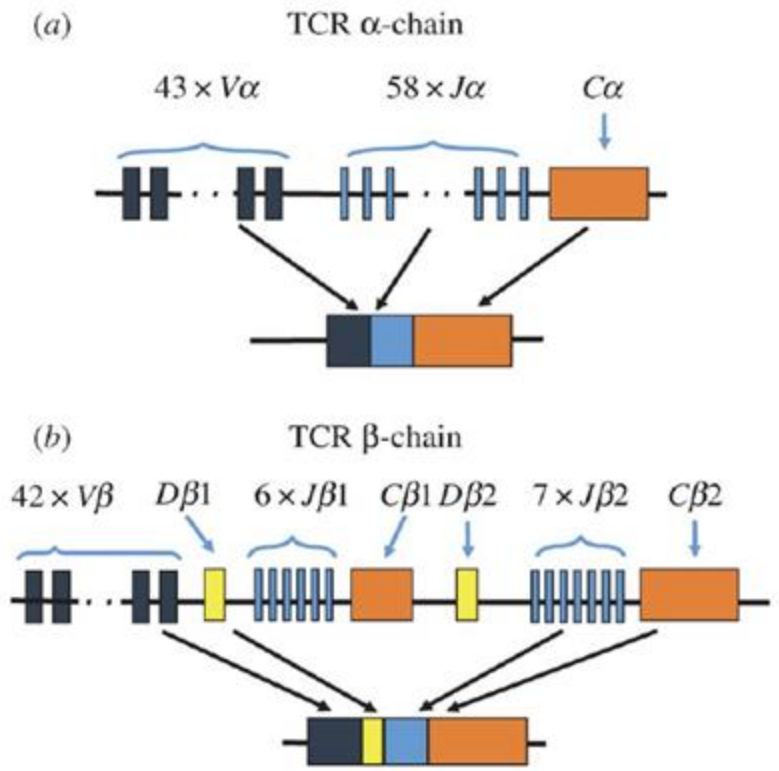
constant region

coded by...



TCRs and BCRs (AIRR): keystones of adaptive immunity





Each cell

T cell

Each cell

T cell

Somatic recombination

Pictures: IMGT.org, Janeway

TCRs and BCRs (AIRR): keystones of adaptive immunity

Somatic recombination: stochastic combinations of V, (D) and J gene rearrangements and imprecise joining contribute to the diversity of the TCR repertoire.

	V	J	D	V del	J del	Ins	combinations
α	43	58	-	15	12	15	4.3×10^{14}
β	42	13	3	10	12	18	5.1×10^{15}

$\alpha\beta = 10^{30}$

At any given time: 5×10^{11} T cells, 100g*; 10^{8} receptors
All possible 10^{21}g $= 10^{18}$ kg

\+ genetic diversity: alleles of every V, D, J gene → under-researched, most software rely on “known” germline variants

What the data tell us

What can we get from the single cell data?

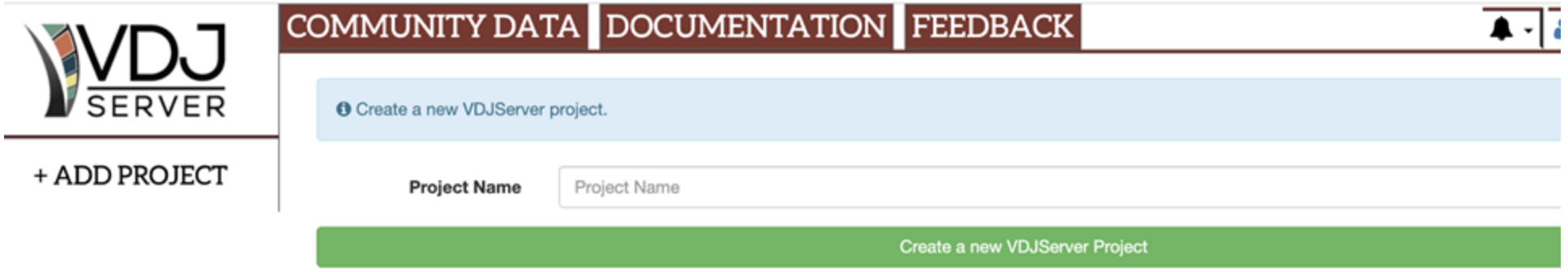
DNA TCR/BCR sequence unique to one cell*
If a cell multiplies (eg infection), more cells with identical receptor

→ We have a barcode for cells of the same origin
→ We can guess that a receptor is involved in something when many cells have the same receptor
→ We might detect an ongoing infection/inflammation
→ We might already know what receptor is against

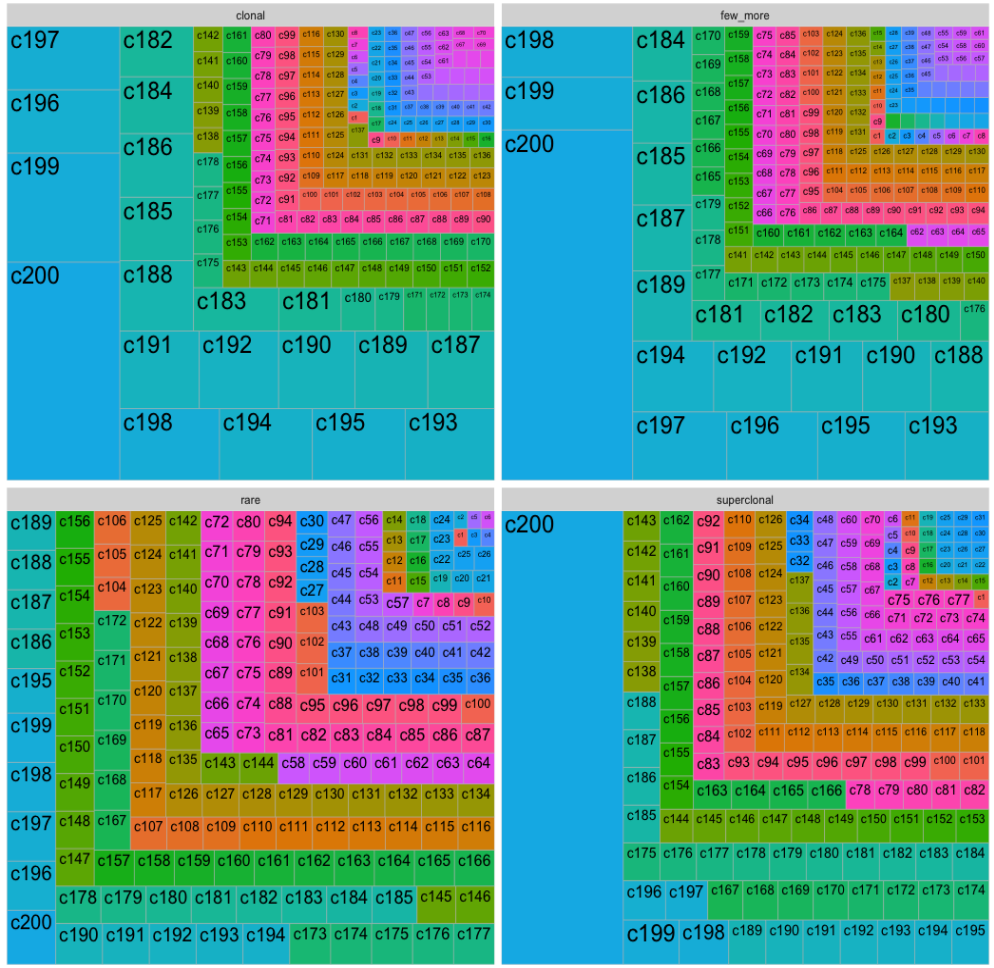
Constraints
→ Limited knowledge about TCR-antigen-(HLA)
→ *Not really unique
→ BCRs undergo the additional process of the somatic hypermutation
→ Very few TCRs/BCRs captured

What the data tell us

VDJdb



What the data tell us



- R = richness – number of unique clonotypes (each might be present in more than 1 cell)
- p_i = relative abundance of clonotype i (0-1)
- q = order of diversity
- N = total number of receptors (cells)

Hill diversity

$$ {}^qD = (\sum_{i=1}^{R} p_i^q)^{1/(1-q)} = exp(\frac{1}{1-q} ln(\sum_{i=1}^{R} p_i^q)) = exp({}^qH) $$

Renyi entropy

$$ H = \frac{1}{1-q} ln(\sum_{i=1}^{R} p_i^q) = ln(D^q) $$

Clonality

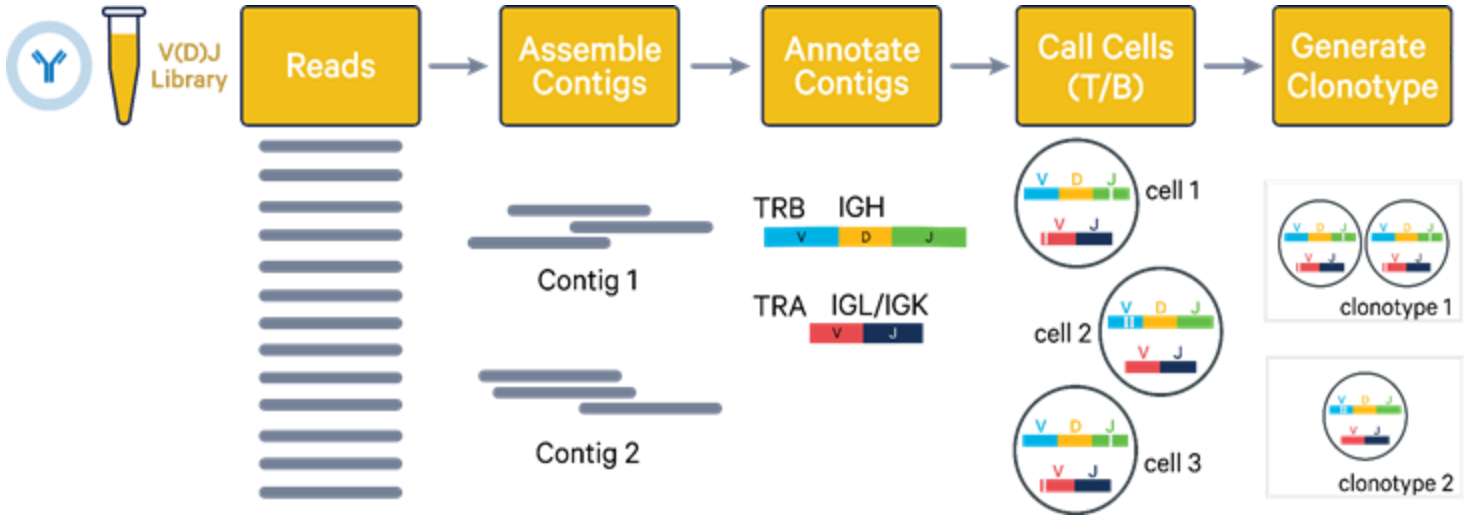
$$ C = 1 - \frac{H}{lnR} $$

Shannon entropy

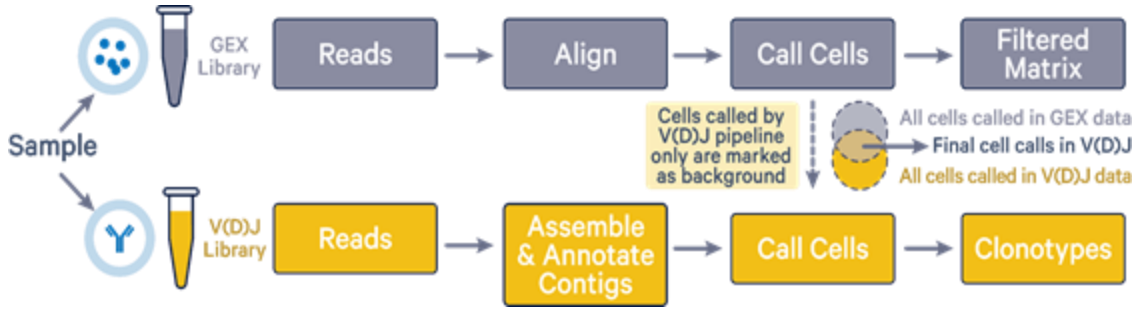
$$ H = -\sum_{i=1}^{R} p_i \ln p_i = ln(D^1) $$

How the data is produced

Within one barcode:



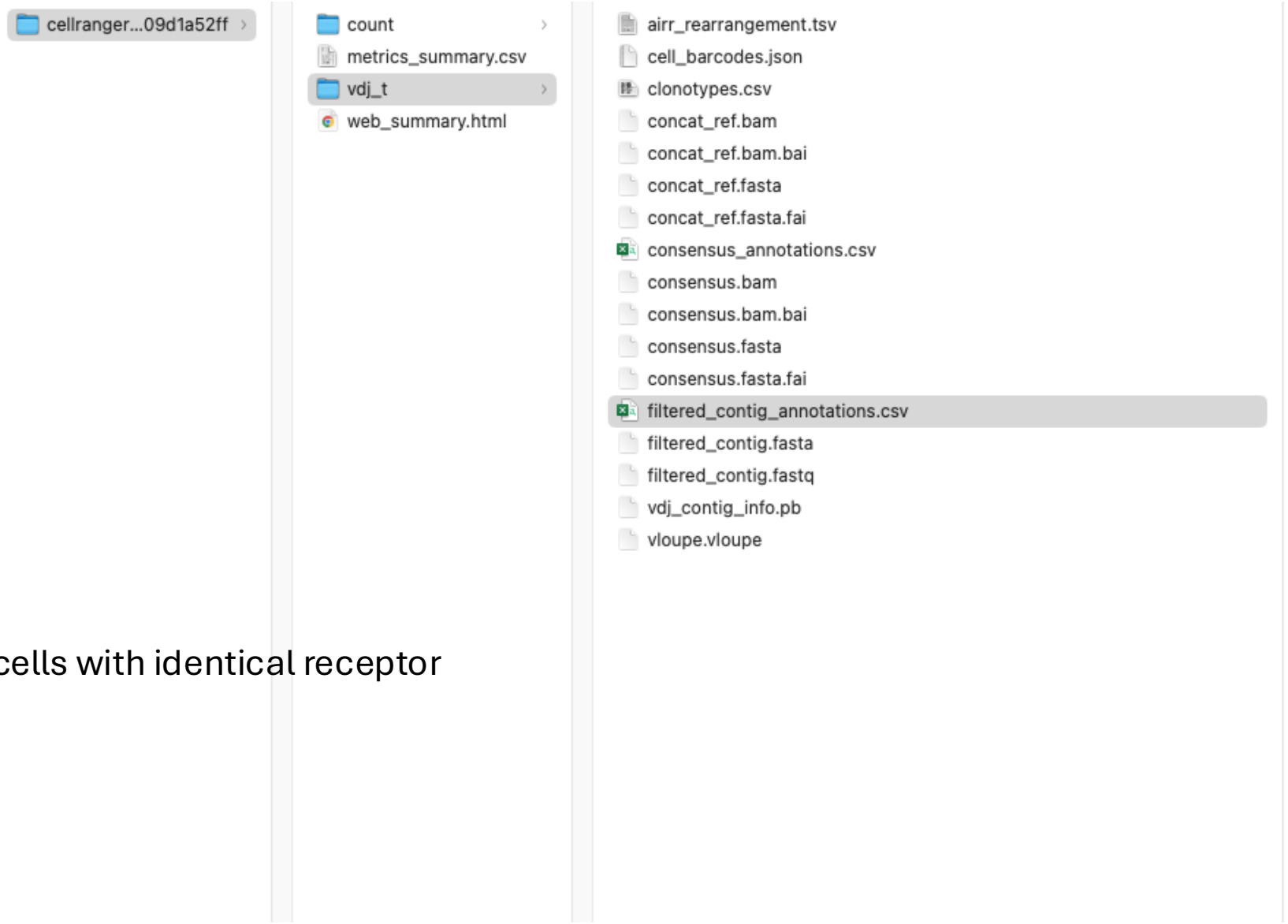
Cell assignment is from GEX



10x GENOMICS™ **CellRanger**

Other technologies similar approach

How the data is produced



Per identified chain info
Alignments to the reference
Per clonotype info
Per cell info

Clonotype = all cells with identical receptor

questions

NCBI > GEO > **Accession Display** Not logged in | Lo

GEO help: Mouse over screen elements for information.

Scope: Self Format: HTML Amount: Quick GEO accession: GSE145926 GO

Series GSE145926 Query DataSets for GSE145926

Status	Public on Apr 22, 2020
Title	Single-cell landscape of bronchoalveolar immune cells in COVID-19 patients
Organism	Homo sapiens
Experiment type	Expression profiling by high throughput sequencing Other
Summary	Immune characteristics associated with Coronavirus Disease-2019 (COVID-19) severity are currently unclear. We characterized bronchoalveolar lavage fluid (BALF) immune cells from patients with varying severity of COVID-19 disease and from healthy subjects using single-cell RNA-sequencing. Proinflammatory monocyte-derived macrophages were abundant in the BALF from severe COVID-9 patients. Moderate cases were characterized by the presence of highly clonally expanded tissue-resident CD8+ T cells. This atlas of the bronchoalveolar immune-microenvironment suggests potential mechanisms underlying pathogenesis and recovery in COVID-19.
Overall design	Using 10x genomics to measure single-cell RNA sequence (scRNA-seq)/TCR-seq to comprehensively characterize the lung immune microenvironment in the bronchoalveolar lavage fluid (BALF) from 6 severe and 3 moderate COVID-19 patients and 3 healthy control.
Contributor(s)	Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, Li J, Wang X, Wang F, Liu L, Amit I, Zhang S, Zhang Z
Citation(s)	Liao M, Liu Y, Yuan J, Wen Y et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med* 2020 Jun;26(6):842-844. PMID: 32398875 Zhang Z, Zhang L, Wang K, Xie T et al. Single-cell landscape of bronchoalveolar immune cells in patients with immune checkpoint inhibitor-related pneumonitis. *NPJ Precis Oncol* 2024 Oct 5;8(1):226. PMID: 39369126 medRxiv: https://doi.org/10.1101/2020.02.23.20026690
Submission date	Feb 25, 2020
Last update date	Nov 05, 2024
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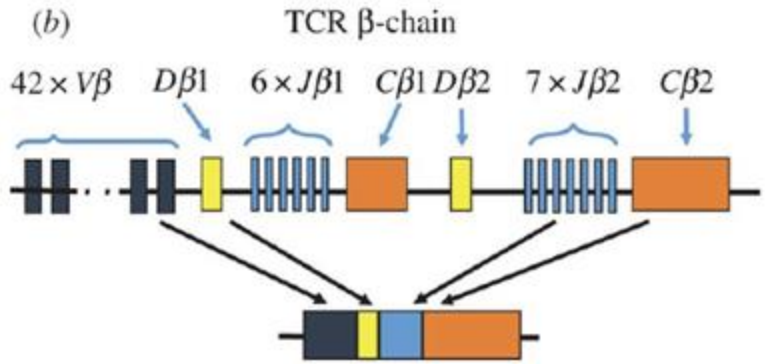
City	Shenzhen
State/province	Guangdong
ZIP/Postal code	454171
Country	China
Platforms (1)	GPL23227 BGISEQ-500 (Homo sapiens)
Samples (21) More...	GSM4339769 BALF, C141 (scRNA-seq) GSM4339770 BALF, C142 (scRNA-seq)

GSM4339769 BALF, C141 (scRNA-seq)
GSM4339770 BALF, C142 (scRNA-seq)
GSM4339771 BALF, C143 (scRNA-seq)
GSM4339772 BALF, C144 (scRNA-seq)
GSM4339773 BALF, C145 (scRNA-seq)
GSM4339774 BALF, C146 (scRNA-seq)
GSM4385990 BALF, C141 (TCR-seq)
GSM4385991 BALF, C142 (TCR-seq)
GSM4385992 BALF, C143 (TCR-seq)
GSM4385993 BALF, C144 (TCR-seq)
GSM4385994 BALF, C145 (TCR-seq)
GSM4385995 BALF, C146 (TCR-seq)
GSM4475048 C51 (scRNA-seq)
GSM4475049 C52 (scRNA-seq)
GSM4475050 C100 (scRNA-seq)
GSM4475051 C148 (scRNA-seq)
GSM4475052 C149 (scRNA-seq)
GSM4475053 C152 (scRNA-seq)
GSM4475054 C148 (TCR-seq)
GSM4475055 C149 (TCR-seq)
GSM4475056 C152 (TCR-seq)

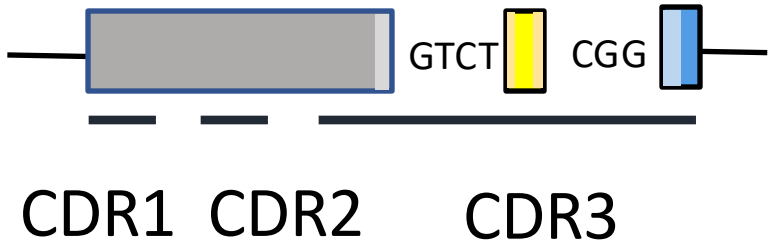
barcode	is_cell	contig_id	high_confide	length	chain	v_gene	d_gene	j_gene	c_gene	full_length	productive	cdr3	cdr3_nt	reads	umis	raw_clonotype_id	raw_
AAACCTGCATGGTCAT-1	TRUE	AAACCTGCATGGT	TRUE	492	TRA	TRAV13-2	None	TRAJ10	TRAC	TRUE	TRUE	CAEKSSGGG	TGTGCAGAG	37974	11	clonotype129	clon
AAACCTGCATGGTCAT-1	TRUE	AAACCTGCATGGT	TRUE	501	TRB	TRBV6-6	None	TRBJ2-5	TRBC2	TRUE	TRUE	CASSYGTTGI	TGTGCCAGC	12806	7	clonotype129	clon
AAACCTGCATGGTCAT-1	TRUE	AAACCTGCATGGT	TRUE	509	TRA	TRAV6	None	TRAJ40	TRAC	TRUE	TRUE	CALRSGTYKY	TGTGCTCTAC	17114	5	clonotype129	clon
AAACCTGCATGGTCAT-1	TRUE	AAACCTGCATGGT	TRUE	318	TRA	None	None	TRAJ5	TRAC	FALSE	FALSE	None	None	6288	2	clonotype129	None
AAACCTGGTTTAGCTG-1	TRUE	AAACCTGGTTTAG	TRUE	494	TRB	TRBV7-6	None	TRBJ2-3	TRBC2	TRUE	TRUE	CASRSIEADT	TGTGCCAGC	57140	6	clonotype130	clon
AAACCTGGTTTAGCTG-1	TRUE	AAACCTGGTTTAG	TRUE	497	TRA	TRAV17	None	TRAJ21	TRAC	TRUE	TRUE	CATDGDNFN	TGTGCTACG	8332	4	clonotype130	clon
AAACCTGTCAATCACG-1	TRUE	AAACCTGTCAATCA	TRUE	471	TRB	TRBV29-1	None	TRBJ2-7	TRBC2	TRUE	TRUE	CSVEGTATYE	TGCAGCGTT	39068	10	clonotype131	clon
AAACCTGTCAATCACG-1	TRUE	AAACCTGTCAATCA	TRUE	576	TRA	TRAV8-4	None	TRAJ32	TRAC	TRUE	TRUE	CAVSDGFGG	TGTGCTGTGA	30316	11	clonotype131	clon
AAACGGGAGAACTCGG-1	TRUE	AAACGGGAGAACT	TRUE	634	TRA	TRAV8-2	None	TRAJ41	TRAC	TRUE	TRUE	CVGNSGYALI	TGTGTTGGGA	6840	4	clonotype25	clon
AAACGGGAGAACTCGG-1	TRUE	AAACGGGAGAACT	TRUE	471	TRB	TRBV10-2	None	TRBJ2-3	TRBC2	TRUE	TRUE	CASNLAGPTI	TGCGCCAGC	38840	17	clonotype25	clon
AAACGGGAGAACTCGG-1	TRUE	AAACGGGAGAACT	TRUE	387	TRB	None	None	TRBJ1-5	TRBC1	FALSE	FALSE	None	None	16520	5	clonotype25	None
AAACGGGAGAACTCGG-1	TRUE	AAACGGGAGAACT	TRUE	607	TRB	None	None	TRBJ1-5	TRBC1	FALSE	FALSE	None	None	11888	3	clonotype25	None
AAACGGGAGAACTCGG-1	TRUE	AAACGGGAGAACT	TRUE	510	TRA	TRAV1-2	None	TRAJ6	TRAC	TRUE	FALSE	CAVPHQEEA	TGTGCTGTC	3442	3	clonotype25	None

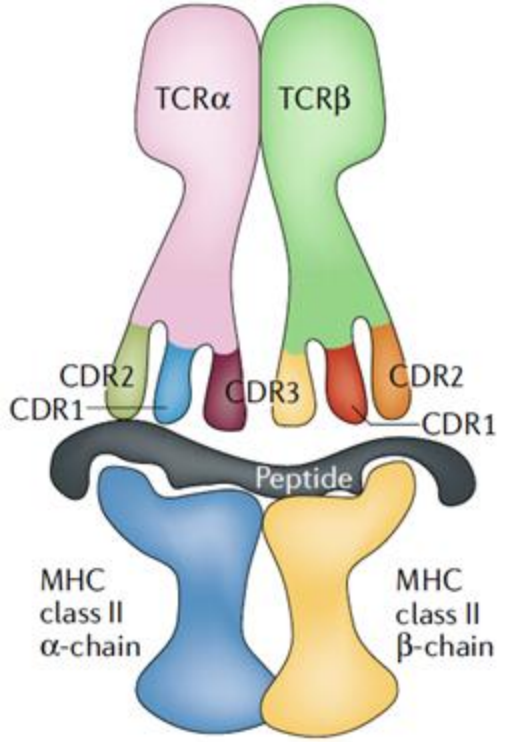
Additional slides

Most variable parts - **C**omplementarity **D**etermining **R**egions – interact with pMHC complex



Janeway





La Gruta *Nat Rev Imm 2018*

Final CDR3 length: 7-40 aminoacids
Median 13

IMGT – source of references

References
www.IMGT.org

TRBV subgroup	TRBV gene name	Fct	TRBV allele name	Accession number	Confirmed by genetics and/or data	
2	2	F	V2*01	L36092/U66059	+	g65 ,R22 |a237 |
		(F)	V2*02	M62379		g65>a,R22>H|
		(F)	V2*03	M64351		|a237>g|
3	3-1	F	V3-1*01	U07977	+	t174 |c181 ,L61 |c225 |c256 ,c258 ,H86 |
		(F)	V3-1*02	L06889		t174>c|c181>a,L61>I|c225>a|c256>a,c258>a,H86>K|
4	4-1	F	V4-1*01	U07977	+	t93 |
		(F)	V4-1*02	M13855		t93>a|
	4-2	F	V4-2*01	U07975	+	t263 ,F88 |
		(F)	V4-2*02	X58811		t263>g,F88>C|
	4-3	F	V4-3*01	U07978	+	t84 |g183 |t263 ,F88 |
		(F)	V4-3*02	X58812		|t263>c,F88>S|
		(F)	V4-3*03	L06888		|g183>t|
		(F)	V4-3*04	X57616		t84>g|
5	5-1	F	V5-1*01	L36092/U66059	+	a2 ,K1 |a9 |t28 ,Y10 |a64 ,S22 |c137 ,P46 |c
		(F)	V5-1*02	M14271		a2>g,K1>R|a9>g|t28>c,Y10>H|a64>g,S22>G|c137>t,P46>L|c
	5-3	ORF	V5-3*01	X61439	+	g254 ,C85 |
		ORF	V5-3*02	AF009660	+	g254>a,C85>Y|
	5-4	F	V5-4*01	L36092/U66060	+	t60 |t212 ,71F |g257 ,86S |
		(F)	V5-4*02	X57615		|g257>a,86S>N|
		(F)	V5-4*03	S50547		t60>a|
		(F)	V5-4*04	X58804		|t212>c,71F>S|

Practicalities: not always possible/necessary, different references!
Underrepresentation of Non-Europeans

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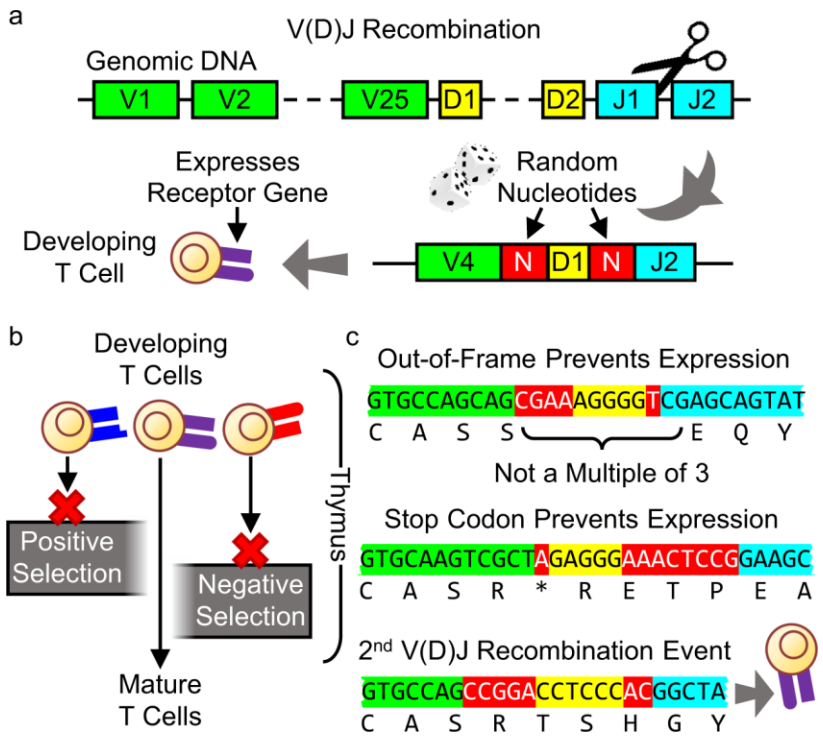
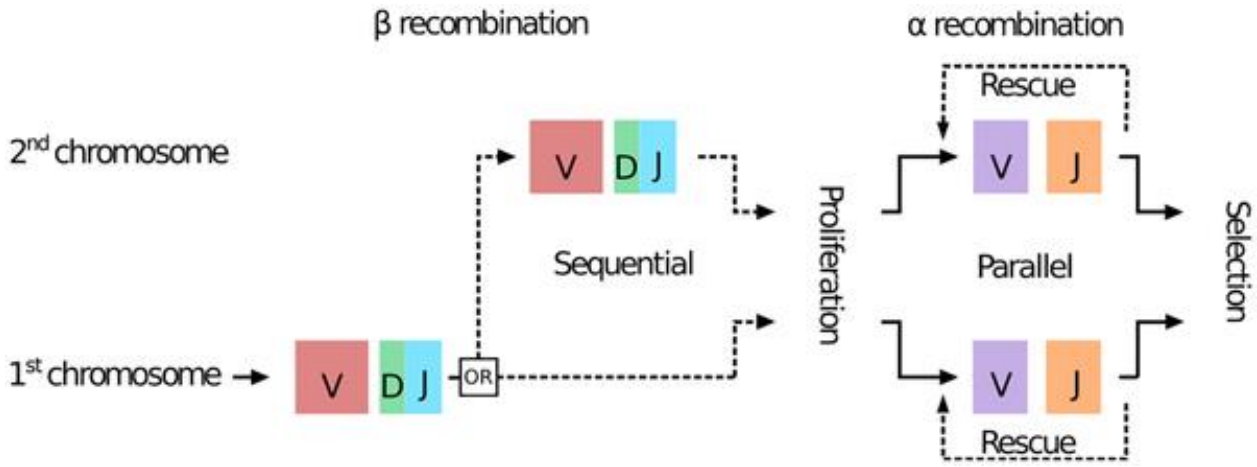
DNA level





Ostmeyer et al 21

Dupic et all, Plos Comp Biol 2019

- Nonproductive sequences

Bulk methods do not see it!

- Allelic exclusion is leaky: ~7% of cells with 2x TCRβ (1% both expr), 7-30% with 2xTCRα
- Final length: few to ~40 aas, majority 13aa
- Non-random frequency of specific recombination events → pgen

Some practical hints

The same VDJ – different CDR3
Different VDJ – the same CDR3

Convergence:

aa1==aa2
nuc1 != nuc2

Functional comparison: aminoacid level
“Tag” analysis: DNA level

HLA-dependence

Not always full TRB reconstruction possible

Sampling issues: →

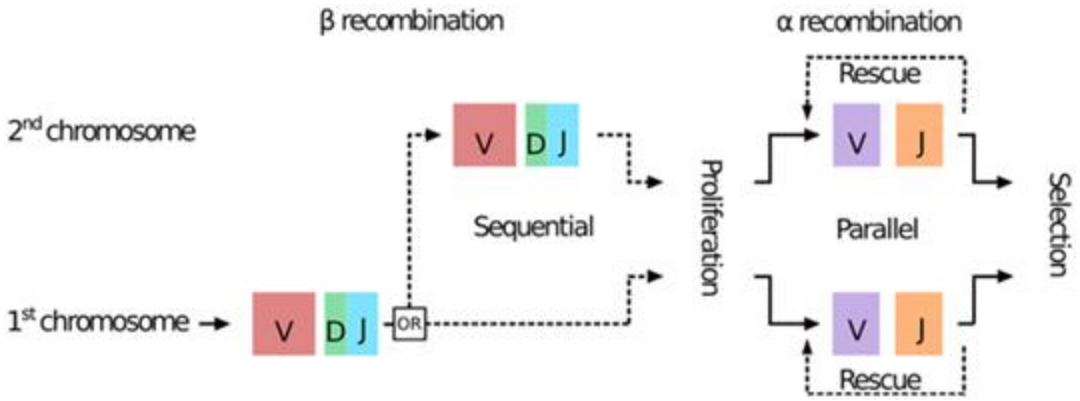
- Size of the repertoire
- Cell-type dependency
- Tisssue dependency
- Clonotypes of importance might be rare

The naive T-cell receptor repertoire has an extremely broad distribution of clone sizes
https://doi.org/10.7554/eLife.49900

Known TCR sequences: selection bias

Differences in recombination of TRA and TRB

- Both alleles recombine at the same time
- Rescue mechanism
- 1-2 recombined alleles (2 in 7-30% of cells)
- Positive/negative selection



Dupic et all, Plos Comp Biol 2019

- Has to interact with MHC in the thymus
- If recombination unsuccessful, another round
- Single product, leakiness <1%

Very very very very wasteful

- Recombination products Out of frame and with stop codons (non-productive sequences)
- Negative/positive selection

Both together: LOWER estimate of selection factor is 99.9%

We can estimate probability of a recombination-derived TCR

Select a V gene

↓ P1(V=V_i)

Select a J gene

↓ P2(J=J_i)

Select number of V deletions

↓ P3(m=1,2,3….n)

Select number of J deletions

↓ P4(m=1,2,3,…)

Select no. of nucleotide additions

→ P4(Nt=A,T,C,G)

p5,p6 ….

↺ Select nucleotide

Murugan et al. Statistical inference of the generation probability of T-cell receptors from sequence repertoires. Proc Natl Acad Sci U S A. 2012 Oct 2;109(40):16161-6.

Sethna et al. OLGA: fast computation of generation probabilities of B- and T-cell receptor amino acid sequences and motifs. Bioinformatics. 2019 Sep 1;35(17):2974-2981

$$P_{\text{recomb}}(E) = P(V)P(D,J) \times P(\text{del}V|V)P(\text{del}J|J)P(\text{del}5'D, \text{del}3'D|D) \times P(\text{ins}VD)\prod_{i=1}^{\text{ins}VD} p_{VD}^{(2)}(x_i|x_{i-1})P(\text{ins}DJ)\prod_{i=1}^{\text{ins}DJ} p_{DJ}^{(2)}(y_i|y_{i-1}).$$

Probabilities to obtain from non-productive sequences

https://github.com/statbiophys/OLGA

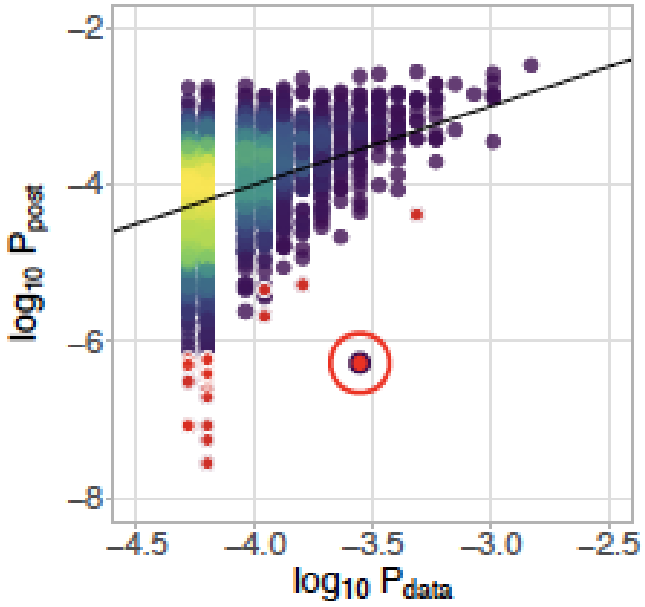
welcome sanger institute

We can estimate probability of a recombination-derived TCR

A nucleotide sequence with low probability of generation and shared between individuals

An aminoacid sequence with low probability of generation, coded by many nucleotides

- putatively selected sequences

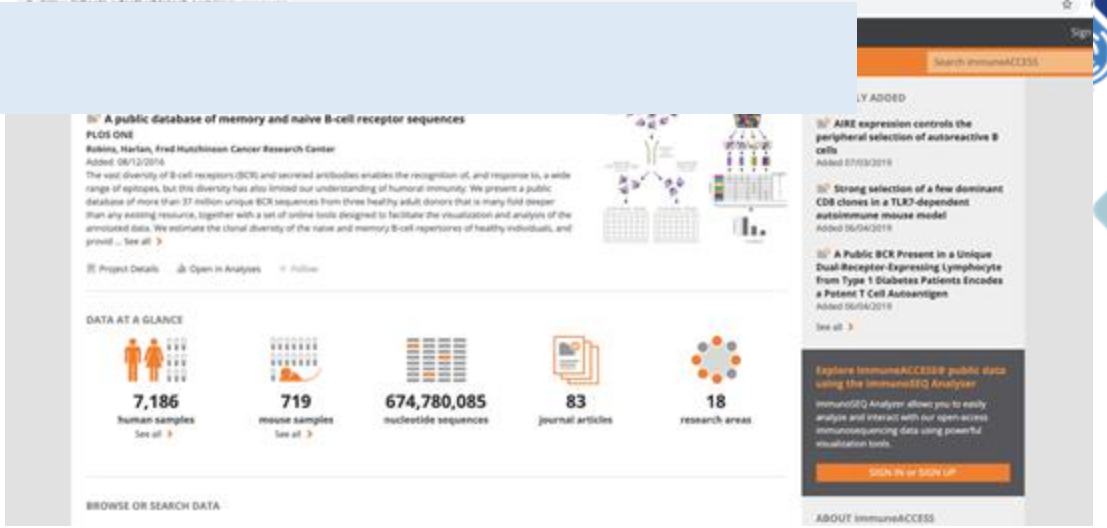


Pogorelyy, Elife 2018

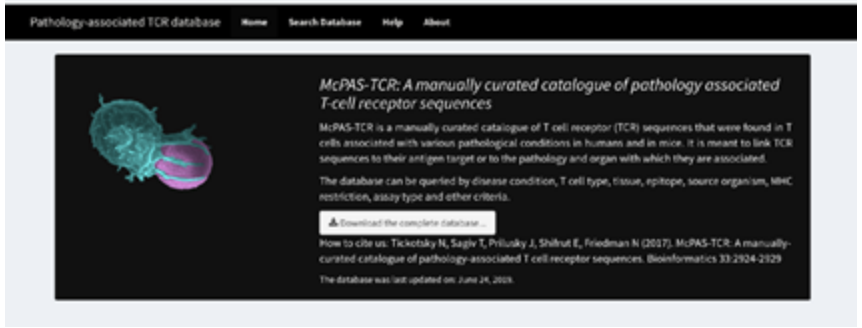
Databases and repositories

VDJdb

ImmuneACCESS



McPass-TCR



FASTQ: NCBI SRA