Single-Cell TCR/BCR Analysis

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Leiden Computational Biology Center, LUMC, Leiden

Delft Bioinformatics Lab, Delft

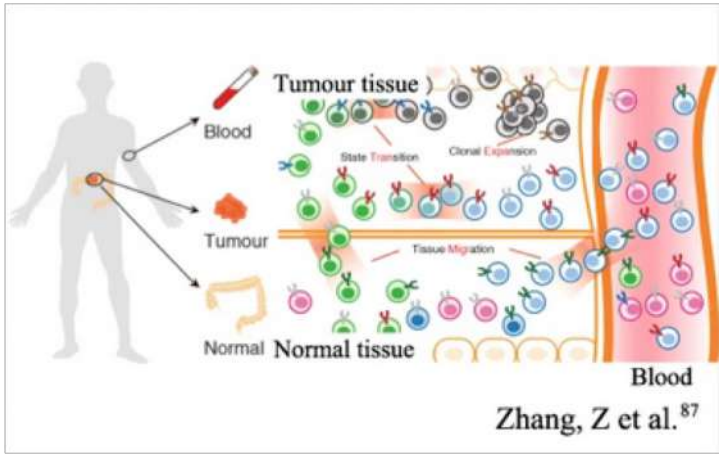
Single-Cell Course

Leiden Computational Biology Center

LUMC

TU Delft

Immunology questioned with single-cell RNA sequencing



Understanding regional immunity in tumor regions

Source: Chen et al, Cellular and Molecular Immunology, 2019

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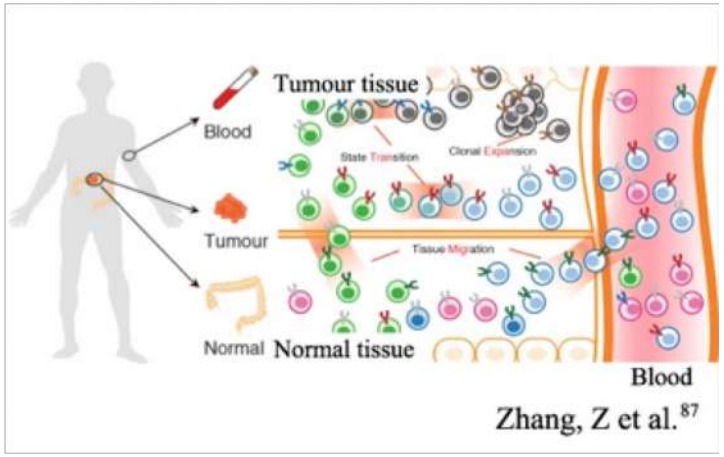

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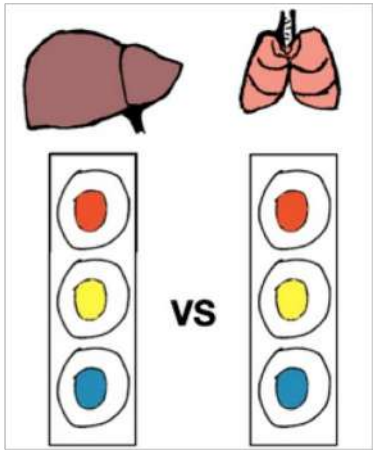

Comparing immune Microenvironment across tissues

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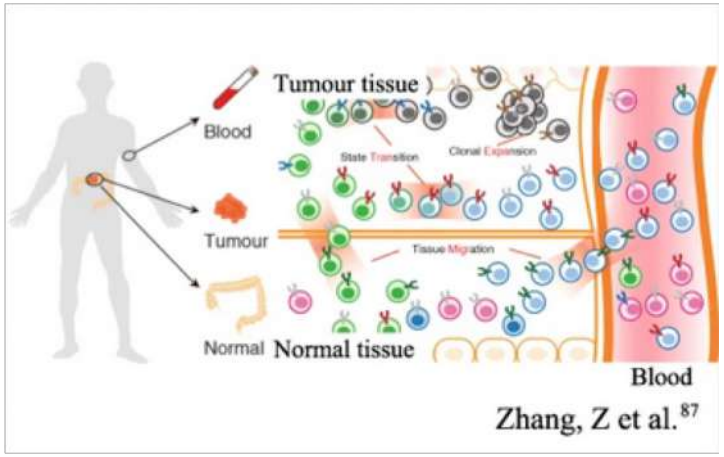

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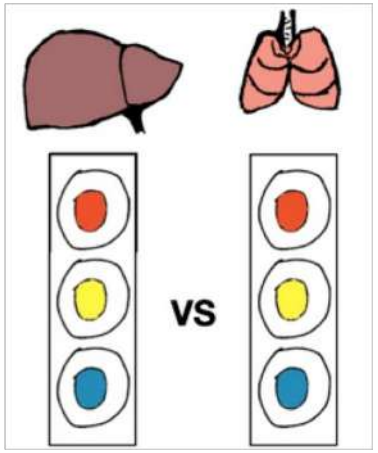

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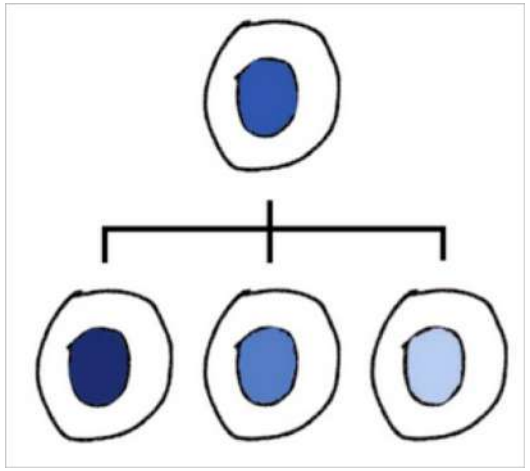

Identifying novel Immune cell subtypes

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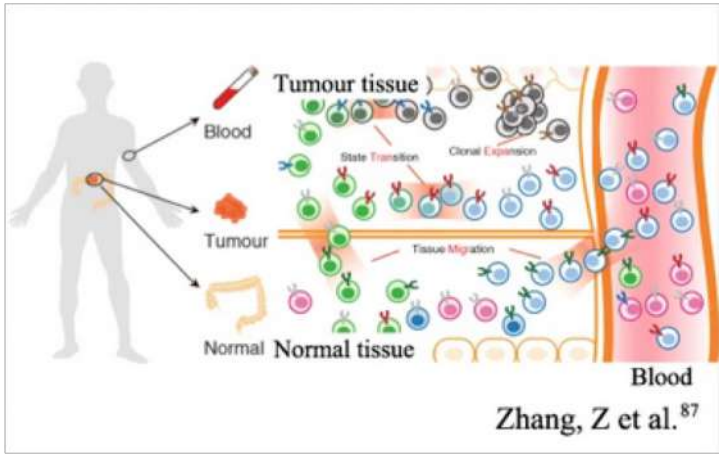

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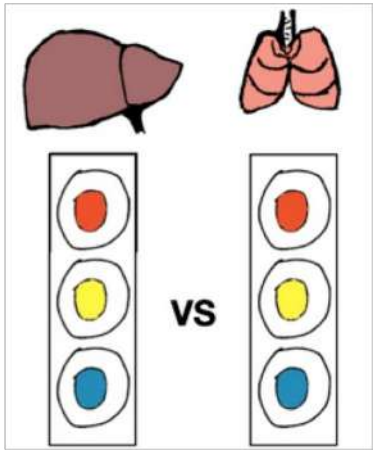

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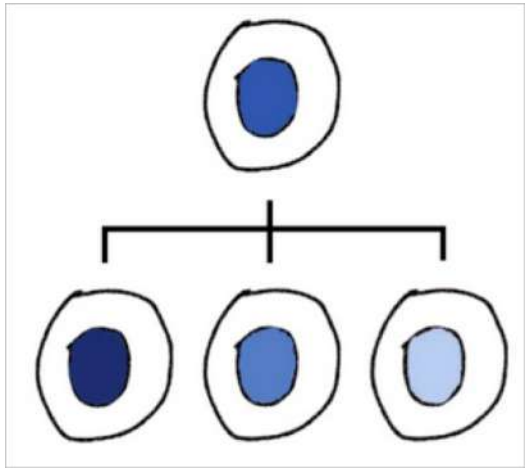

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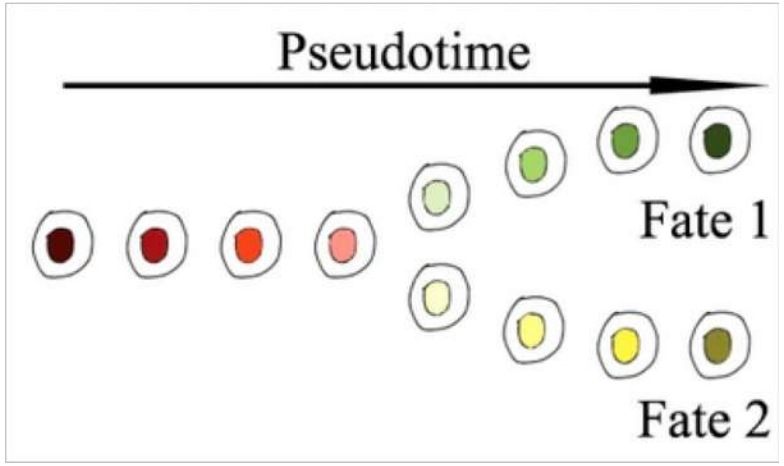
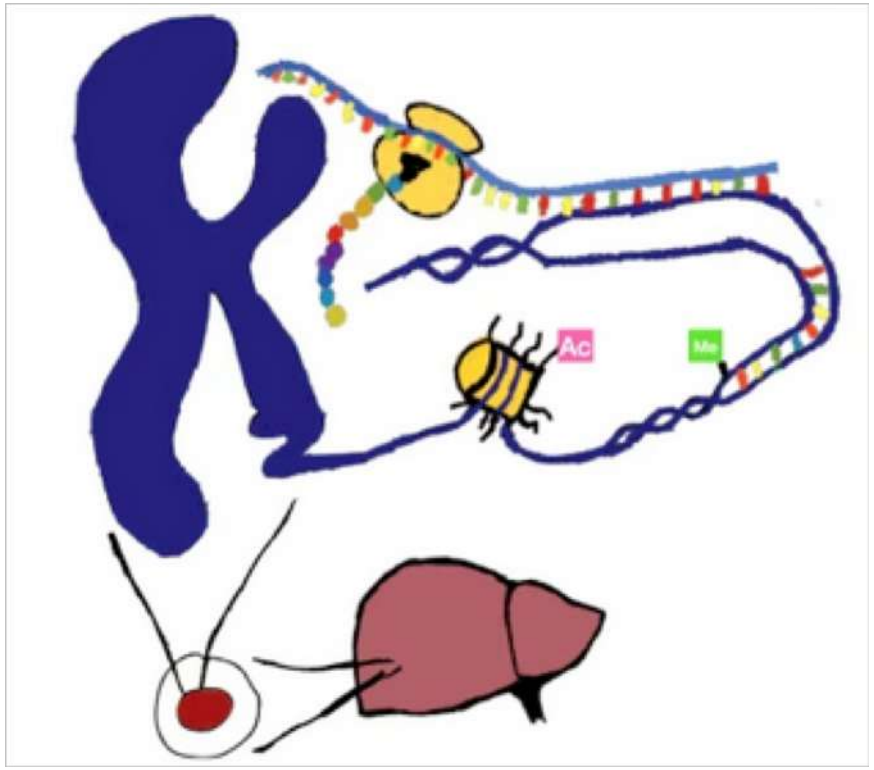


Reconstructing the trajectory of immune cell development and differentiation

Source: Chen et al, Cellular and Molecular Immunology, 2019

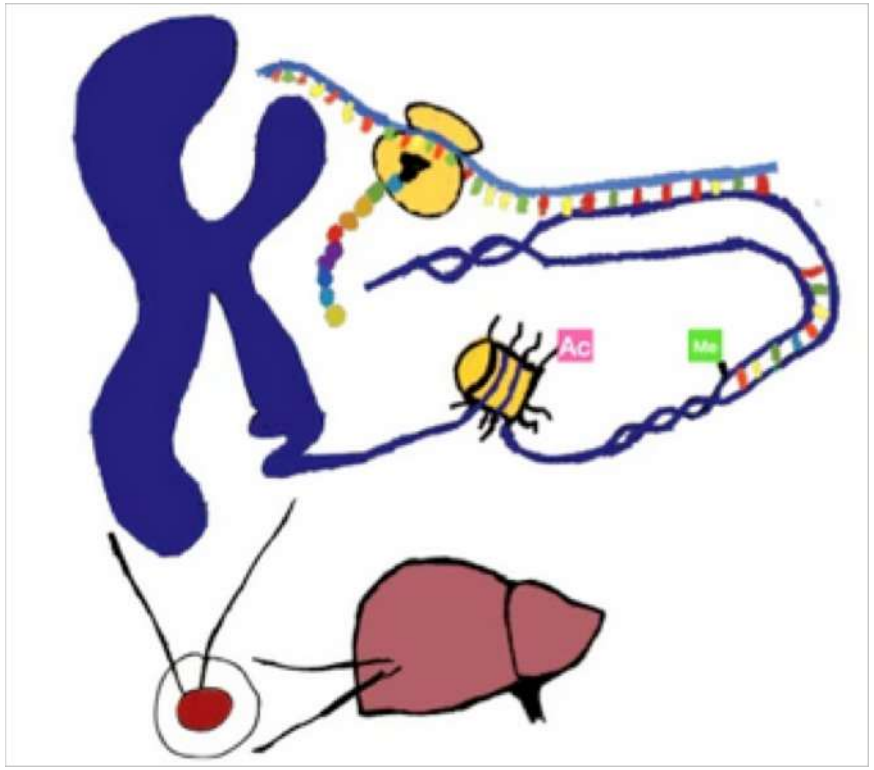
Advanced methods of integrating data types



Integrating single-cell
multi-omics and spatial analysis

Source: Chen et al, Cellular and Molecular Immunology, 2019

Questioning immune processes with multiple factors involved



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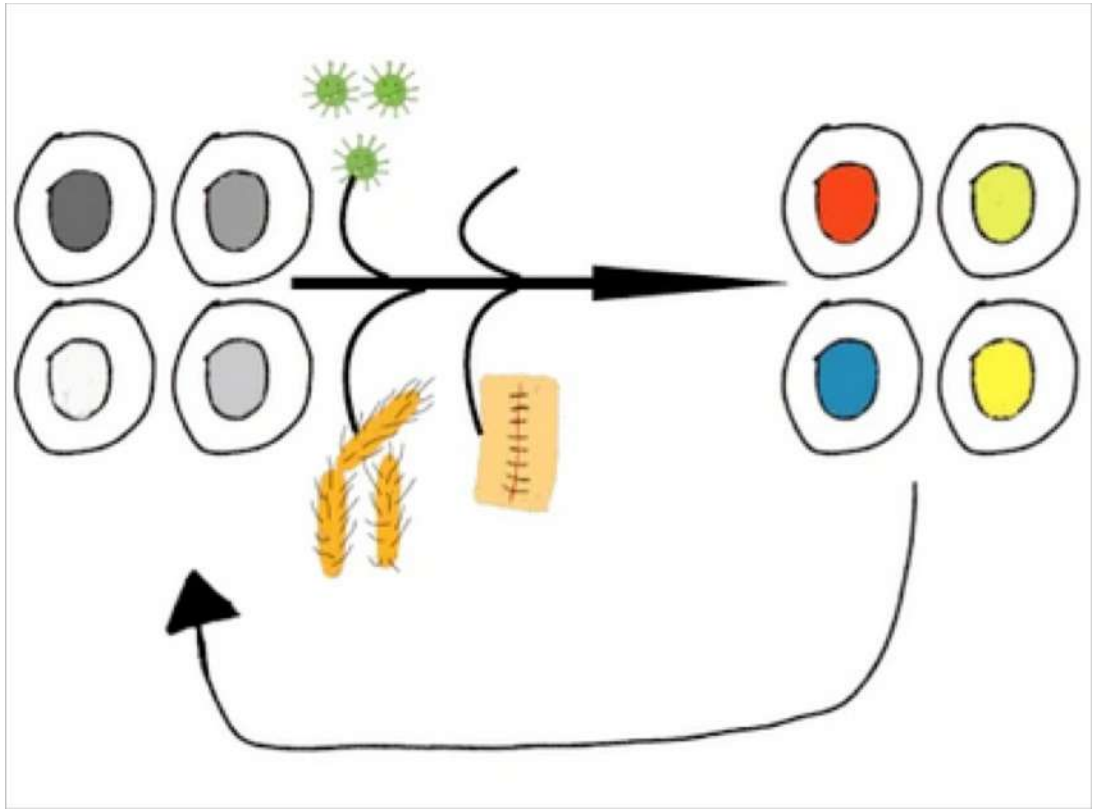
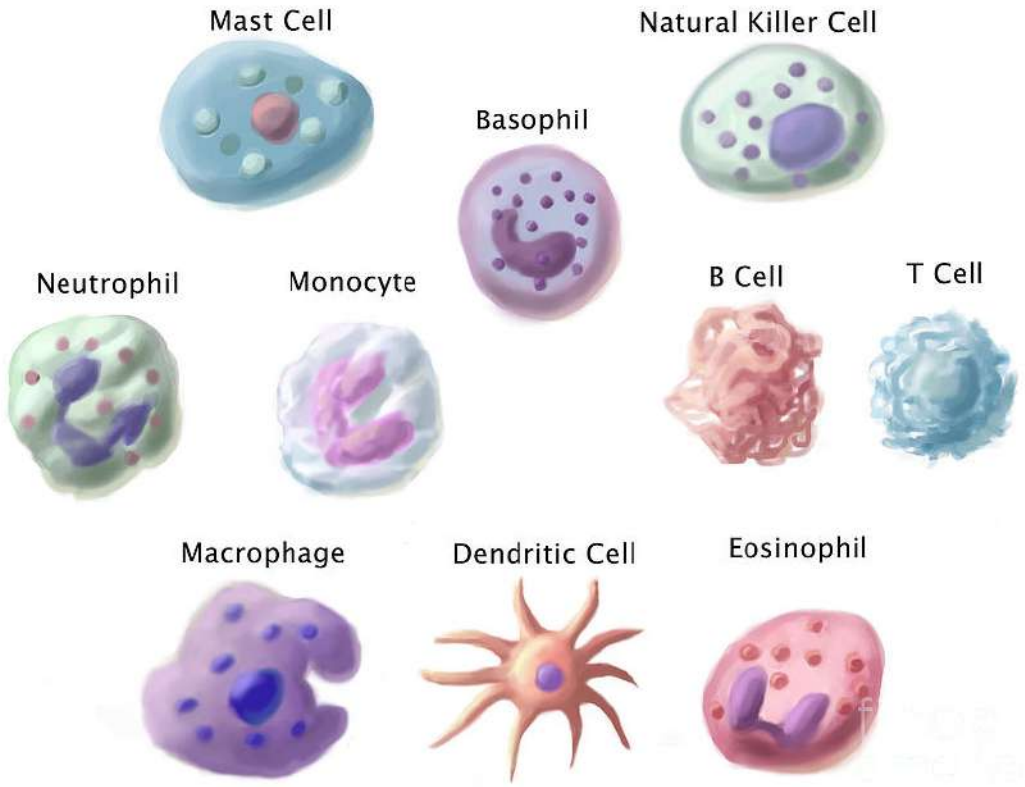


Studying immune processes

Source: Chen et al, Cellular and Molecular Immunology, 2019

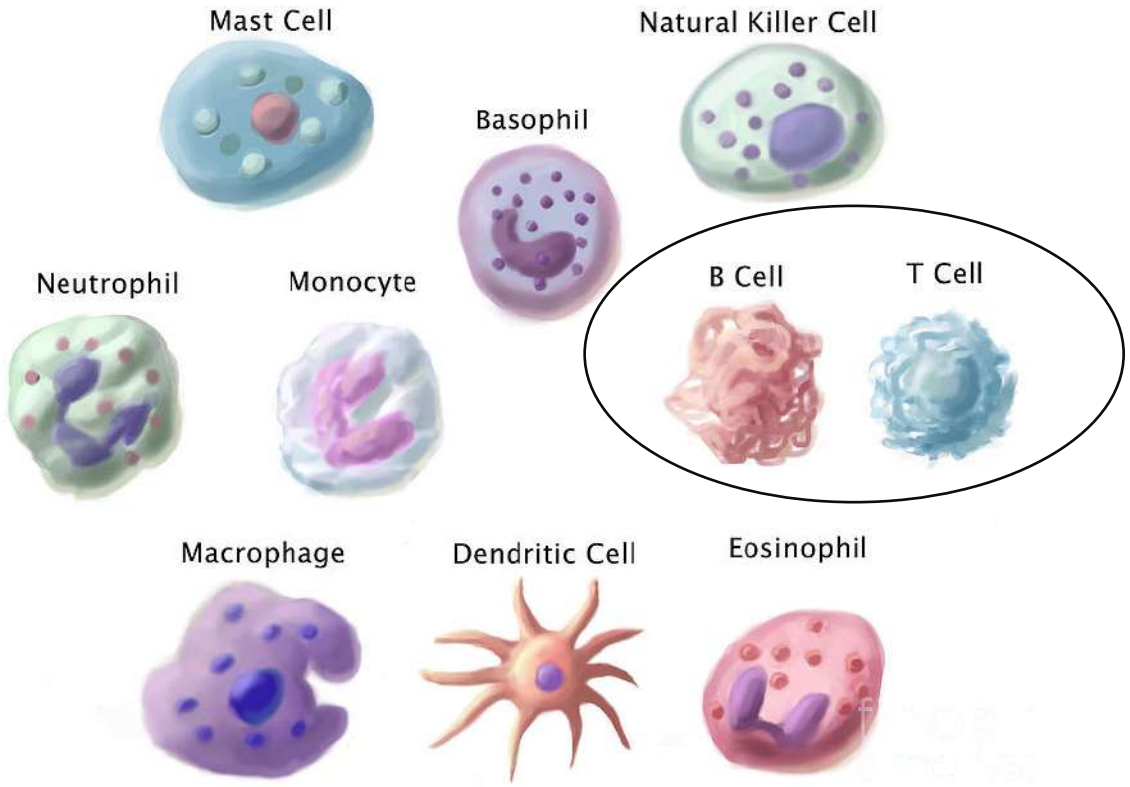
Different immune cells

Immune cells



AIM

Immune cells



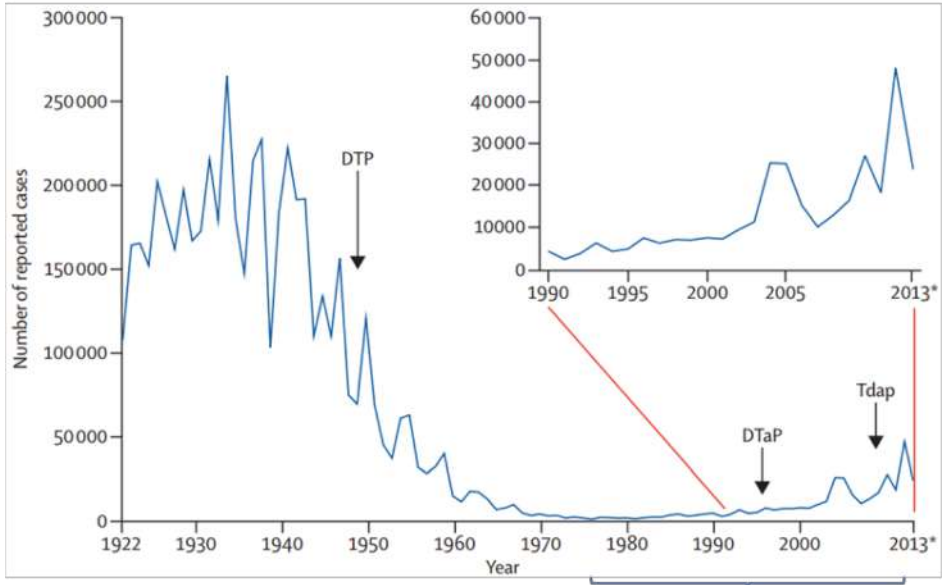
1. T/B-cell receptor
2. T/B-cell binding to antigen
3. Diversity of T/BCR
4. Structure of receptors

Pertussis: vaccination and resurgence

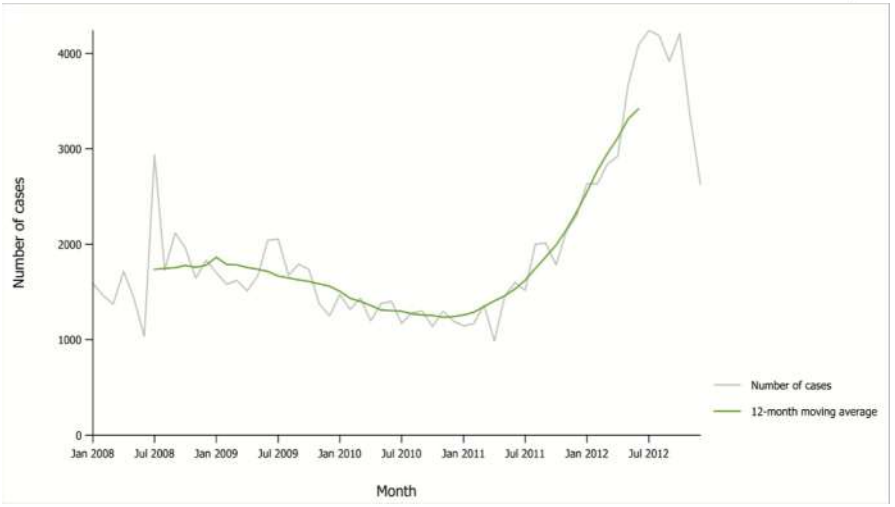
Bordetella pertussis

- Whooping cough/pertussis
- Gram negative, aerobic bacterium
- Airborne infection (droplets)
- Infection mostly affects young children
- Important virulence factors:
  - FHA (filamentous hemagglutinin)
  - PTx (pertussis toxin)
  - Prn (pertactin)

US pertussis cases



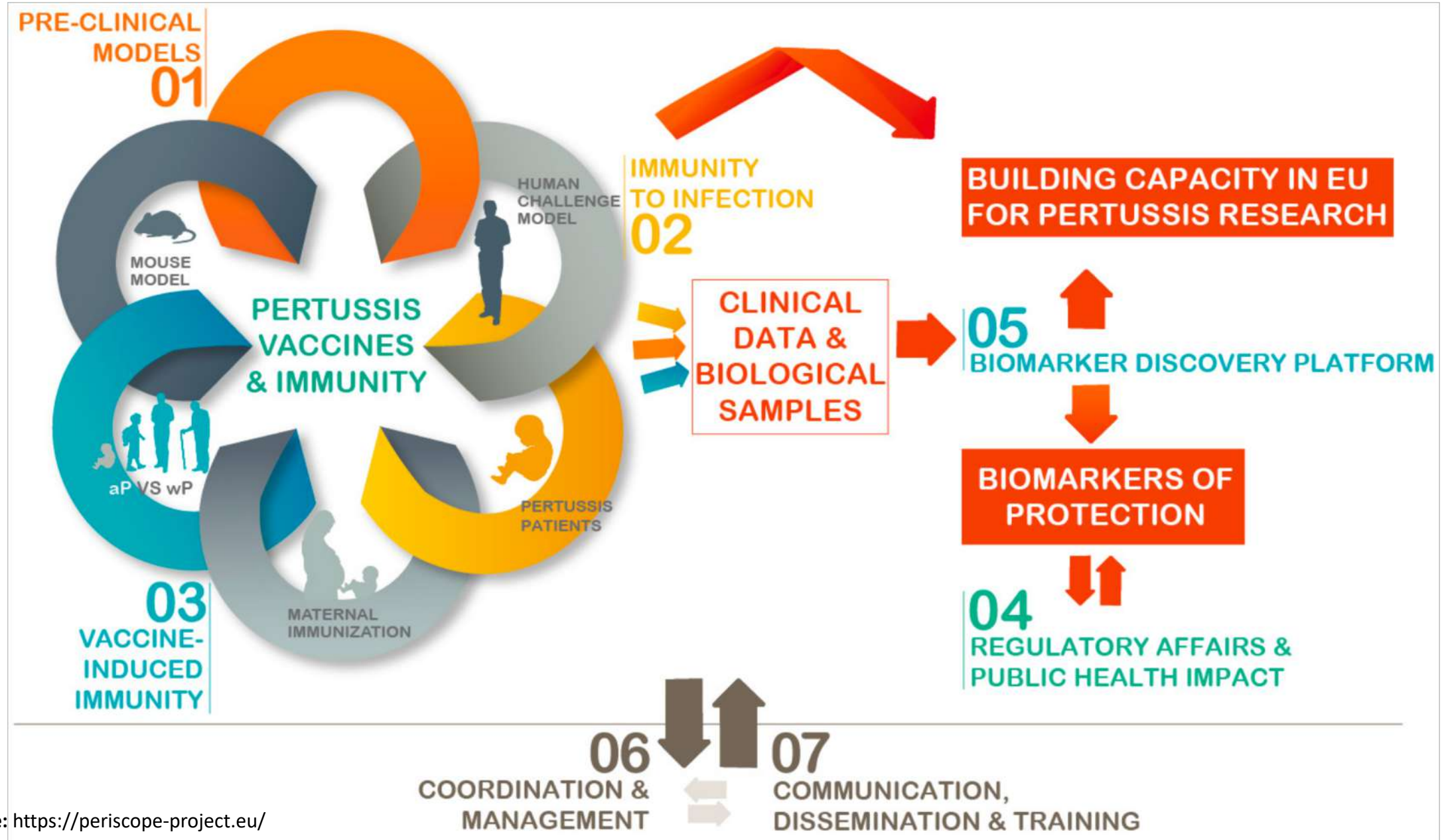
50 fold increase



Pertussis vaccines

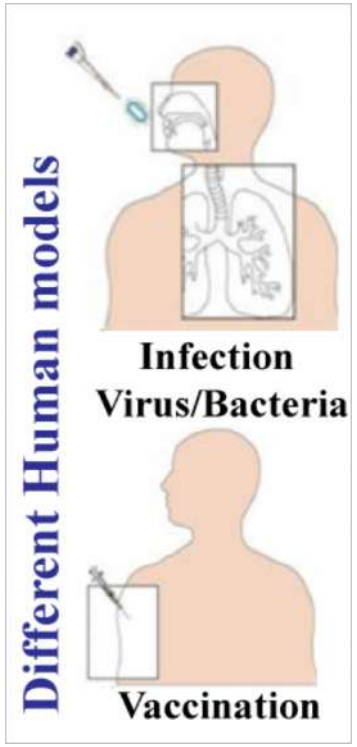
- whole-cell vaccine (wP)
- acellular vaccine (aP)

PERISCOPE EU Project



Source: https://periscope-project.eu/

Unique human samples to answer waning of immunity



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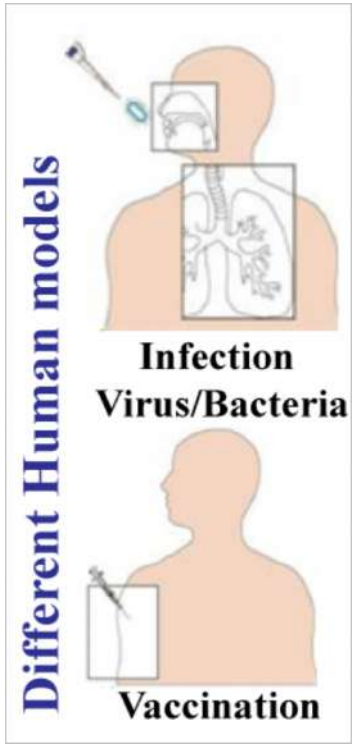
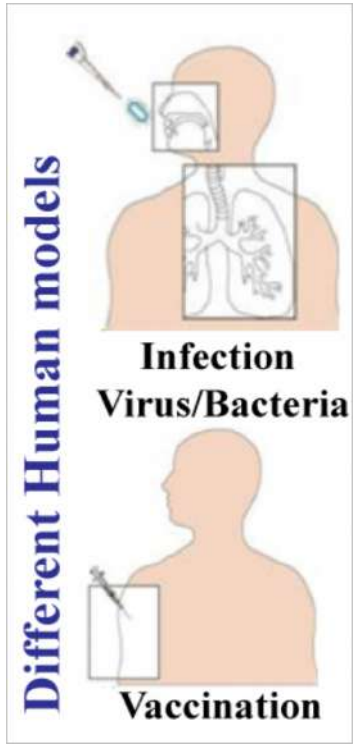


Can We Improve Vaccine Efficacy by Targeting T and B Cell Repertoire Convergence?

*Katja Fink**

Singapore Immunology Network, Agency for Science, Technology and Research, Singapore, Singapore

Using BCRs to assess vaccine efficacy



Compare the immune repertoire between infection and vaccination settings

Immune repertoire

No memory, no attack mode

Naïve B

Naïve T

Naïve B and T cells are produced in immune system

Immune repertoire

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Naïve B

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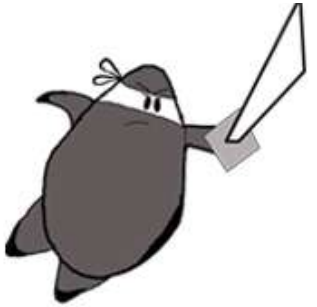
Naïve B and T cells are produced in immune system

After antigen encounter cells mature

Antigen experienced B-cell



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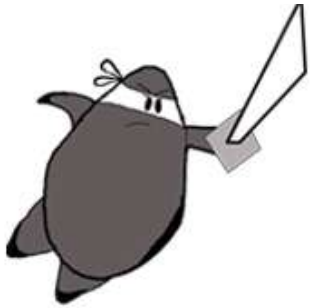
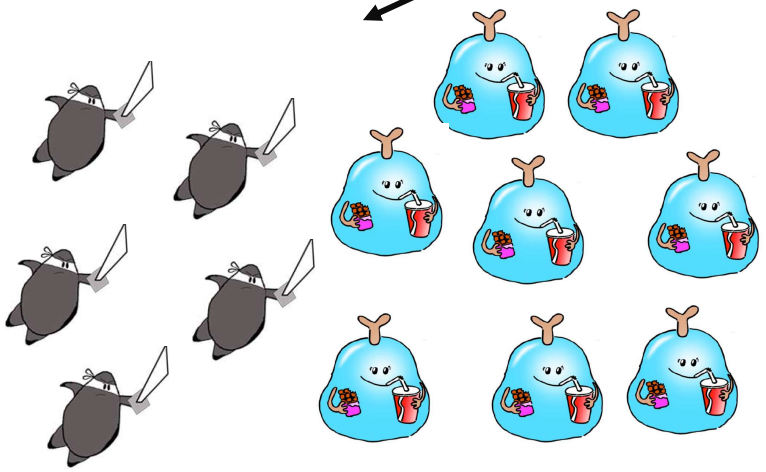


Antigen experienced T-cell

Proliferation



Immune repertoire

Immune repertoire

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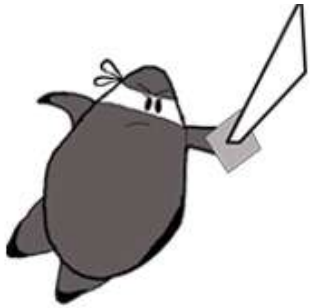
Naïve B and T cells are produced in immune system

Memory of antigen and Attack mode is on

After antigen encounter cells mature

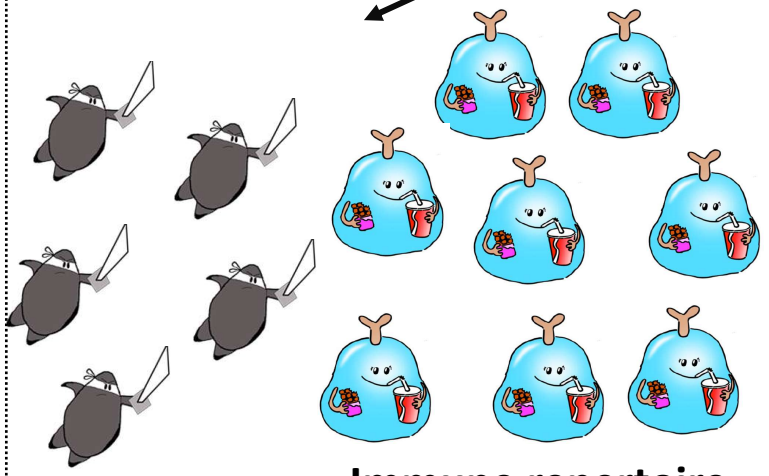
Antigen experienced B-cell



Antigen experienced T-cell

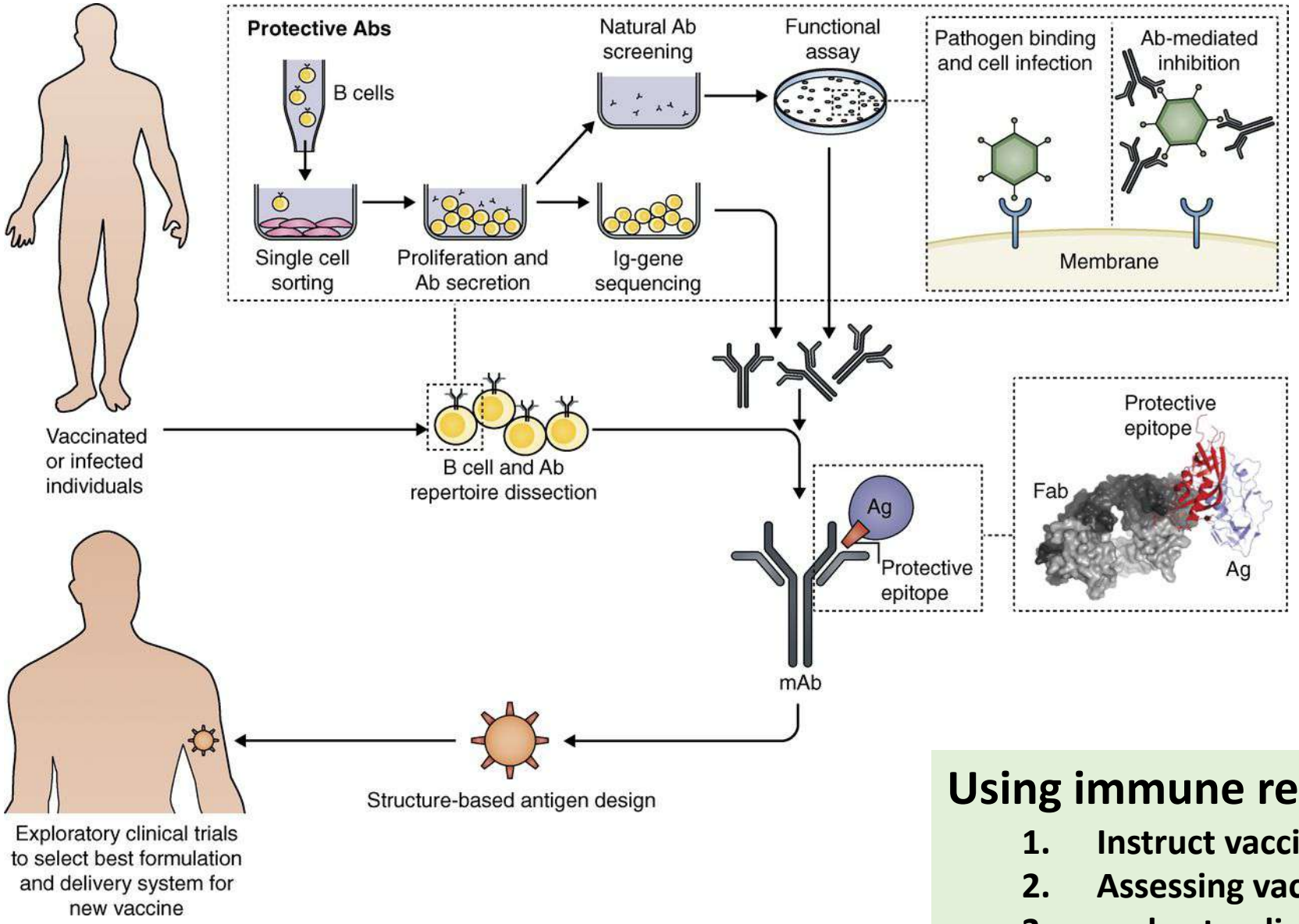
Proliferation

Memory



Immune repertoire

Reverse Vaccinology: Human immunology instructs vaccine antigen design

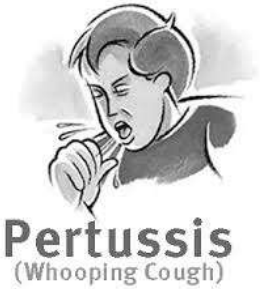


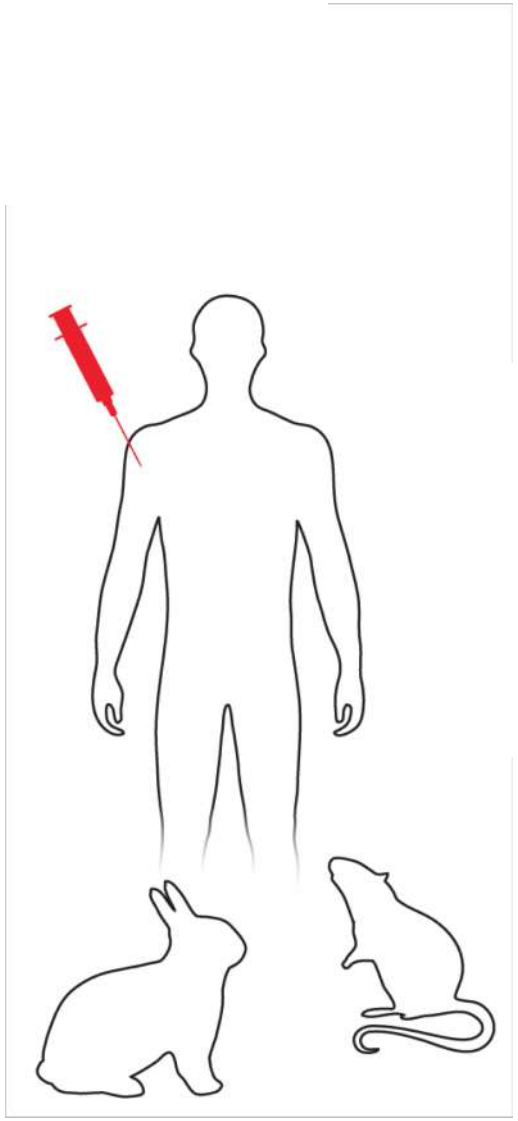
Source: Rappuoli et al., Journal of Experimental Medicine, 2016

Using immune response to

1. **Instruct vaccine design**
2. **Assessing vaccine efficacy**
3. **understanding immune mechanisms**

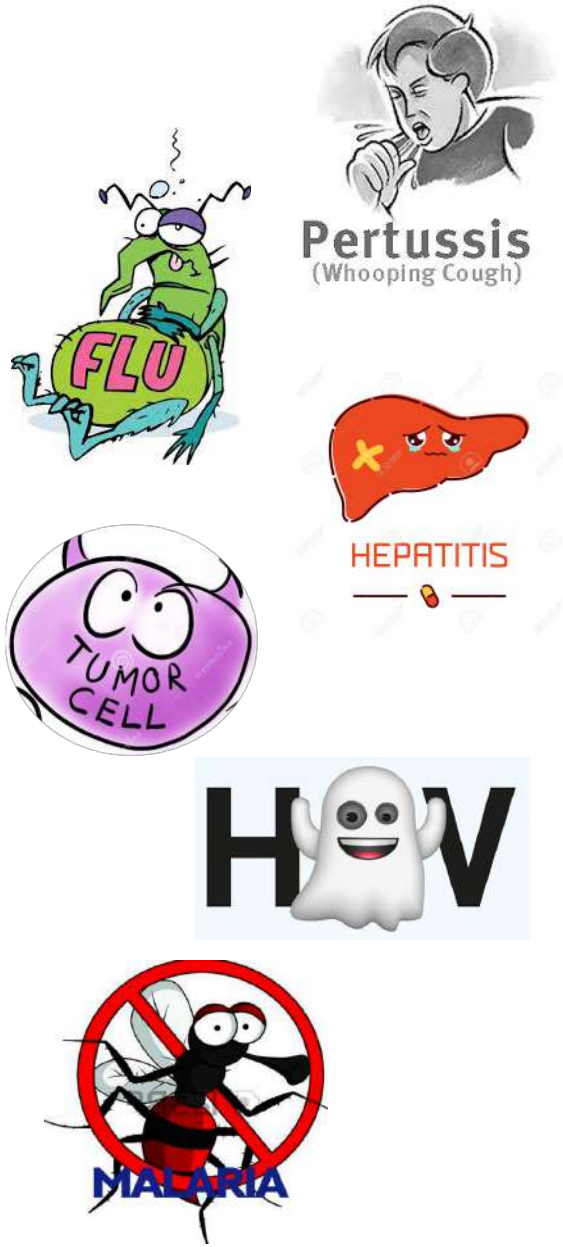
Immune system fighting against infections

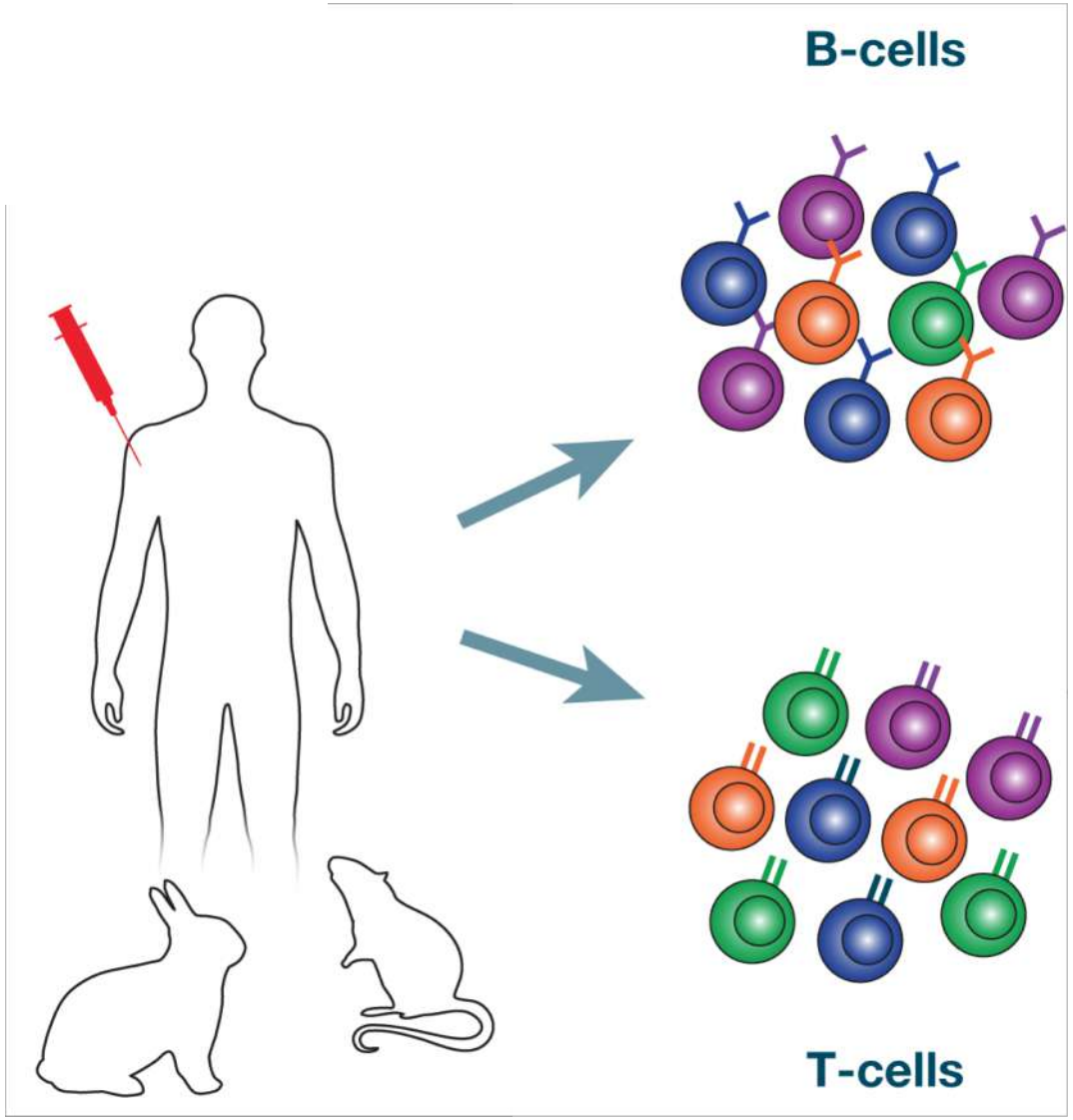




Source: https://ngsdataanalysis.com/technology/immune-repertoire/

Immune system fighting against infections





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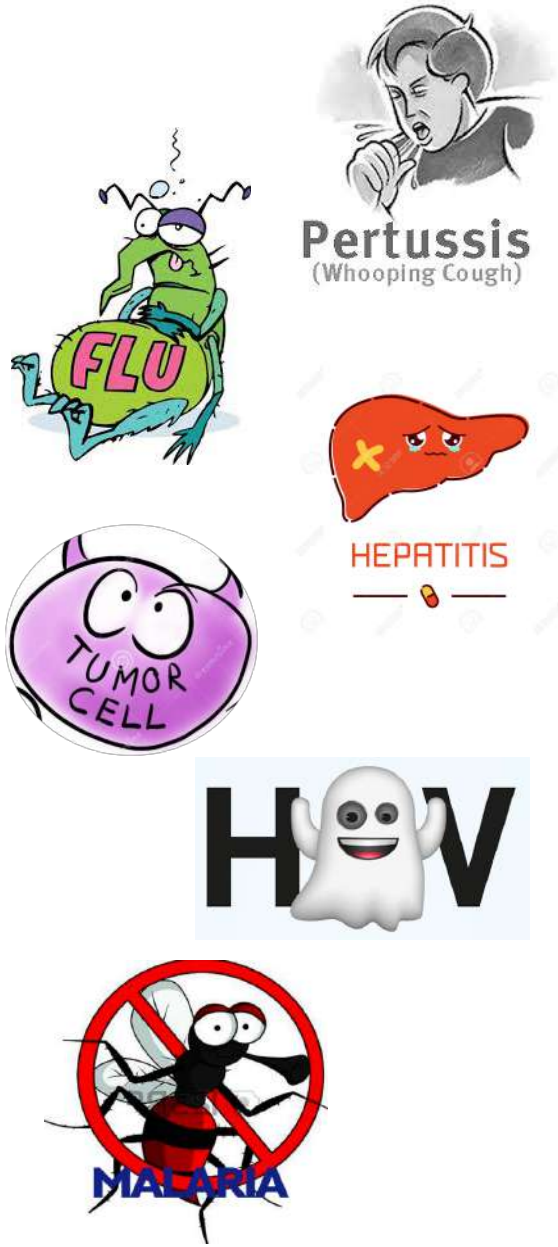

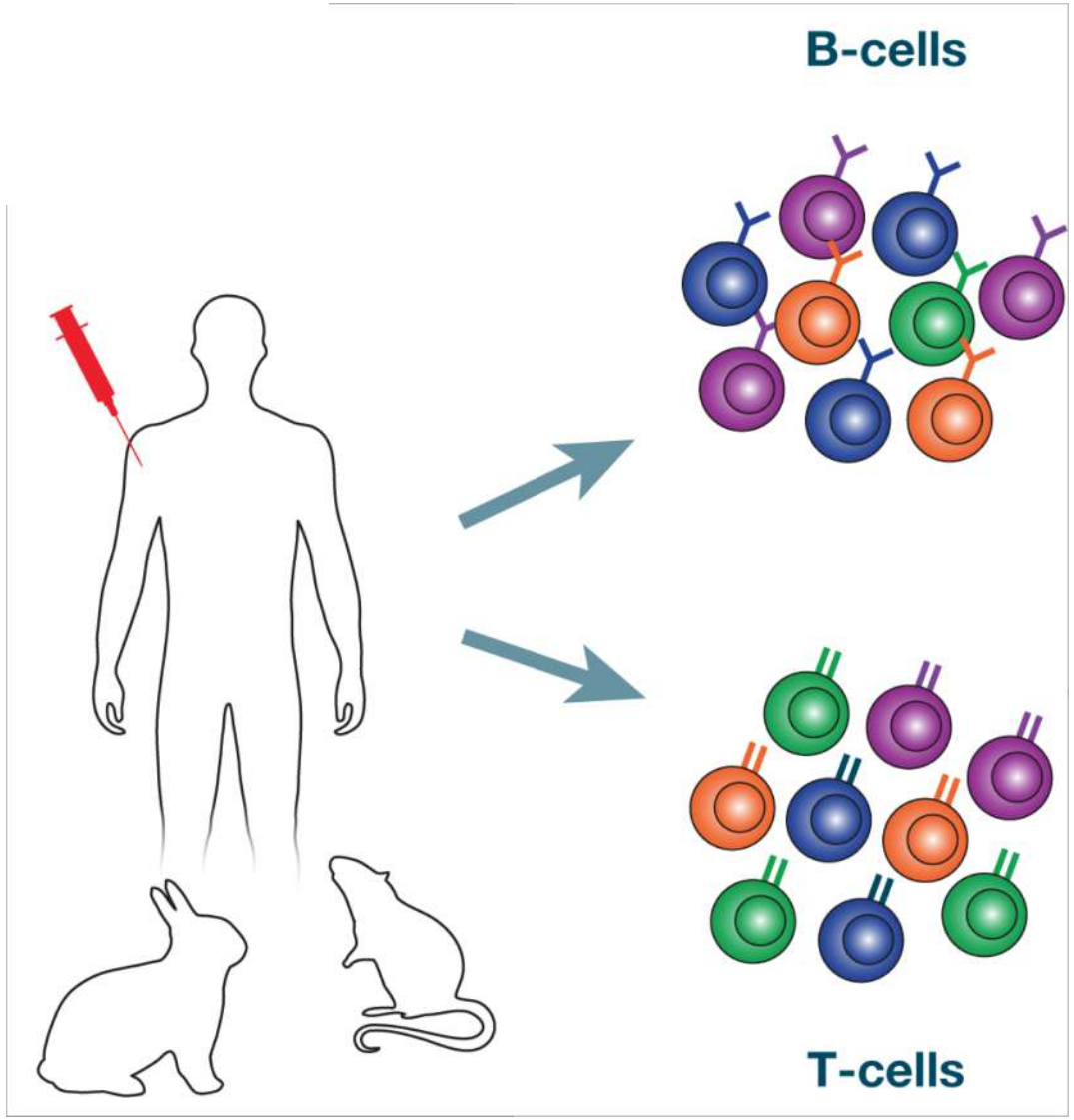

Gene Expression

Receptors

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Receptors

Source: https://ngsdataanalysis.com/technology/immune-repertoire/

How does our immune system can recombine to millions of receptors

By recombining between different genes and chains

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Each receptor is composed of 2 chains: Heavy and light

Each chain is generated by a series of recombination events

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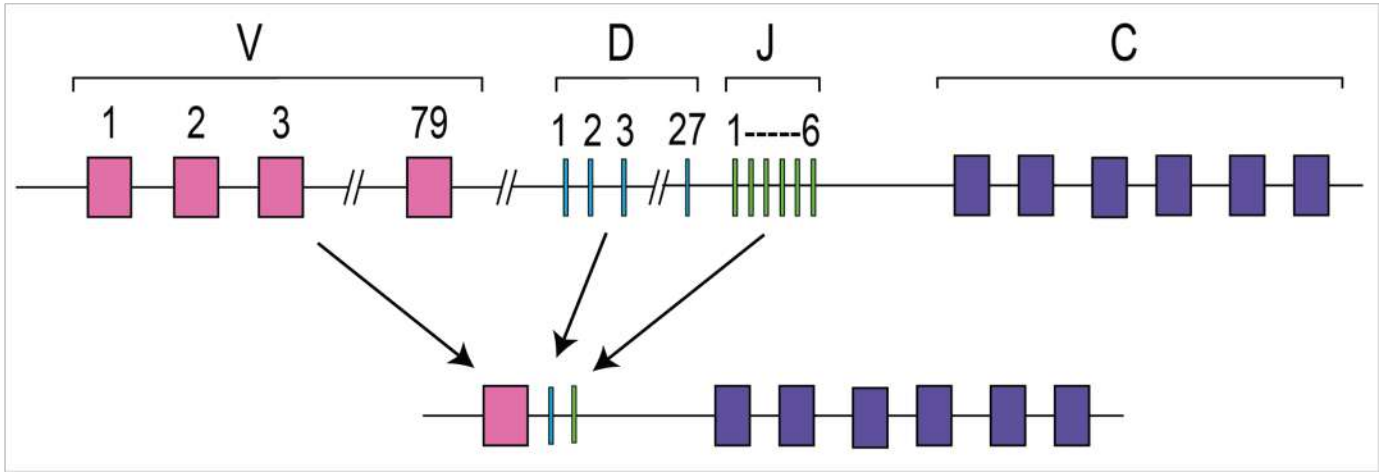
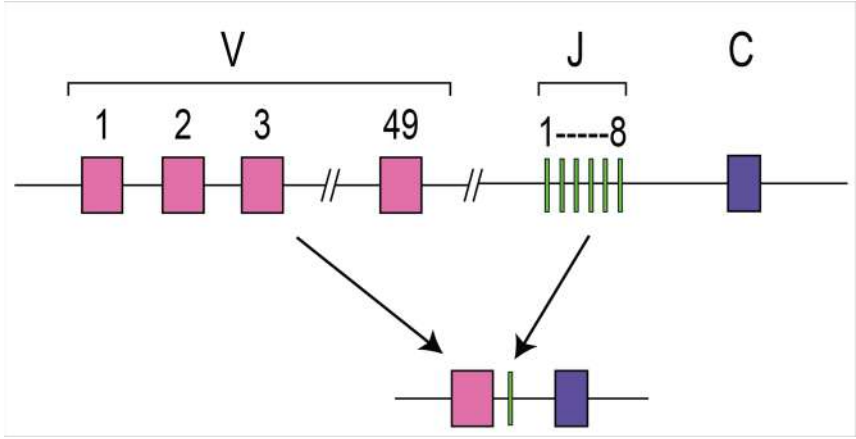
Heavy locus



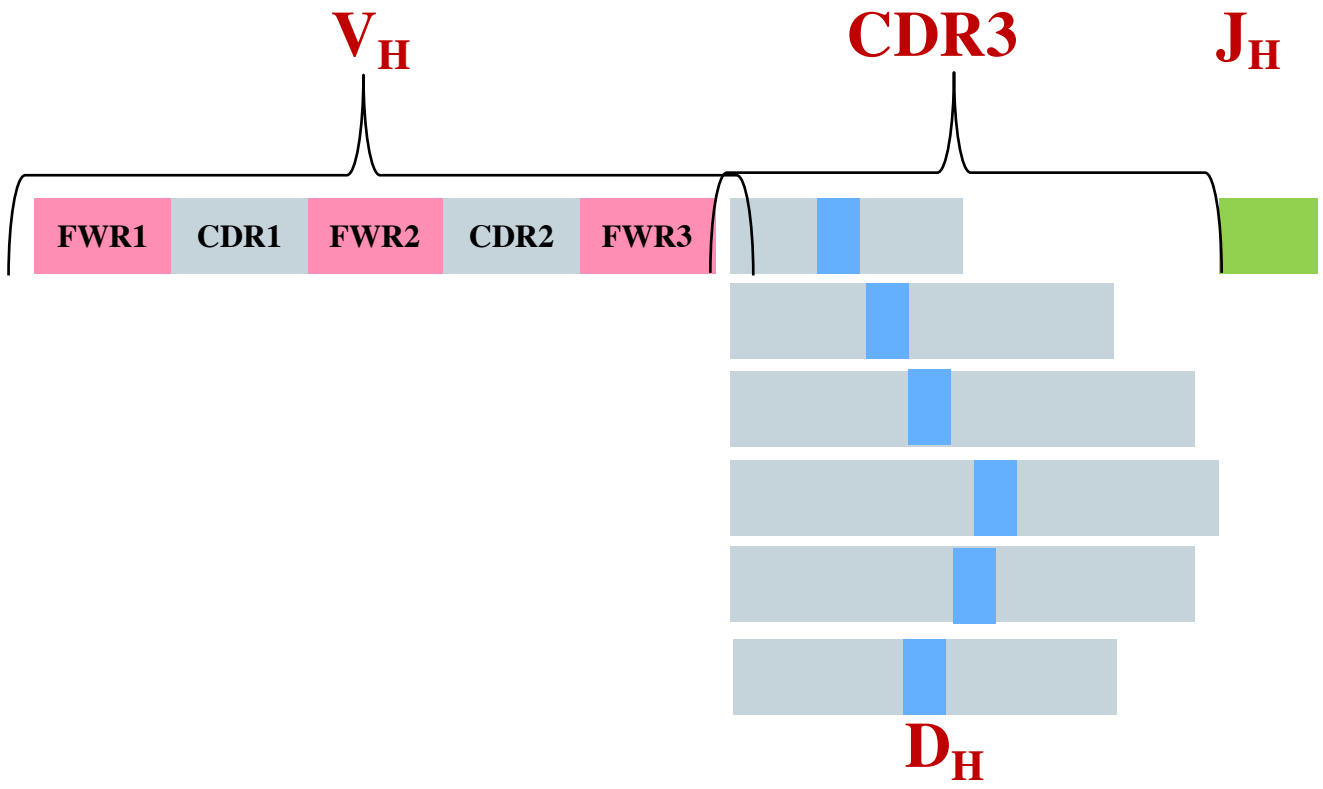
Light locus



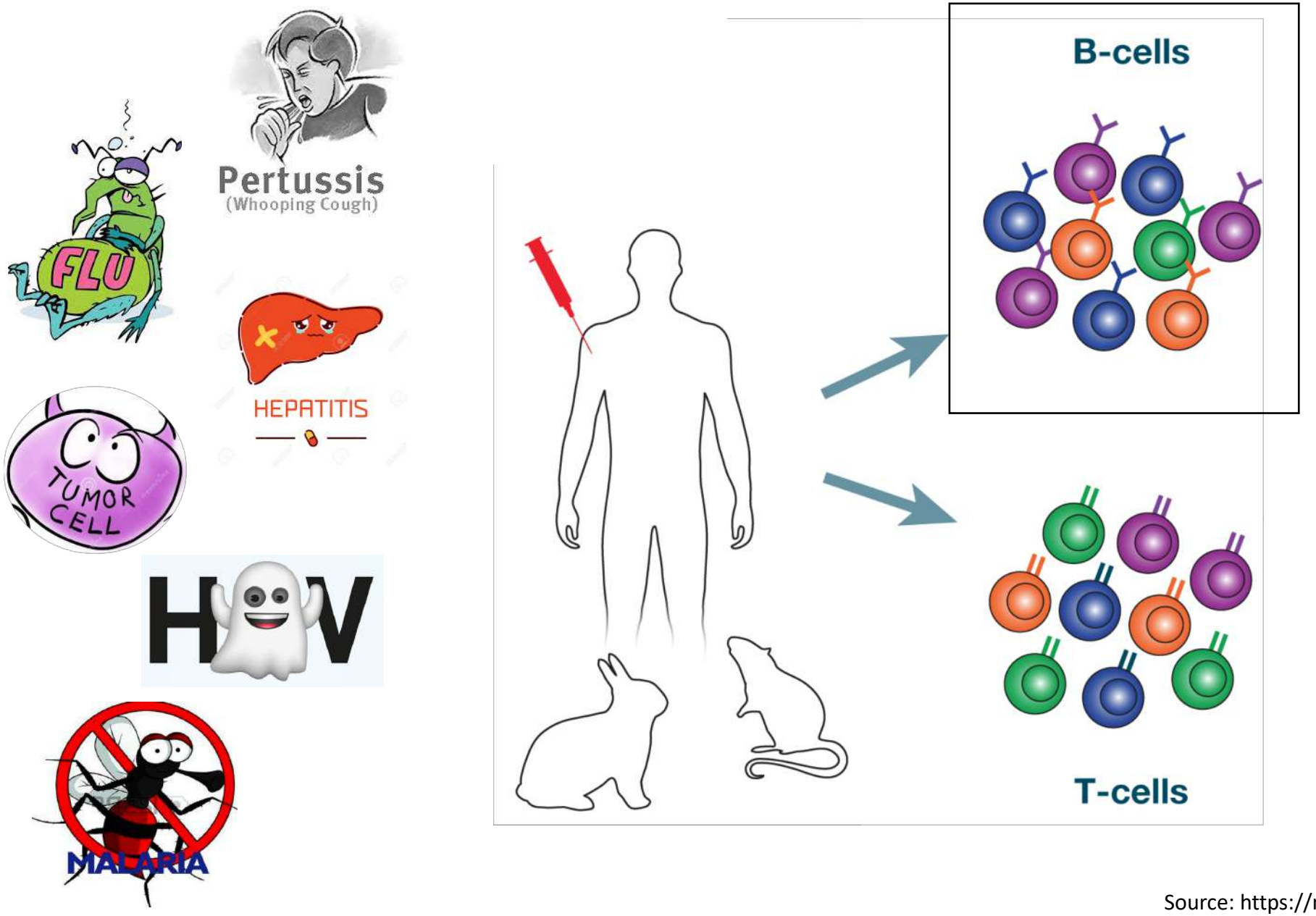
***V(D)J* Recombination**

Domain structure of Naïve B/TCR

Naïve B/T cell



BCR are different from TCRs

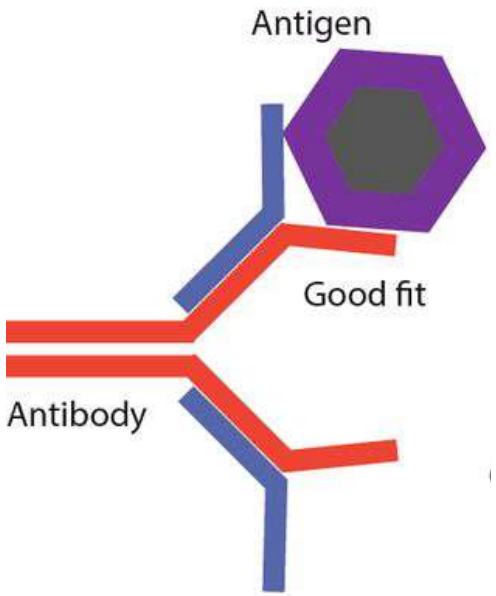


B-cells have another level of diversity

Source: https://ngsdataanalysis.com/technology/immune-repertoire/

BCR can mutate their receptor to fit antigen

Antibody binds to antigen



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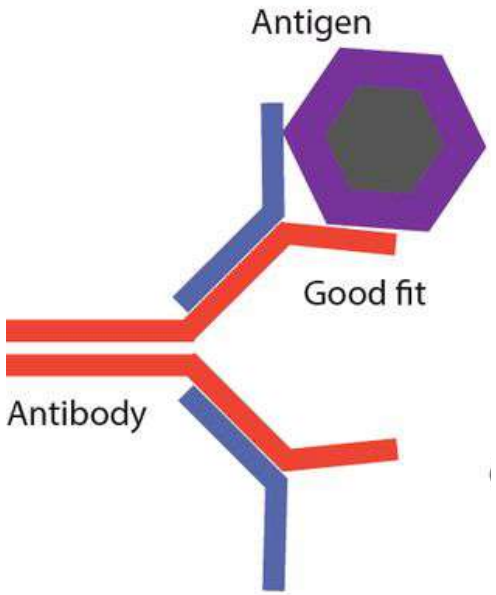
Antigen is foreign protein from bacteria/ virus/ infecting agent



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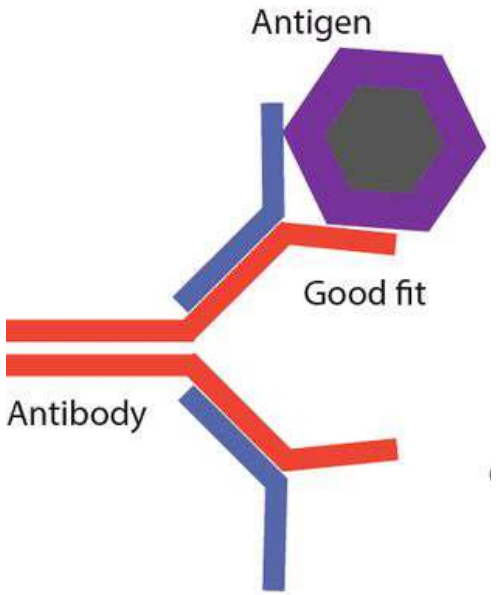

Our immune system generates millions of antibodies

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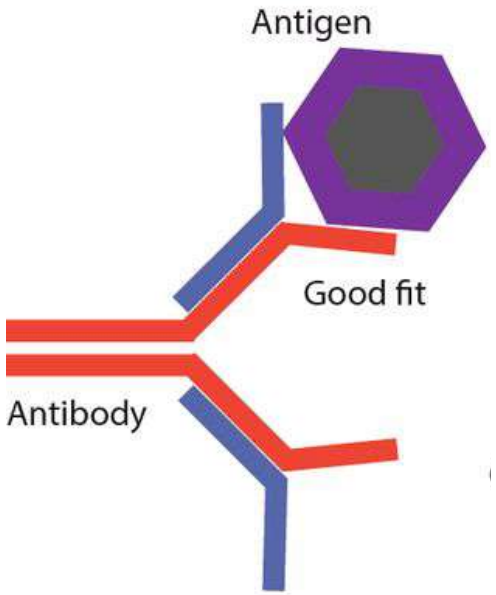

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HOW

By mutating Binding regions

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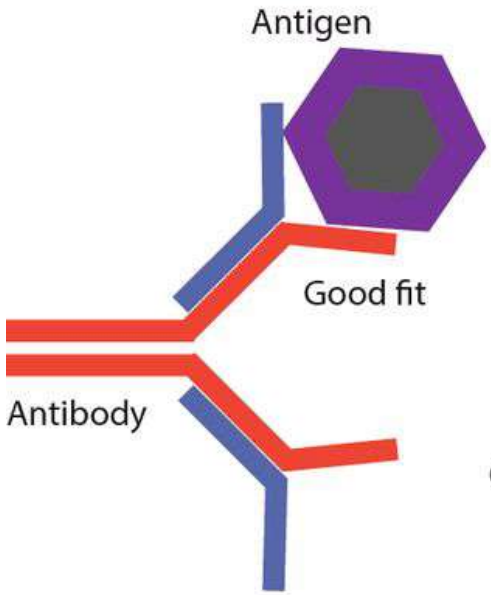

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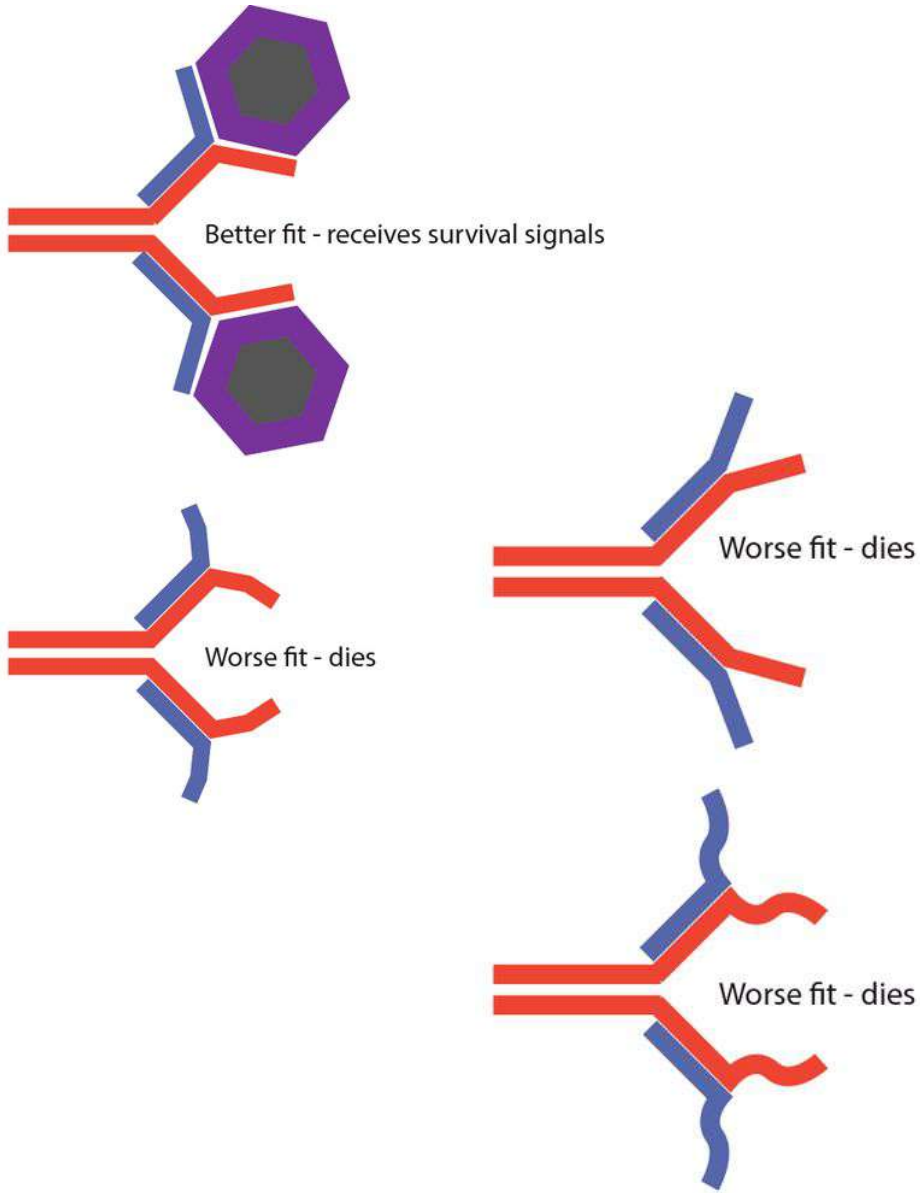
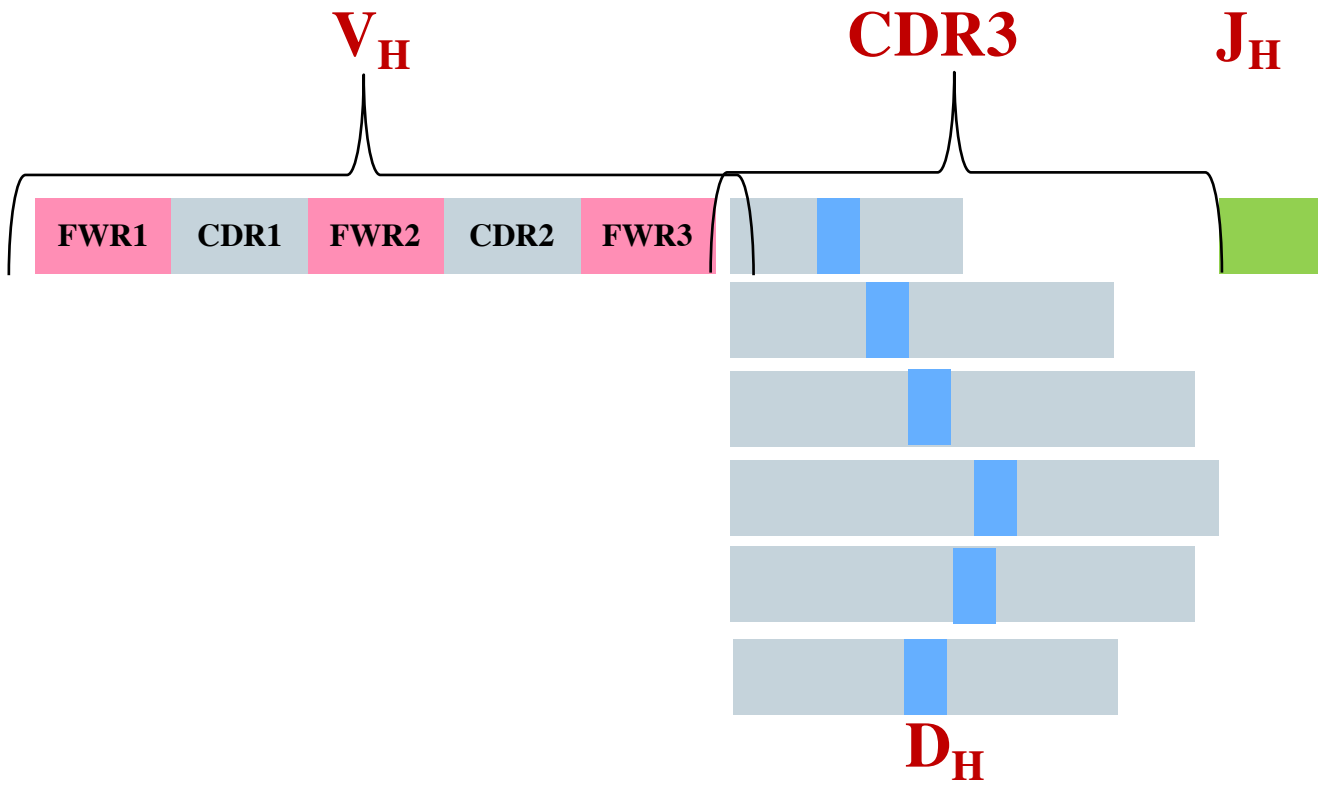


Domain structure of Antigen experienced BCR

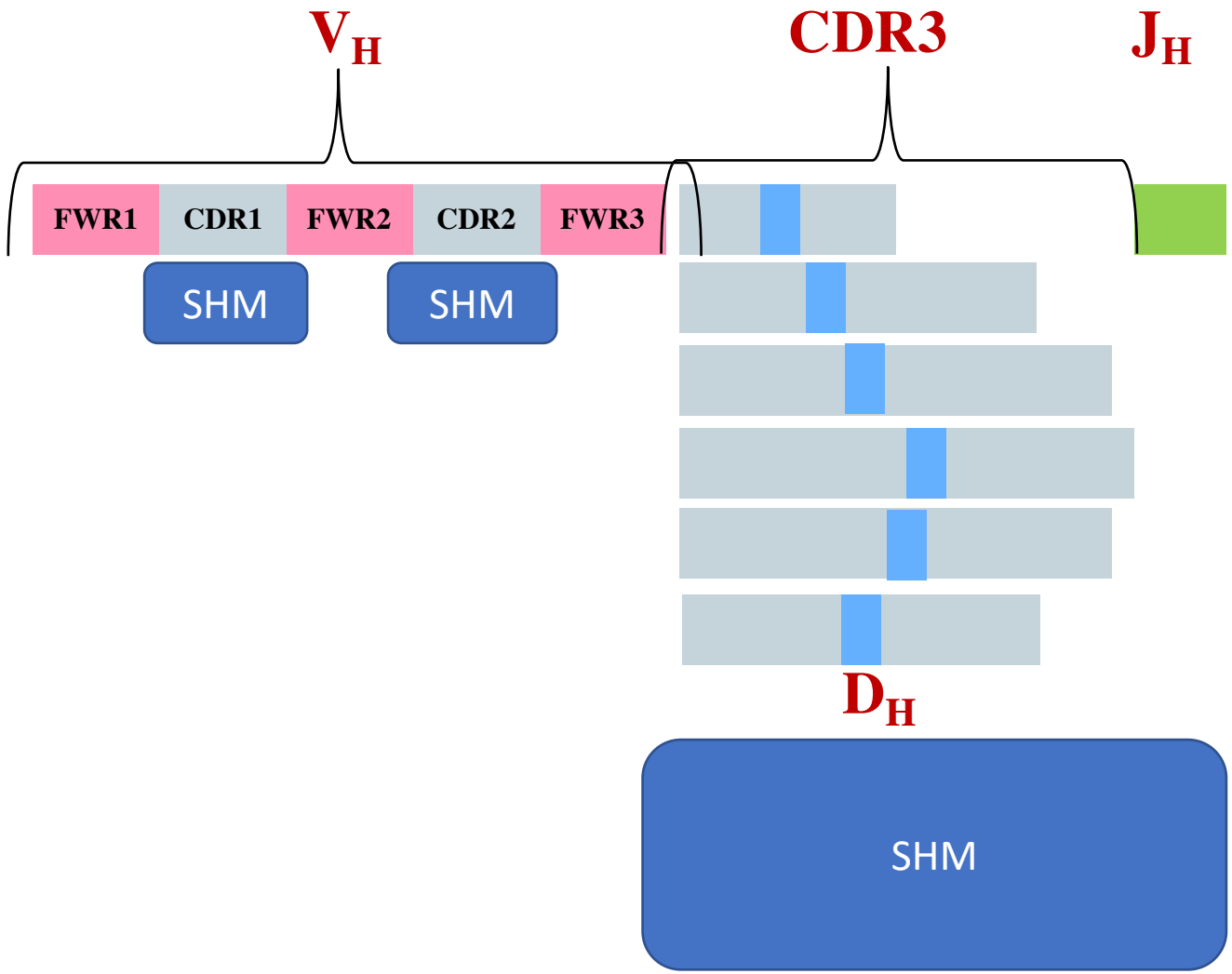
Antigen Encountered



Highly hypermutated by Somatic hypermutation process

Domain structure of Antigen experienced BCR

Antigen Encountered



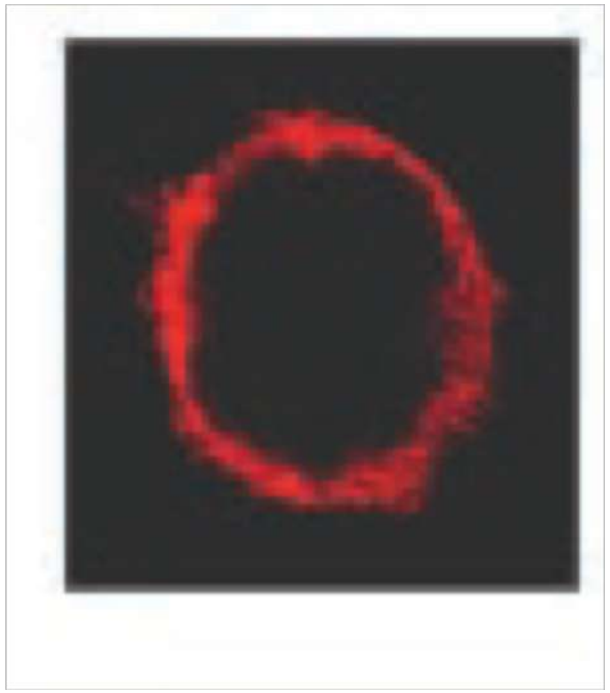
Highly hypermutated by Somatic hypermutation process

How many T/BCRs can a cell have?

How many T/BCRs can a cell have?

Thousands….

TCR



BCR

Identical receptor

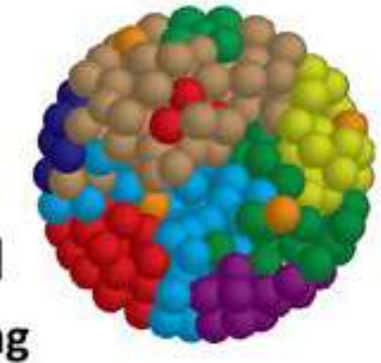
Confocal images

High throughput sequencing of T/BCRs

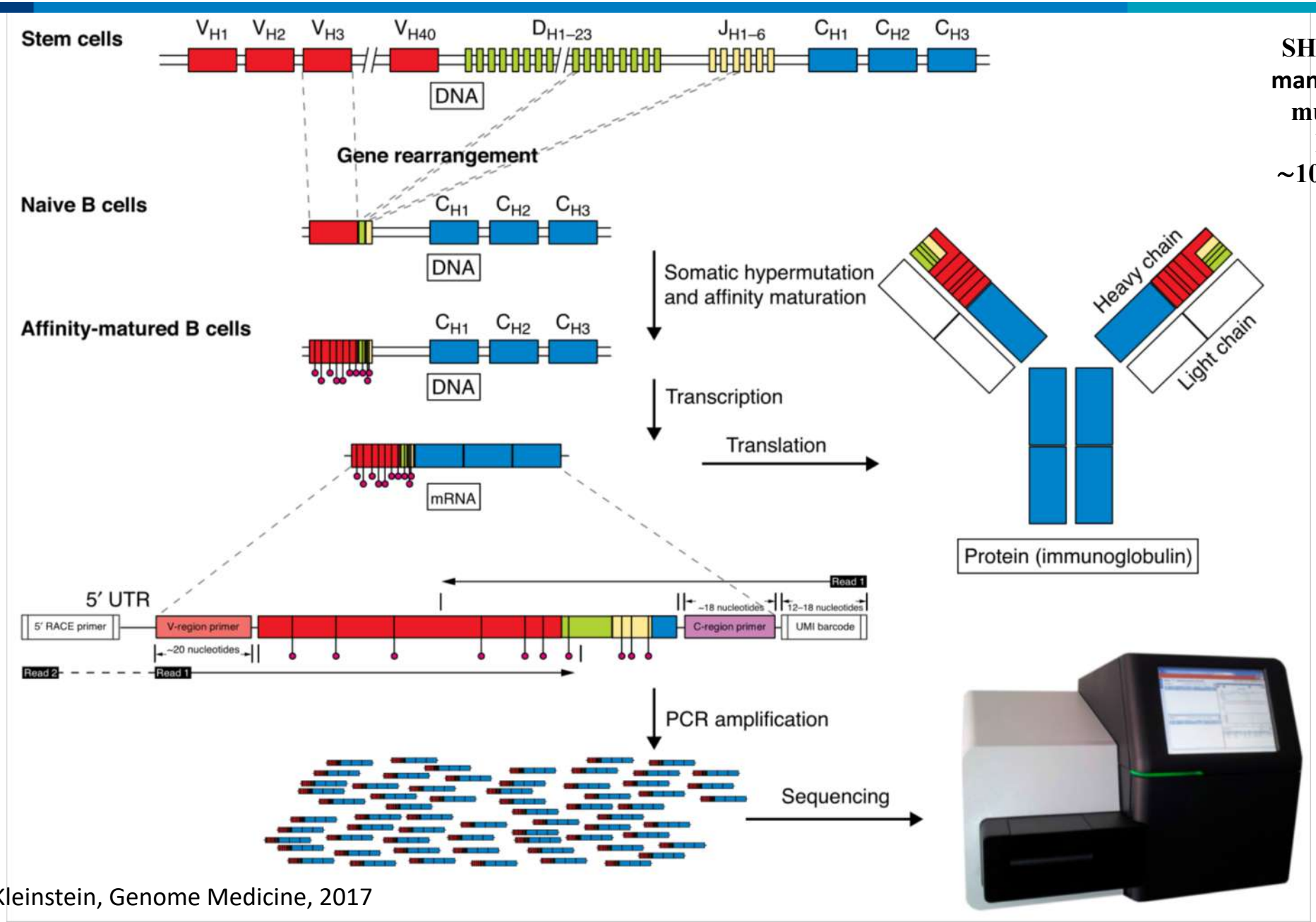
Length of receptor: 500-700 bases

Bulk Sequencing

Single-cell Sequencing



High-throughput sequencing of T/BCR



SHM in an error-prone manner introduces point mutations into the Ig locus at a rate of $\sim 10^{-3}$ per base-pair per division

Source: Yaari and Kleinstein, Genome Medicine, 2017

Short read versus Long read

Short reads

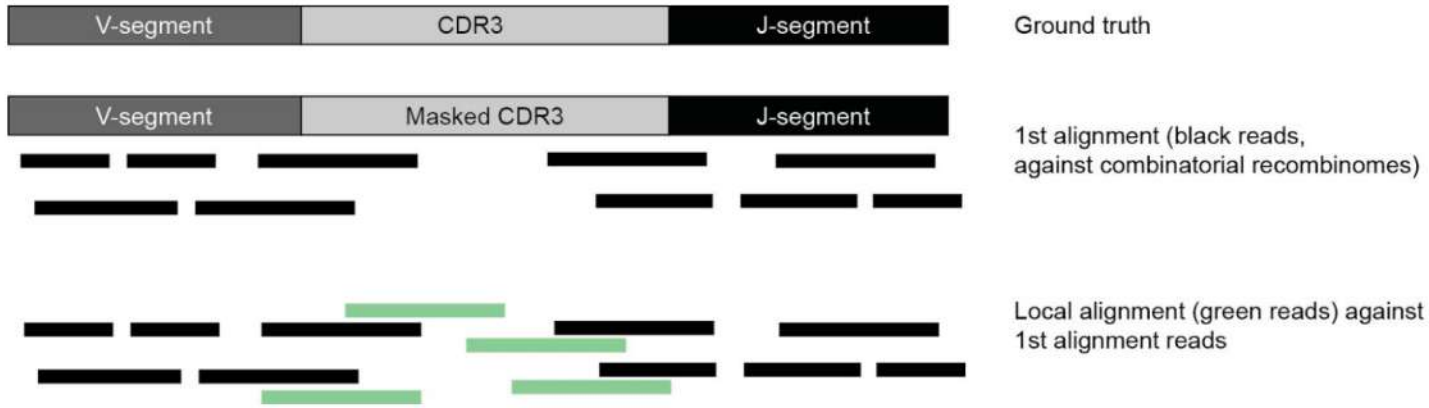


Illustration of the two alignment steps for heavy chain when reads are 50 bp or shorter.

Short read versus Long read

Short reads

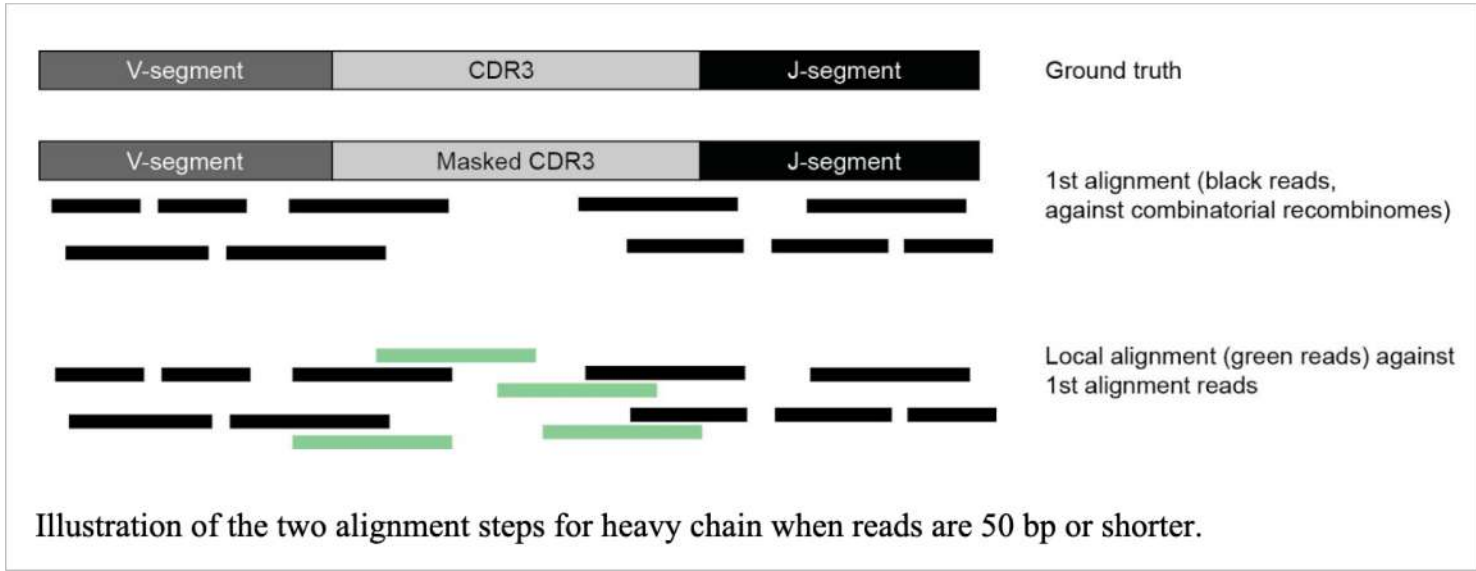
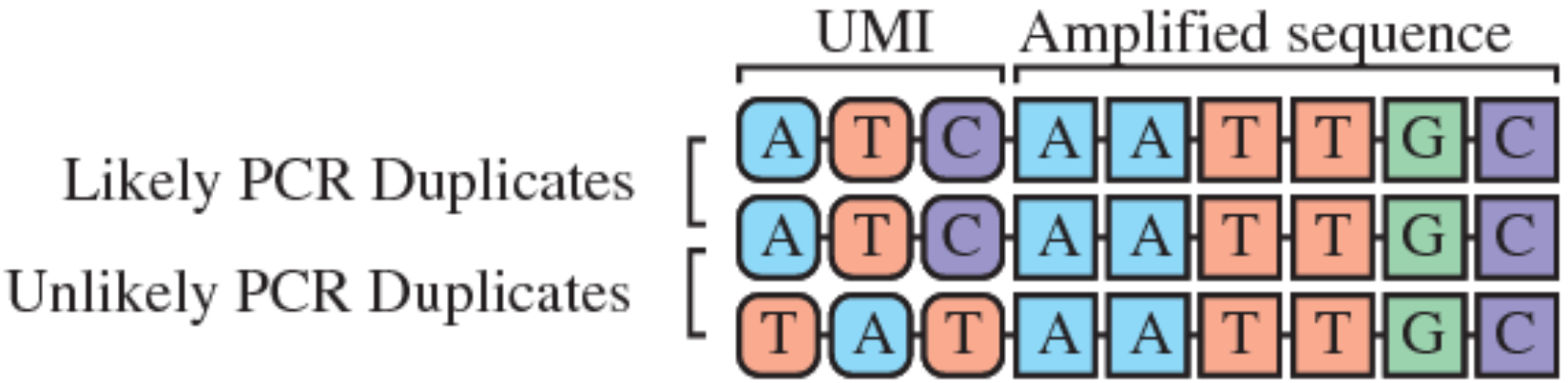


Illustration of the two alignment steps for heavy chain when reads are 50 bp or shorter.

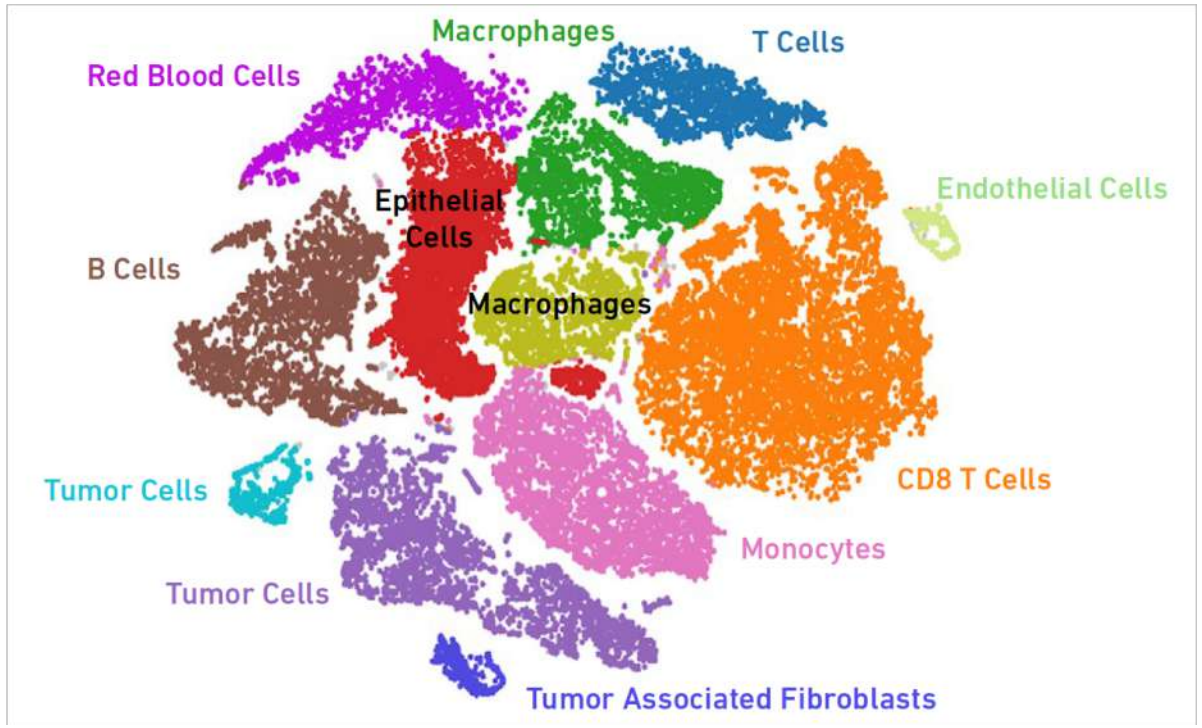
long reads

Bulk versus UMI based methods

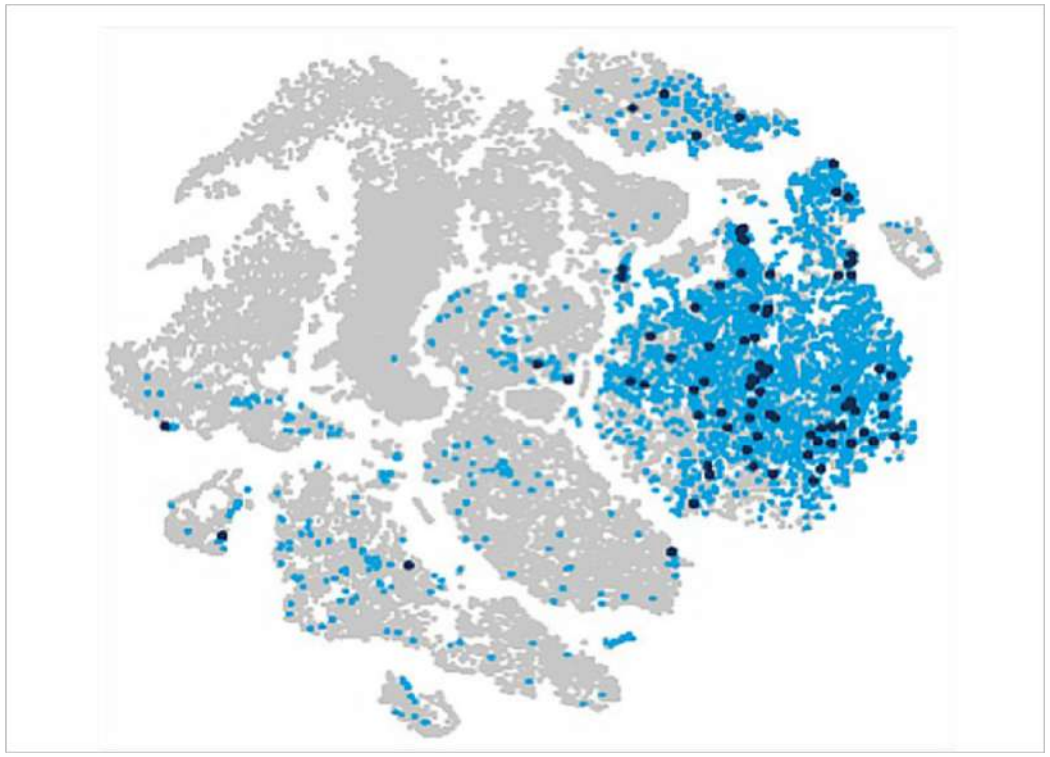
UMI: Unique molecular identifier



10X Genomics for ScRep-Seq offer



Simultaneous assessment of Gene Expression & V(D)J Repertoire



Expression of T-cell receptor in blue

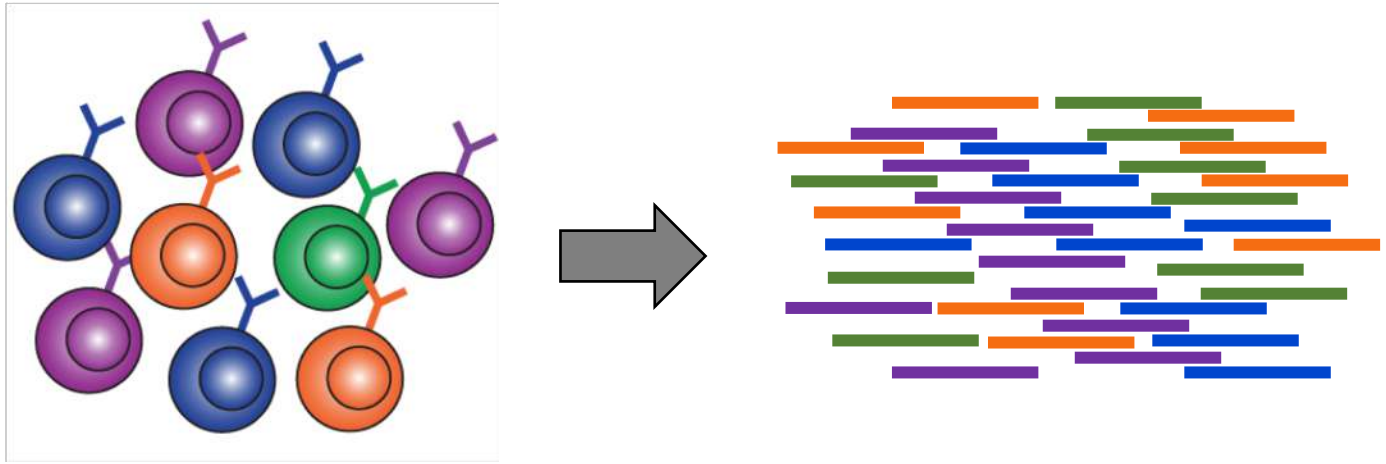
Source: go.10xgenomics.com/ps-vdj-nextgem

scRep-Seq data analysis

Preprocessing and Downstream Analysis:

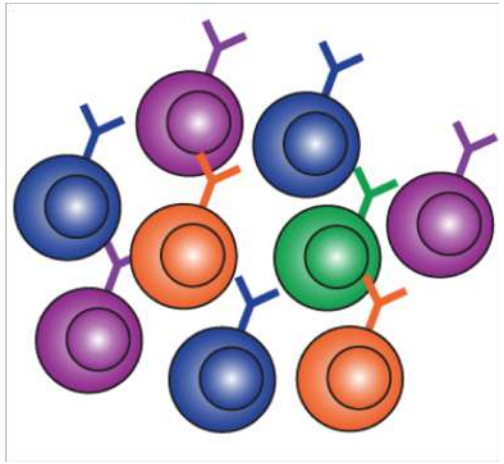
- Quality Control (QC)
- Map to IG/TR Germline databases
- Filter in productive rearrangements
- Cluster BCR/TCR sequences: B-cell clonal groups
- Build clonal lineages

Quality Control and assembling BCR/TCR from single-cells

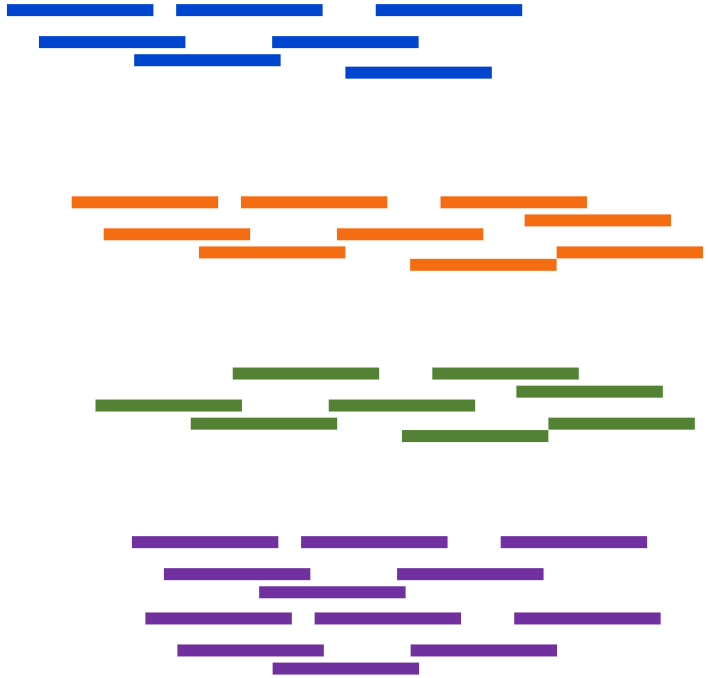


Single-cell Sequencing

Quality Control and assembling BCR/TCR from single-cells

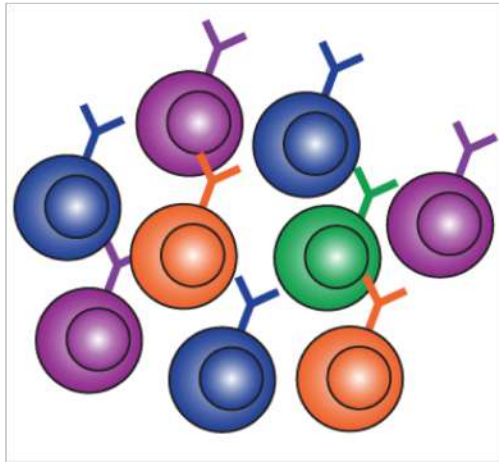


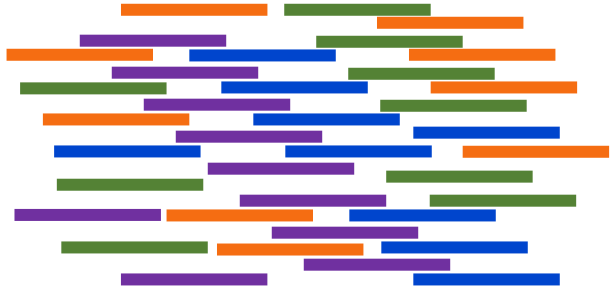
Single-cell Sequencing



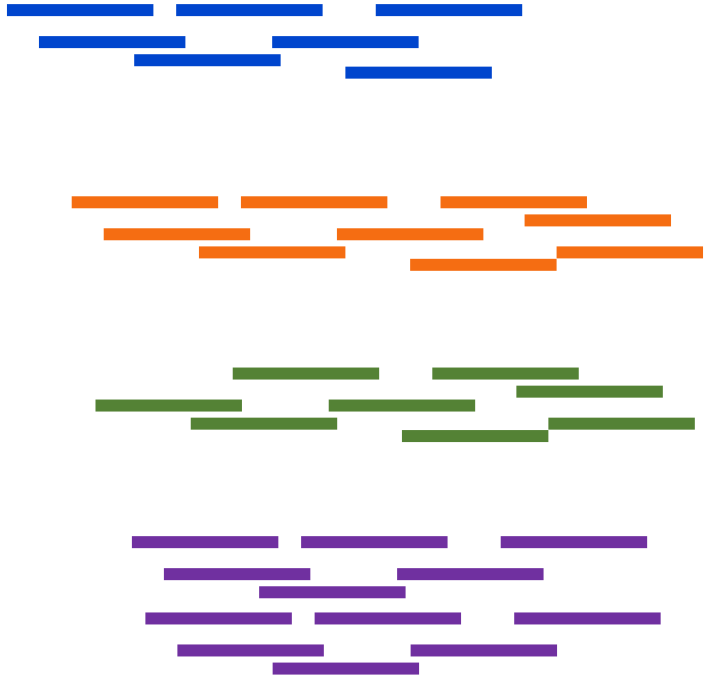
1. Quality Filtering
2. Demultiplexing
3. Assembling/Mapping reads
4. Functional rearrangements

Quality Control and assembling BCR/TCR from single-cells





Single-cell Sequencing



Mapping of assembled contigs is done to germline database

1. Quality Filtering
2. Demultiplexing
3. Assembling/Mapping reads
4. Functional rearrangements

Tools for QC and Assembling T/BCRs

BASIC: BCR and TCR assembly from single cell RNA-seq

BASIC is a semi-de novo assembly method for assembling BCR and TCR genes from single cell RNA-seq data.

BASIC – B-cell receptor assembly from single cells

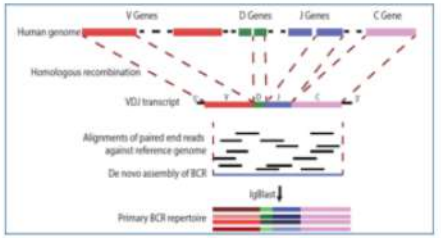
November 10, 2016 Leave a comment 4,313 Views

The B-cell receptor enables individual B cells to identify diverse antigens, including bacterial and viral proteins. While advances in RNA-seq have enabled high throughput profiling of transcript expression in single cells, the unique task of assembling the full-length heavy and ...

Read More »

VDJPuzzle – B-cell receptor reconstruction from single-cell RNA-seq

April 18, 2018 Leave a comment 2,911 Views



The B-cell receptor (BCR) performs essential functions for the adaptive immune system including recognition of pathogen-derived antigens. The vast repertoire and adaptive variation of BCR sequences due to V(D)J recombination and somatic...

Read More »

Source: https://www.rna-seqblog.com/tag/b-cell-receptor/

TraCer: T-cell-receptor reconstruction and clonality inference from single-cell RNA-Seq

BraCer: B-cell-receptor reconstruction and clonality inference from single-cell RNA-Seq

Source: https://github.com/Teichlab/tracer
Source: https://github.com/Teichlab/bracer

Assigning VDJ genes to the receptors

Role in clinical diagnostics

IMMUNOBIOLOGY AND IMMUNOTHERAPY

Biased IGH VDJ gene repertoire and clonal expansions in B cells of chronically hepatitis C virus–infected individuals

Felicia A. Tucci,[1] Simo Kitanovski,[2] Patricia Johansson,[1,3] Ludger Klein-Hitpass,[1] Alisan Kahraman,[4] Jan Dürig,[3] Daniel Hoffmann,[2,5] and Ralf Küppers[1,5]

Brief Report | Full Access

Rapid Capture Next-Generation Sequencing in Clinical Diagnostics of Kinase Pathway Aberrations in B-Cell Precursor ALL

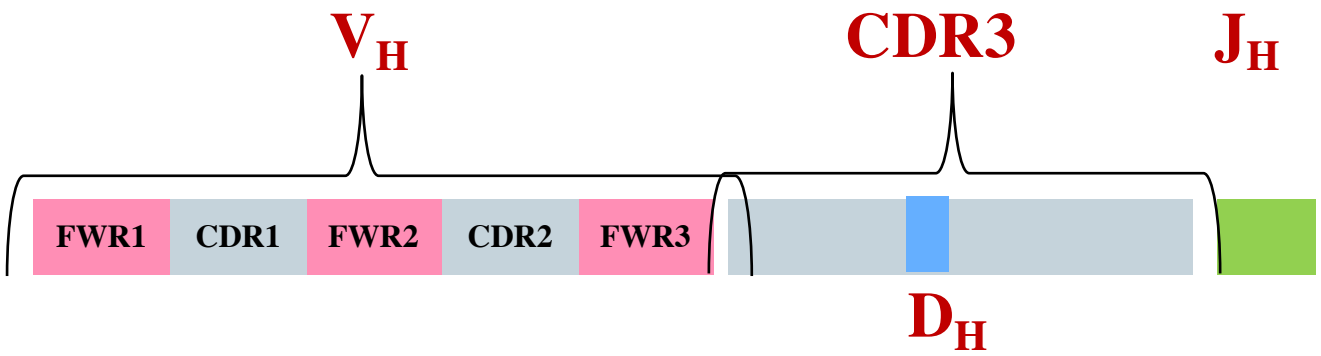
Udo zur Stadt PhD, Gabriele Escherich MD, Daniela Indenbirken PhD, Malik Alawi MSc, Manuela Adao, Martin A. Horstmann MD

blood 1988 71: 1495-1498

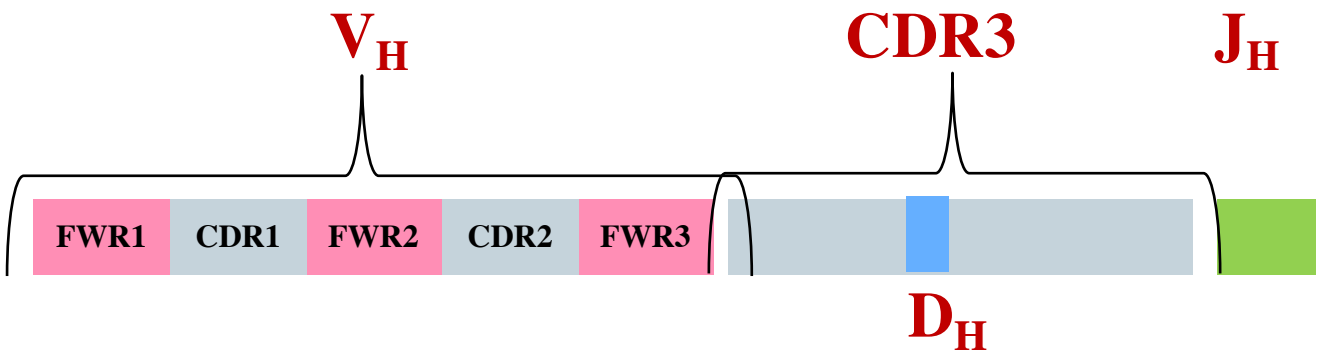
Molecular analysis of clonality and bcr rearrangements in Philadelphia chromosome-positive acute lymphoblastic leukemia

AG Turhan, CJ Eaves, DK Kalousek, AC Eaves and RK Humphries

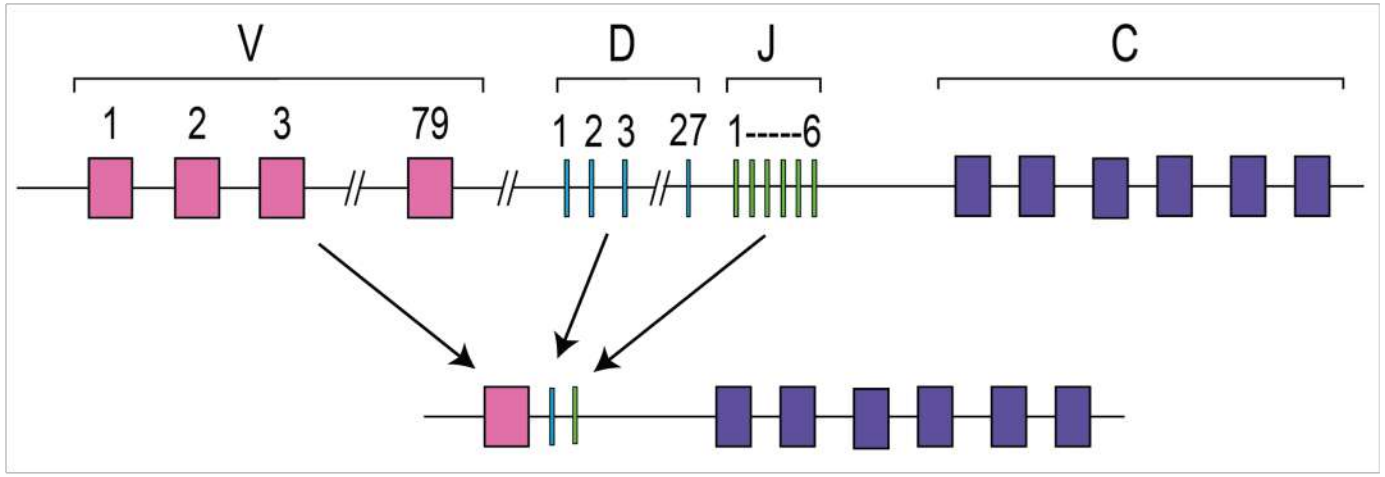
V(D)J Assignment



V(D)J Assignment



Which V, D and J genes recombined?



Tools for V(D)J assignments

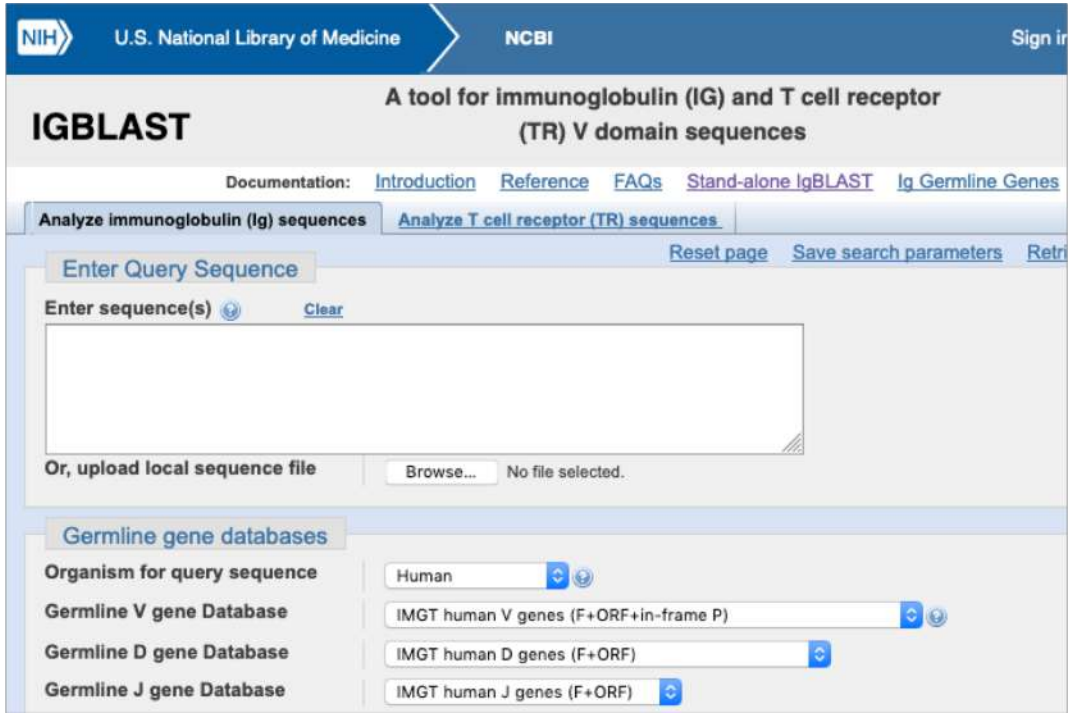
IMGT/HighV-QUEST

IMGT/HighV-QUEST[1] (V-QUERy and STandardization) is part of IMGT®, the international ImMunoGeneTics information system® http://www.imgt.org [2].

IMGT/HighV-QUEST is the high-throughput version of IMGT/V-QUEST [3,4] for the analysis of thousands of immunoglobulin (IG) and T cell receptor (TR) rearranged nucleotide sequences (up to 150 000 sequences) per run.

IMGT/HighV-QUEST has been developed by IMGT®, the international ImMunoGeneTics Information System® to answer the problematic of the analysis of the antigen receptor data from Next-Generation Sequencing (NGS).

Source: http://www.imgt.org/IMGTindex/IMGTHighV-QUEST.php



Source: https://www.ncbi.nlm.nih.gov/igblast/

Assigning clonal groups and analysis

Targeted reconstruction of T cell receptor sequence from single cell RNA-seq links CDR3 length to T cell differentiation state

Shaked Afik, Kathleen B. Yates, Kevin Bi, Samuel Darko, Jernej Godec, Ulrike Gerdemann, Leo Swadling, Daniel C. Douek, Paul Klenerman, Eleanor J. Barnes ... Show more
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Brief Report | Full Access

Rapid Capture Next-Generation Sequencing in Clinical Diagnostics of Kinase Pathway Aberrations in B-Cell Precursor ALL

Udo zur Stadt PhD, Gabriele Escherich MD, Daniela Indenbirken PhD, Malik Alawi MSc, Manuela Adao, Martin A. Horstmann MD

Autoimmune, Cholestatic and Biliary Disease | Open Access

Immunoglobulin G4$^+$ B-cell receptor clones distinguish immunoglobulin G 4-related disease from primary sclerosing cholangitis and biliary/pancreatic malignancies

Marieke E. Doorenspleet, Lowiek M. Hubers, Emma L. Culver, Lucas J. Maillette de Buy Wenniger, Paul L. Klarenbeek, Roger W. Chapman, Frank Baas ... See all authors

Immune deficiencies, infection, and systemic immune disorders

Clonal expansion of CD4$^+$ cytotoxic T lymphocytes in patients with IgG$_4$-related disease

Hamid Mattoo PhD [a, *], Vinay S. Mahajan MBBS, PhD [a, *], Takashi Maehara DDS, PhD [a, b], Vikram Deshpande MD [a], Emanuel Della-Torre MD [a], Zachary S. Wallace MD [a], Maria Kulikova BS [a], Jefte M. Drijvers MD [a], Joe Daccache BS [a], Mollie N. Carruthers MD [a], Flavia V. Castelino MD [a], James R. Stone MD, PhD [a], John H. Stone MD, MPH [a], Shiv Pillai MBBS, PhD [a]

Clonal Grouping

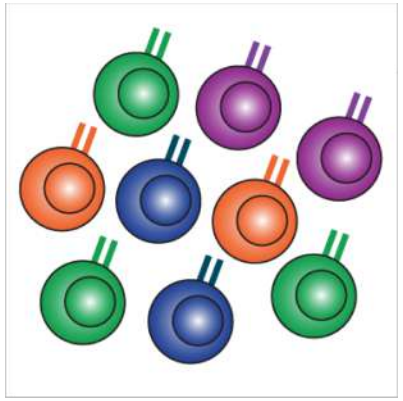
Which T/B-cell clones are selected

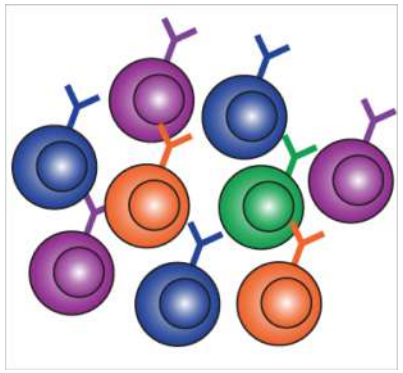
Clonal Grouping

Which T/B-cell clones are selected

What are clones?

Naïve cells
Parent Population

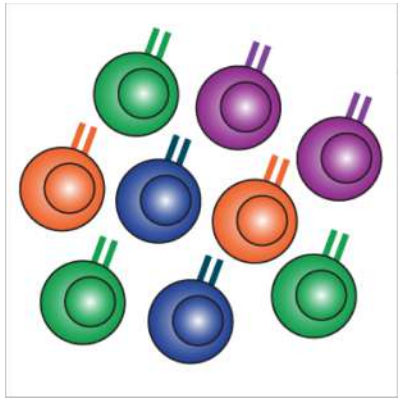


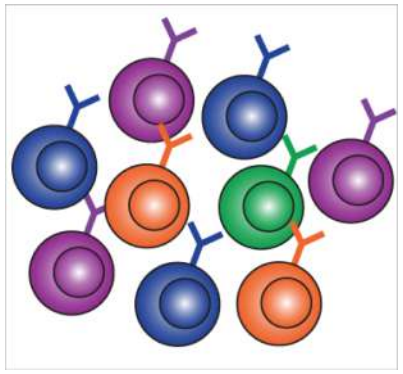


Clonal Grouping

Which T/B-cell clones are selected

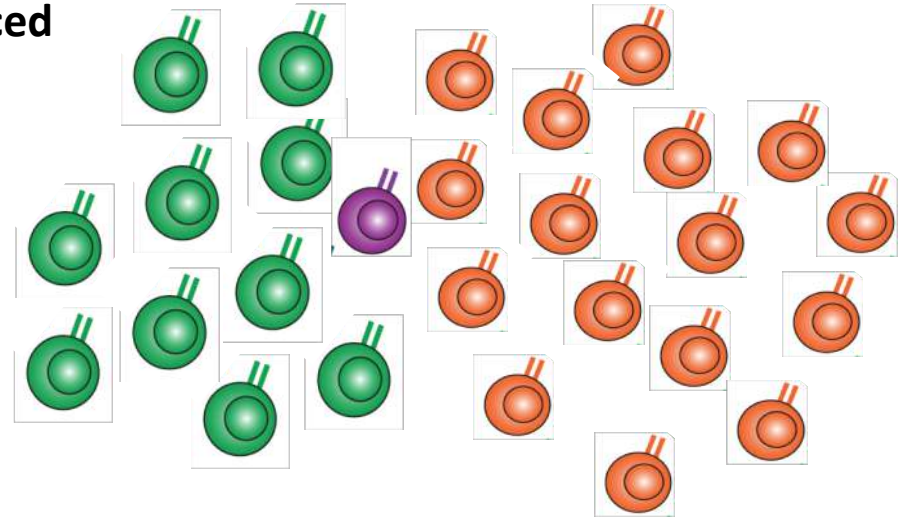
What are clones?





Naïve cells
Parent Population

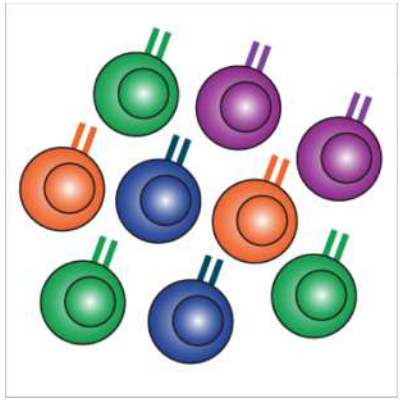
Antigen-Experienced

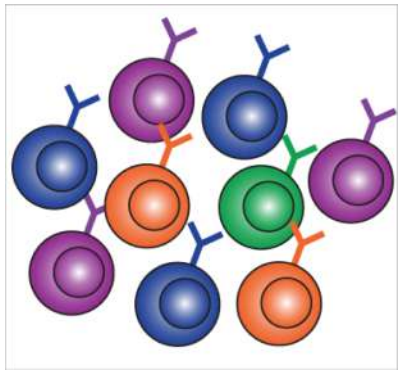


Clonal Grouping

Which T/B-cell clones are selected

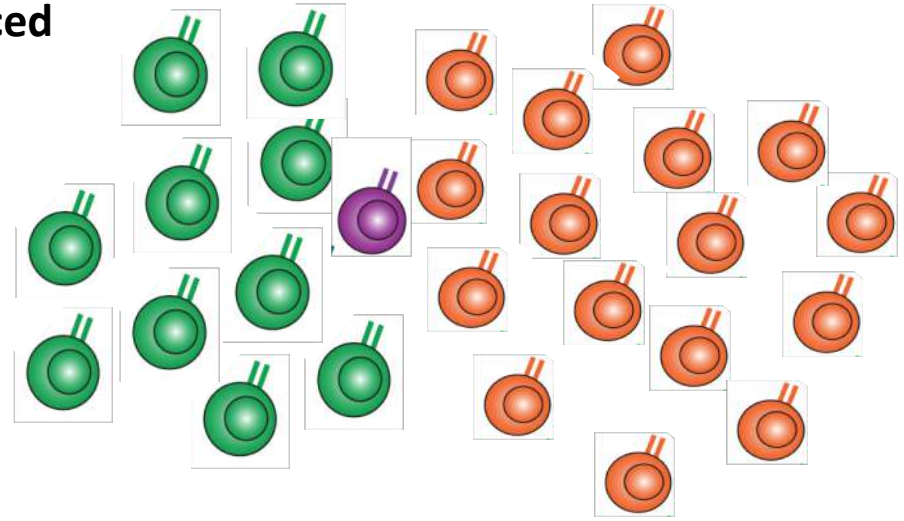
What are clones?

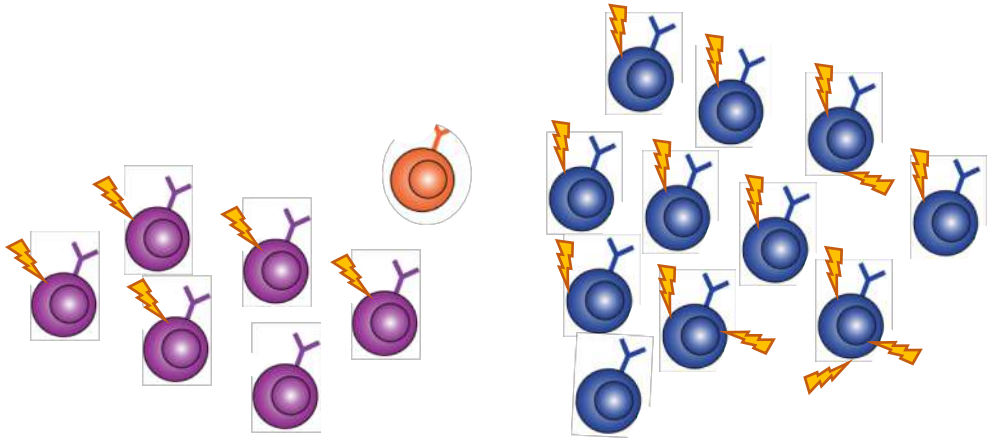




Naïve cells
Parent Population

Antigen-Experienced

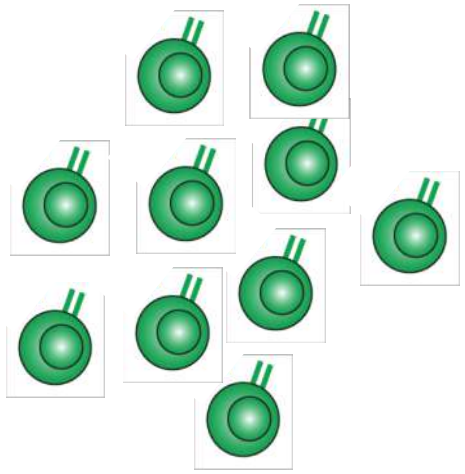




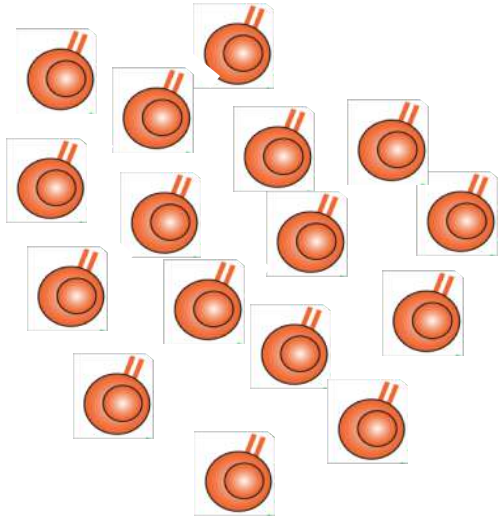
Mutations

Ad-hoc Clustering: Clonal grouping

T-cells



Same V gene
Same J gene
Equal CDR3 length



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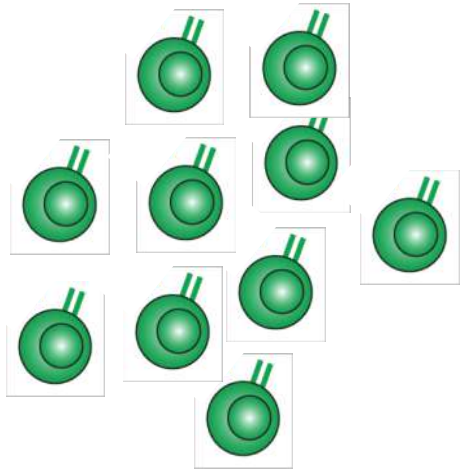
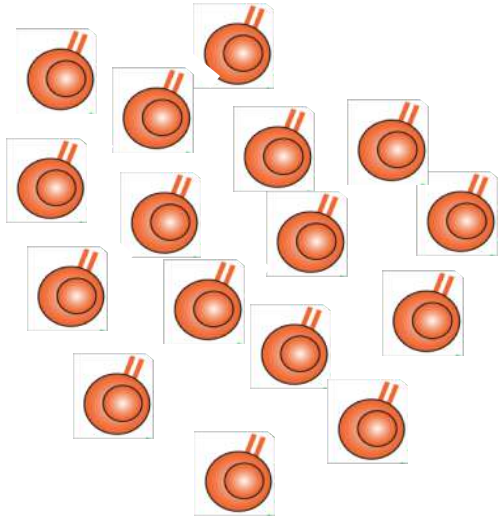
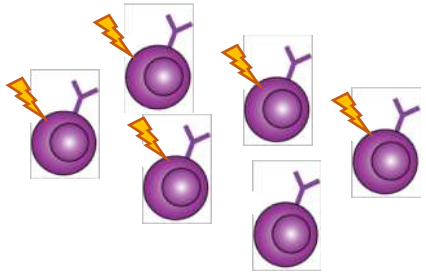
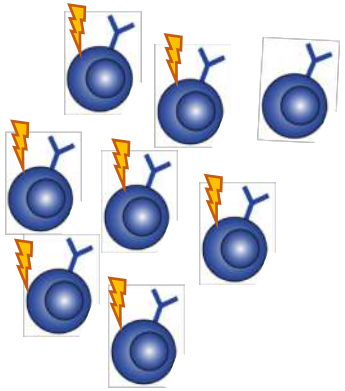


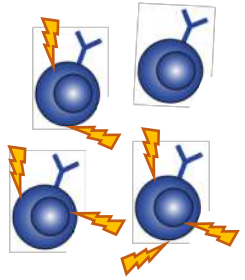


B-cells

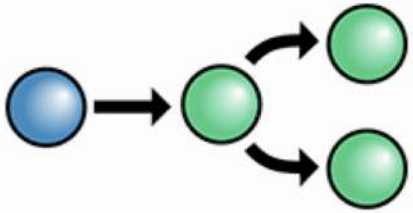
Same V gene
Same J gene
Equal CDR3 length
Sequence Identity of CDR3





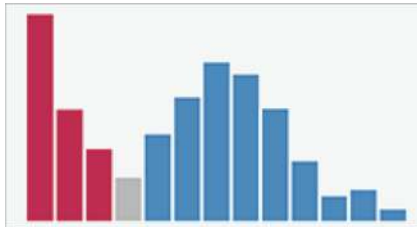


Tools for clonal grouping



Change-O

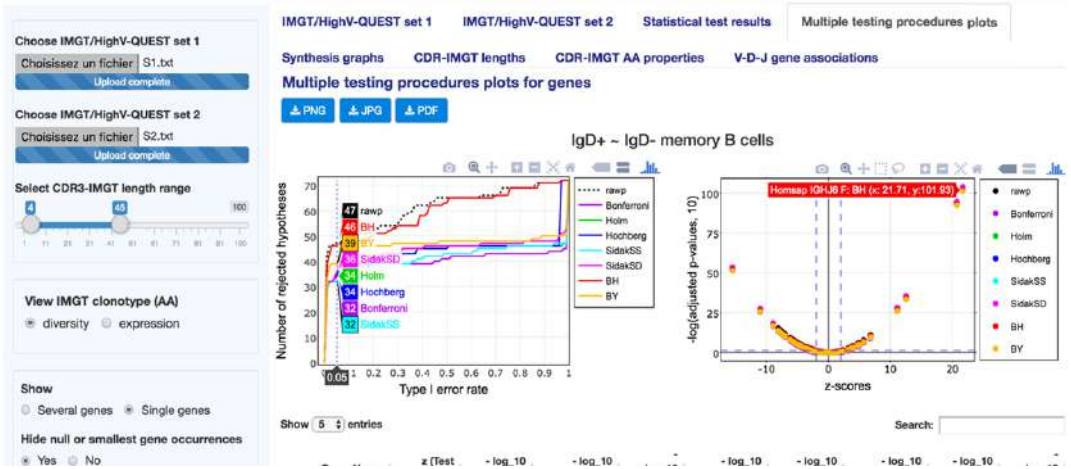
- V(D)J reference alignment standardization
- Clonal clustering
- Germline reconstruction
- Conversion and annotation



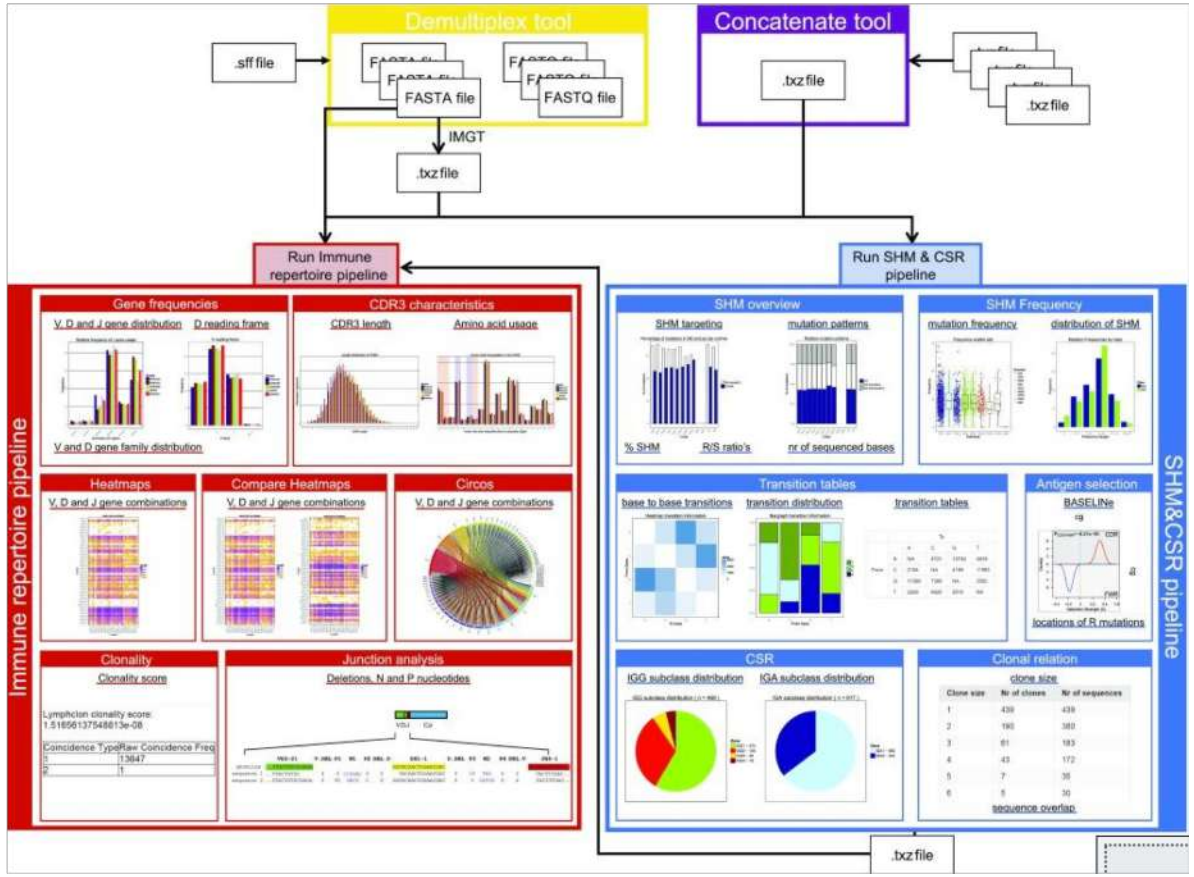
SCOPer

- Spectral clonal clustering methods

IMGT-Clonotype

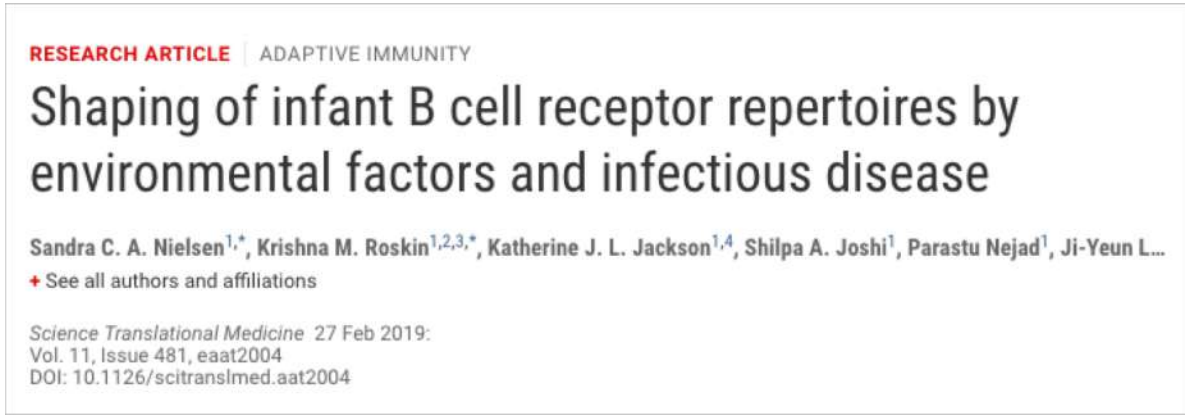


Statistical analysis of clonal groups



Applications in time-series response data

Long-term or short-term effects on T/B-cell clones



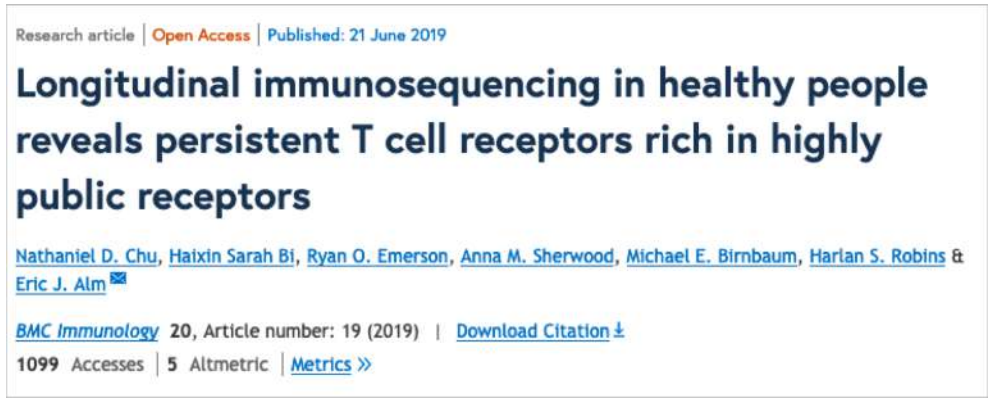
RESEARCH ARTICLE | ADAPTIVE IMMUNITY

Shaping of infant B cell receptor repertoires by environmental factors and infectious disease

Sandra C. A. Nielsen[1,*], Krishna M. Roskin[1,2,3,*], Katherine J. L. Jackson[1,4], Shilpa A. Joshi[1], Parastu Nejad[1], Ji-Yeun L...

+ See all authors and affiliations

Science Translational Medicine 27 Feb 2019:
Vol. 11, Issue 481, eaat2004
DOI: 10.1126/scitranslmed.aat2004



Research article | Open Access | Published: 21 June 2019

Longitudinal immunosequencing in healthy people reveals persistent T cell receptors rich in highly public receptors

Nathaniel D. Chu, Haixin Sarah Bi, Ryan O. Emerson, Anna M. Sherwood, Michael E. Birnbaum, Harlan S. Robins & Eric J. Alm

BMC Immunology 20, Article number: 19 (2019) | Download Citation

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Time as an important factor in Rep-Seq studies

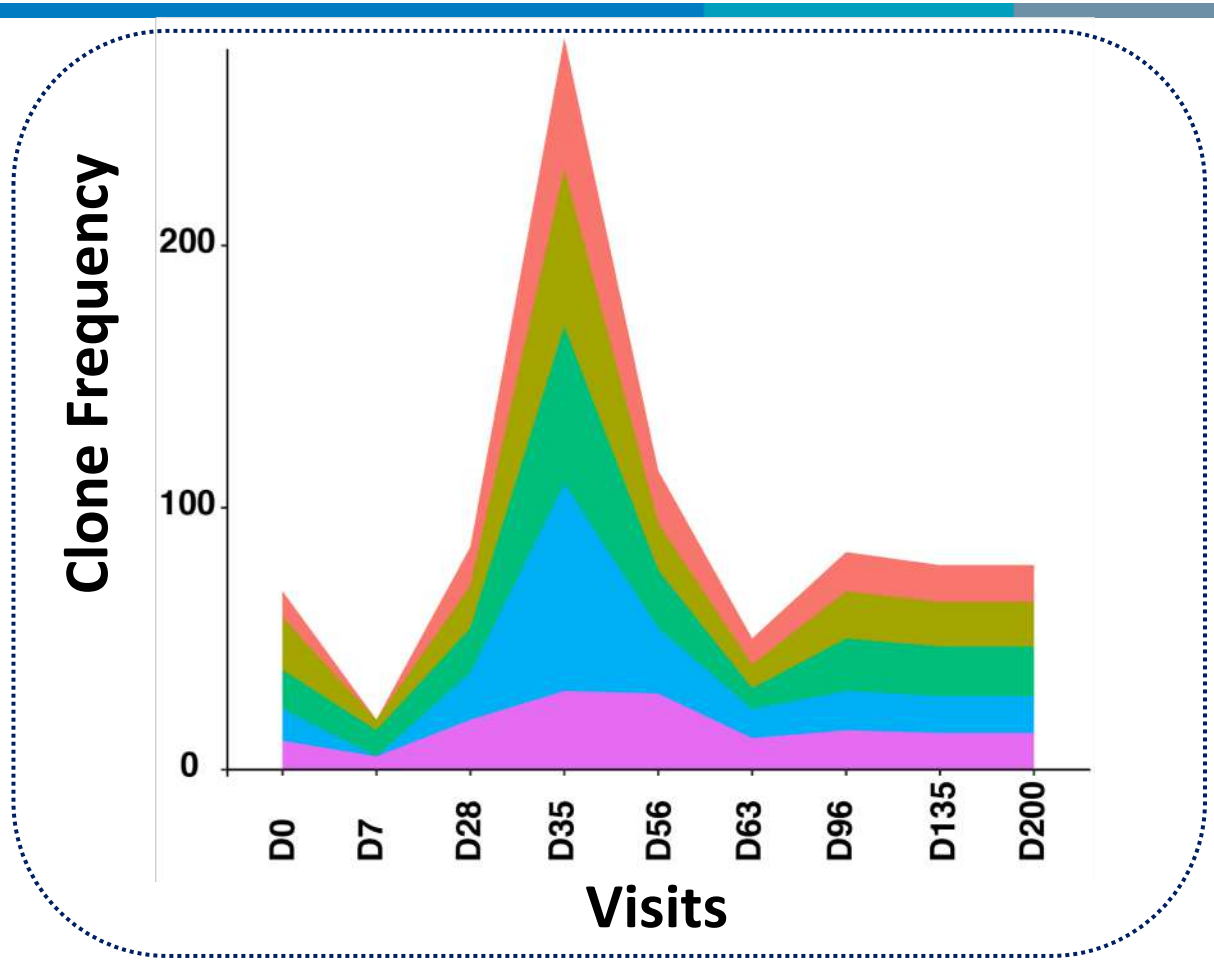
Infection

Vaccine

Day0	Day7	Day28	Day35	Day56	Day63	Day96	Day135	Day200
Visit1	Visit2	Visit3	Visit4	Visit5	Visit6	Visit7	Visit8	Visit9

Sequencing BCRs on all the visits

Clonal frequencies in time-response data



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Sequencing BCRs on all the visits

Time plays role in tumor settings too

Healthy versus Tumor

Sequencing TCRs where T cell clones migrate to metastasize

Time plays role in tumor settings too

Healthy versus Tumor

Sequencing TCRs where T cell clones migrate to metastasize

Treatment efficacy in immunodeficiency diseases

Sequencing TCRs where the deficiency is in generating diversity of receptors namely leukemia, SCID, vaccines

Clonal evolution of B-cells

Constructing clonal lineages

New Results

Comment on this paper

Clonal replacement of tumor-specific T cells following PD-1 blockade

Kathryn E. Yost, Ansuman T. Satpathy, Daniel K. Wells, Yanyan Qi, Chunlin Wang, Robin Kageyama, Katherine McNamara, Jeffrey M. Granja, Kavita Y. Sarin, Ryanne A. Brown, Rohit K. Gupta, Christina Curtis, Samantha L. Bucktrout, Mark M. Davis, Anne Lynn S. Chang, Howard Y. Chang

Br J Haematol. 2019 Mar;184(5):829-833. doi: 10.1111/bjh.15179. Epub 2018 Mar 13.

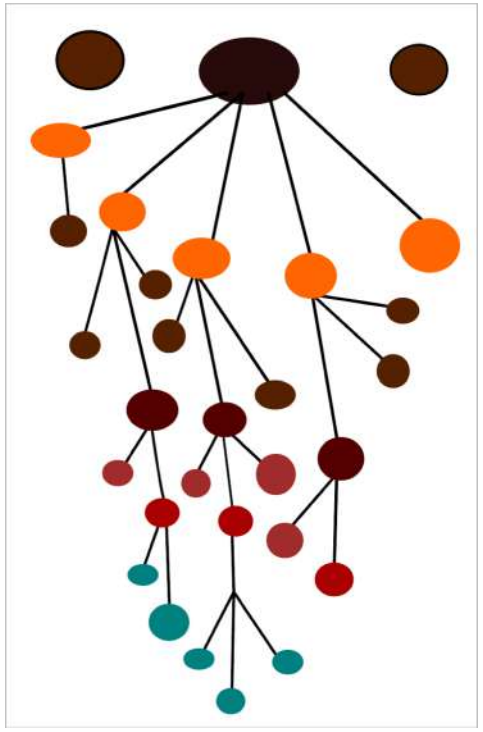
Clonality and clonal evolution analysis of paediatric ALL based on B-cell receptor/T-cell receptor rearrangement.

Ding LW[1], Tan KT[1], Sun QY[1], Lao ZT[1,2], Yang H[1], Jiang N[3], Chien W[4], Xiao JF[1], Loh XY[1], Huang ML[1], Lill M[4], Lin DC[4], Yeoh AEJ[1,3], Koeffler HP[1,4].

Clonal evolution of B cells in transformation from low- to high-grade lymphoma

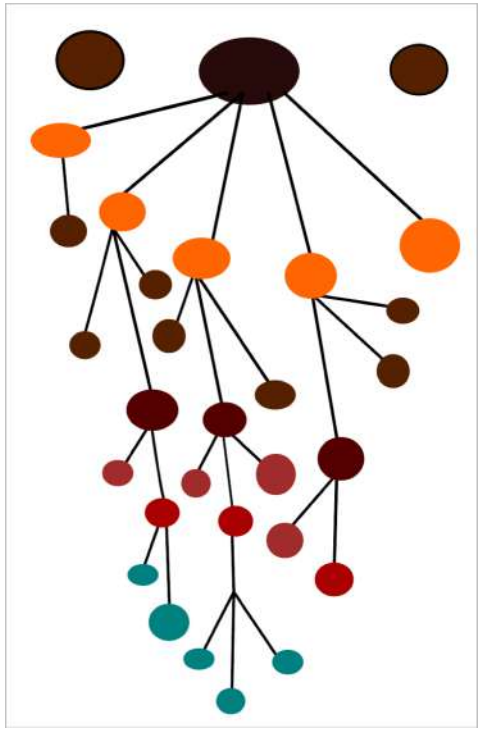
András Matolcsy,[1,2] Elaine J. Schattner,[3,4] Daniel M. Knowles,[1] and Paolo Casali[1,4]

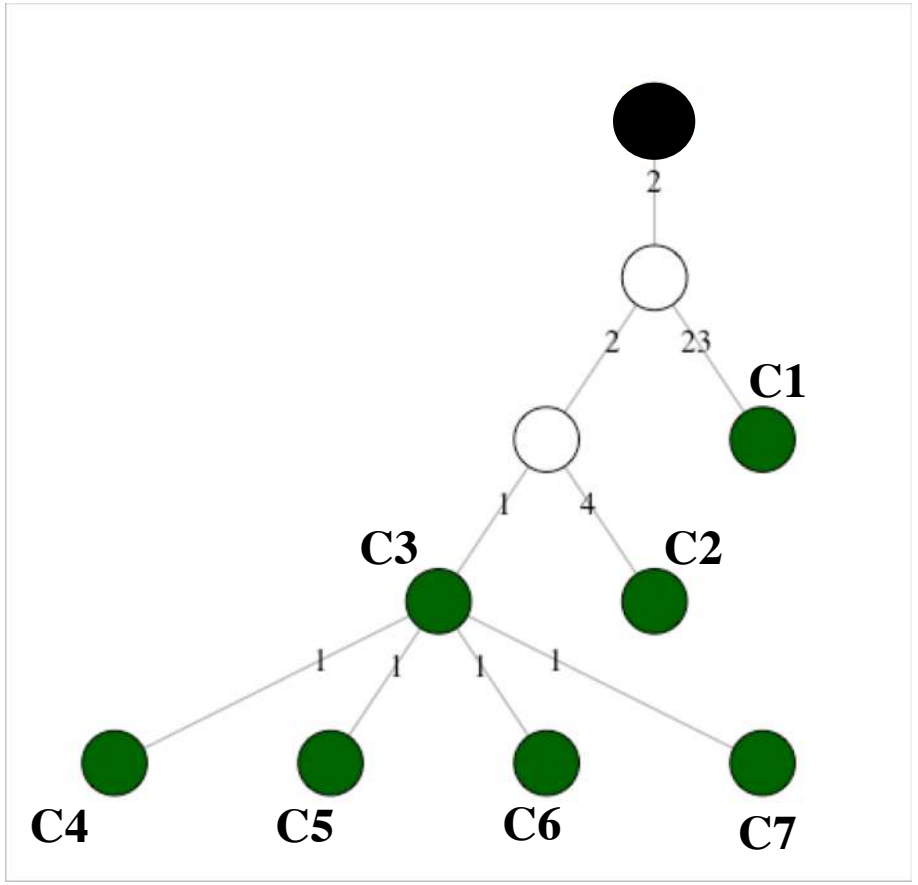
Construction of Clonal lineages Similar to fate mapping or pseudotime trajectories



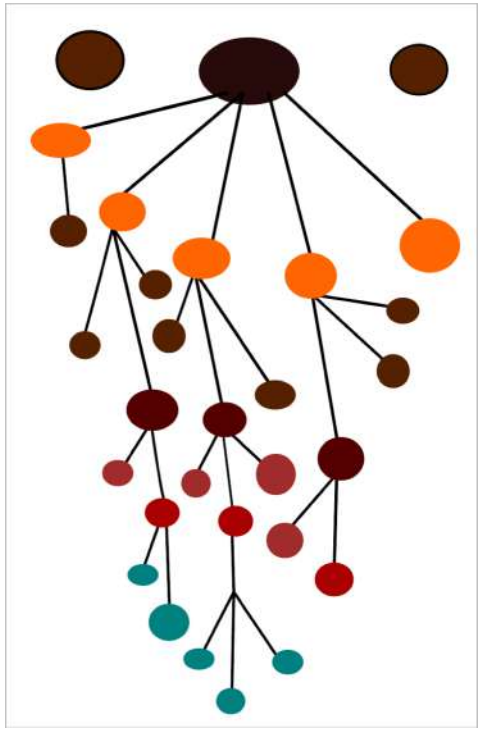
Phylogenetically tracing the path of somatic hypermutations in Antibodies

Construction of Clonal lineages





Construction of Clonal lineages



```
Germline    CACTG--GGCTC--AGCAGTGGTAGTTACTA--GCT--GG--TGGAGTGGATTGGGTATATCTATTACAGTG
C1          CACTG--GGTTC--AATACTGATCAGTACCA--GTT--GC--TGCAGTGGCTAGGCCATACTTATTATGGTA
C2          ---------------------------------------------------------------------
C3          CACTG--GGCTC--AGGAATGATAGTTCCTA--GTT--GG--TGGAGTGGATTGGGTATATCTATTACAGTG
C4          ---------------------------------------------------------------------
C5          CAGTG--GGCTC--AGGAATGATAGTTCCTA--GTT--GG--TGGAGTGGATTGGGTATATCTATTACAGTG
C6          CACTG--GGCTC--AGGAATGATAGTCCCTA--GTT--GG--TGGAGTGGATTGGGTATATCTATTACAGTG
C7          CAATG--GGCTC--AGGAATGATAGTTCCTA--GTT--GG--TGGAGTGGATTGGGTATATCTATTACAGTG


Germline    GGAGCACCAAC--AGTCGAGTCACCAT--CGAG--CAGTAGA--AAG--AGTTC--AAGCTGAGCTC--CTC
C1          GGAGCACCACC--AGTCGCGTCACCAT--CGCG--CCATTGA--AAG--AGTTC--ACGTGAGCCC--CTC
C2          ------------AGGCGAGCCGTCAT--CGAG--CAGTTGA--AAG--AATTC--AAGCTGACCTC--CTC
C3          GGAGTACCAGC--AGTCGAGTCACCAT--CGAG--CAGTAGA--AGG--AATTC--AAGCTGACCTC--CTC
C4          ----------------------------CCATTGA--AGG--AATTC--AAGCTGACCTC--CCC
C5          GGAGTACCAGC--AGTCGAGTCACCAT--CGAG--CAGTAGA--AGG--AATTC--AAGCTGACCTC--CTC
C6          GGAGTACCAGC--AGTCGAGTCACCAT--CGAG--CAGTAGA--AGG--AATTC--AAGCTGACCTC--CTC
C7          GGAGTACCAGC--AGTCGAGTCACCAT--CGAG--CAGTAGA--AGG--AATTC--AAGCTGACCTC--CTC
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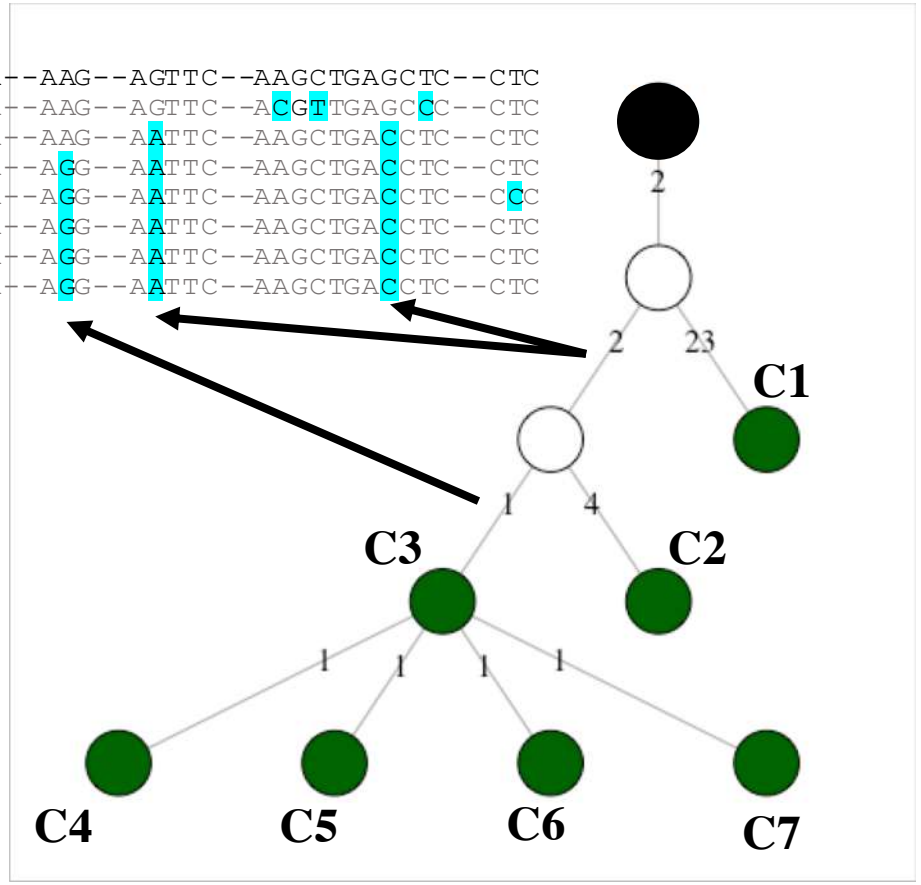
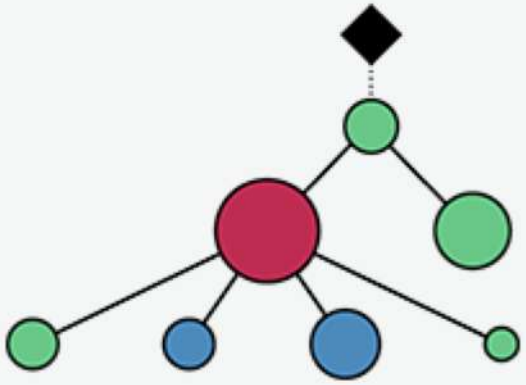
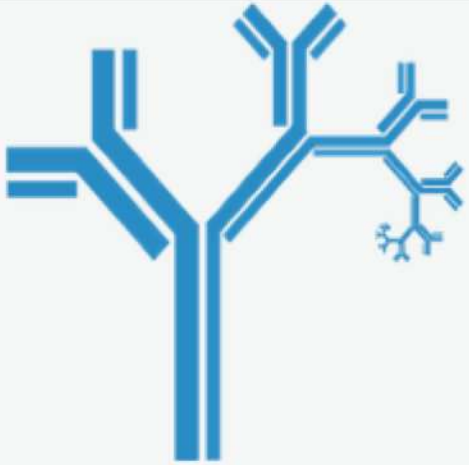


Tools for clonal lineages



Alakazam

- Clonal lineage reconstruction
- Lineage topology analysis
- Repertoire diversity
- V(D)J gene usage
- Physicochemical property analysis



IgPhyML

- Clonal lineage tree contruction
- Mutation/selection hypothesis testing

Adding structure information

Antigen–antibody interface properties: Composition, residue interactions, and features of 53 non-redundant structures

Thiruvarangan Ramaraj[a,1], Thomas Angel[b], Edward A. Dratz[c], Algirdas J. Jesaitis[d], and Brendan Mumey[a,*]

Antibody Specific B-Cell Epitope Predictions: Leveraging Information From Antibody-Antigen Protein Complexes

Martin Closter Jespersen[1], Swapnil Mahajan[2], Bjoern Peters[2], Morten Nielsen[1,3] and Paolo Marcatili[1]**

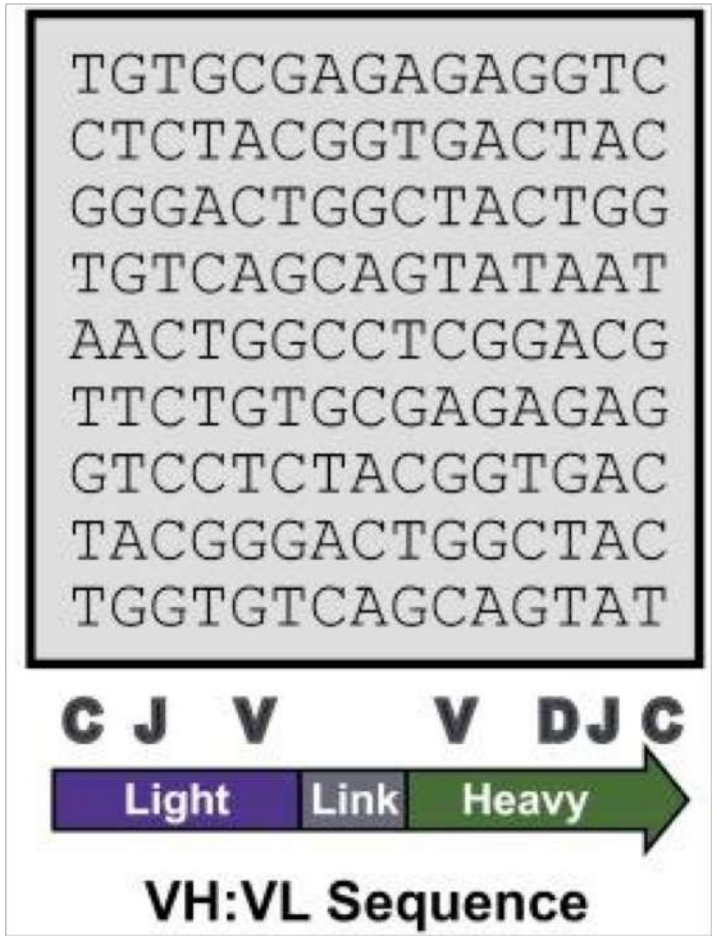
The Journal of Immunology

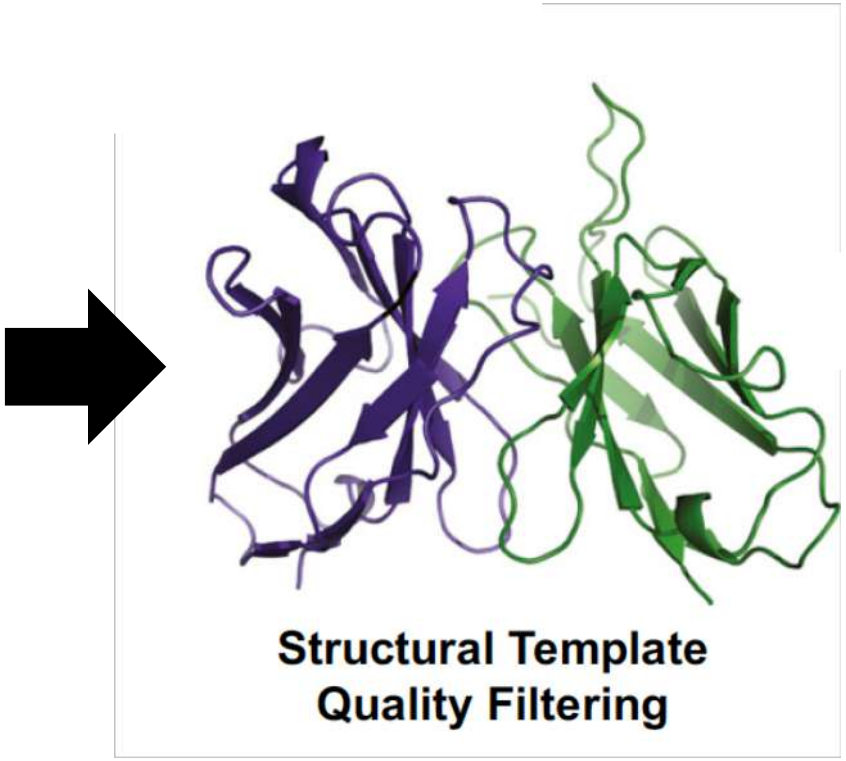
Antibody and Antigen Contact Residues Define Epitope and Paratope Size and Structure

James W. Stave and Klaus Lindpaintner

J Immunol 2013; 191:1428-1435; Prepublished online 24

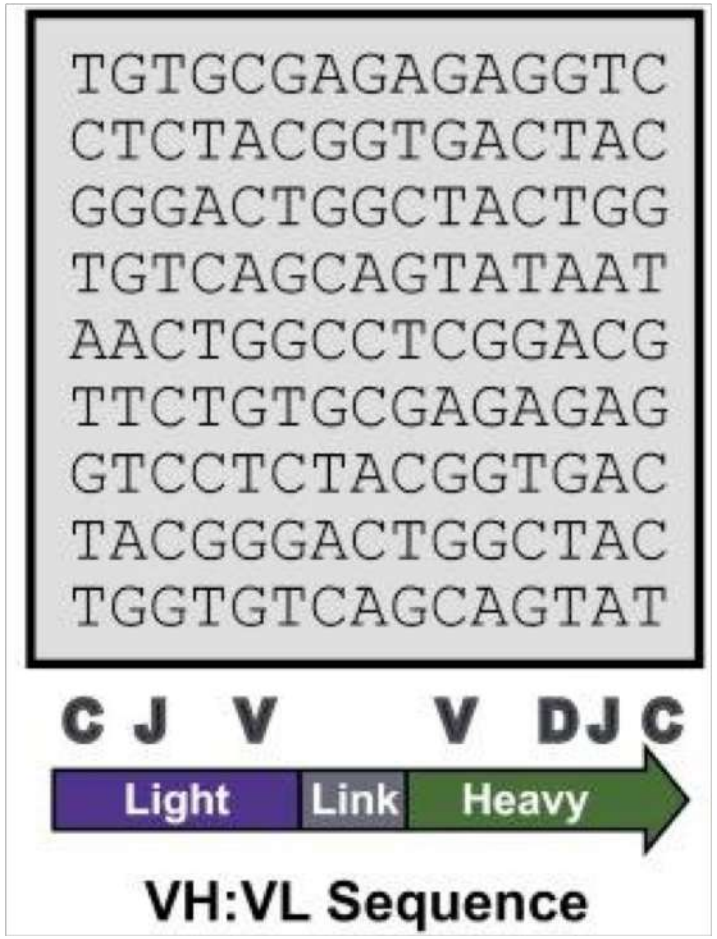
Structure of Receptors: facilitated by ScRep-Seq

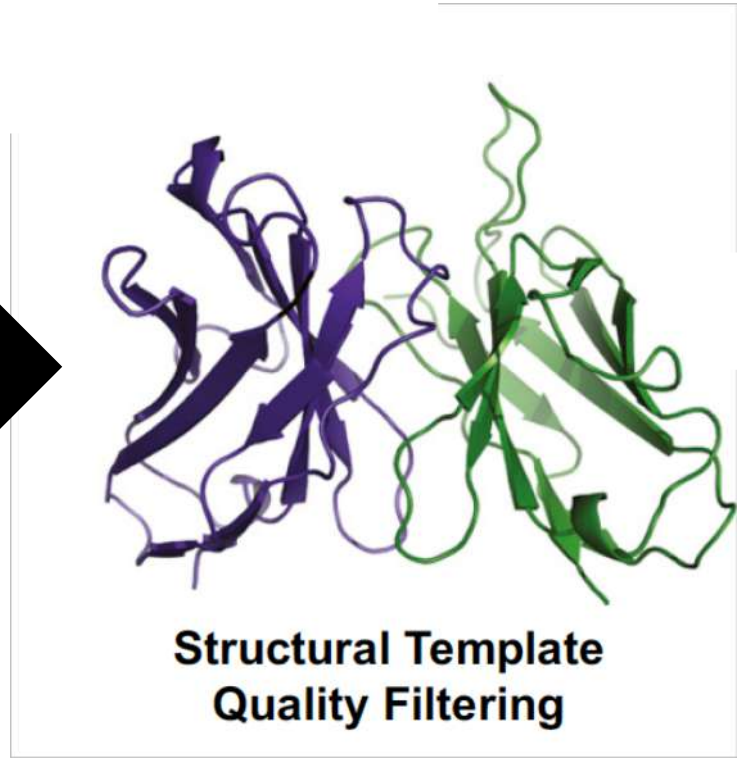




Source: DeKosky BJ et al., PNAS, 2016

Structure of Receptors: facilitated by ScRep-Seq



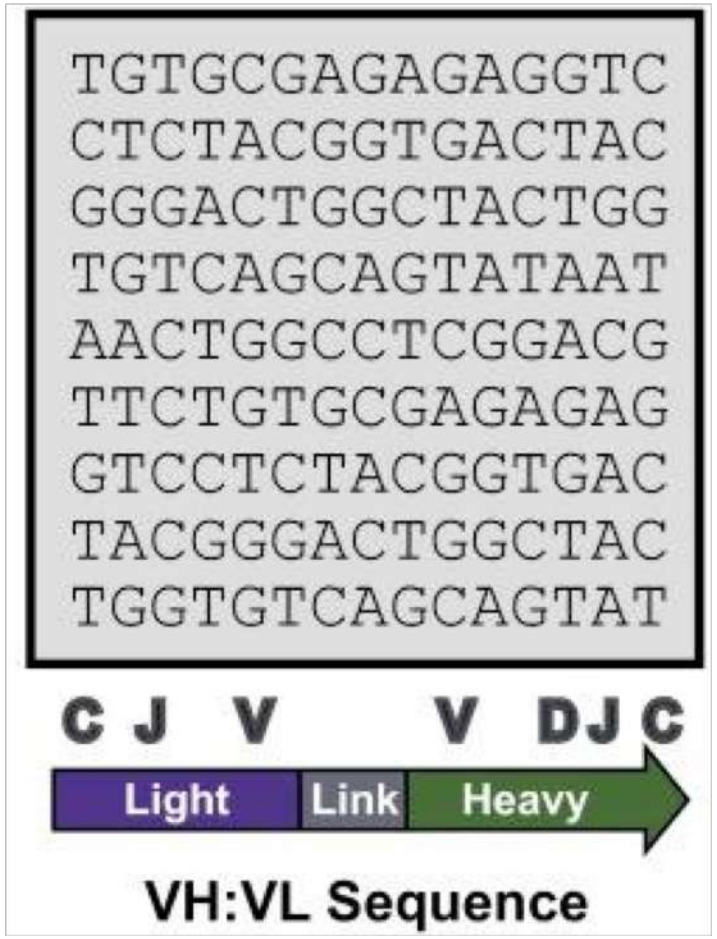


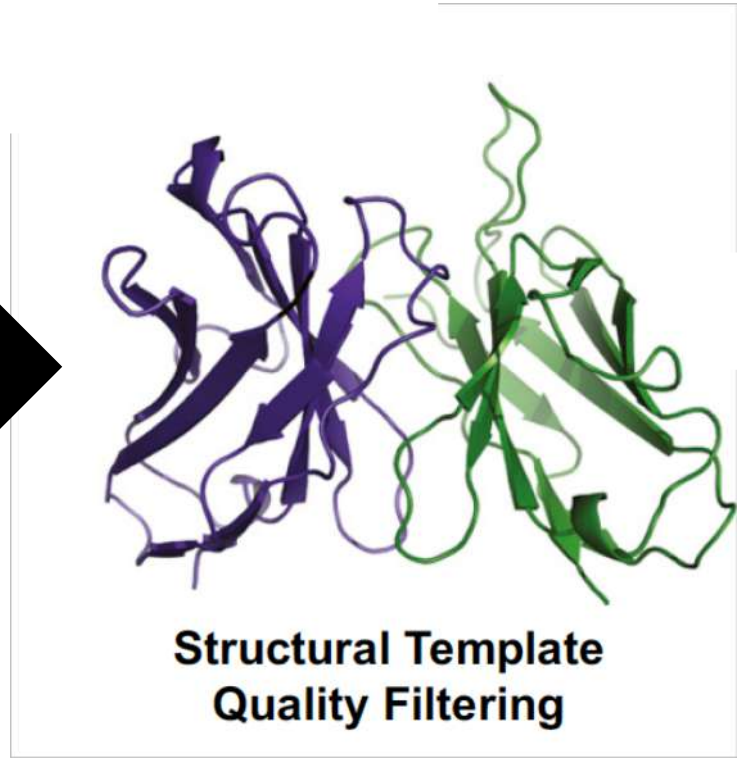
RosettaAntibody
***In-silico* computational modeling**

1. **Template grafting**
2. **CDR-H3 *de novo***
3. **Loop modeling**

Source: DeKosky BJ et al., PNAS, 2016

Structure of Receptors: facilitated by ScRep-Seq





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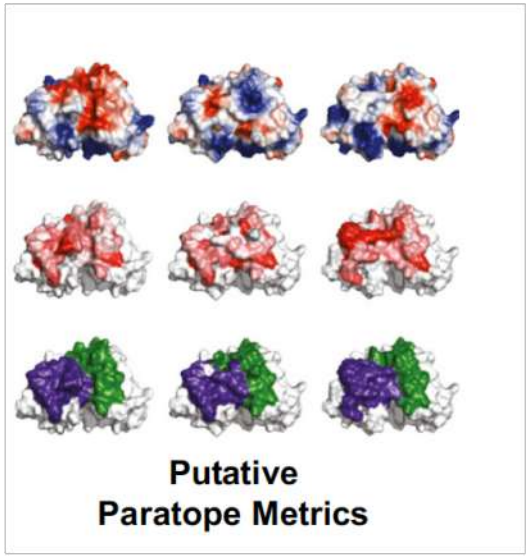

Source: DeKosky BJ et al., PNAS, 2016

Helpful resources

- tcR: a package for T cell receptor and Immunoglobulin repertoires advanced data analysis

https://cran.r-project.org/web/packages/tcR/vignettes/tcrvignette.html

- bcRep: Advanced Analysis of B Cell Receptor Repertoire Data

https://cran.r-project.org/web/packages/bcRep/vignettes/vignette.html

- Immcantation Portal

https://immcantation.readthedocs.io/en/stable/

Thank You

Questions?