

# Single-cell Immune Profiling

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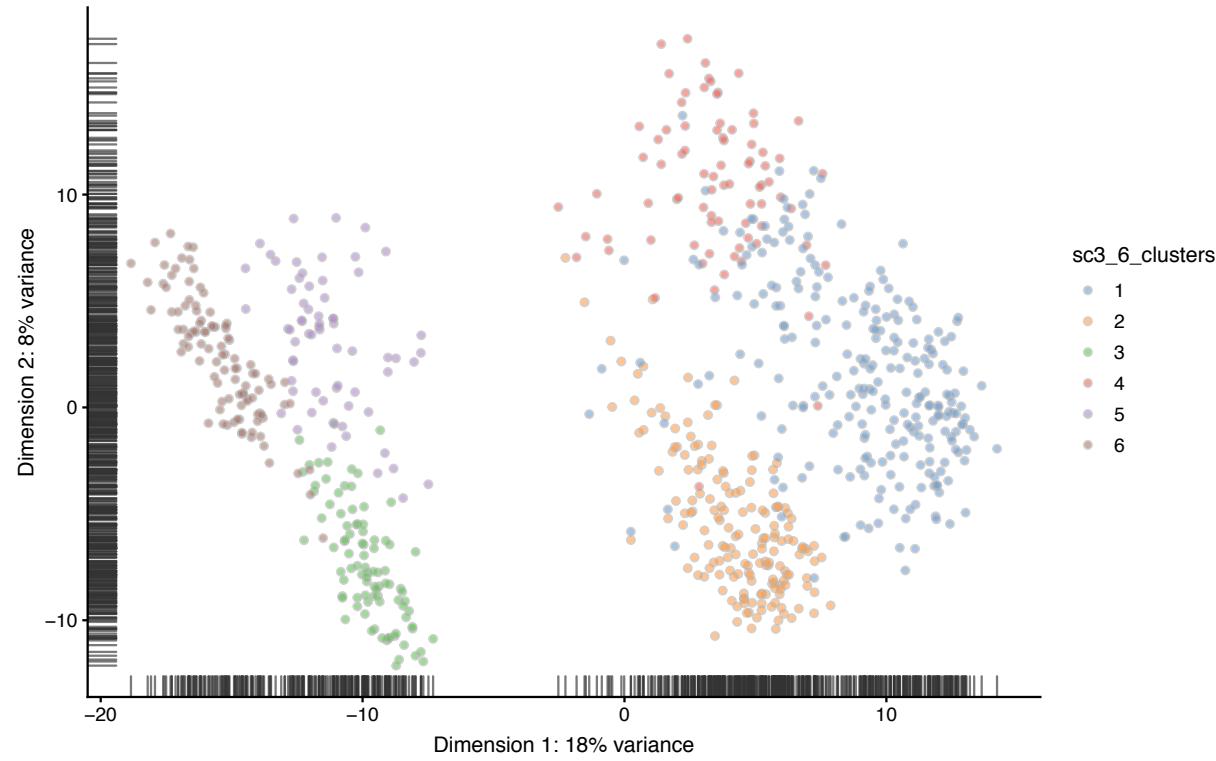
# Why immune cells?

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- The immune system is comprised of a diverse set of adaptable cell types that are responsible for responding to changes or threats in the body.
- Single-cell technologies allow studying the cellular heterogeneity in an unbiased way.
  - Discover novel cell types or cell states.
  - Study cell state transitions.

# Cell type classification

- In order to study cellular heterogeneity, first need to identify what types of cells each cluster represents.
- Usually done by comparing cluster specific DE genes to lists of known marker genes – difficult and time consuming!



# Cell type classification

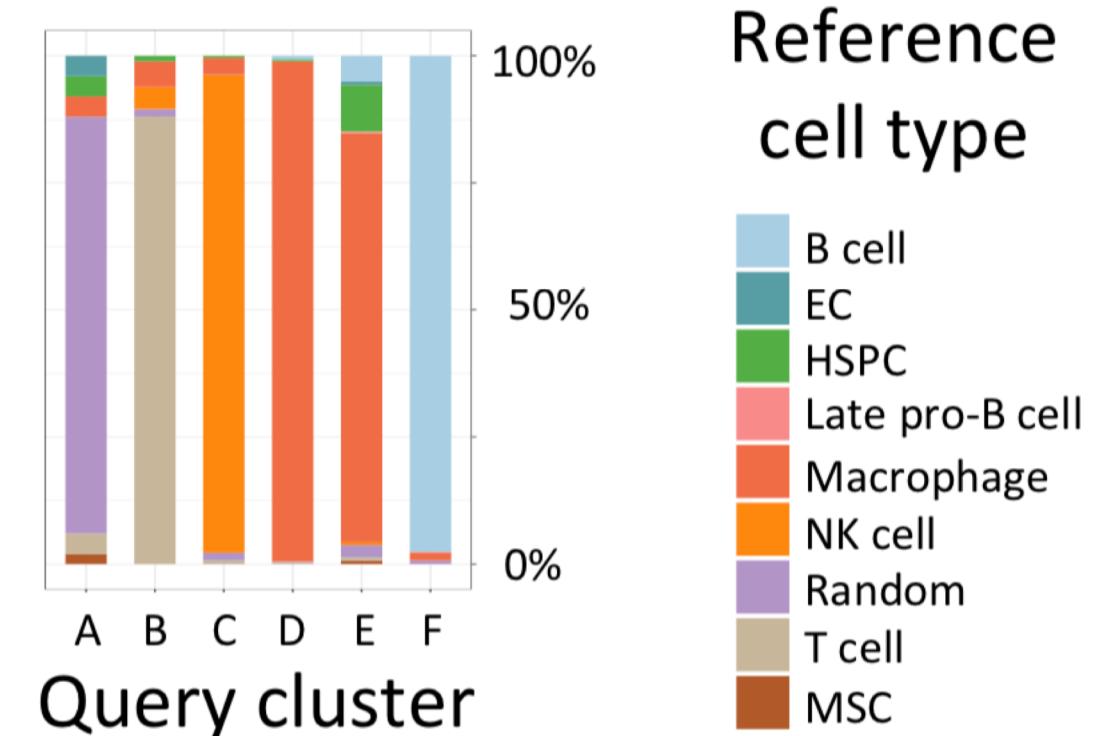
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- Automated cell type identification and classification can help.
- Recent benchmarking preprint by Abdelall and Michielsen et al., “A comparison of automatic cell identification methods for single-cell RNA-sequencing data” on bioRxiv.
- Methods either require a labelled training dataset (or pre-trained classifier) or a list of marker genes.

# Cell type identification – SingleCellNet

## SingleCellNet:

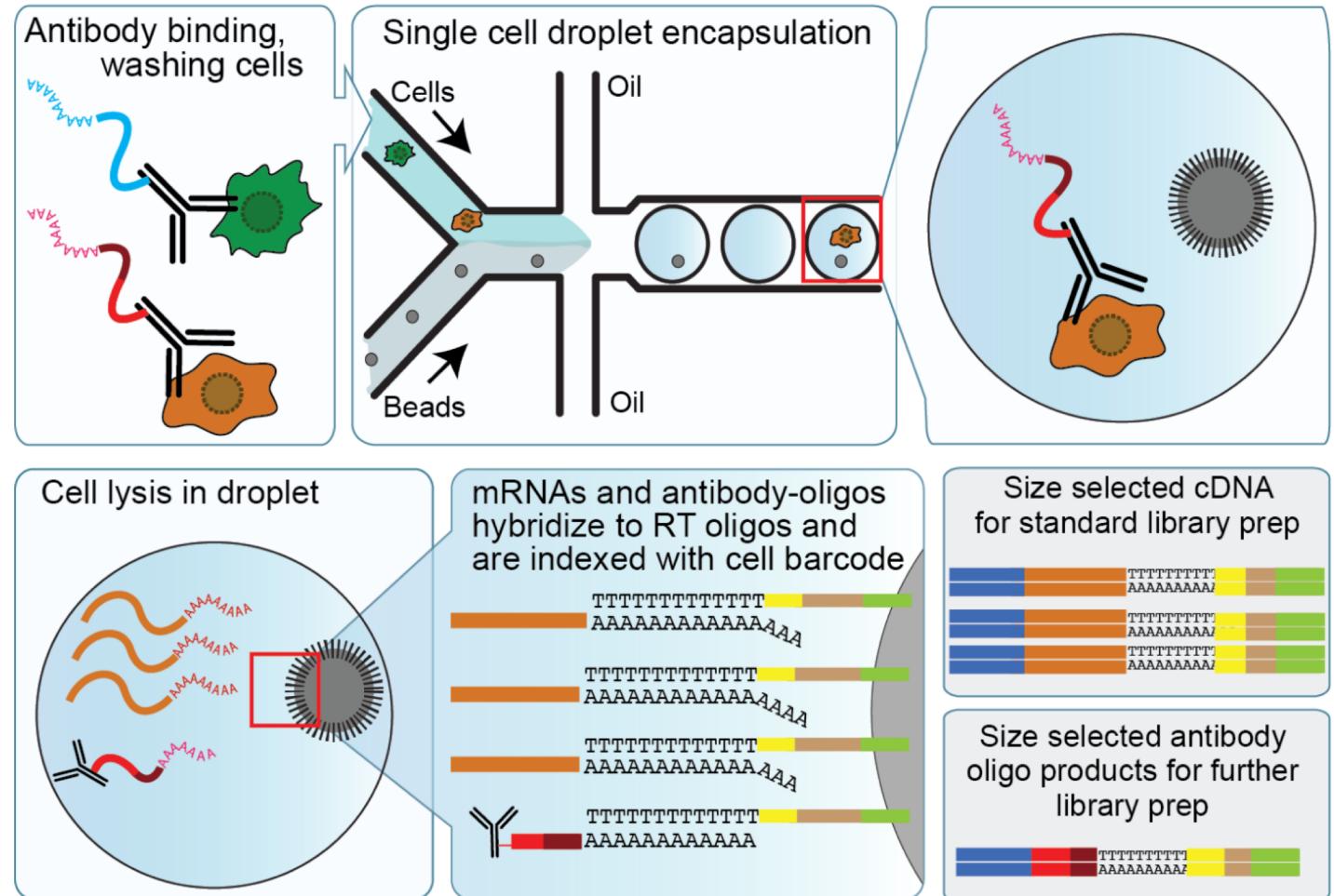
- Requires labelled training data.
- Uses a Random Forest classifier on genes most suitable for classification.
- Outputs a classification score of new cells to the existing cell labels.



Tan and Cahan. bioRxiv. 2018

# CITE-seq

- Multimodal single-cell method to obtain cell surface markers and transcriptome.



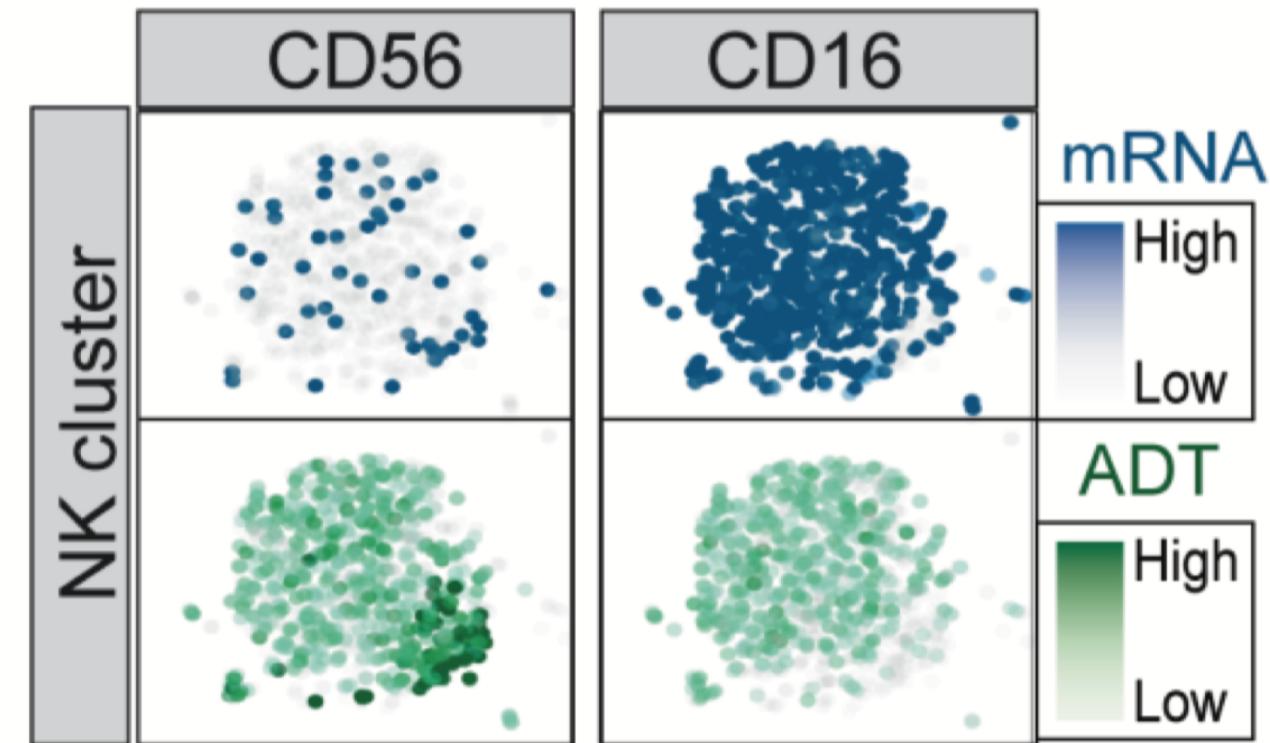
# CITE-seq

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- Allows association of protein levels with mRNA for the same cells.
- Protein has less of a ‘drop-out’ problem.
- Useful for defining/labelling cell types when markers are known.
- Can be used to help cluster cells and differentiate between more subtle differences.

# CITE-seq for immunology

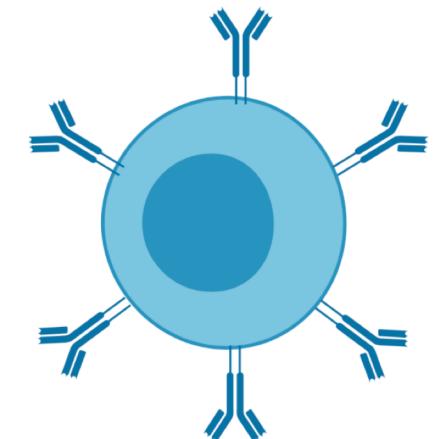
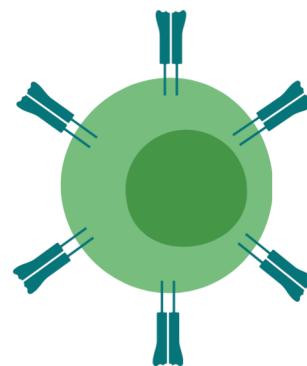
- Immune responses lead to expression of known cell surface markers – immunophenotyping.
- Identify novel cellular phenotypes based on combination of protein and gene expression.



Stoeckius et al. Nature Methods. 2017.

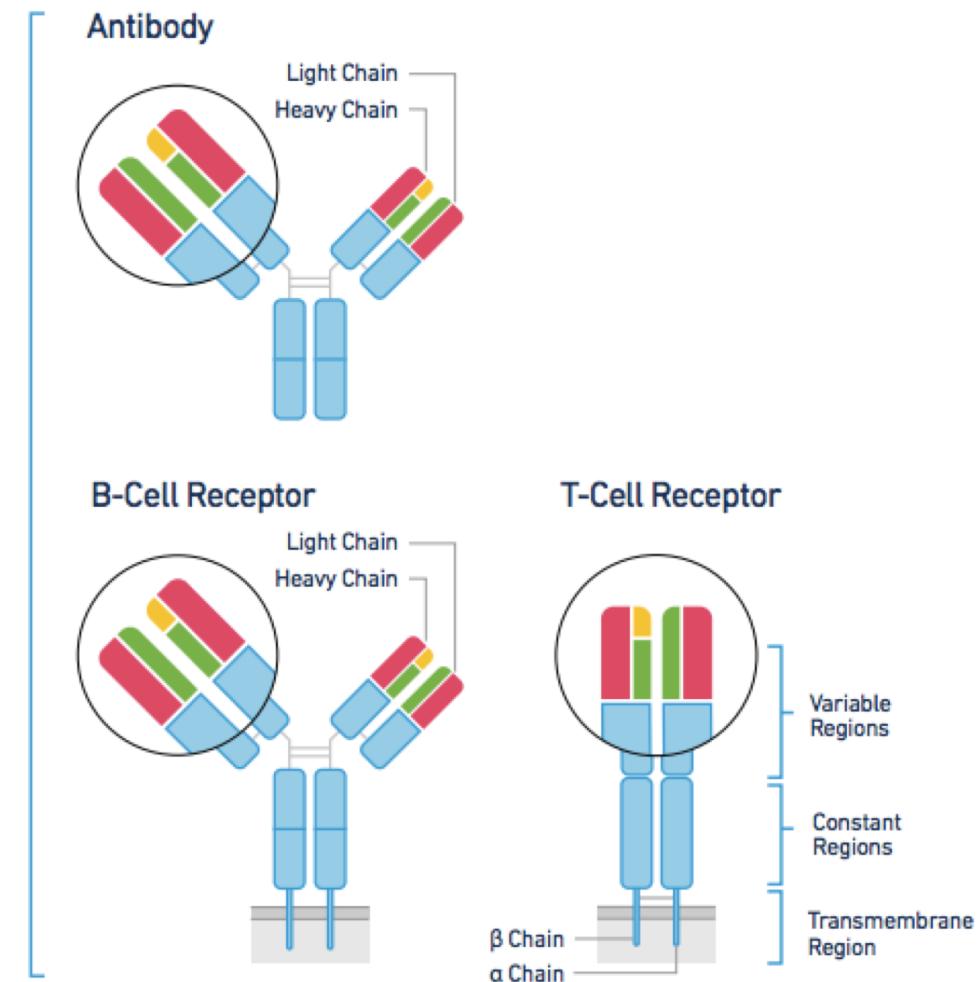
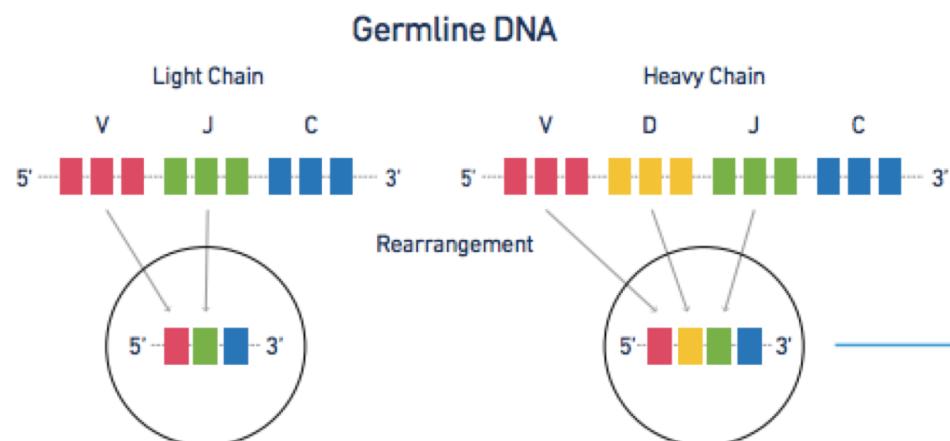
# Why immune cells?

- Specifically, T cells and B cells:
  - Have cell receptor chains on their surface, referred to as TCR and BCR.
  - BCR and TCR are extremely diverse due to somatic gene rearrangement, called V(D)J recombination.



# V(D)J Recombination

## V(D)J Recombination



# Immune repertoire

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- Each T or B cell (and its progeny) will have a unique nucleotide sequence, called a clonotype.
- For T cells, clonotype expansion happens after activation (antigen detection).
  - Expanded cells will share the TCR clonotype.
- Proliferative expansion during T cell development will share the same TCR $\beta$ .

# Single-cell RNA-seq TCR/BCR analysis

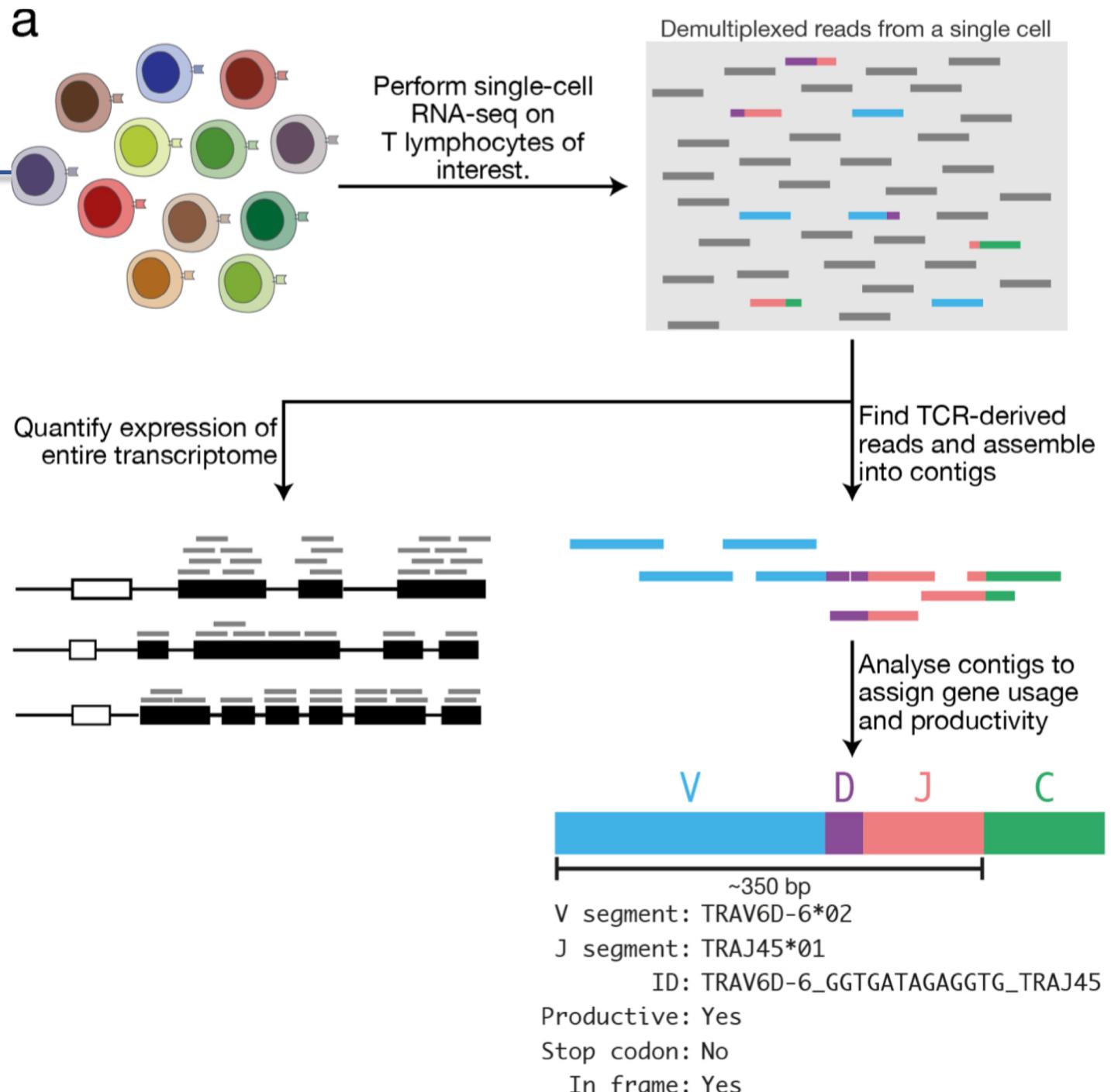
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- Single-cell RNA-seq methods will capture the expressed TCR/BCR genes.
- Can be done using the full length single-cell methods without adjusting the standard protocol.
- Challenge is due to high variability of the region making it difficult to align and distinguish sequencing errors.

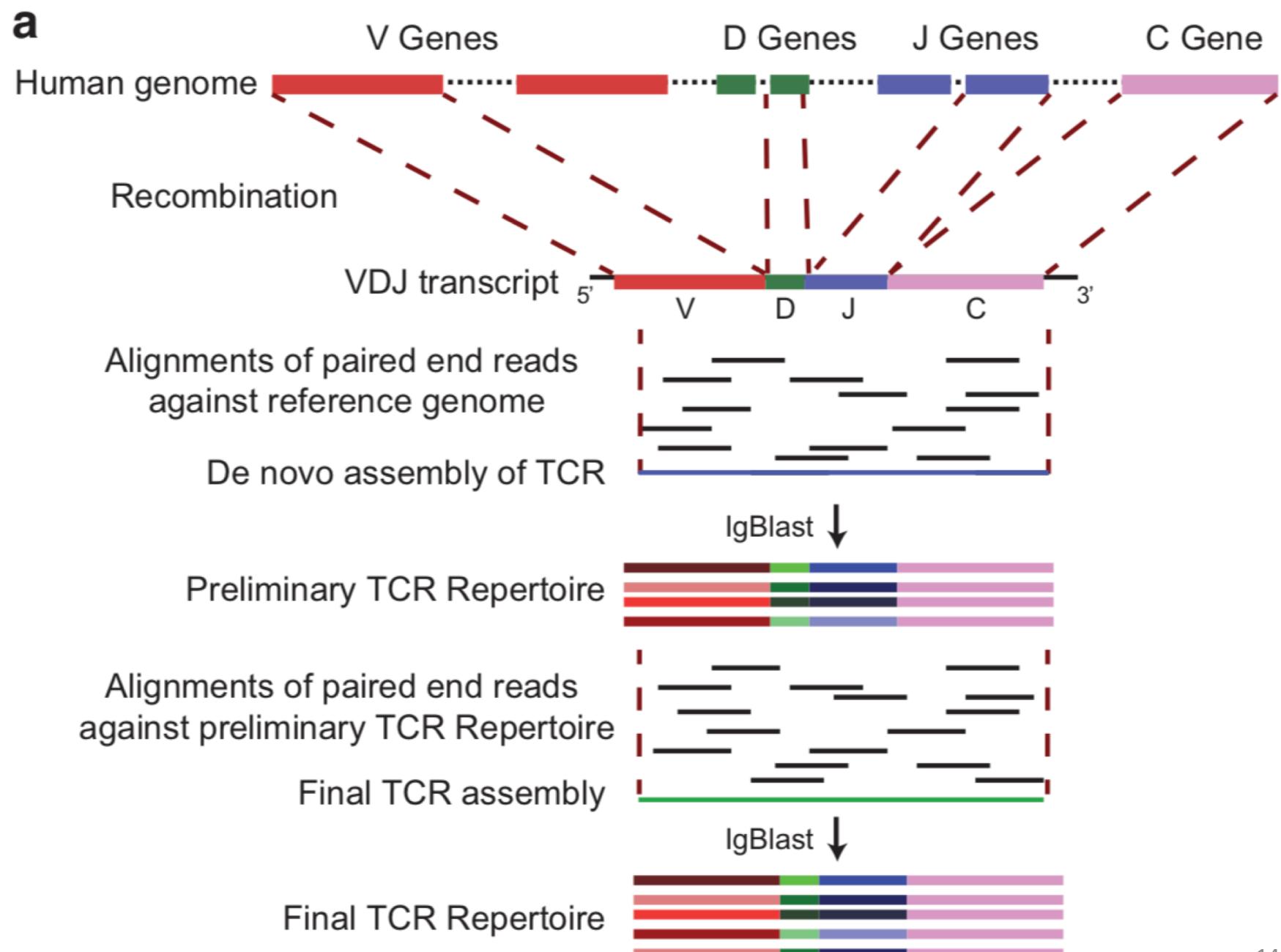
# TraCeR

For each cell:

1. Align TCR reads to a combinatorial reference.
2. Construct contiguous contig to get the full length TCR sequence.
3. Determine productivity status (translatable).



# VDJPuzzle



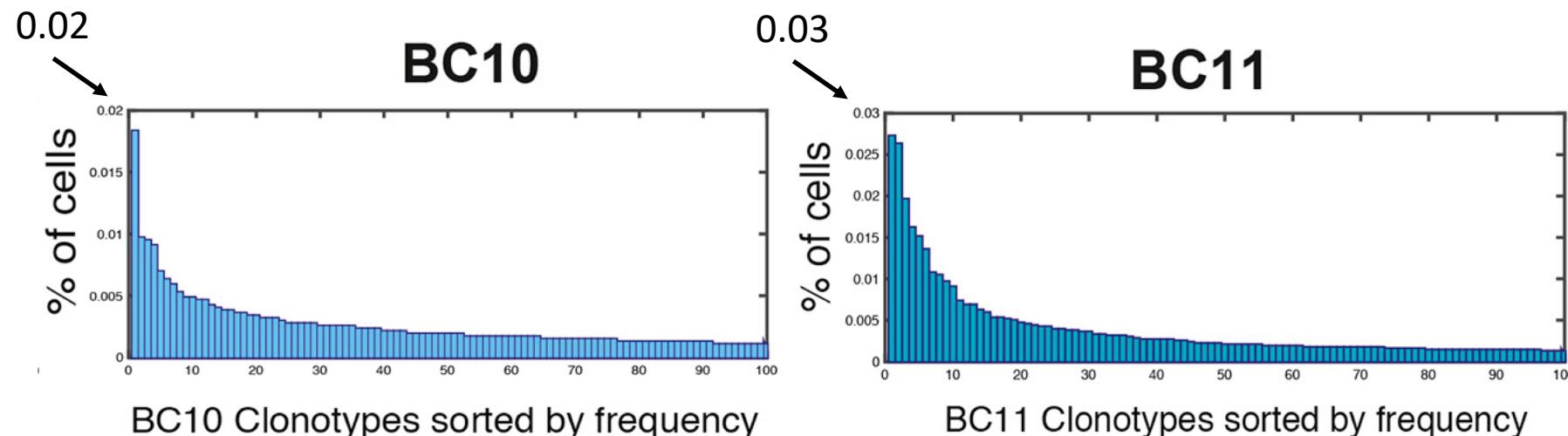
# 10X VDJ and CellRanger

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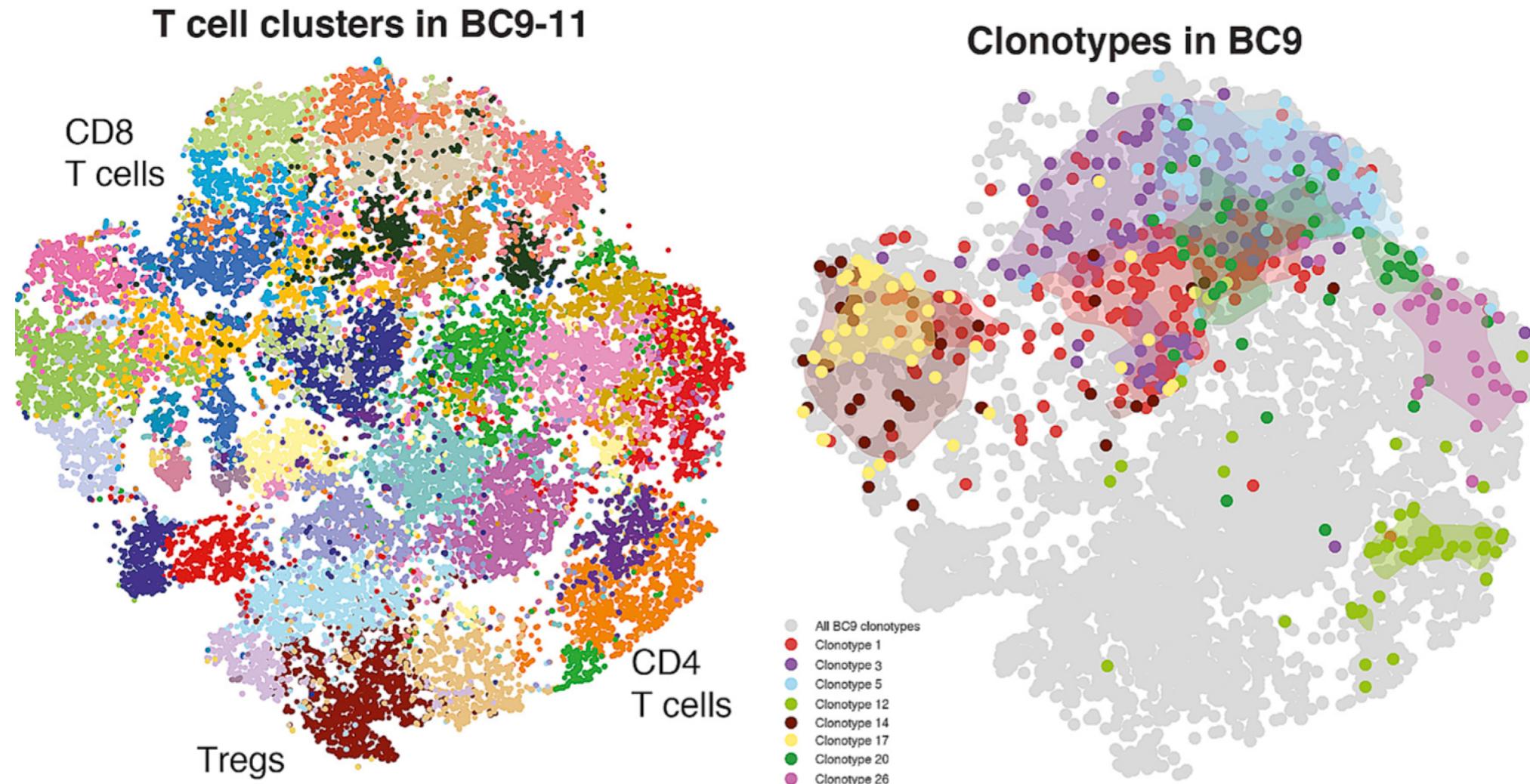
- 10X uses an adapted protocol to obtain tag-based RNA-seq and VDJ sequences.
- Use targeted PCR on all BCR/TCR gene regions to retain the full-length sequences and retain the cell and UMI barcodes.
- Assembles VDJ *de novo* to handle diversity.

# Types of analyses

- Diversity quantification
  - Clonotype lengths, quantity (composition), expression level.
  - V, D, and J alleles in the repertoire, and the most frequent VJ combinations.
  - Total diversity in or across individuals/tissues/tumors.



# Types of analyses

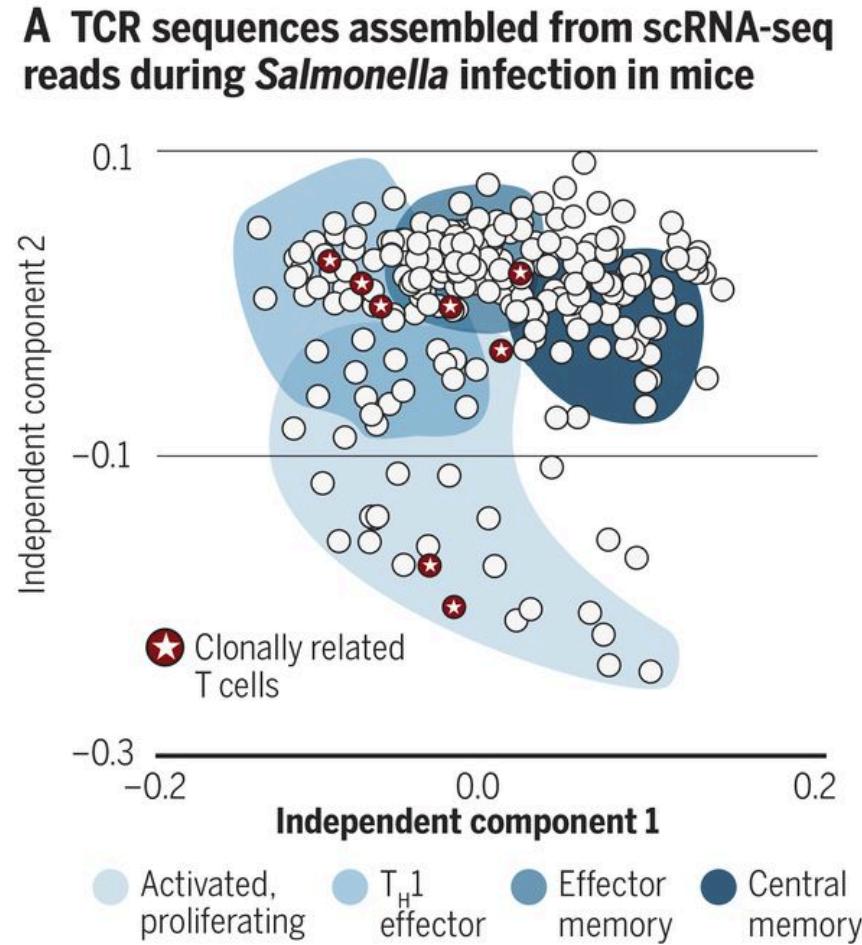


# Types of analyses

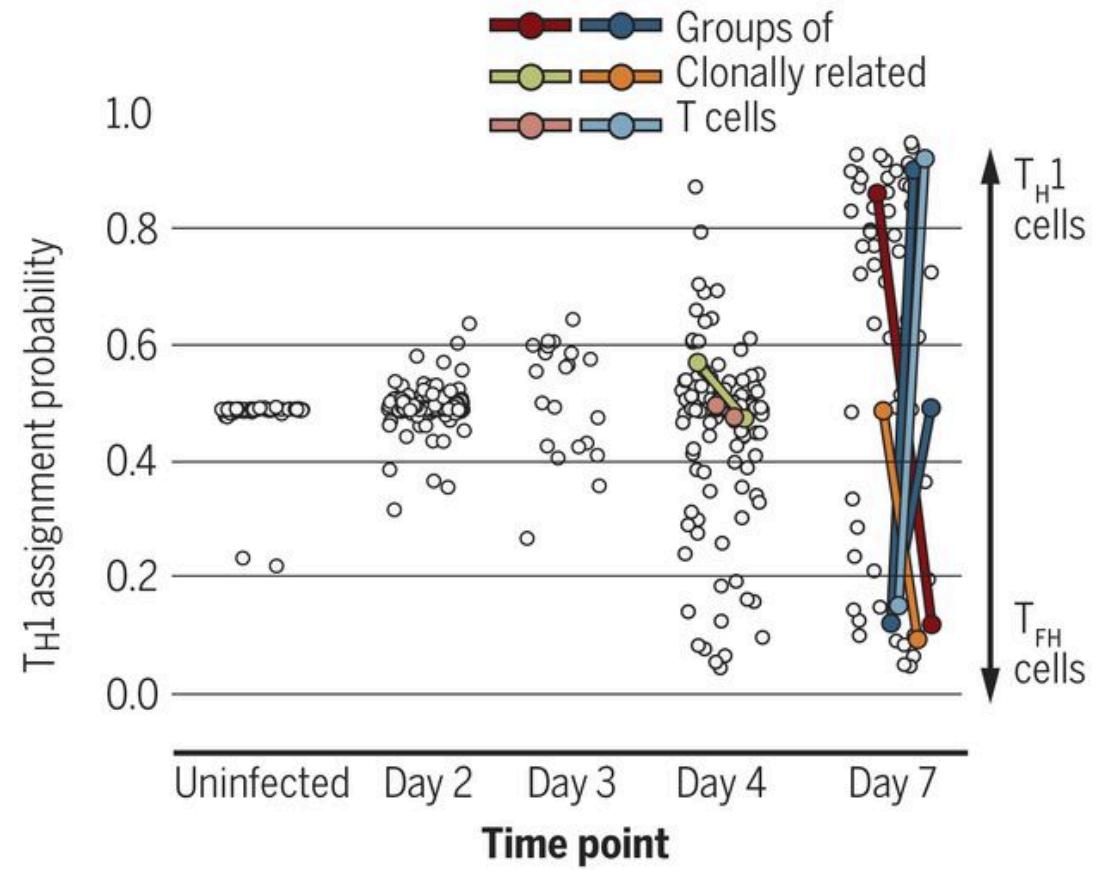
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- Lineage Inference: Characterize the immune response by identifying and quantifying clonotypes present.
  - Expansion represents response to specific antigen.
  - Identify shared clonotypes across types of T cells.

# Clonotype expansions



**B TCR analysis during the immune response to malaria**



# Trajectory inference by shared clonotypes

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