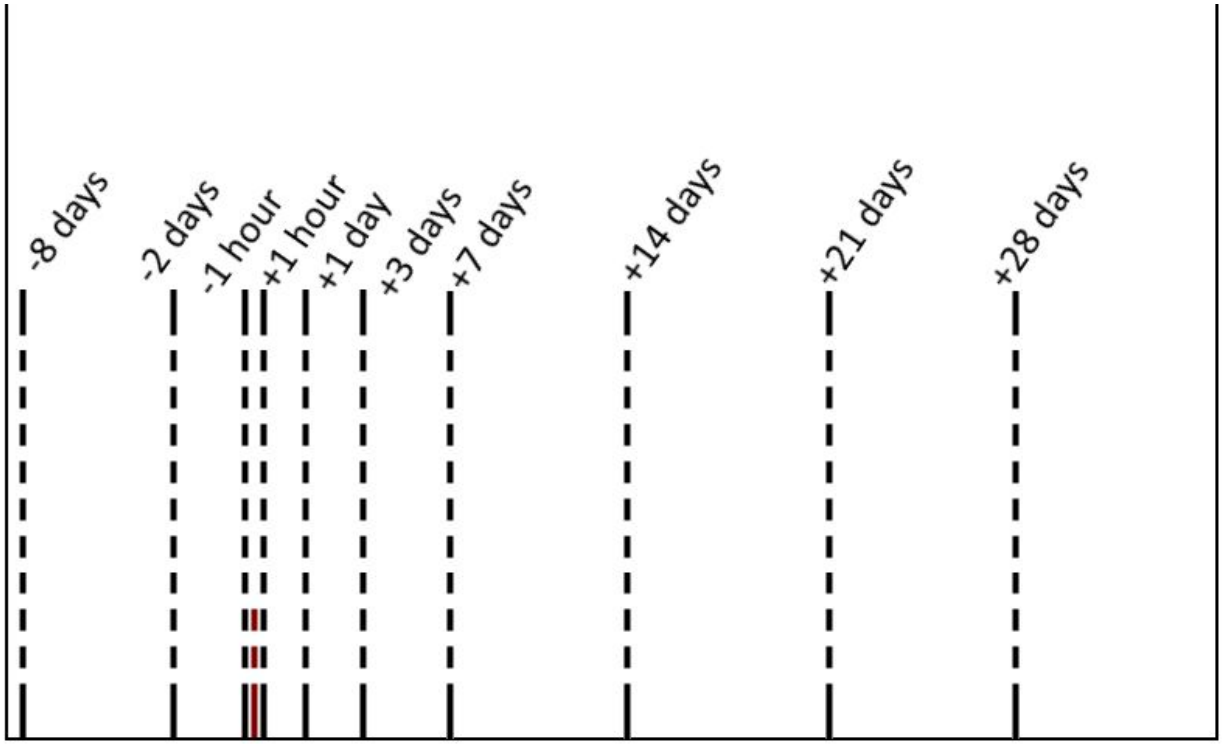
Analysis of immune response dynamics using Rep-seq data

Andrey Slabodkin

Available data:

3 flu vaccinated people with 10 Rep-seq datasets per individual



Approaches

Possible approaches to immune response dynamics analysis:

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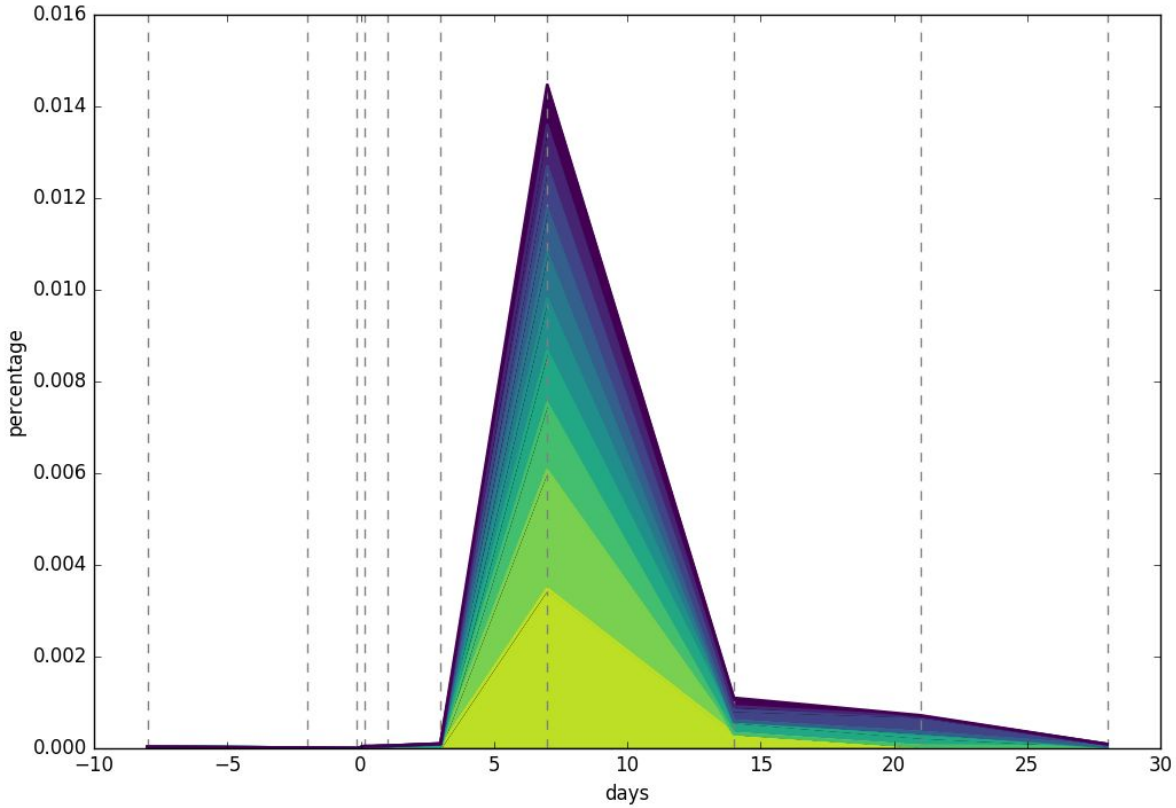
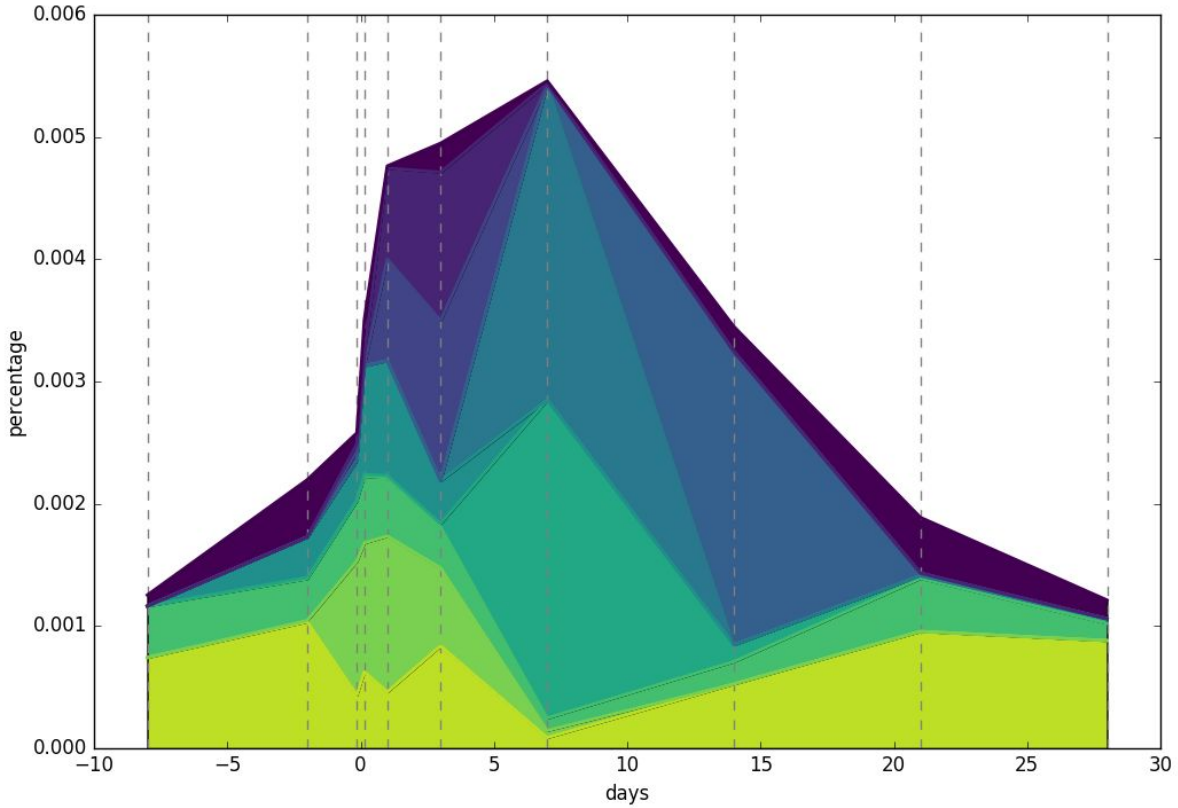
10 clones, most abundant clones on the 7th day



10 most abundant clones during the whole period



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Antevolo

Antevolo algorithm

- Given a dataset of reads of the same isotype
- Split the reads by the VJ-type
- In each component compute the Hamming graph on the CDR3 sequences
- If there is an edge between reads *A* and *B* in the CDR3 graph, we say that *A* and *B* are connected with an edge of one of the following types:

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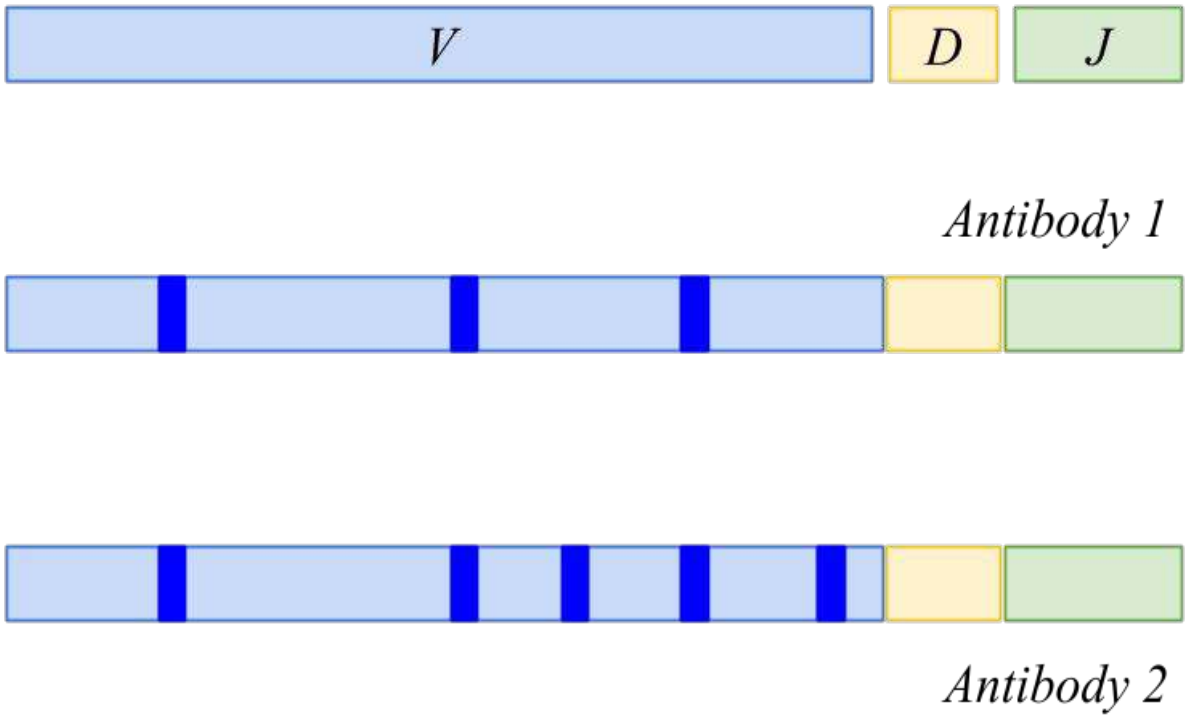

germline segments

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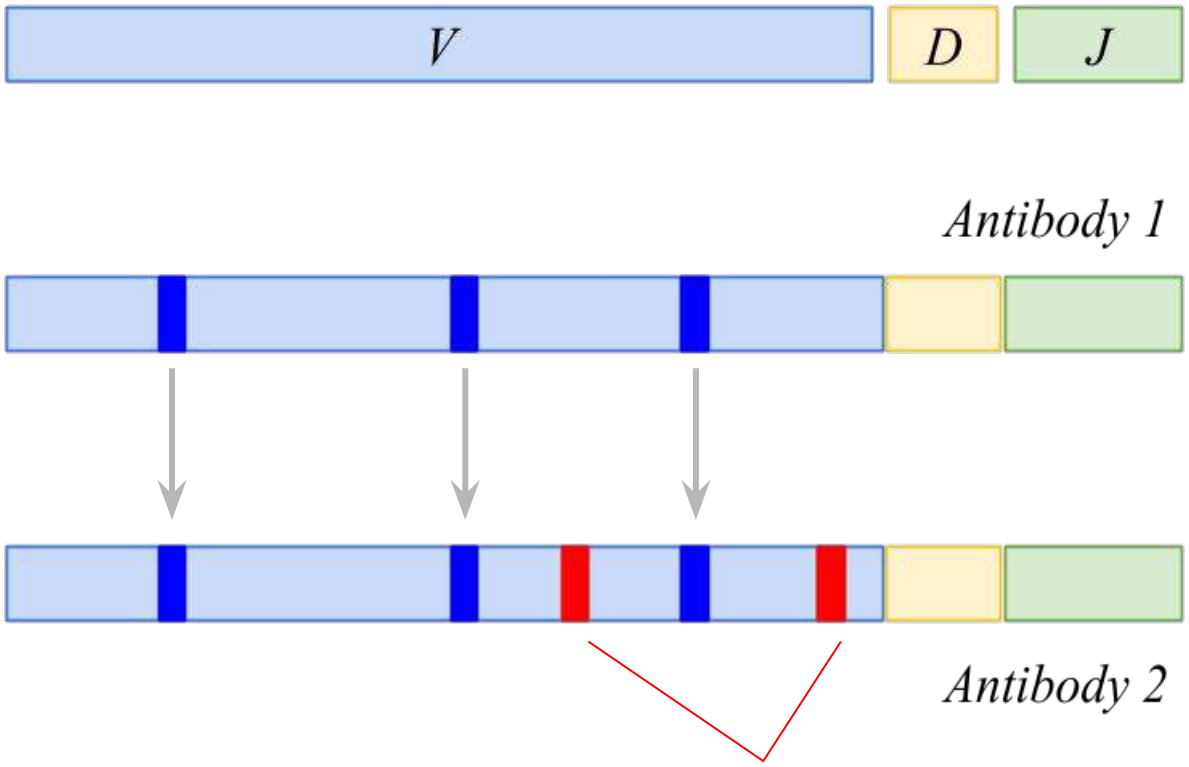

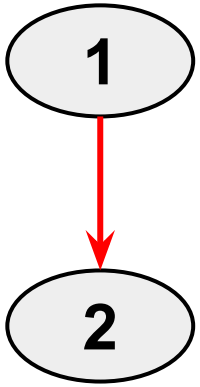

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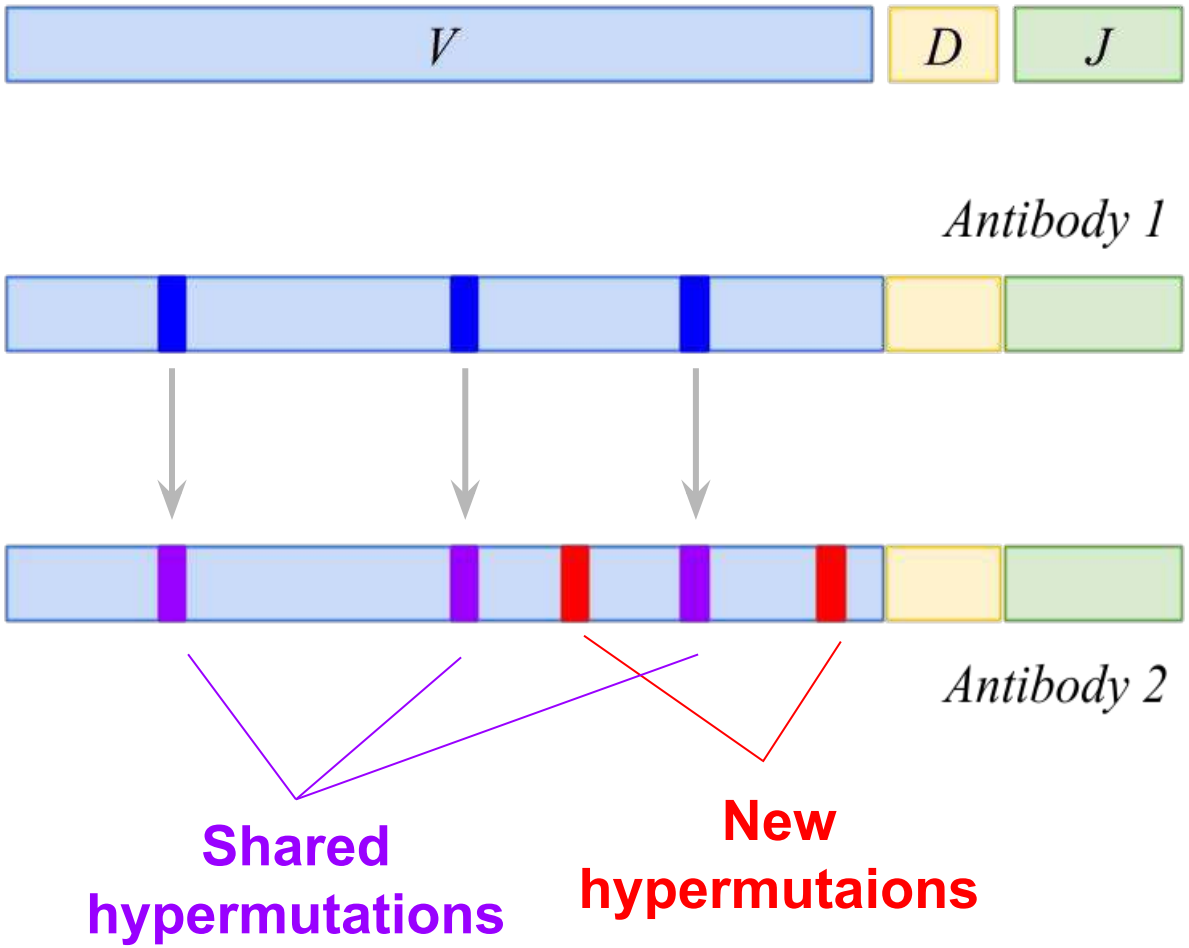

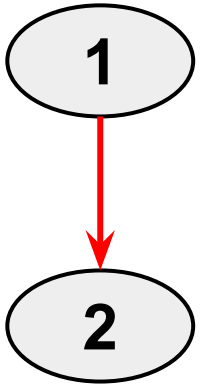

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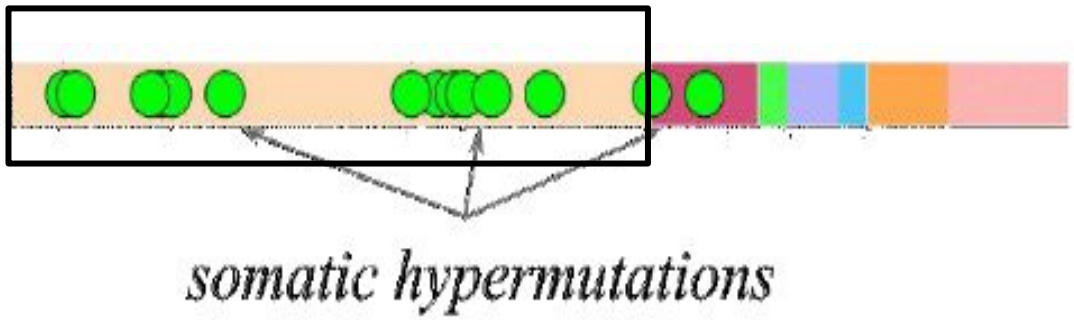
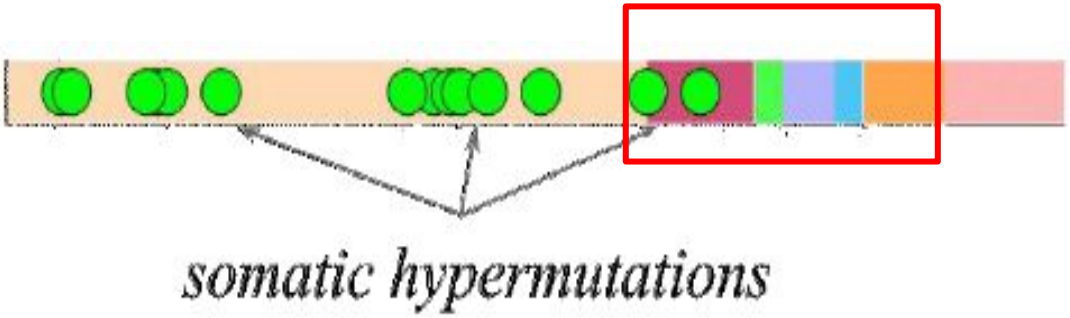
SHMs in V segments are easy to find



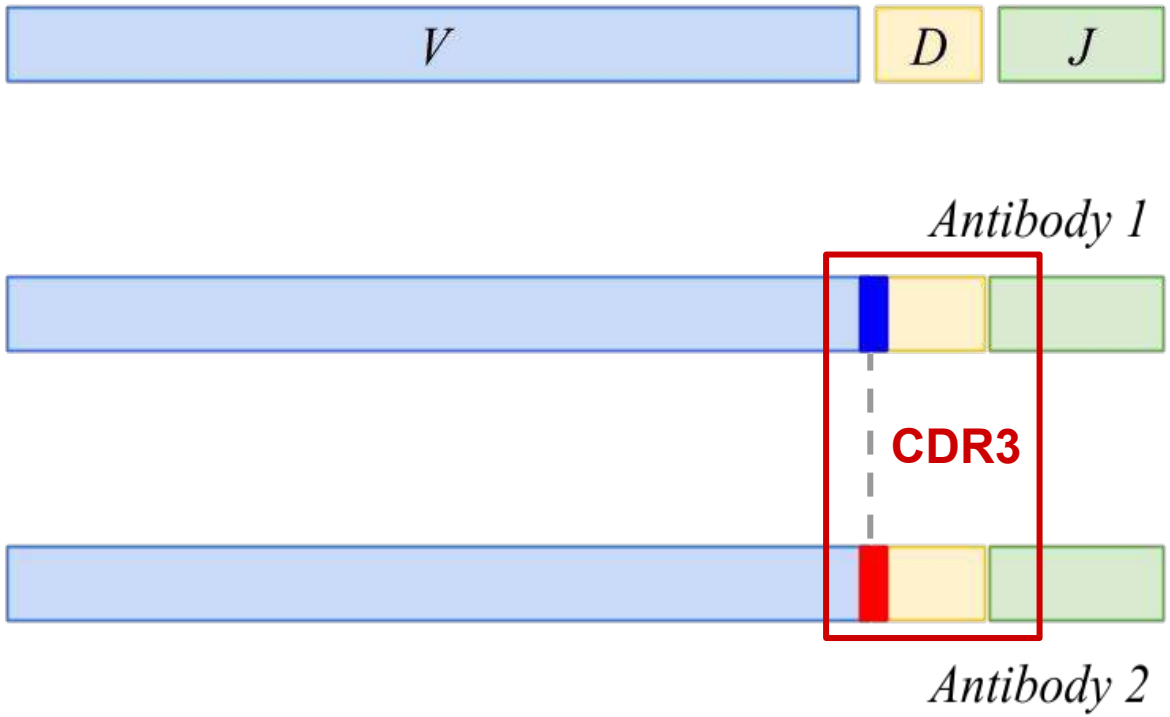
- One can easily identify mutations in the V segment using alignment against the **template** (germline V segment)

SHMs in CDR3 are difficult to identify



- One can easily identify mutations in the V segment using alignment against the **template** (germline V segment)
- **But there is no template for CDR3!**

Undirected edge case



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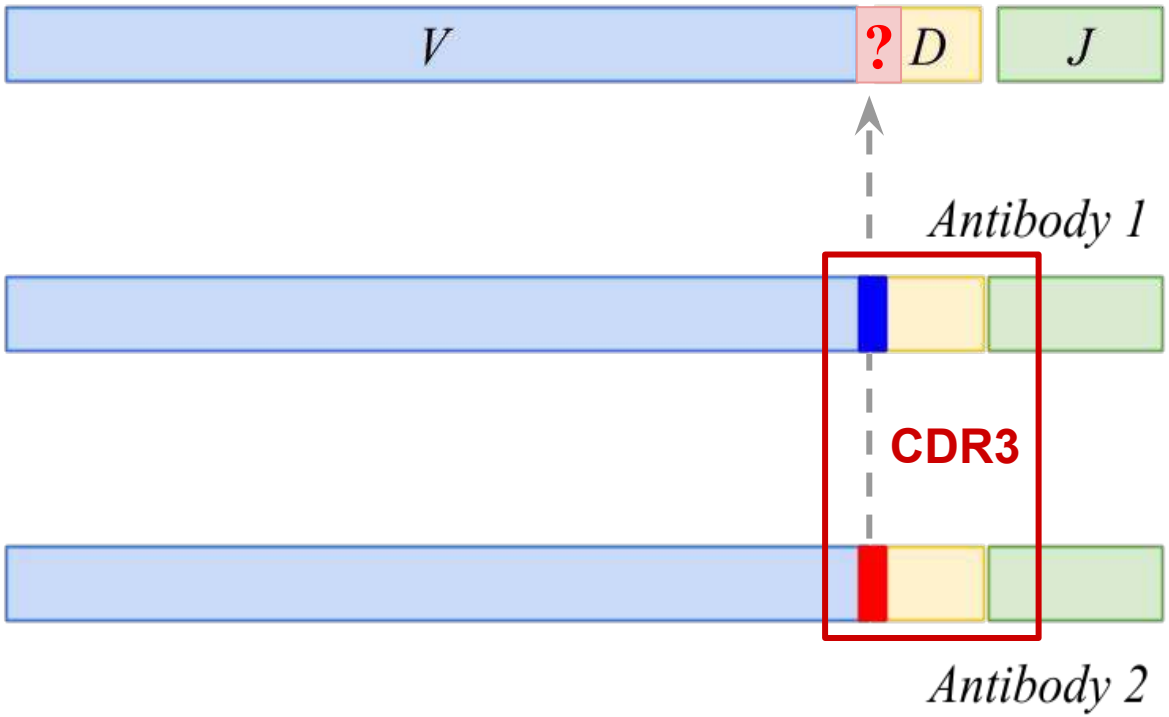

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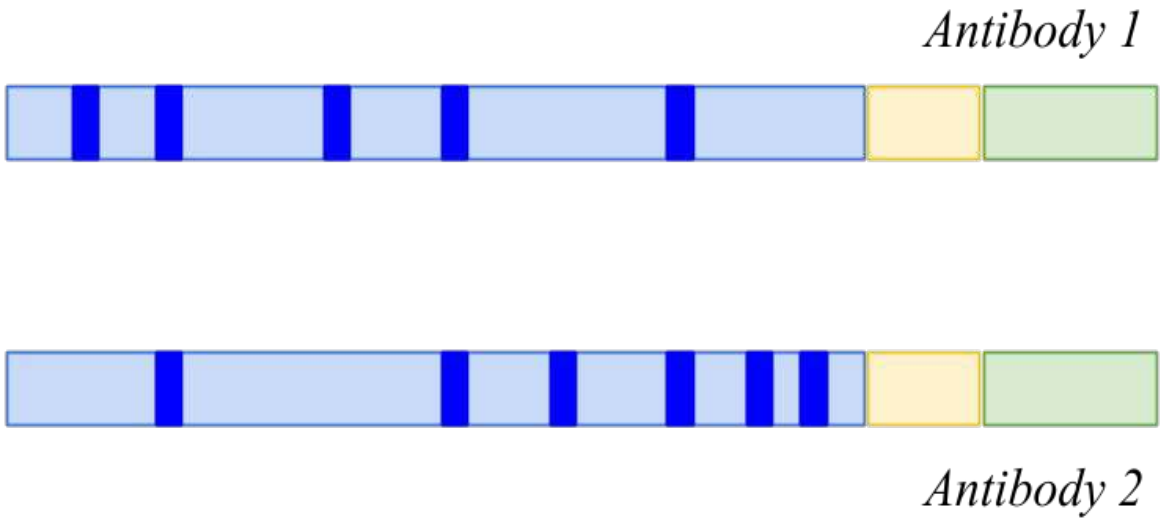

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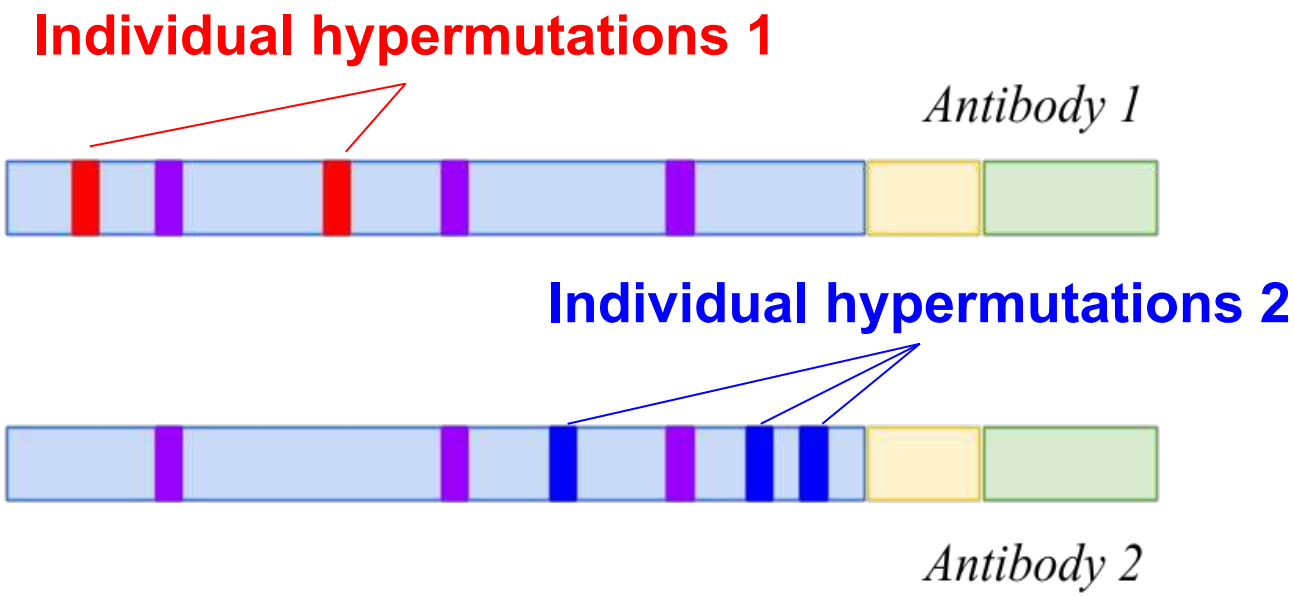

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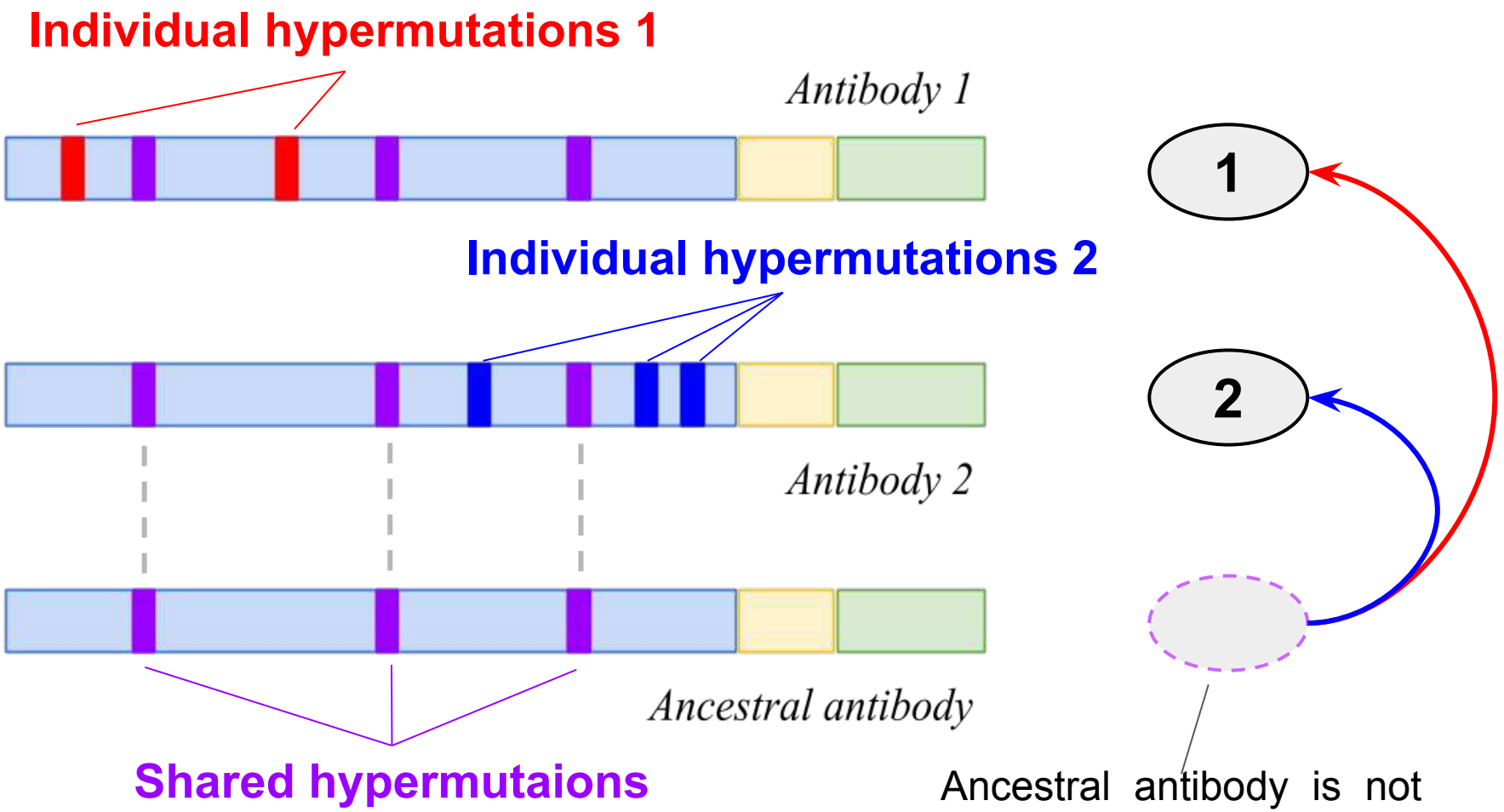
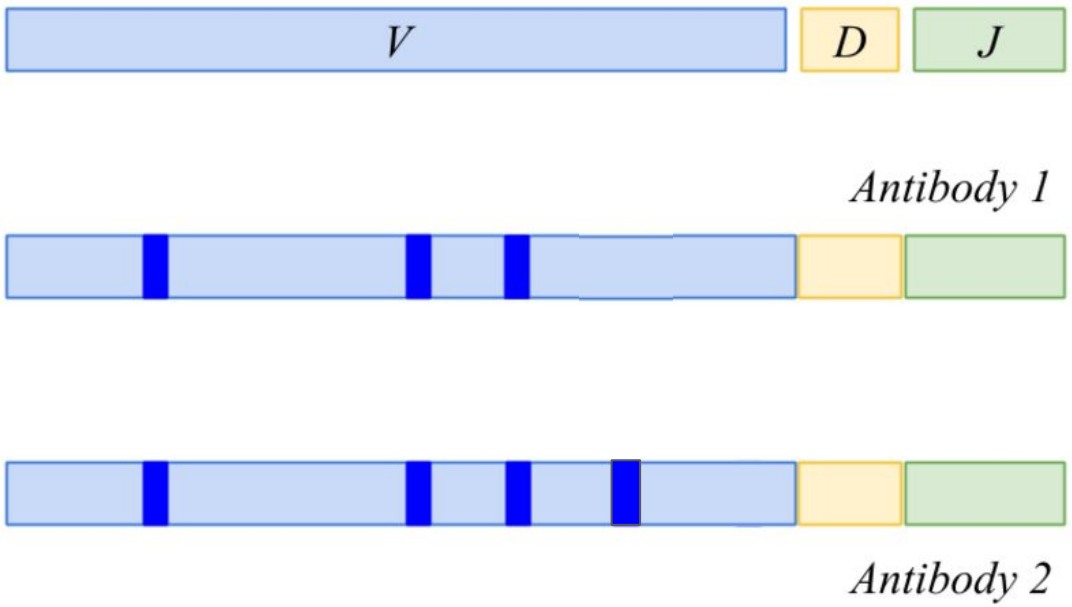


We want to construct a forest using only *directed* and *undirected* edges and then reconstruct some missing vertices with *intersected* edges.

New edge type

- Sometimes not all the VSHMs of B are presented in A, but the individual hypermutations of A and B have the same positions. We call such an edge ***double mutated***

Double mutated edge case



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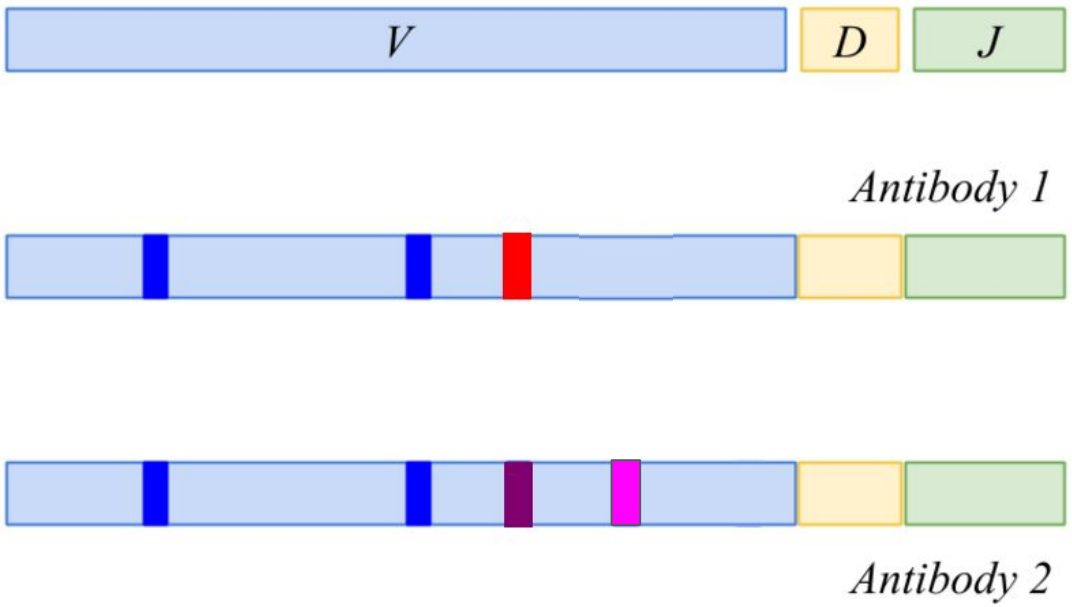

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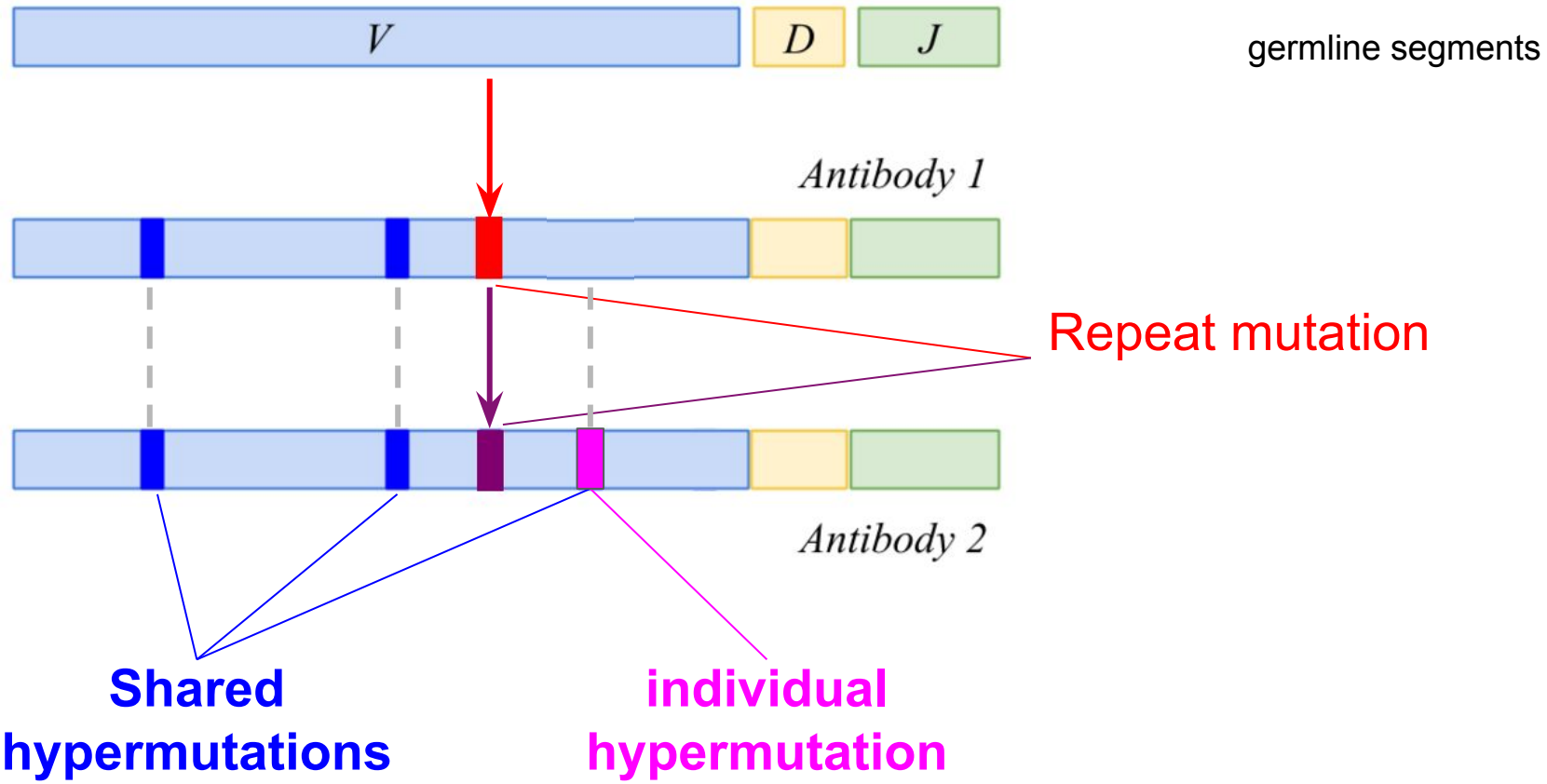

Tree (forest) construction

- Consider a graph containing only ***undirected*** edges. First, we compute connected components of this graph
  - Mutations in the CDR3 are more probable, so we want to draw as many undirected edges as it is possible
  - We will set the directions in the components later
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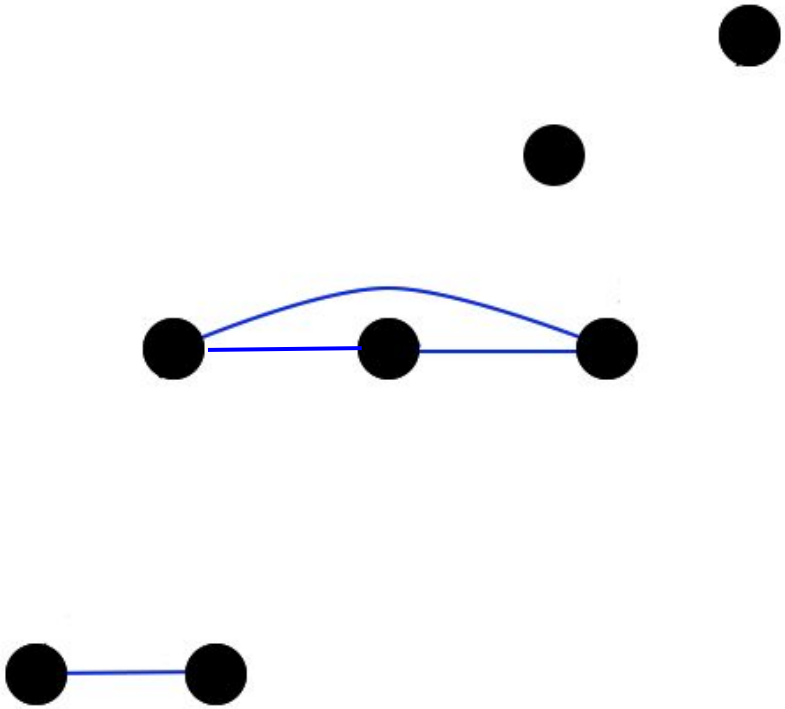

Blue edges are **undirected**.
Black edges are **directed.**

Vertices are ranked by height due to their SHMs number

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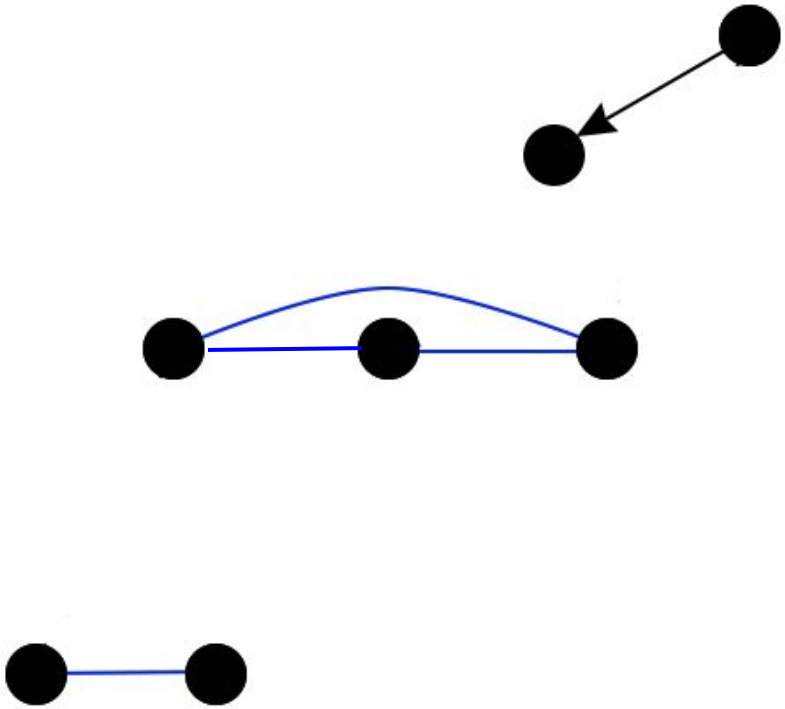

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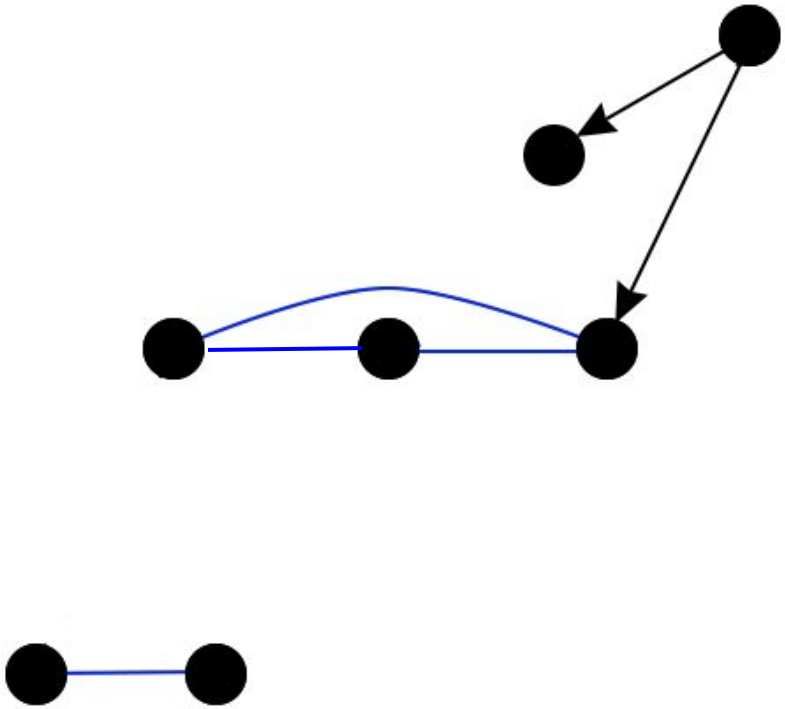

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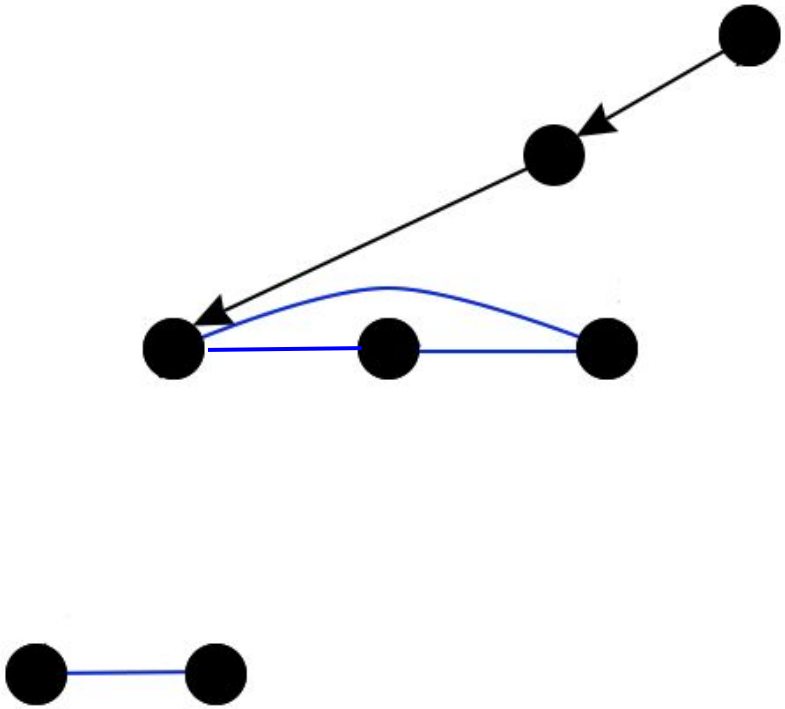

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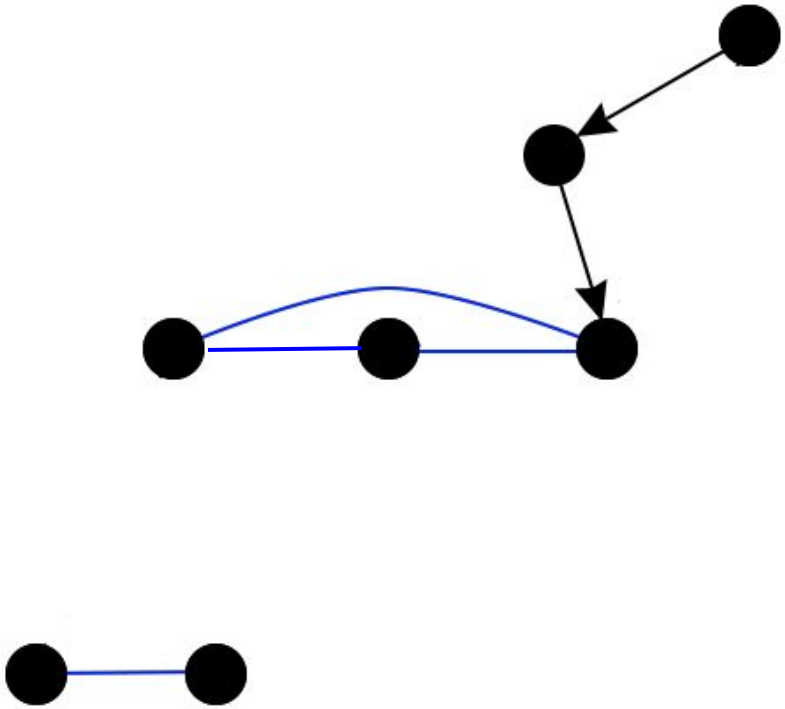

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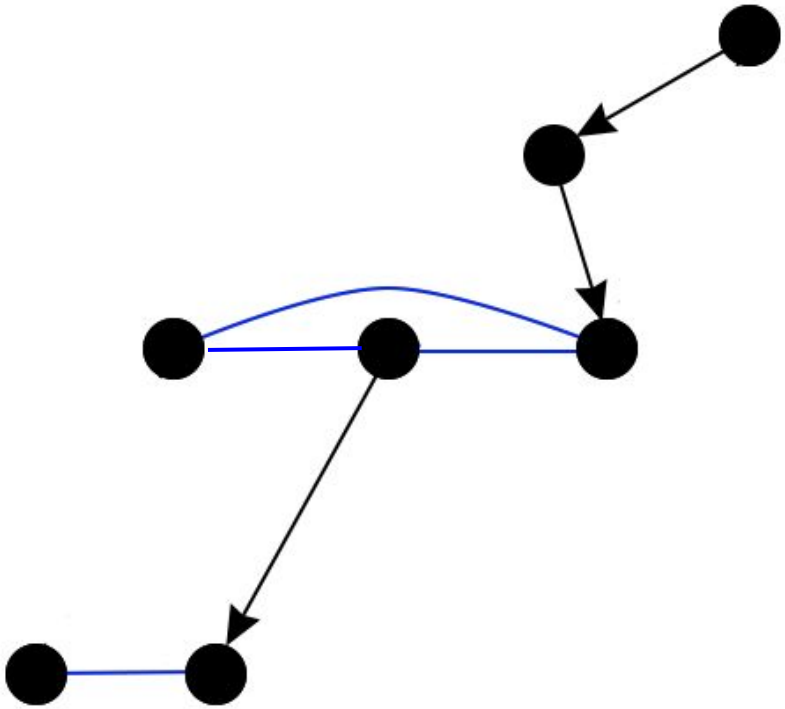

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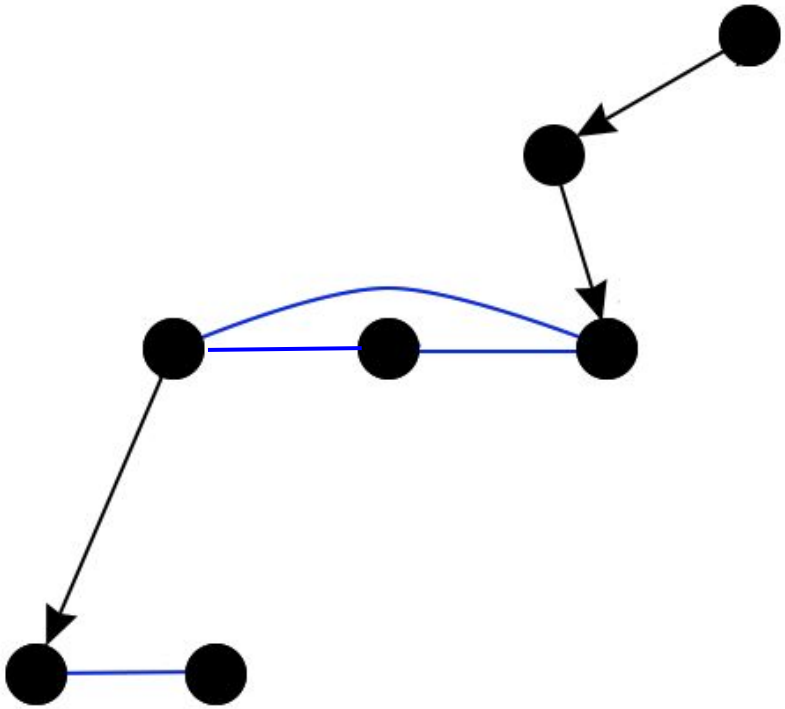

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Probabilistic SHM model

5-mer	Freq	A	C	G	T
ACAAC	**83**	–	0.24	***0.48***	0.28
GGCGT	**1742**	0.22	–	0.12	***0.66***
CCGTC	**12**	0.35	***0.52***	–	0.13
TCTCC	**516**	0.32	***0.54***	0.14	–

- The SHM model takes into account both the mutated nucleotide and its neighbours
- Detect new hot spots and compares SHMs in IG chains

Yaari et al., *Front Immunol,* 2013

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Tree (forest) construction

- We set the inner-components edges directions
  - Consider a connected component on undirected edges.
  - Split each edge (a, b) into a pair of directed edges (a, b) and (b, a)
  - The model gives us the weights of the edges
  - So we simply find the minimum directed spanning tree (also called arborescence) rooted in the endpoint of the parent directed edge on which our component is hanged

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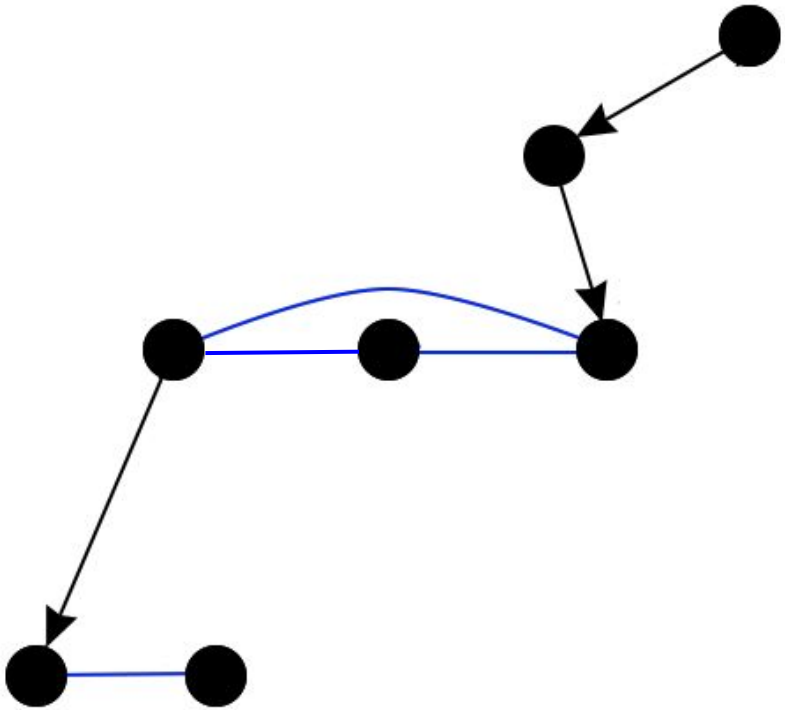

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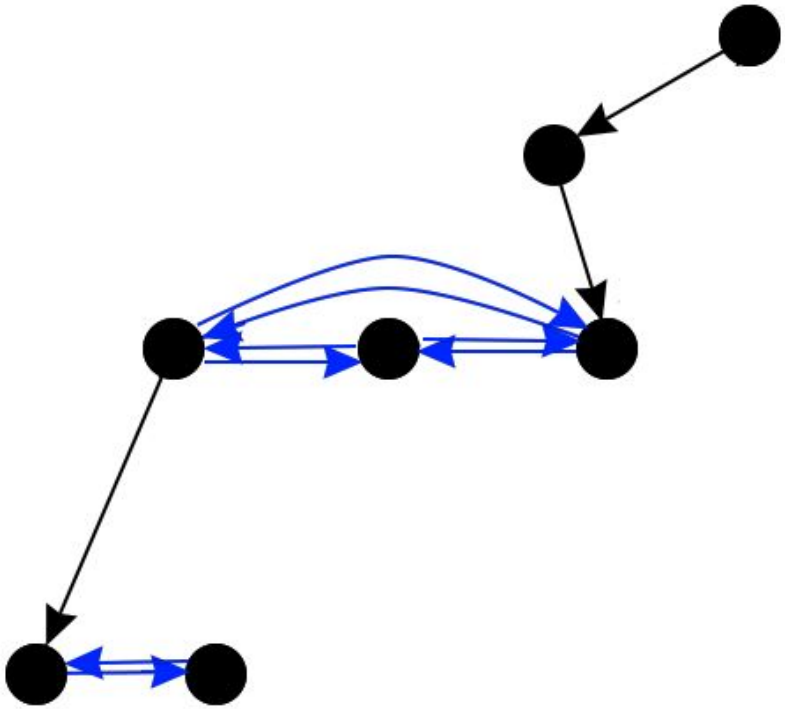

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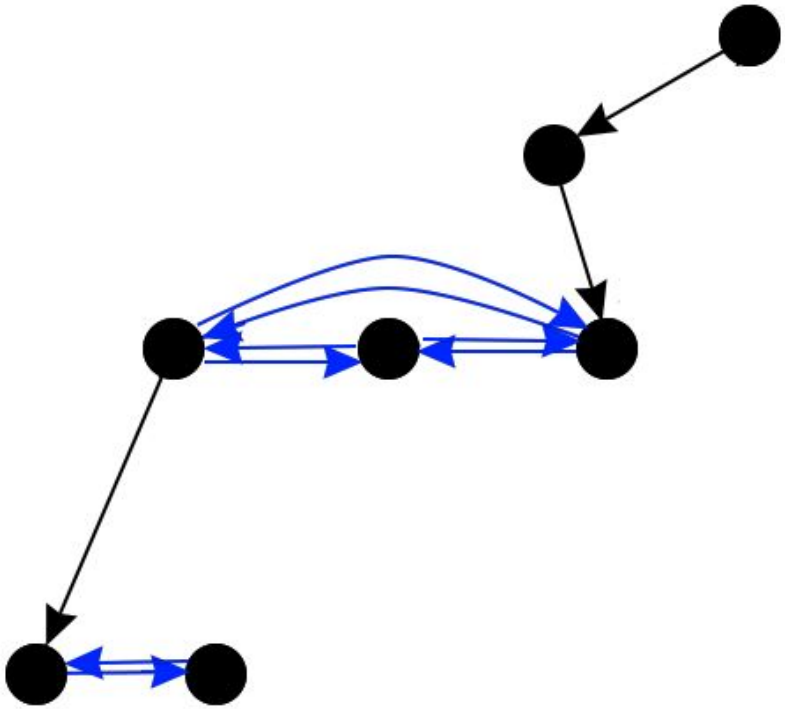

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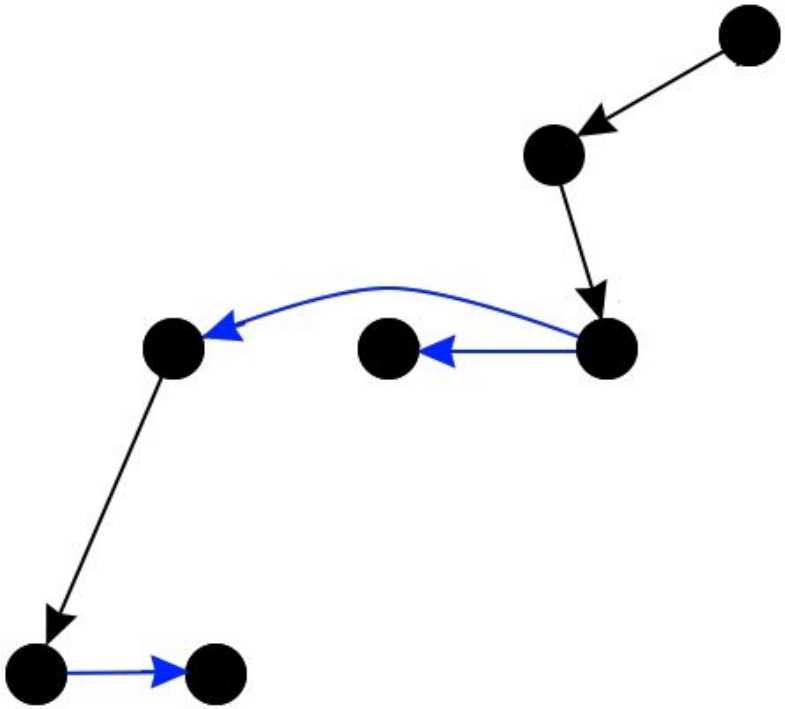

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Repertoire analysis

Nonproductive clones

- A clone is nonproductive if
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  - is out-of-frame.
- It is reasonable to assume that nonproductive clones do not evolve
- But they do!

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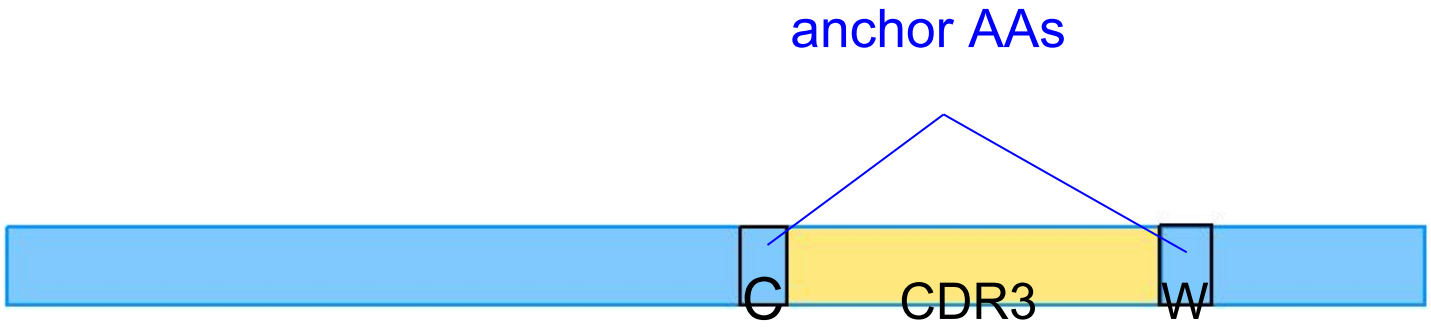
Nonproductive clones

- Mutation probability (non-leaf vertices percentage)
  - for an arbitrary clone: *30±5%* around the datasets
  - for nonproductives:
    - heavy chain: *< 4%* (could be explained with algorithm errors)
    - light chains: *16%* (way too much, may be allelic inclusions)

Anchor amino acids

- Impotrant AA positions around the CDR3
- Change of anchor AAs can totally disrupt the CDR3 structure



Mutation probability

- Mutation probability (non-leaf vertices percentage)
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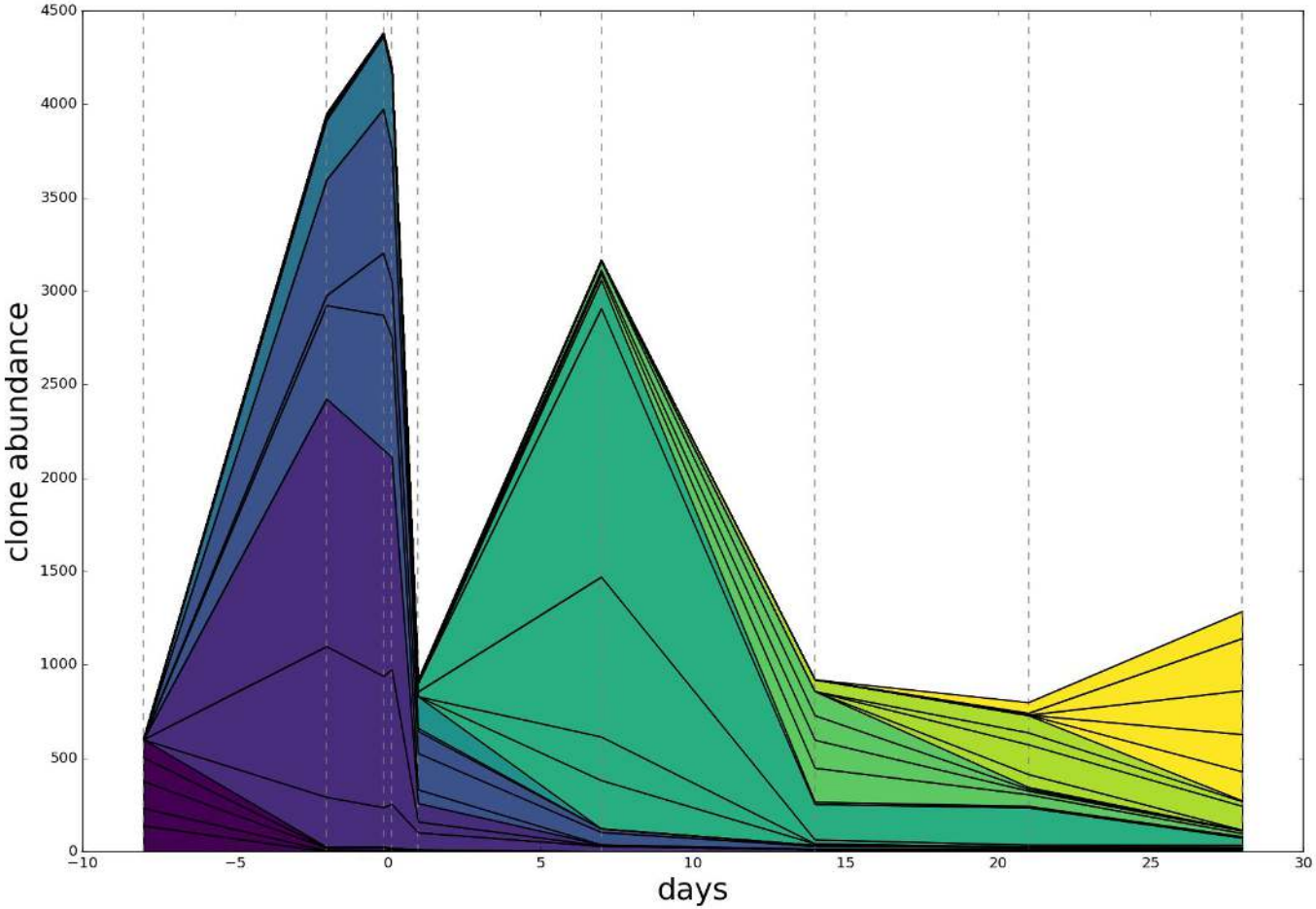
  - for vertices that differ from their parents on at least one anchor AA: *5-6%*

FV, top5 largest clones on each day



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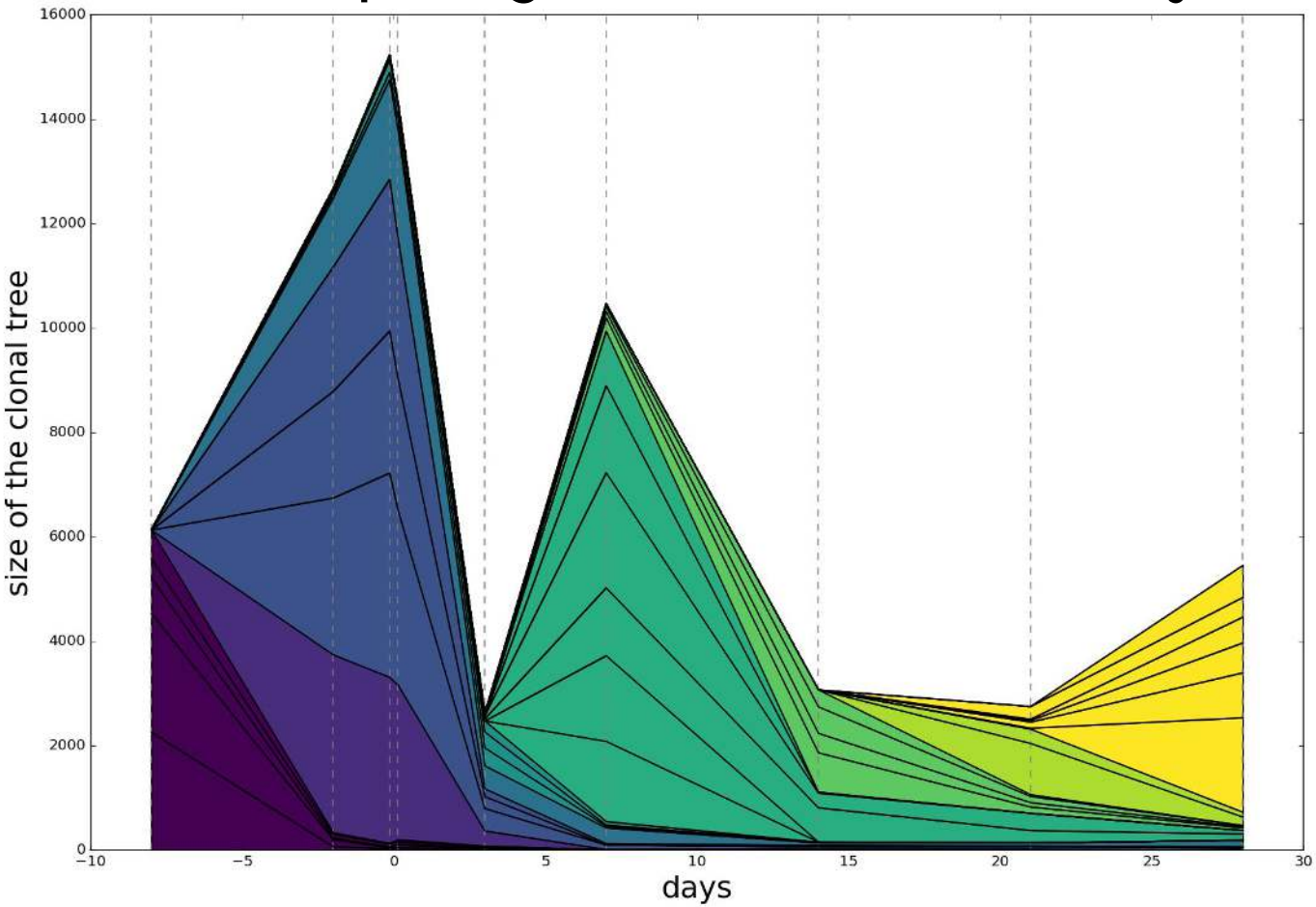

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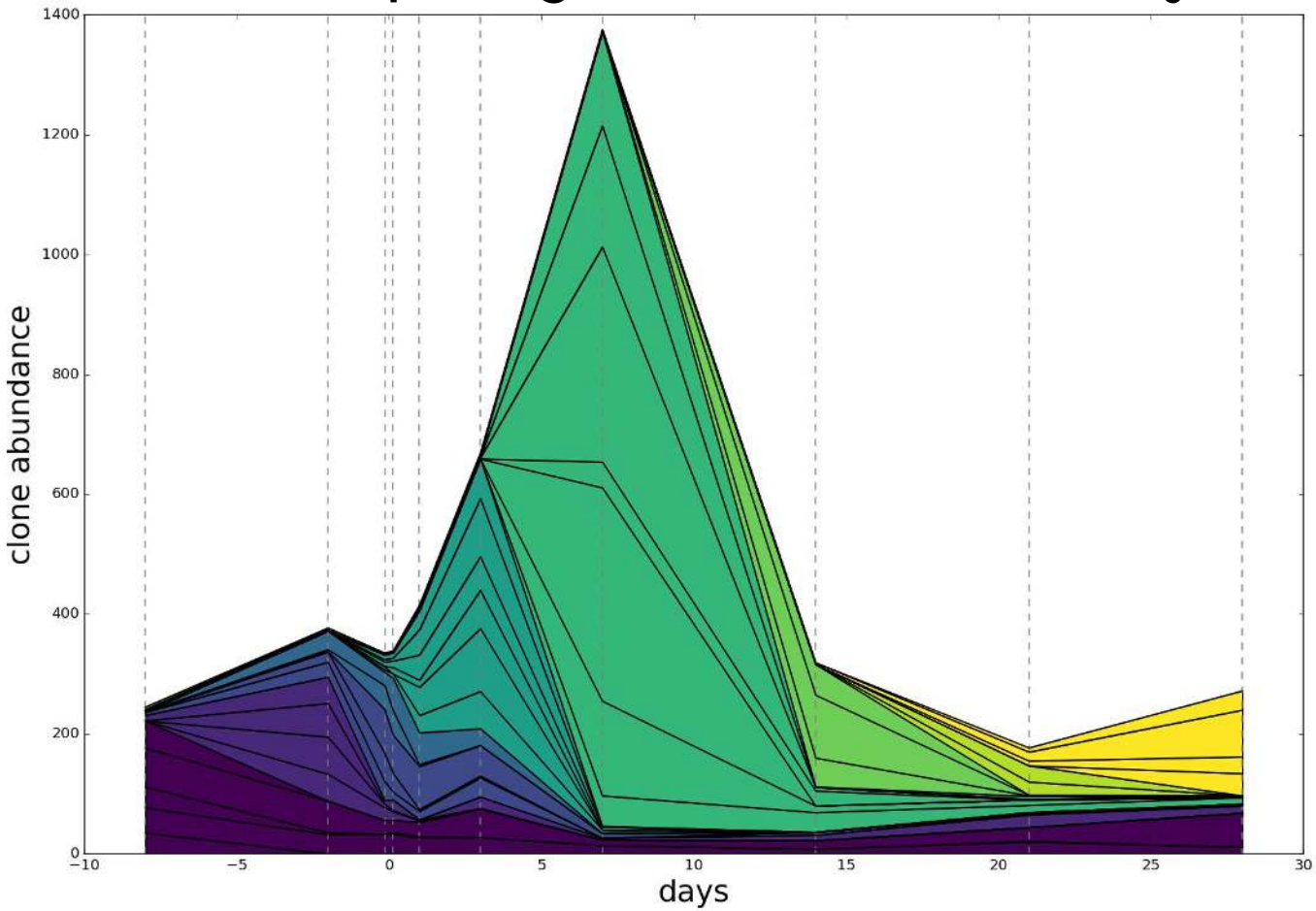

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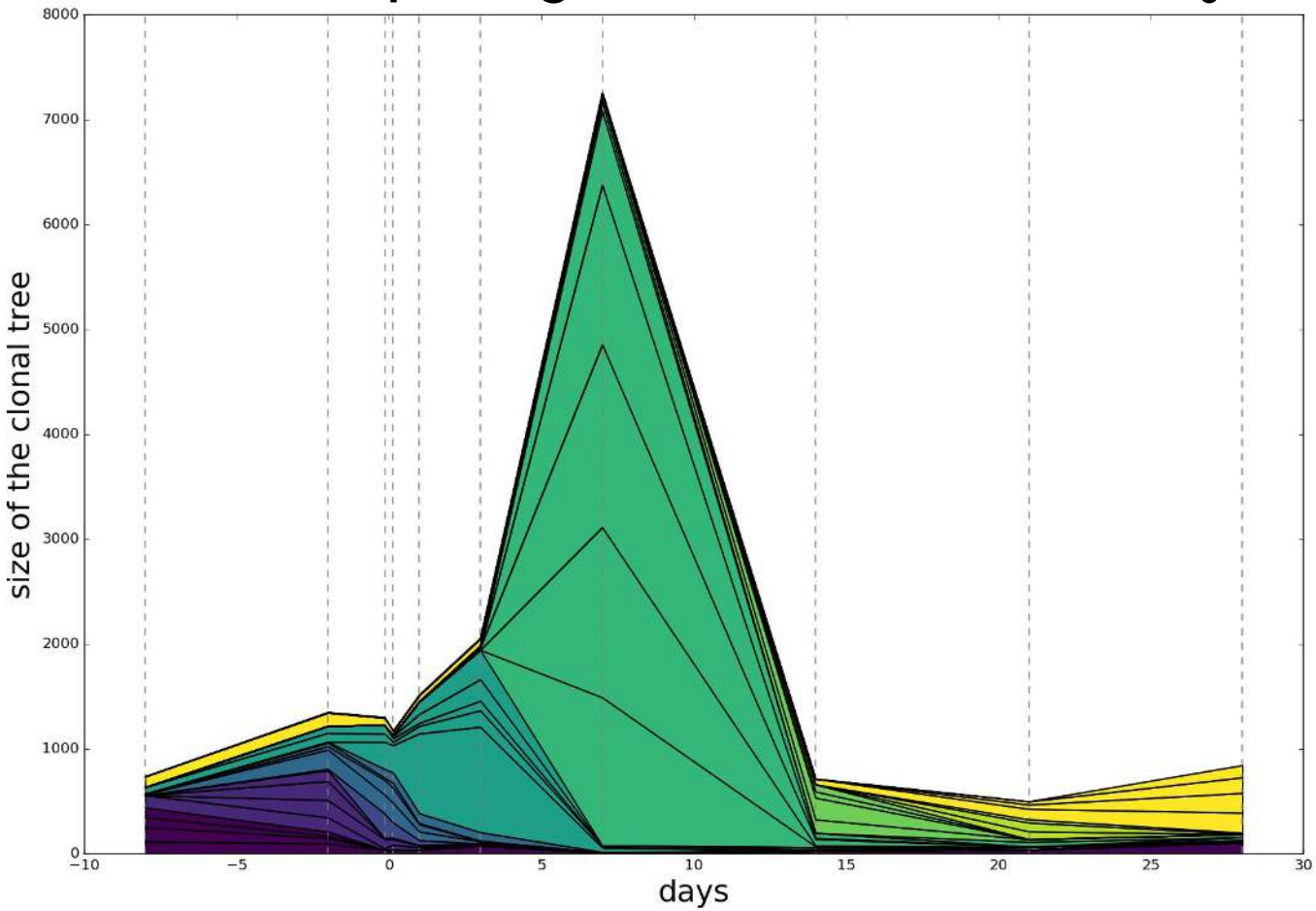

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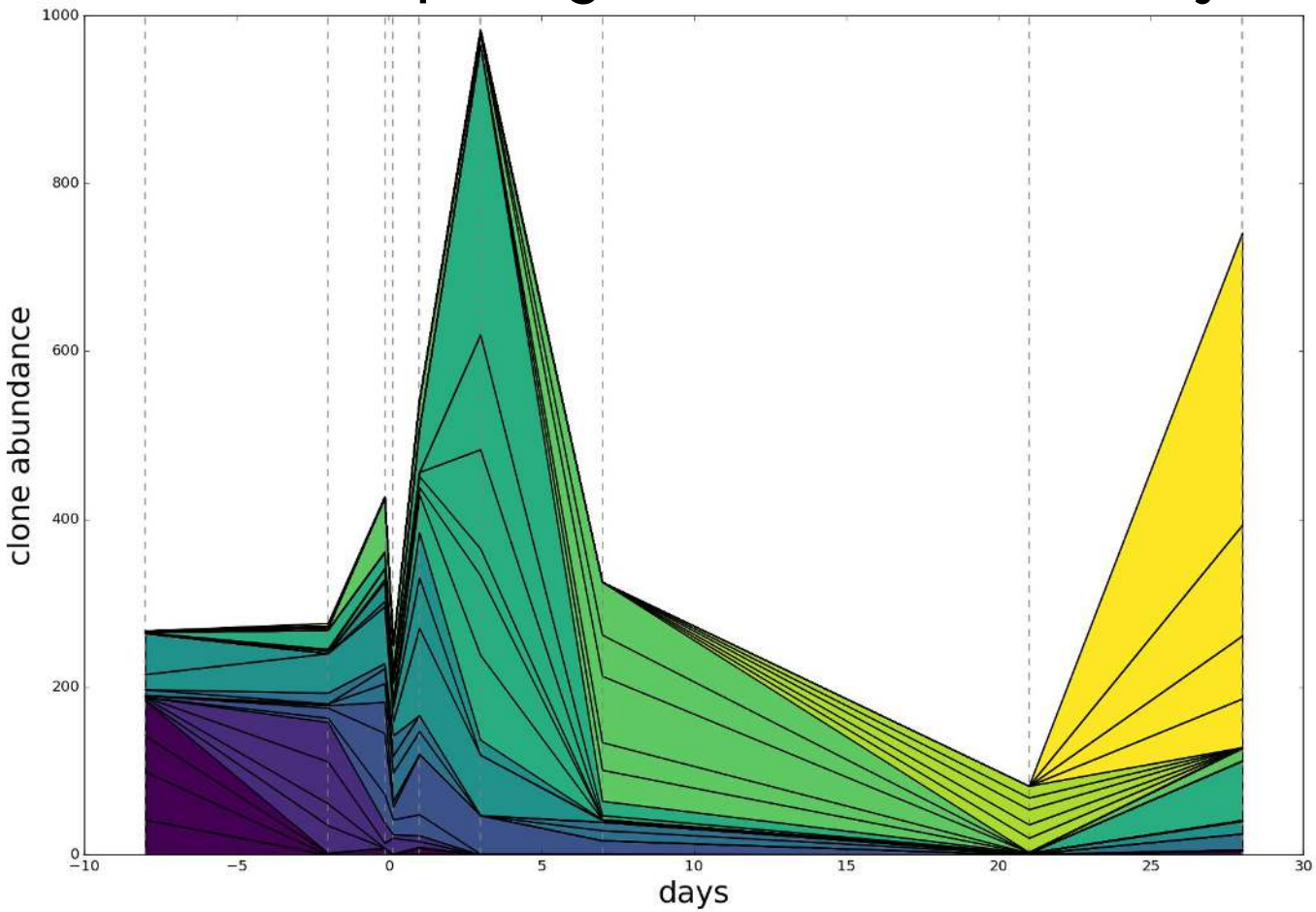

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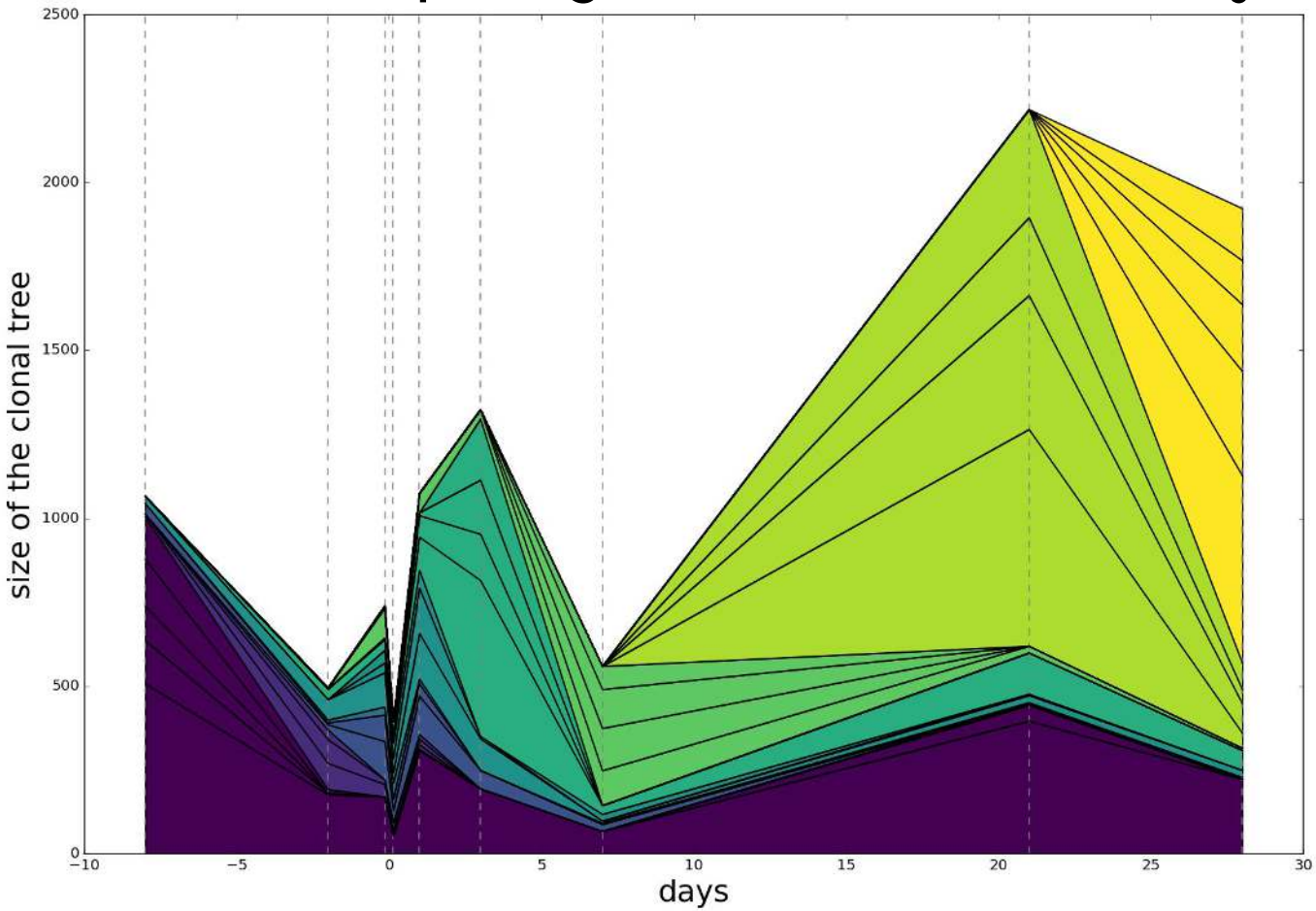

Thank you for your attention!