Towards a comprehensive view of

B cell biology at

single-cell resolution

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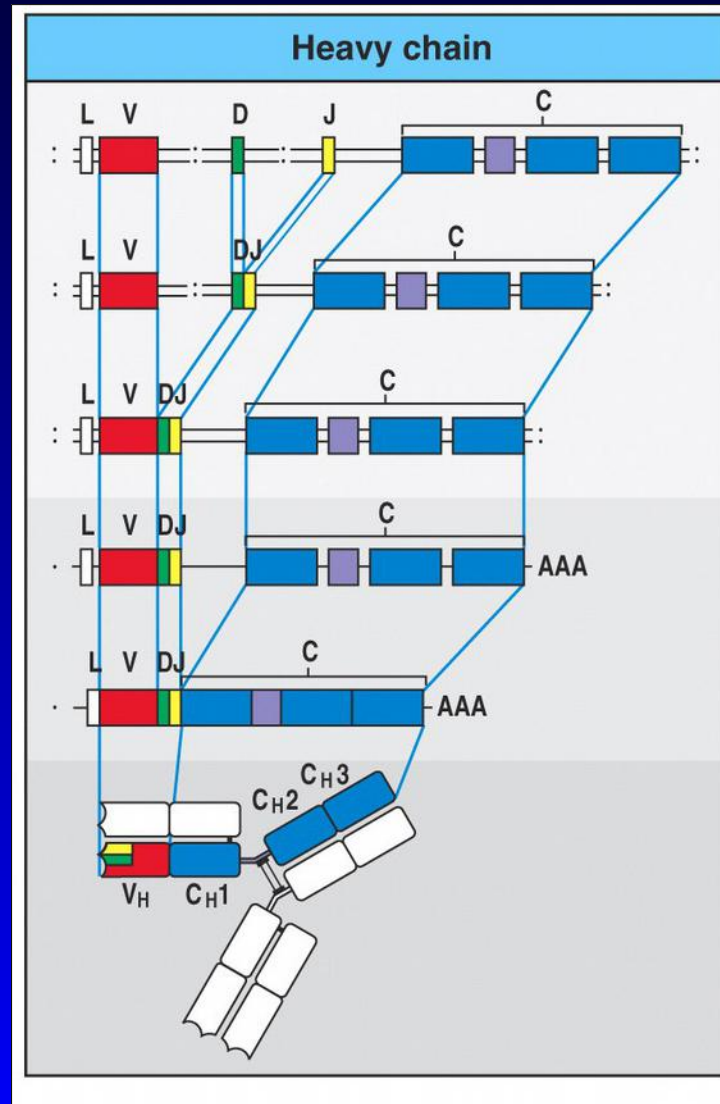
Acknowlegdements

Some years earlier...

What is the size and the structure of the B cell repertoire?

What is the B cell repertoire?

The entireness of B cell antigen receptors that an individual

has at its disposal at a given timepoint.

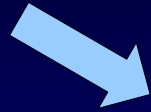# V(D)J recombination generates receptor diversity
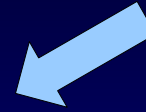
Some years earlier...

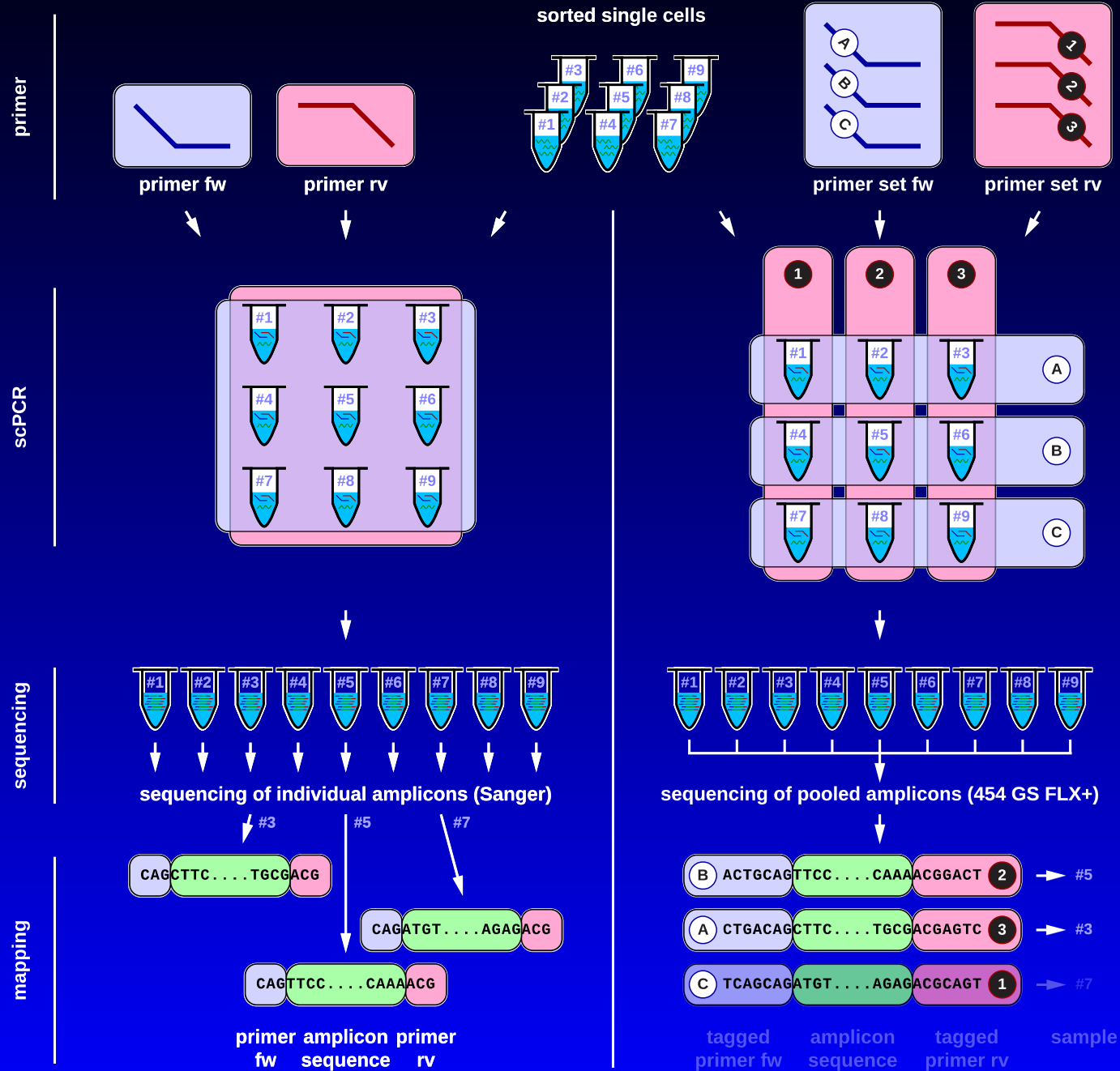What is the size and the structure of the B cell repertoire?

Design criteria

Sequence

Reactivity

100,000x

Phenotype

Conventional scPCR versus primer-matrix scPCR

sorted single cells

primer

primer fw primer rv primer set fw primer set rv

scPCR

sequencing

sequencing of individual amplicons (Sanger) sequencing of pooled amplicons (454 GS FLX+)

mapping

#3
CAG CTTC....TGCG ACG

#5
CAG ATGT....AGAG ACG

CAG TTCC....CAAA ACG

#7

primer amplicon primer
fw sequence rv

B ACTGCAG TTCC....CAAA ACGGACT 2 → #5

A CTGACAG CTTC....TGCG ACGAGTC 3 → #3

C TCAGCAG ATGT....AGAG ACGCAGT 1 → #7

tagged amplicon tagged sample
primer fw sequence primer rv

Experimental workflow

experimental parts

computational parts

animal

tissues

bone marrow · spleen · blood · lymph node · lamina propria · ...

single cell suspensions

single cell FACS sort (indexed)

compensate fluorescence parameters

tissue & phenotype ◄ ► position

single cell RT-PCR

5'-primer (tags encode columns)

3'-primer (tags encode rows)

position ◄ ► tags

sequencing

NGS of pooled & purified products (GS FLX Titanium)

seperate tag & cargo sequence

validate & correct tags

tags ◄ ► cargo sequence

Development cycles

1)   - Matrix-PCR primer panels (mouse)

      - Automated liquid handling (Tecan EVO200 platform)

      - Index Sorting

2)   - Matrix-PCR optimized primer set (human)

      - scPCR efficiency

3)   - Modular data analysis toolkit

      - Relational database

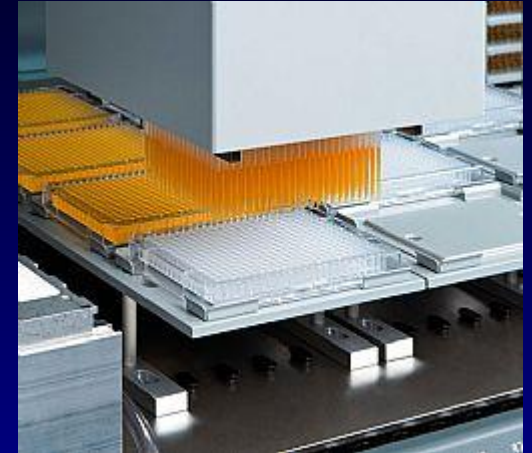sciReptor

[1] Busse et al. (2014) Eur J Immunol

[2] Murugan et al. (2015) Eur J Immunol

[3] Imkeller et al. (accepted) BMC Bioinformatics
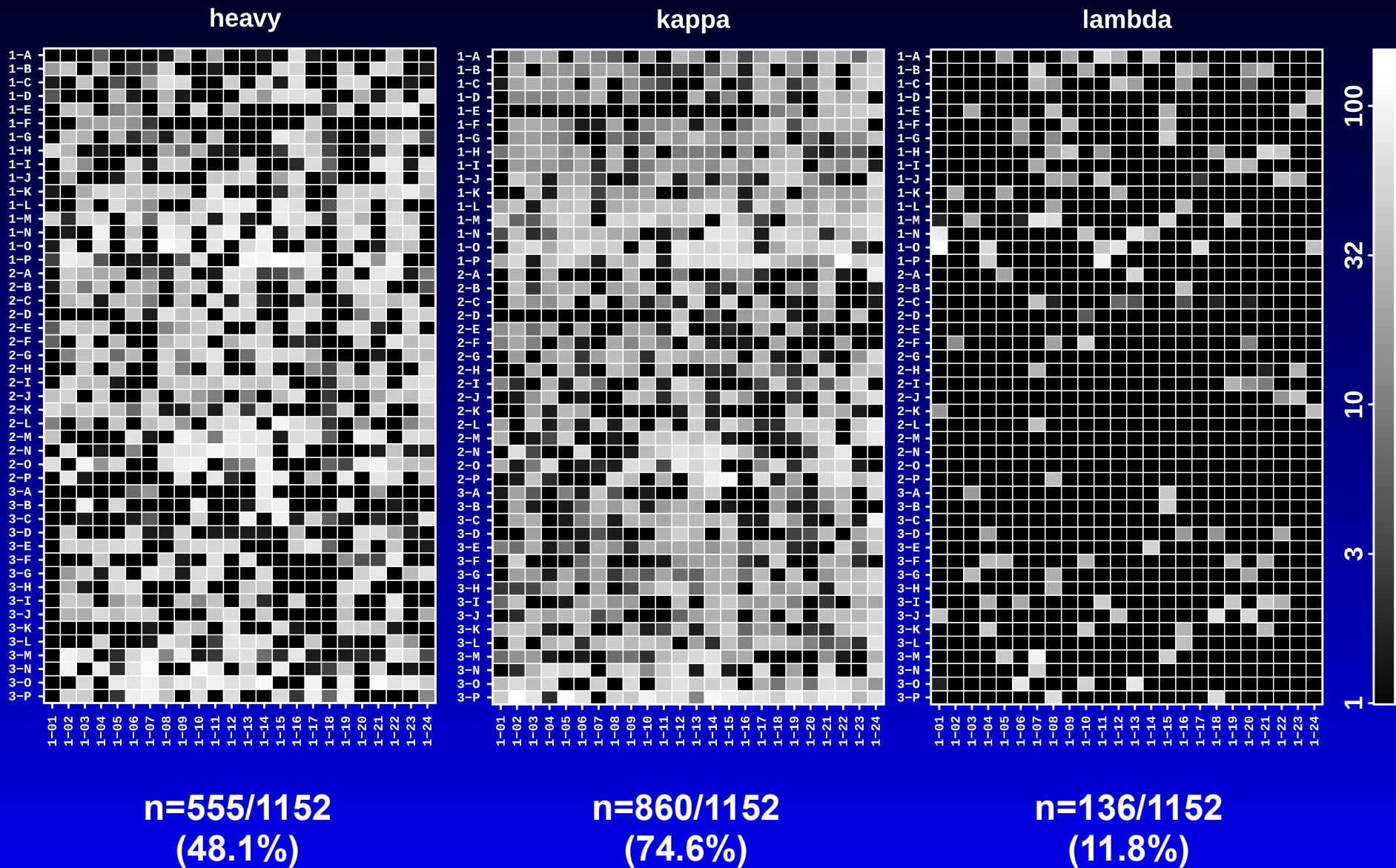
Cost & Throughput

Current state:

• Costs: 1 EUR per cell

• Processing capacity: 10,000 cells per week

• Processing time: 10 days + sequencing time

Ongoing development:

• switch from Roche/454 FLX+ to Illumina MiSeq

• TCR primer sets

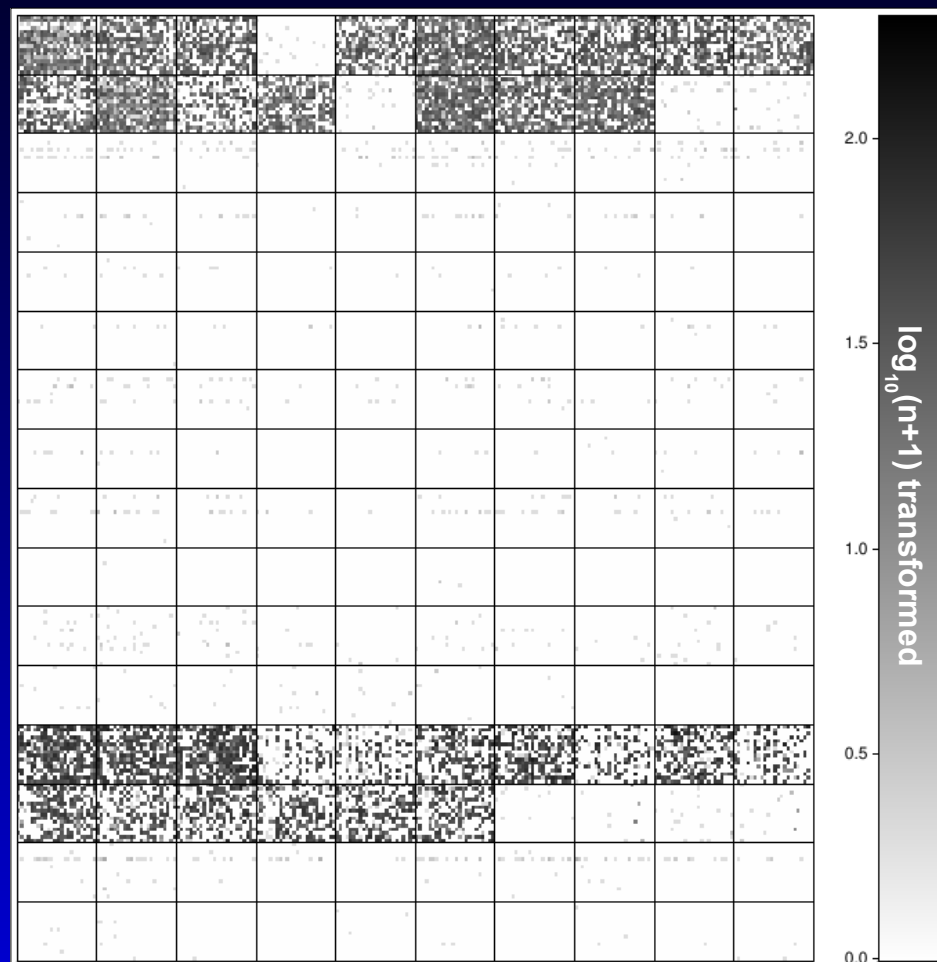# Efficiency of 48x48 (2.25ki) primer matrix scPCR

heavy

kappa

lambda

n=555/1152
(48.1%)

n=860/1152
(74.6%)

n=136/1152
(11.8%)

Efficiency of 240x256 (60ki) primer matrix scPCR
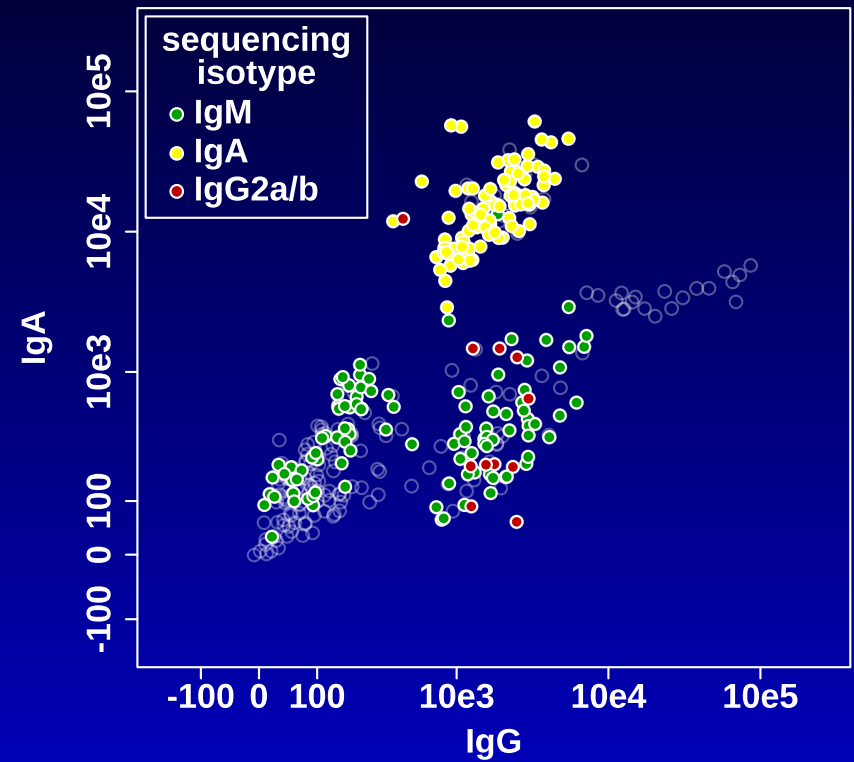
heavy (*Igh*)

kappa (*Igk*)

40.3%
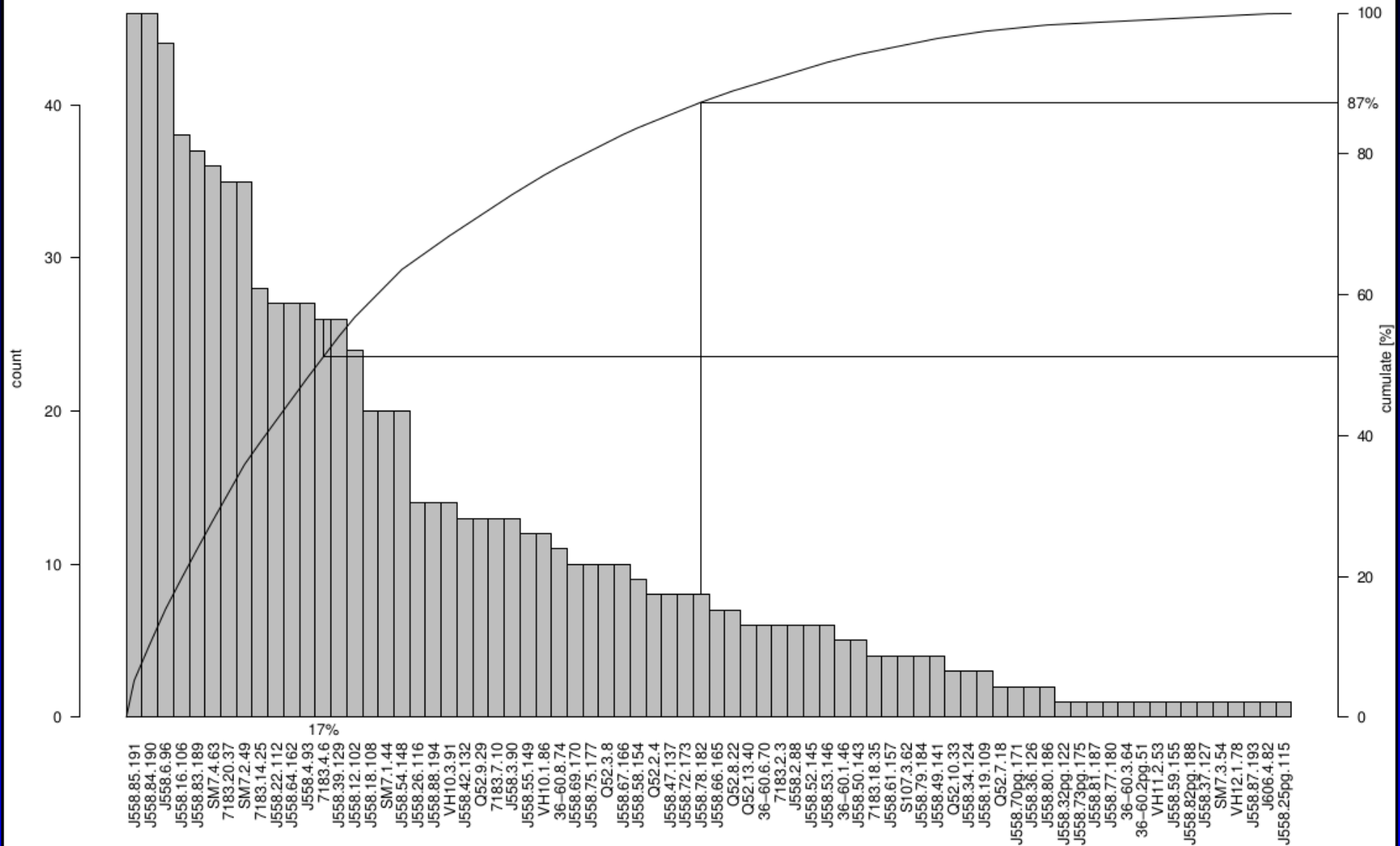**(n=12288)**
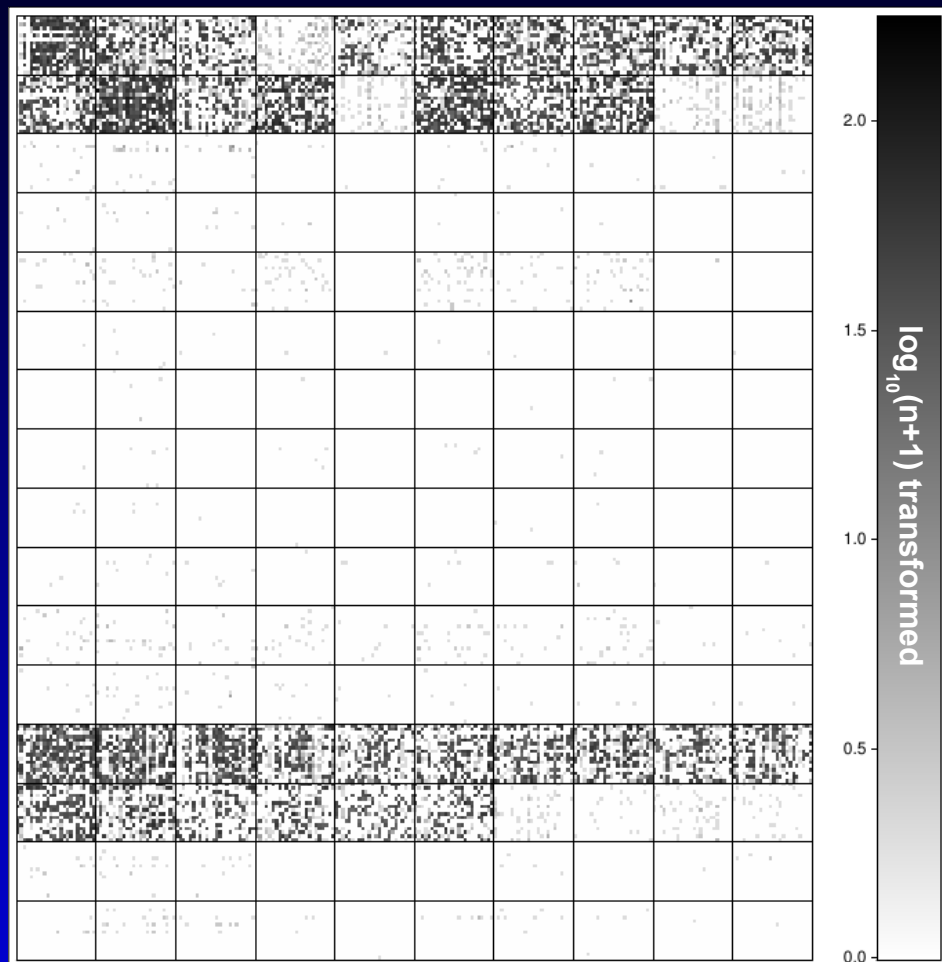
53.1%
(n=12288)

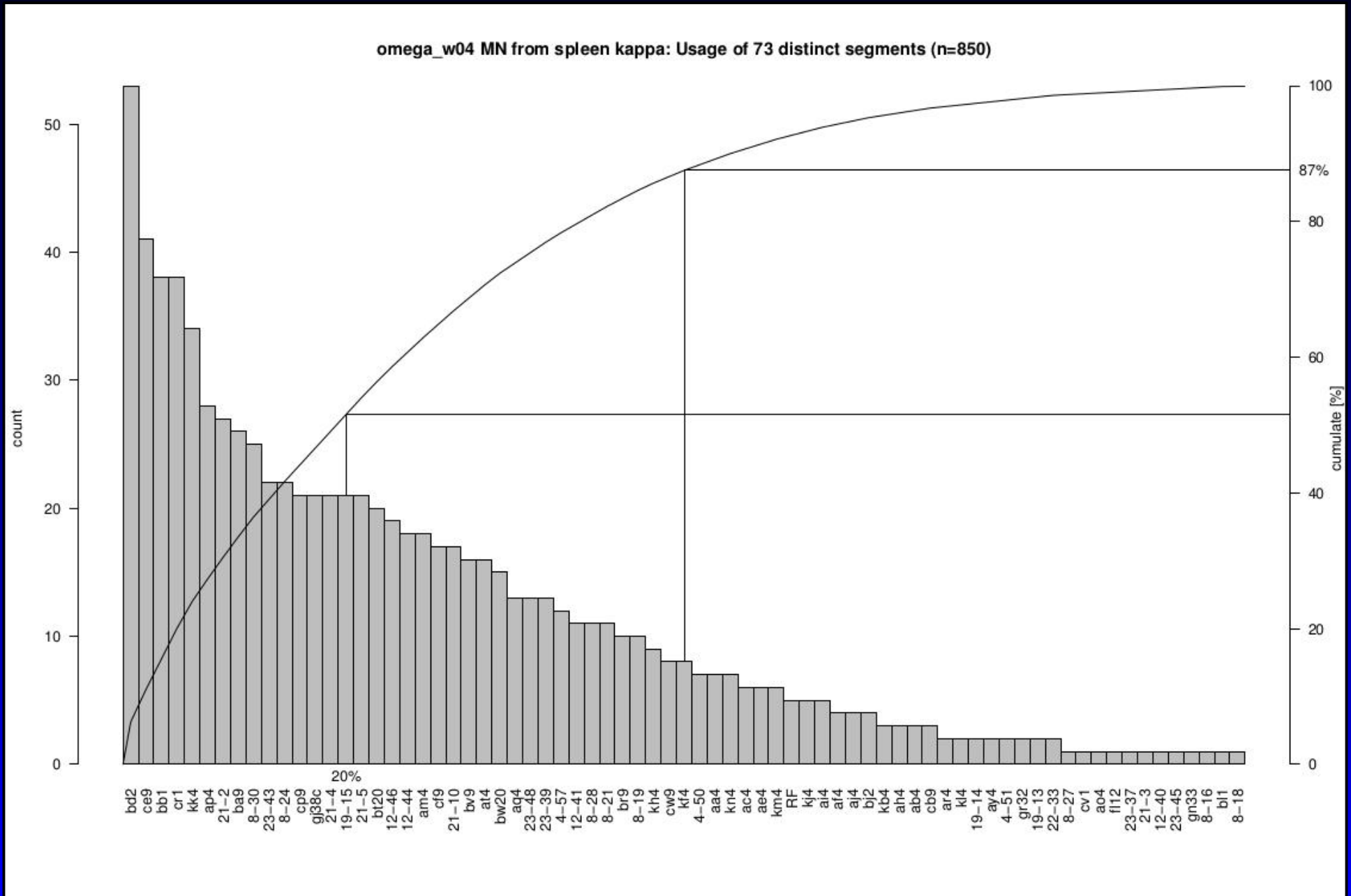# Overlay flow cytometric & sequencing data
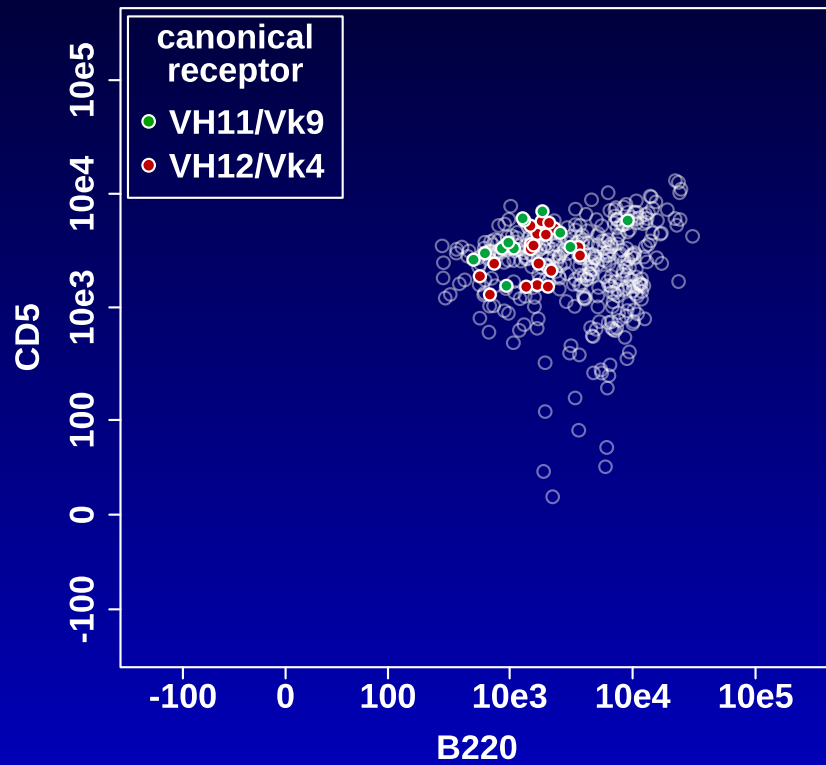
Igh-V segment usage in mature naive B cells

omega_w04 MN from spleen heavy: Usage of 74 distinct segments (n=884)

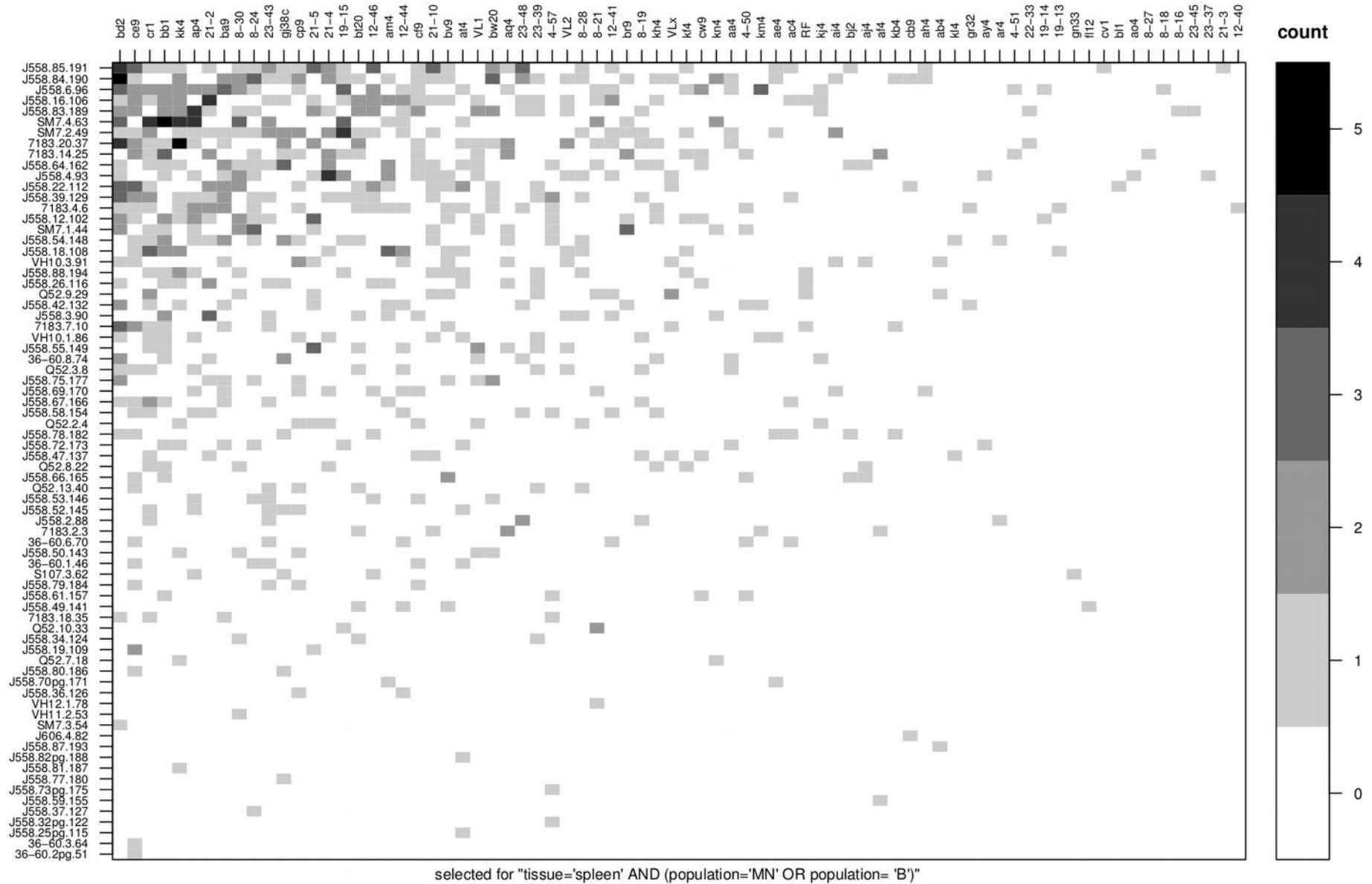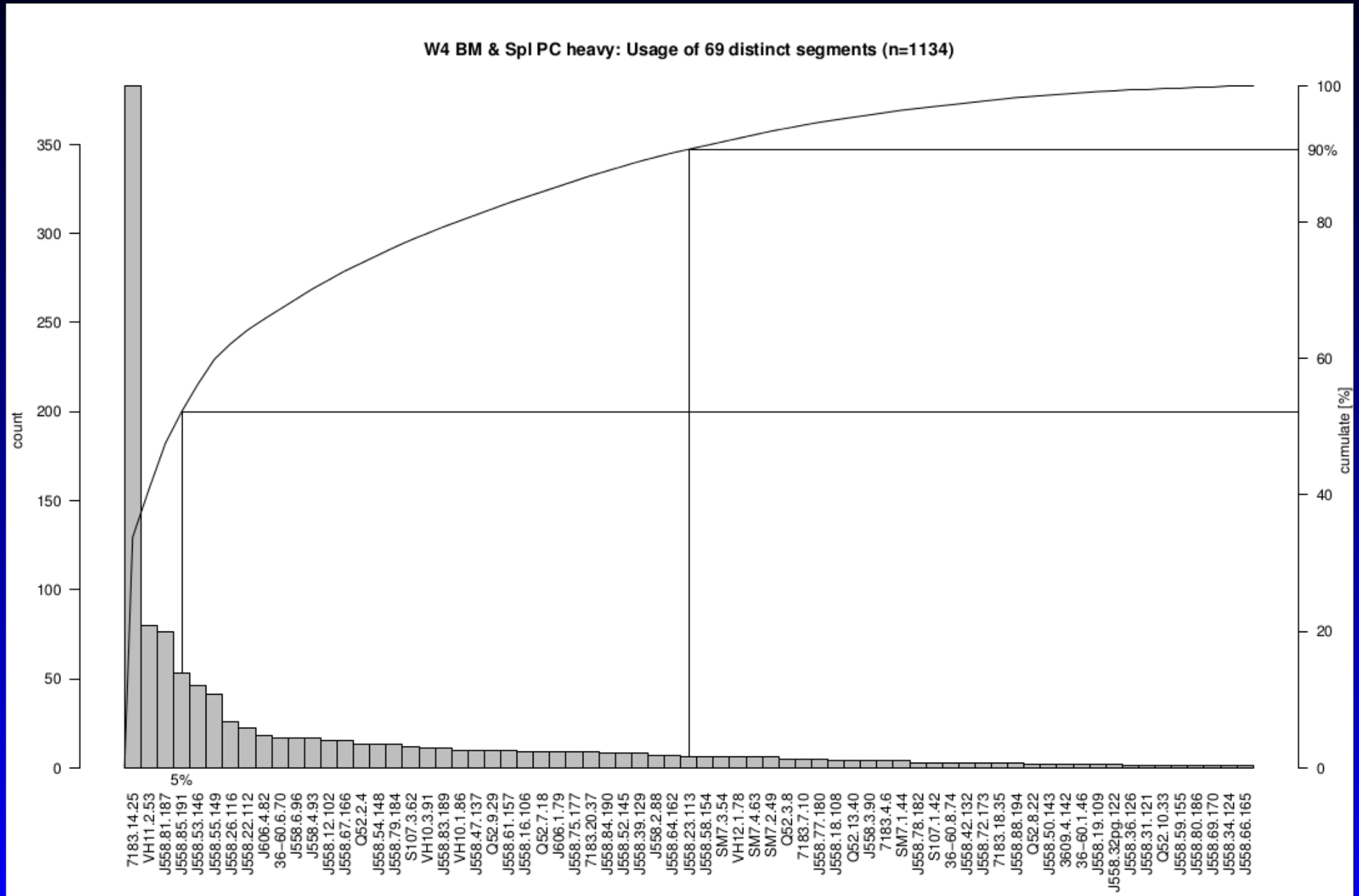# *Igk*-V segment usage in mature naive B cells

omega_w04 MN from spleen kappa: Usage of 73 distinct segments (n=850)

Igh-V/*Igk*-V segment pairing in mature naive B cells

omega_w04 (n=884)

selected for "tissue='spleen' AND (population='MN' OR population= 'B')"

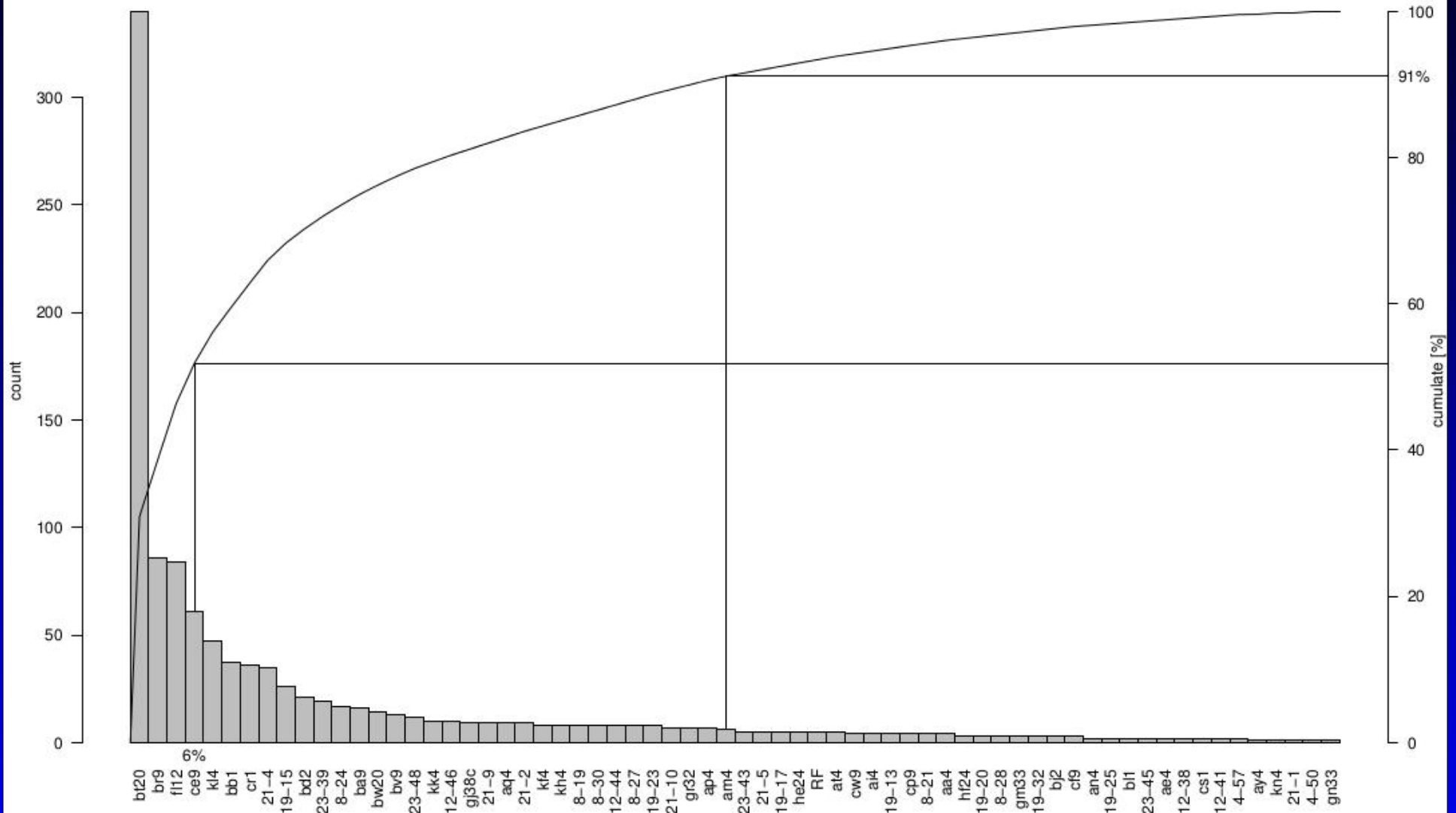# Igh-V segment usage in plasma cells

W4 BM & Spl PC heavy: Usage of 69 distinct segments (n=1134)

Igk-V segment usage in plasma cells

W4 BM & Spl PC kappa: Usage of 66 distinct segments (n=1101)

Phylogeny of a PC cluster (J558.81.187:fl12) (VH+Vk)

Plasma cells, bone marrow and spleen. Clones: J558.81.187:JH2:16:fl12:JK2:9and8, count total 76
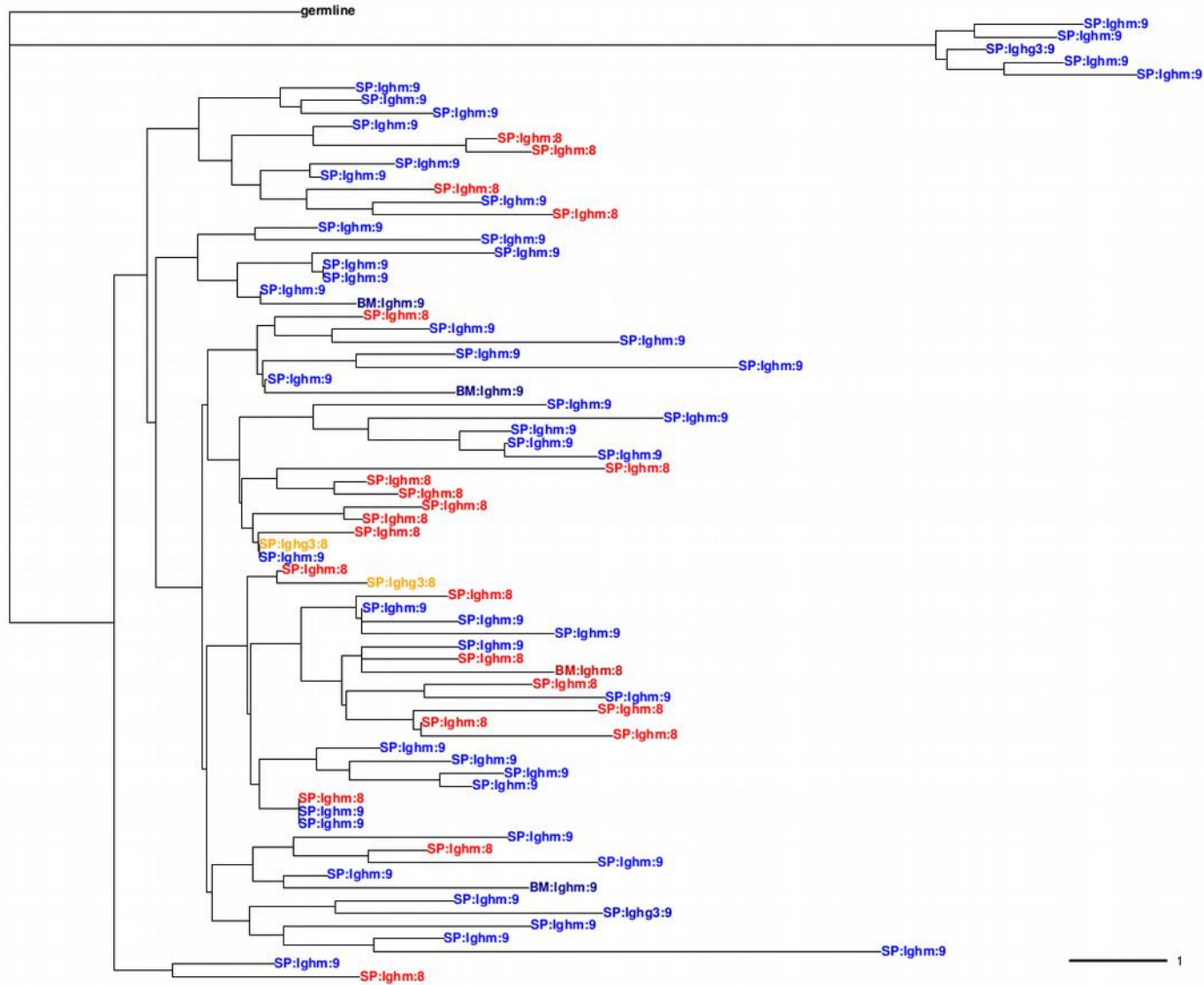
Phylogeny of a PC cluster (J558.81.187:fl12) (VH only)

Plasma cells, bone marrow and spleen. Clones: J558.81.187:JH2:16:fl12:JK2:9and8, count total 76, tree on heavy sequence
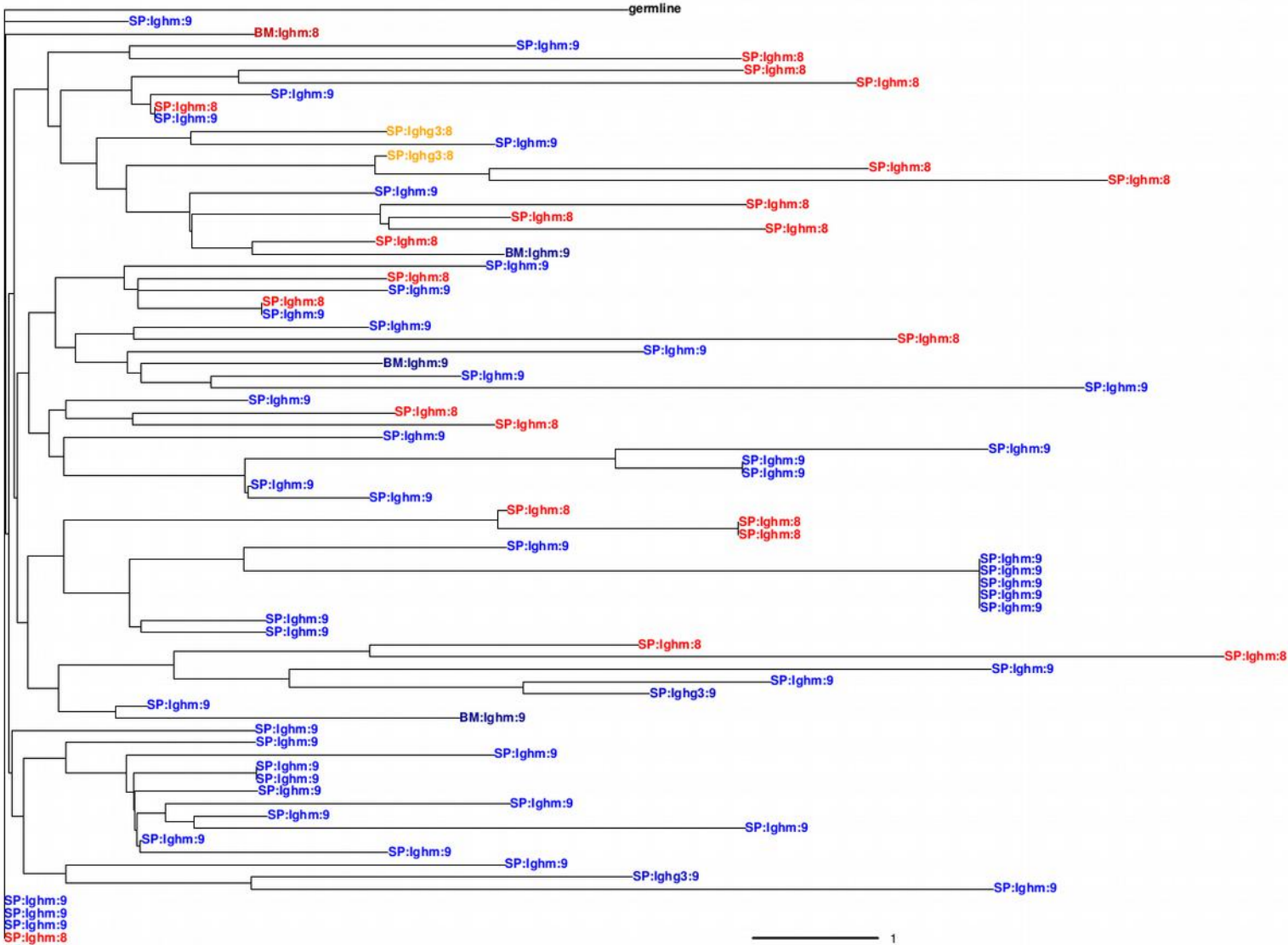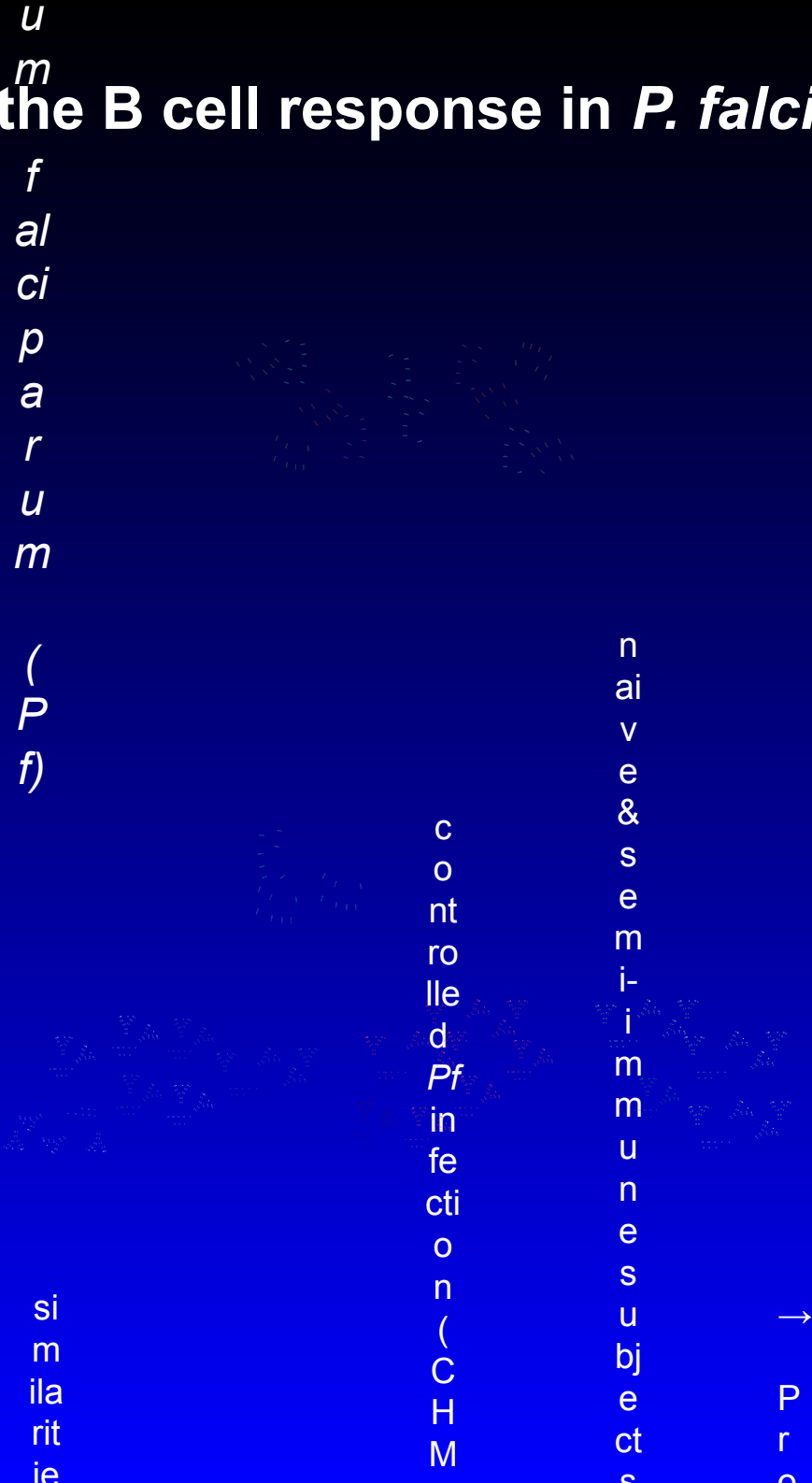
Phylogeny of a PC cluster (J558.81.187:fl12) (*Igk*-V only)

Plasma cells, bone marrow and spleen. Clones: J558.81.187:JH2:16:fl12:JK2:9and8, count total 76, tree on kappa sequence without CDR3

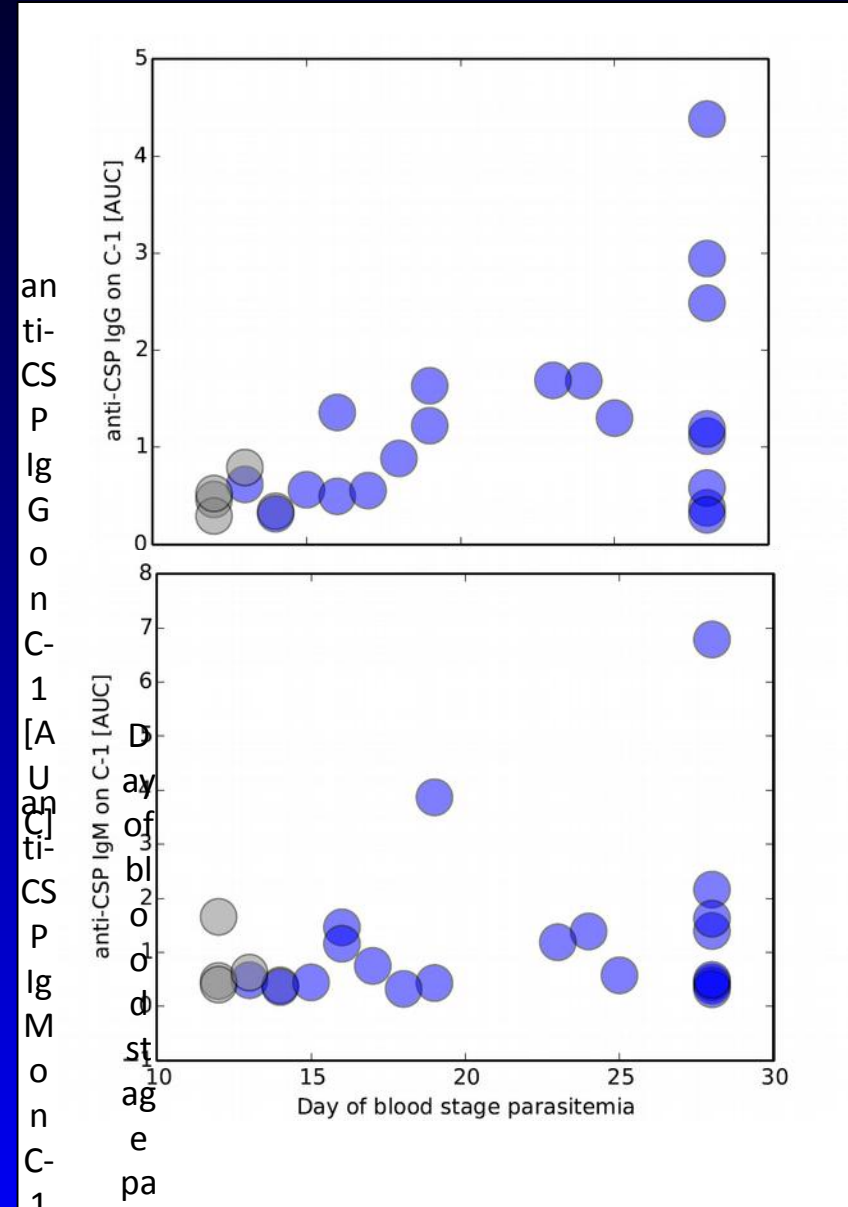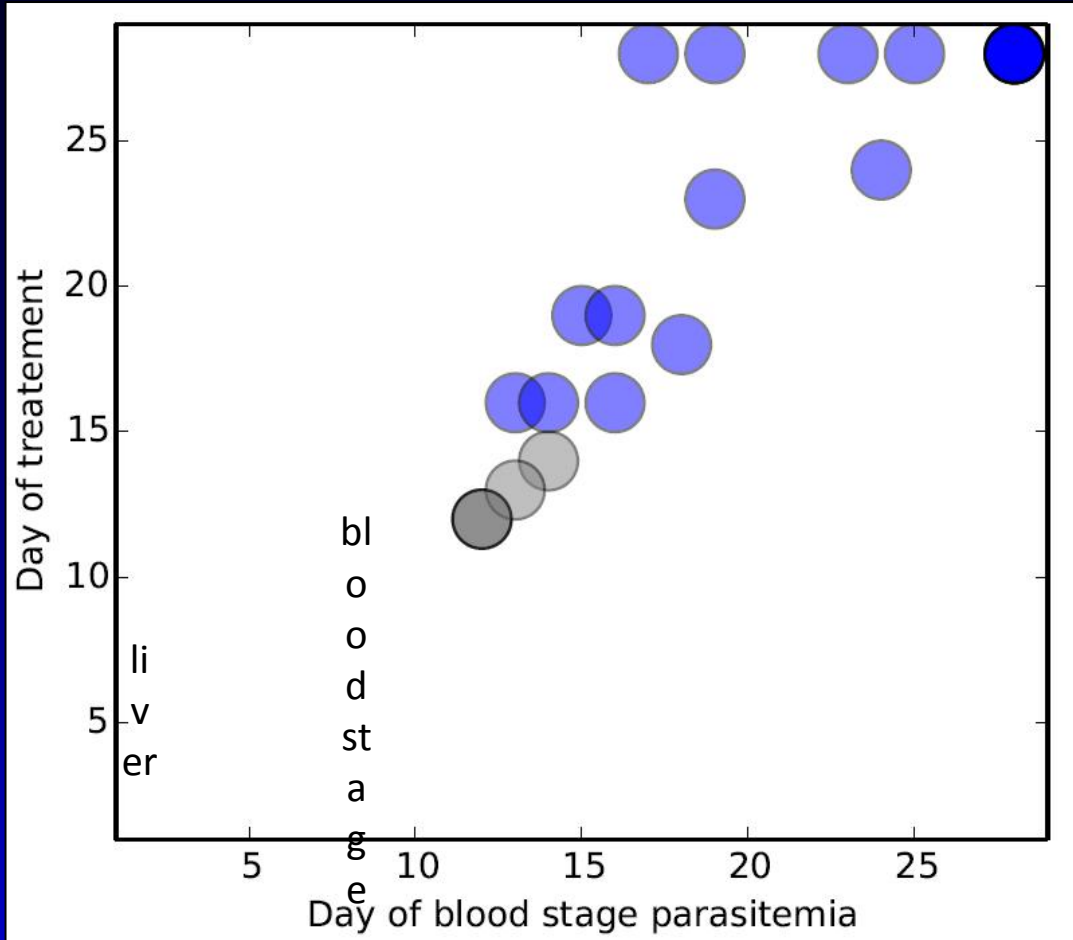# Diversity of the B cell response in *P. falciparum* infection

CHMI in Gabonese subjects shows different levels of protection

Similarity of the antibody response in CHMI donors

- Quantification of si

Legend:
- General memory (black)
- Malaria specific (blue)
- Difference (red)

Heavy chain similarity

Kappa chain similarity

Lambda chain similarity

Shared responses of *Pf* specific B cells
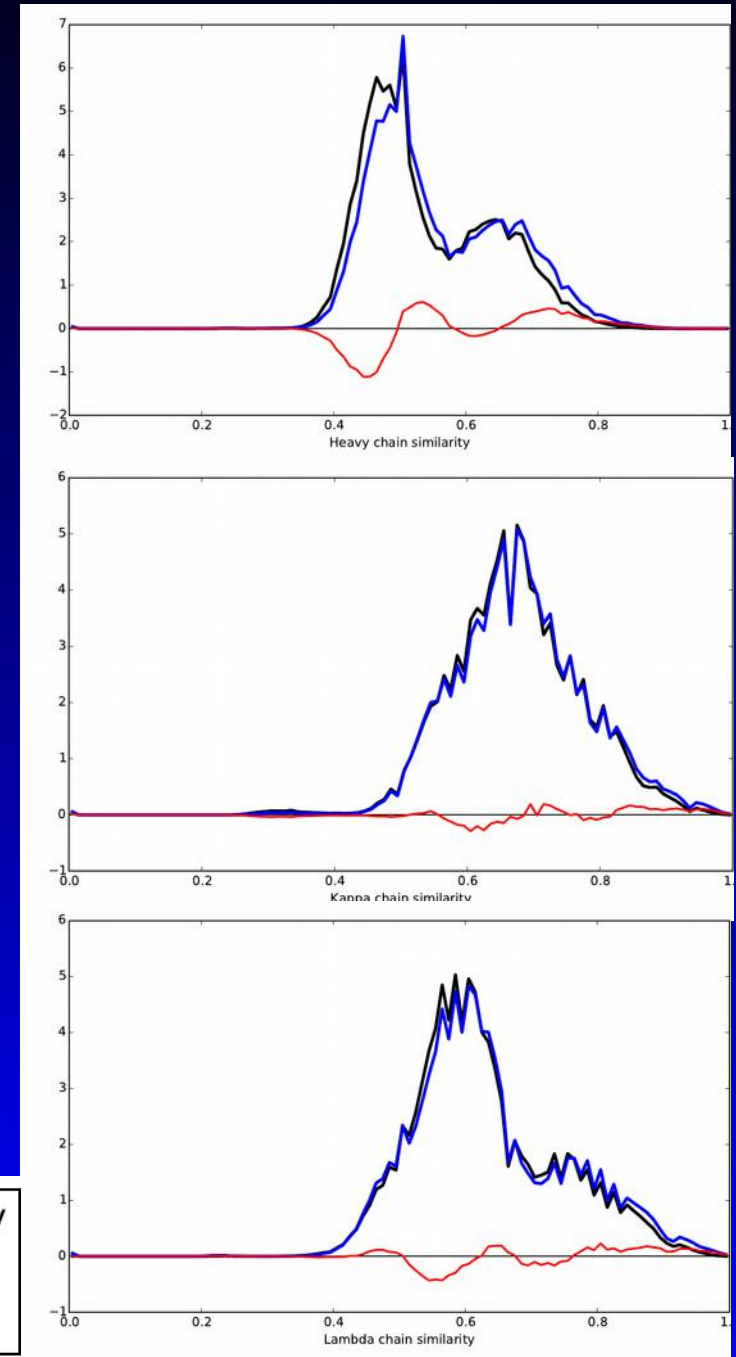
Similarity of the antibody response in CHMI donors
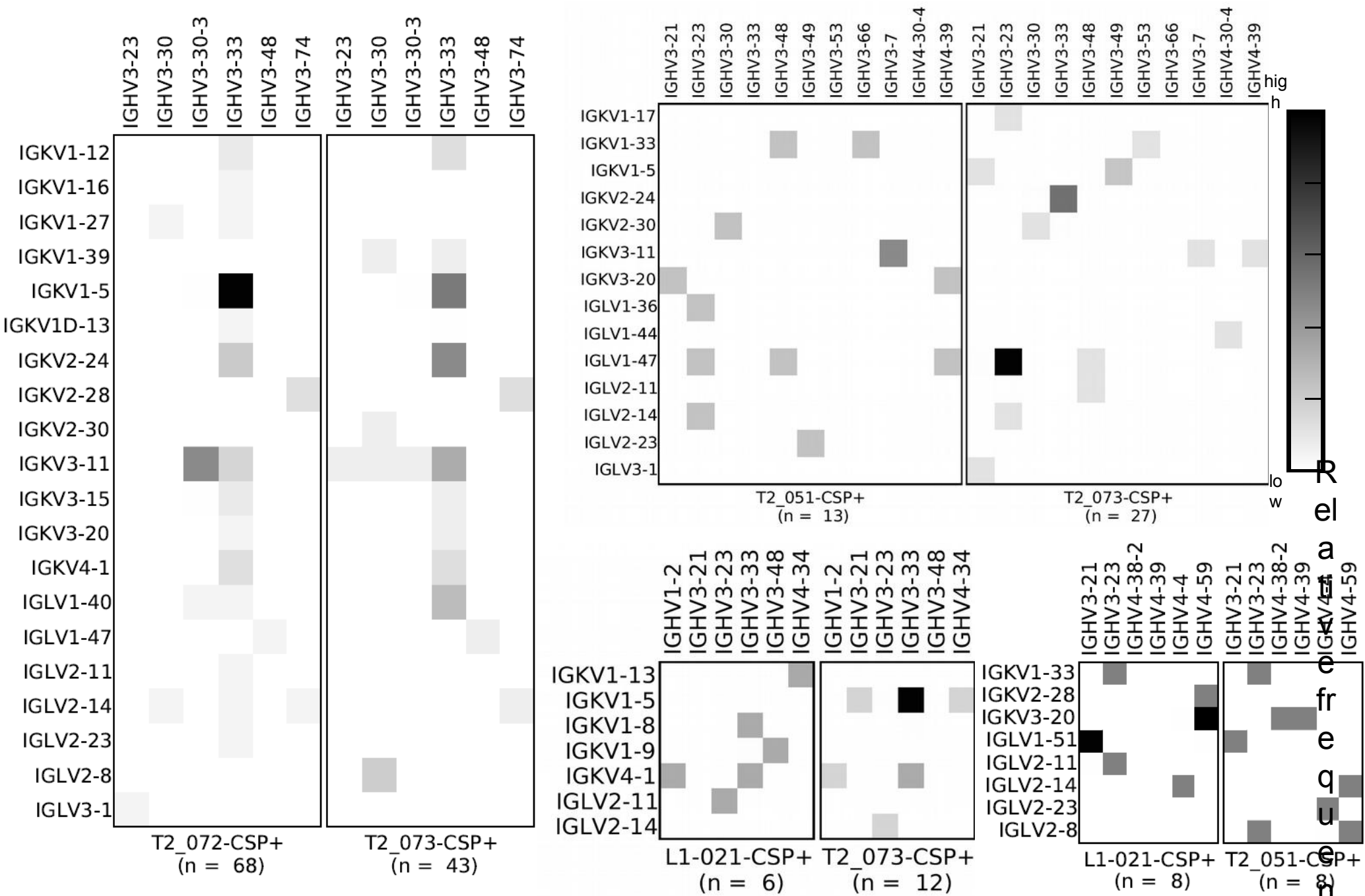
sciReptor is free/open software

- licenced as Affero GPL3
- hosted at Github:

https://github.com/b-cell-immunology

Thank you for your attention!