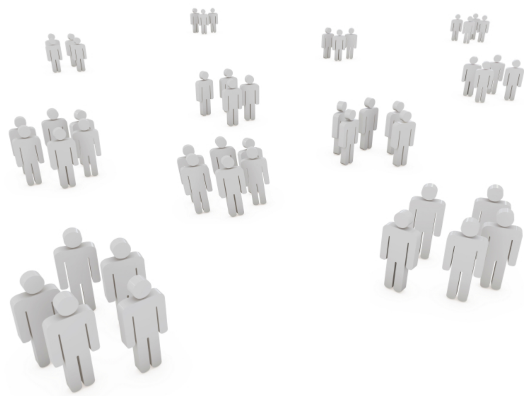
Call of the wild: a new generation of antibody discovery from human populations

Jacob Glanville, CSO
Distributed Bio

Two main types of libraries, and two main ways to create them



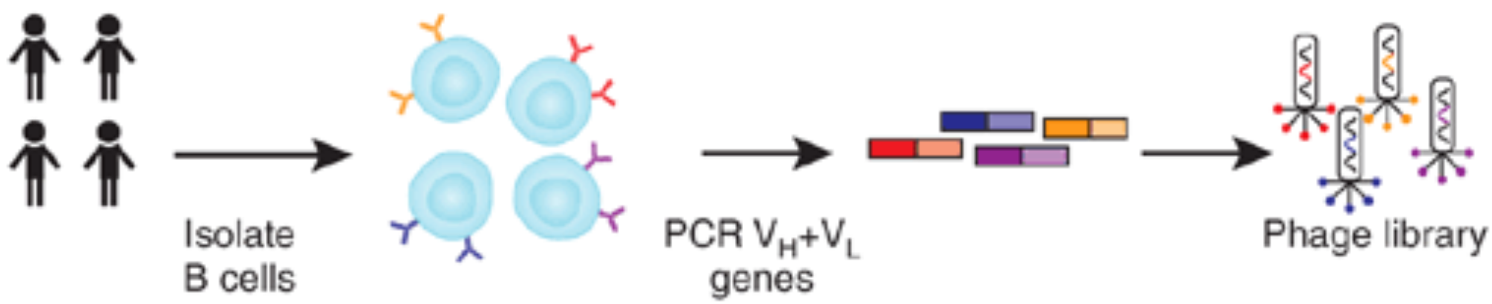
Primary discovery library

Targeted library

Natural diversity

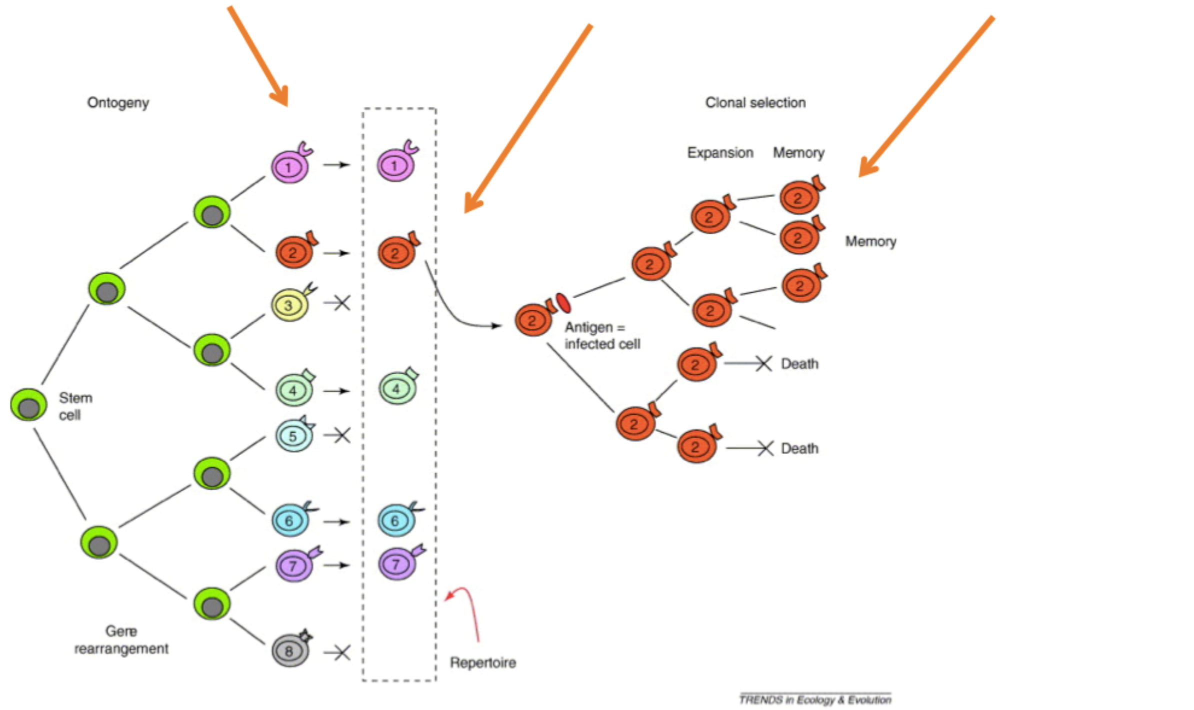
Synthetic diversity

Natural diversity

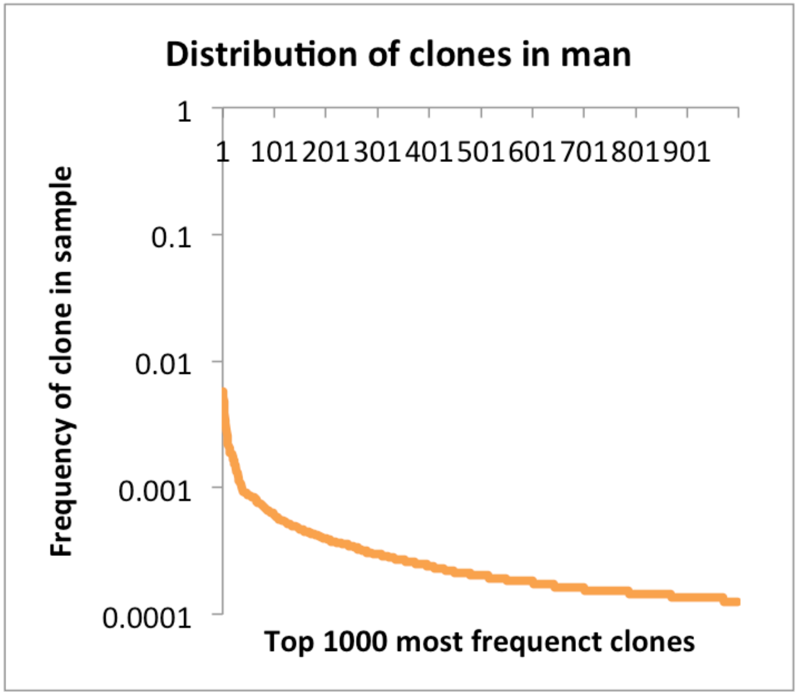


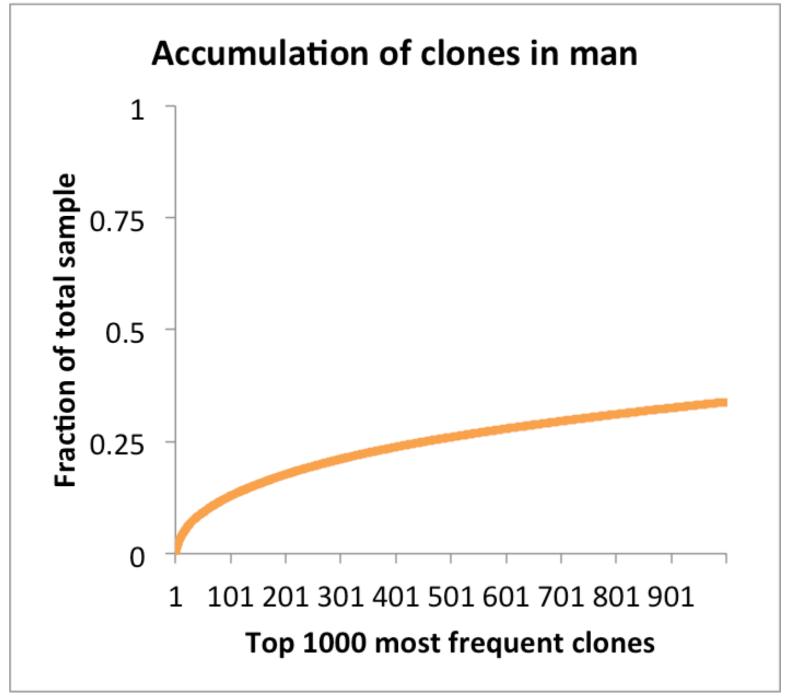
How immunology poisons a natural library

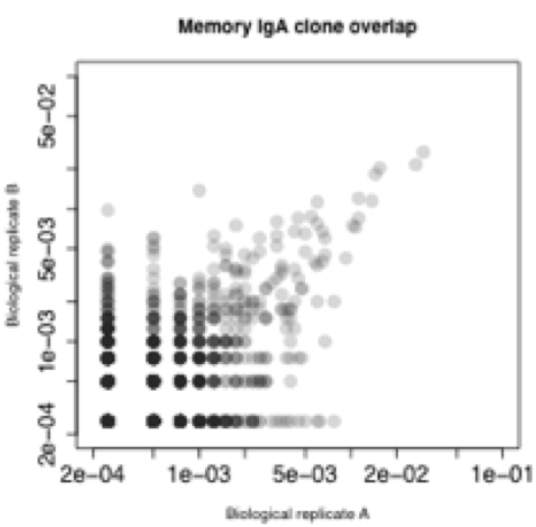
V(D)J recombination antigen selection somatic hypermutation



How immunology poisons a natural library

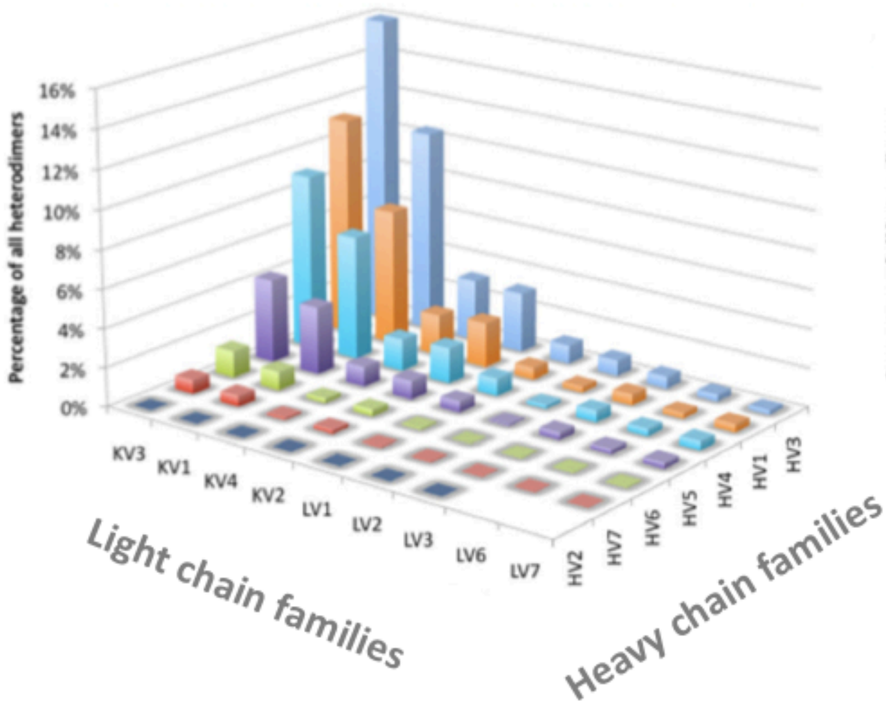




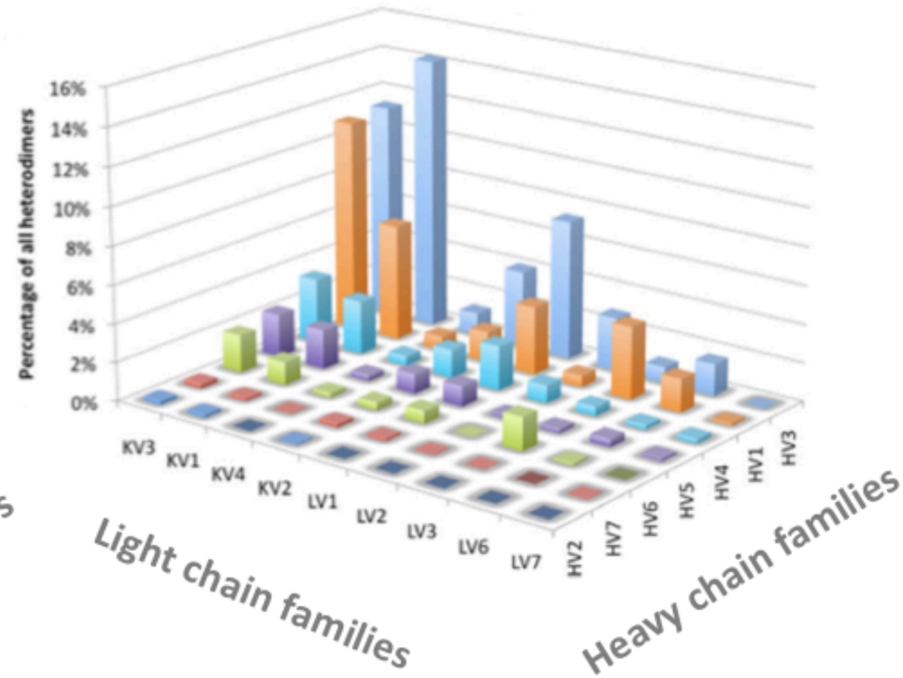


In-vivo immunology translates imperfectly to phage

From NGS sequencing of library



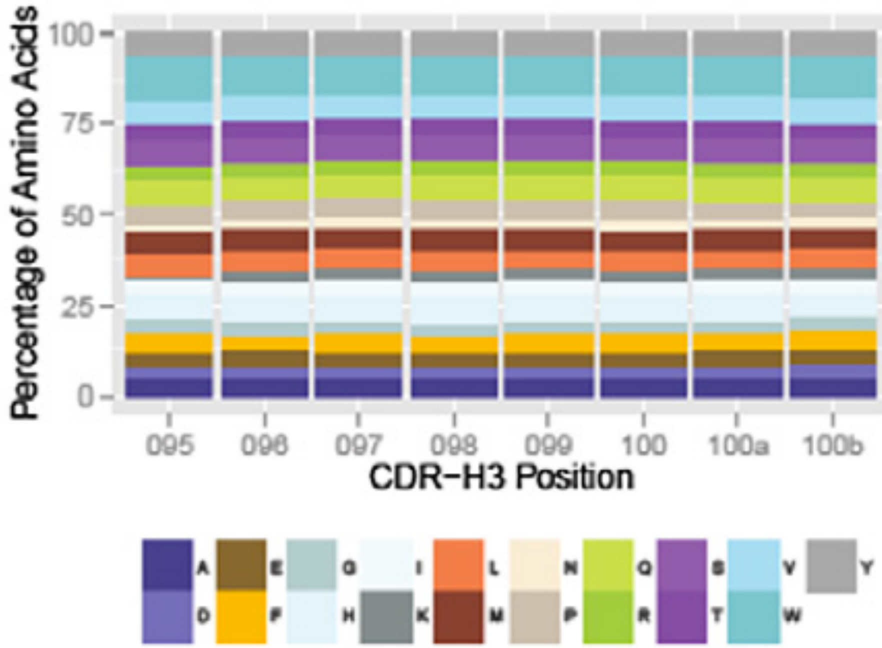
From >20k antigen-specific clones



Glanville et al, 2009 PNAS

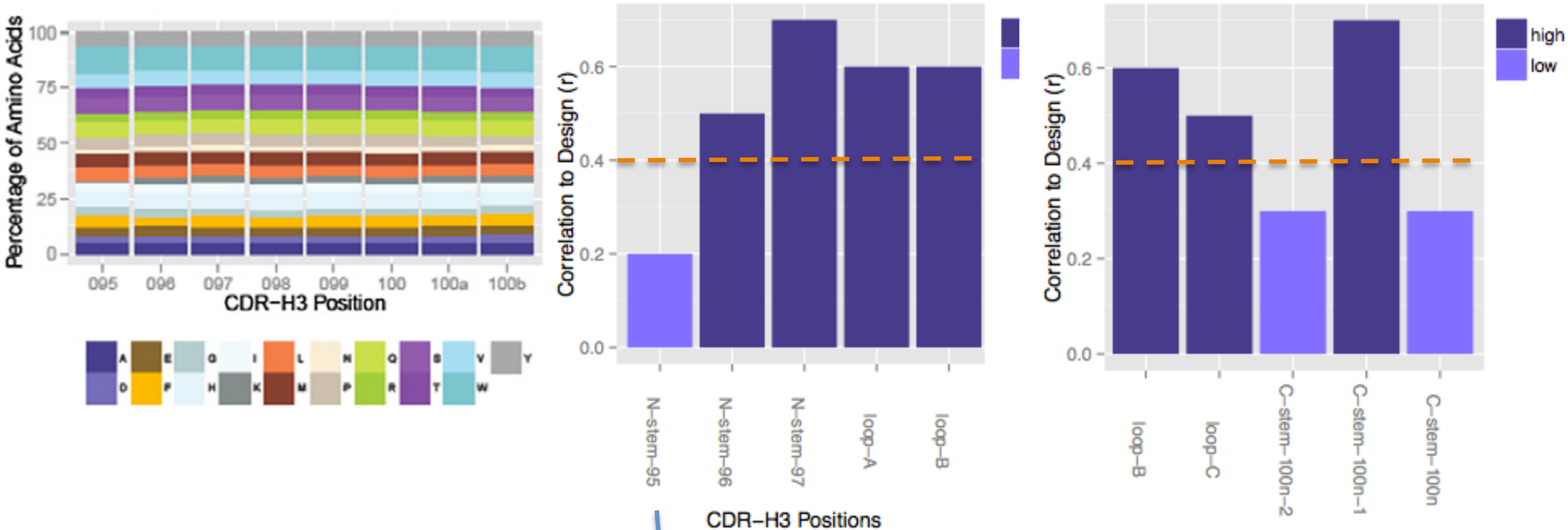
Synthetic diversity

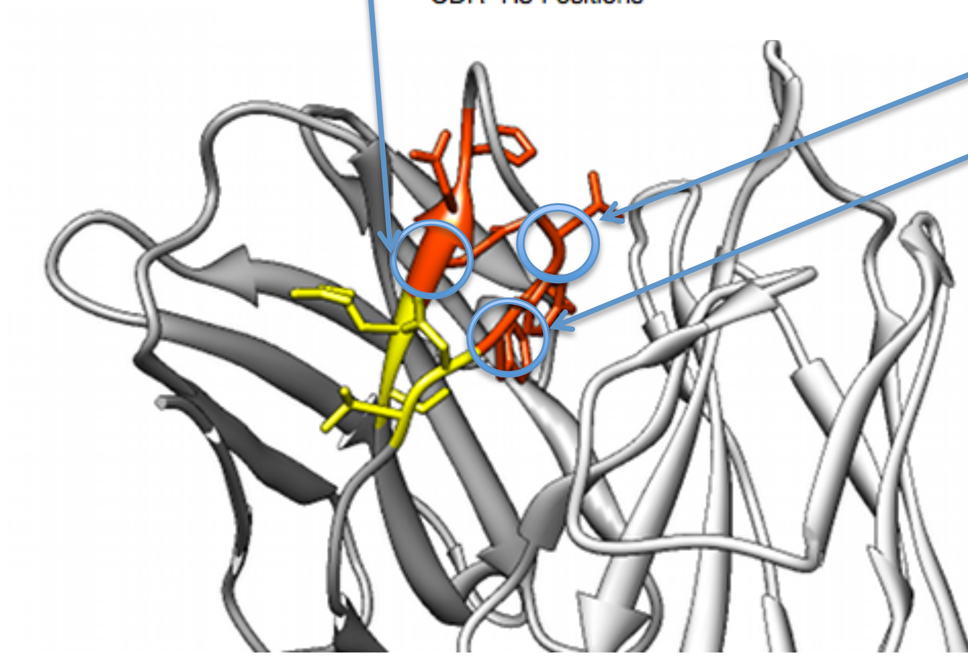
How lack of immunology poisons a synthetic library



Mahon, Laburt, Glanville, et al. "Comprehensive interrogation of a minimalist synthetic CDR-H3 library and its ability to generate antibodies with therapeutic potential." Journal of molecular biology (2013).

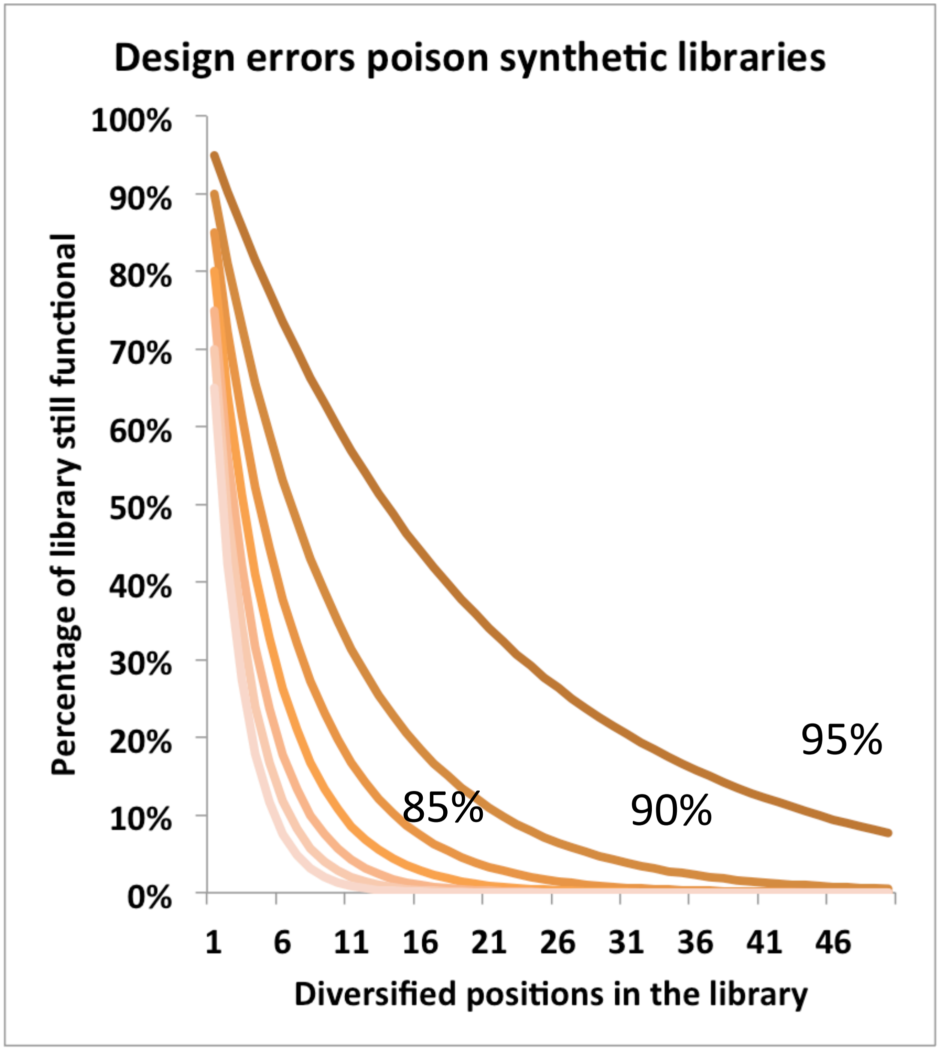
Non-natural diversity not tolerated in many positions

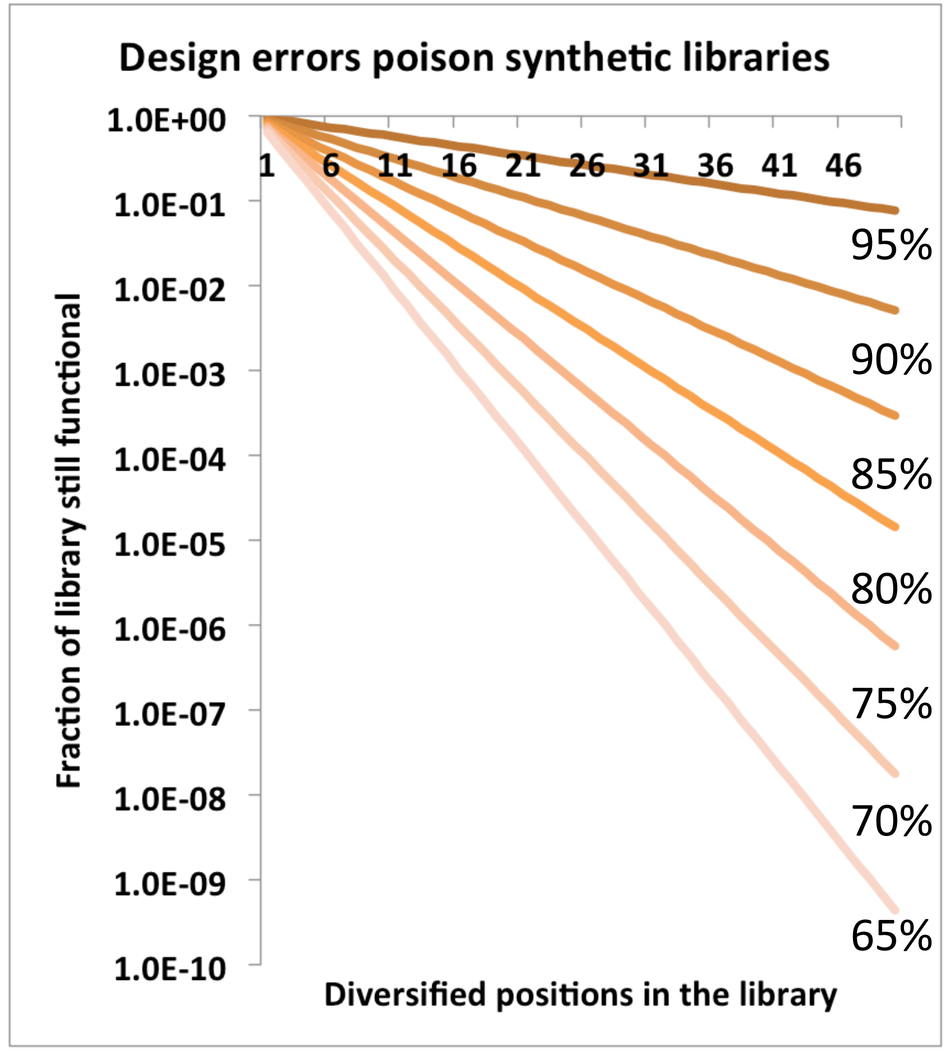


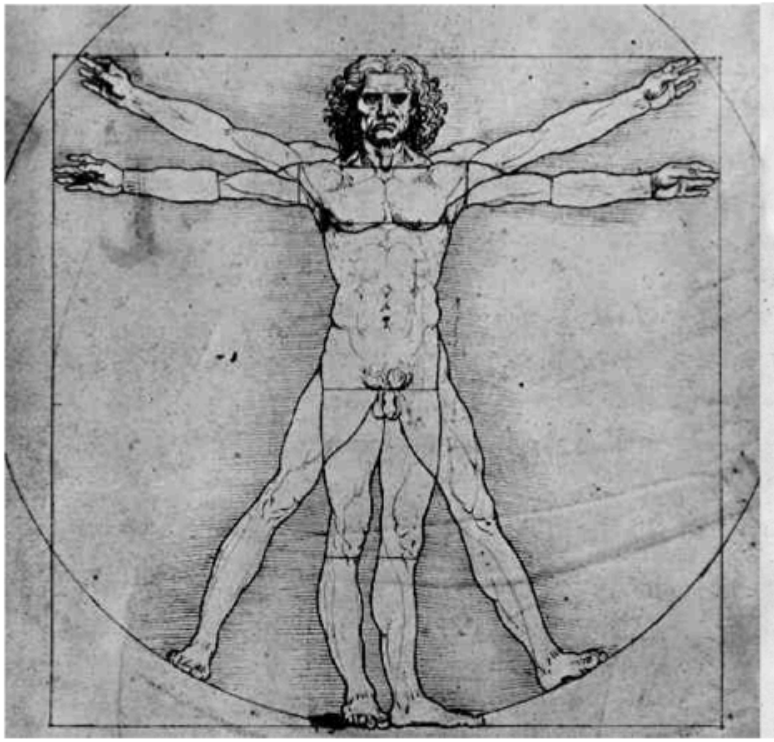


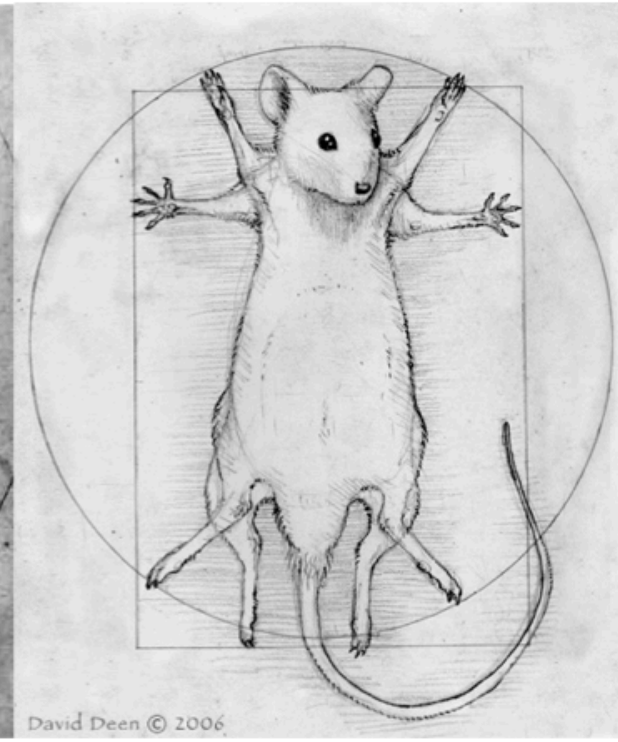
Mahon, Laburt, Glanville, et al. "Comprehensive interrogation of a minimalist synthetic CDR-H3 library and its ability to generate antibodies with therapeutic potential." Journal of molecular biology (2013).

How the accumulation of small errors poisons a synthetic library

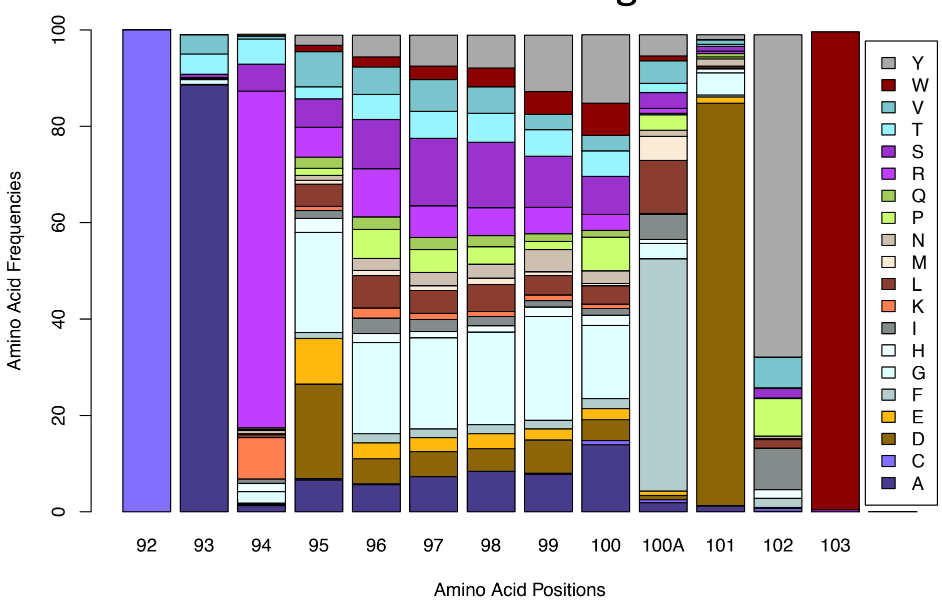




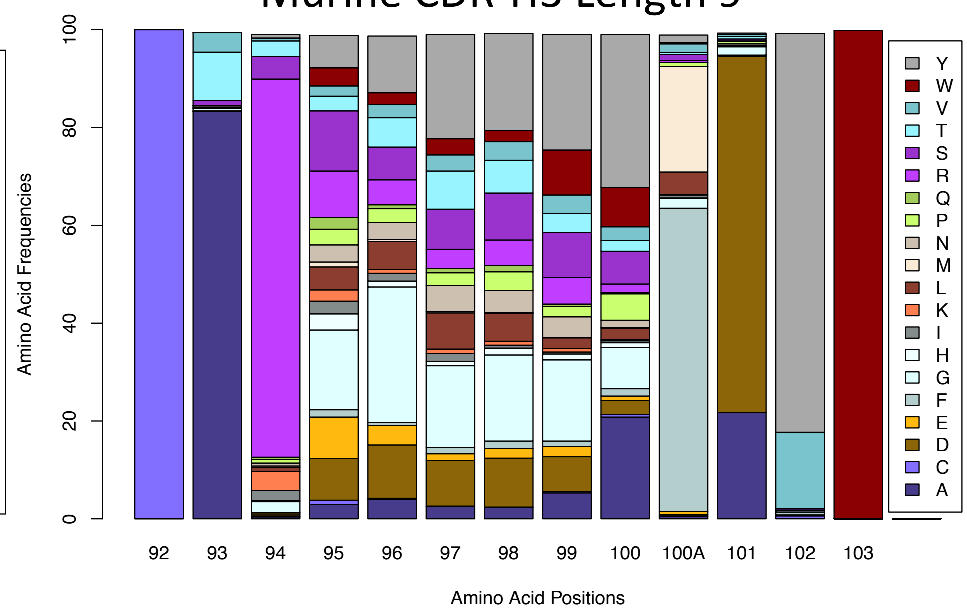




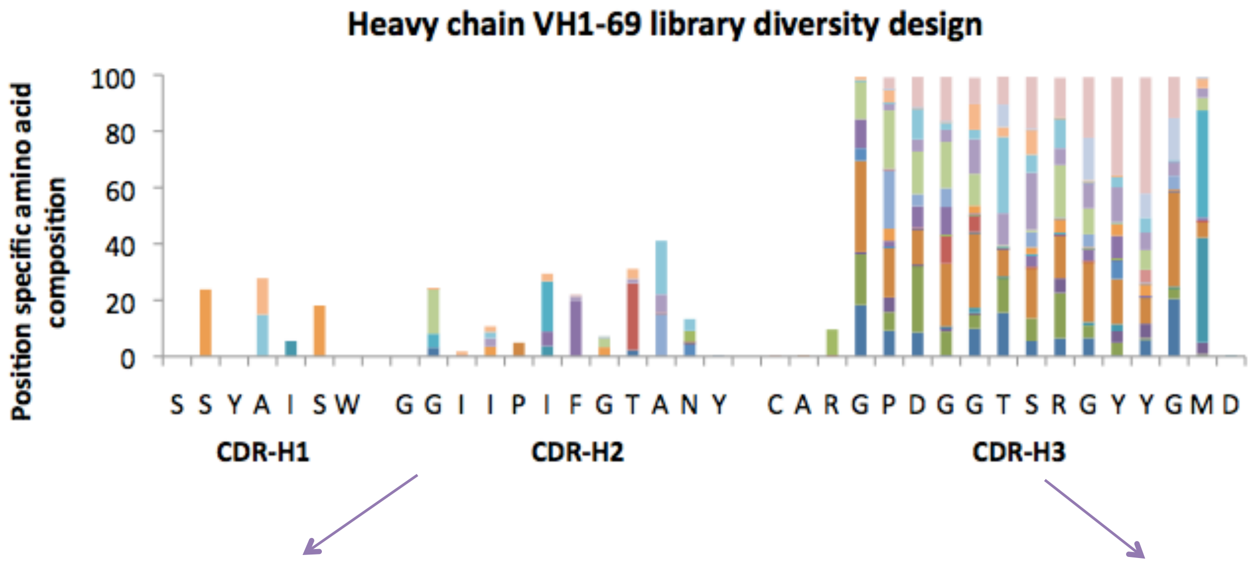
Human CDR-H3 Length 9

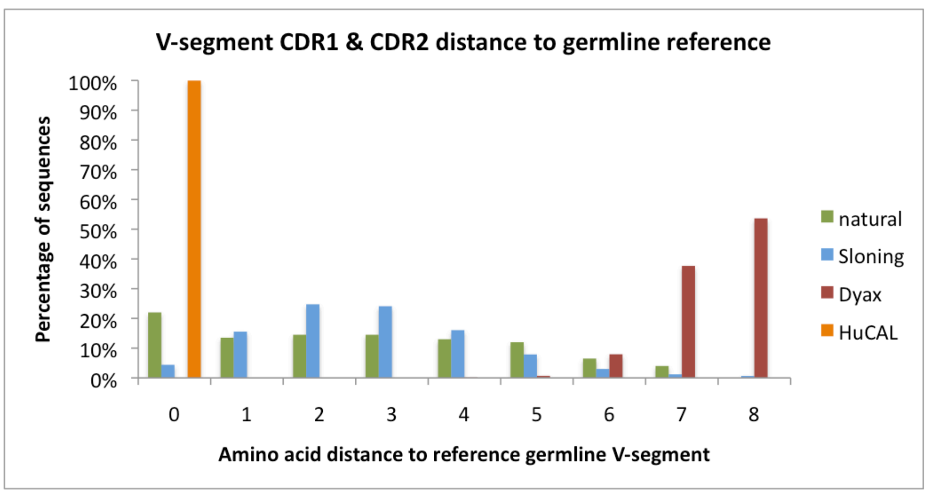


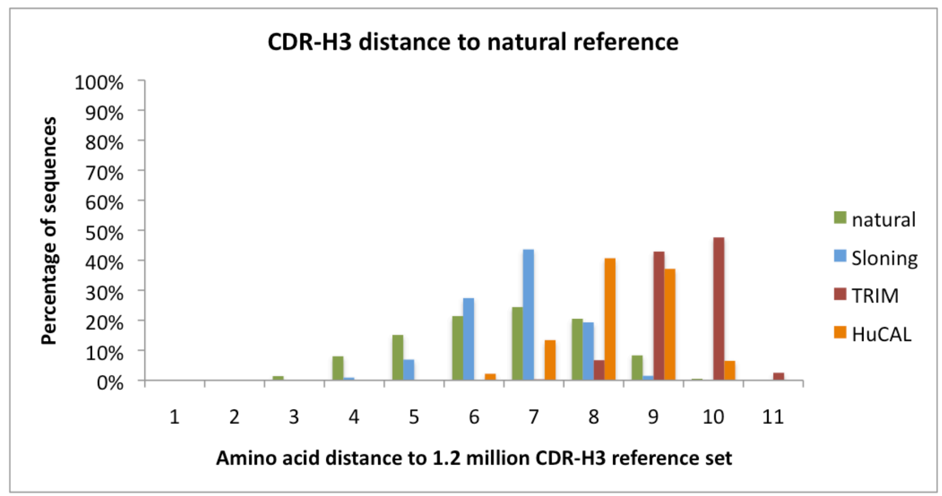
Murine CDR-H3 Length 9



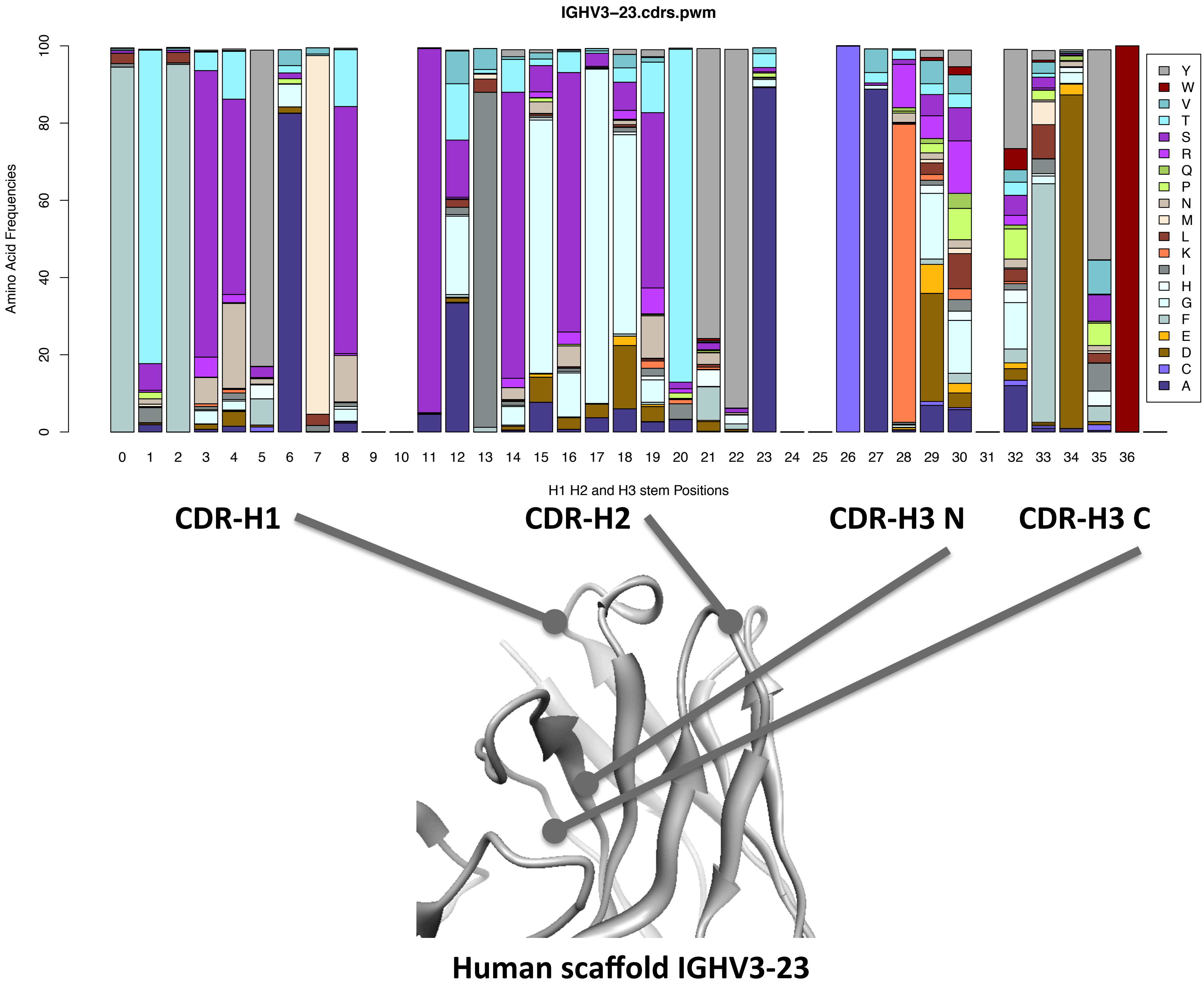
Synthetic libraries designed on natural sequence landscapes



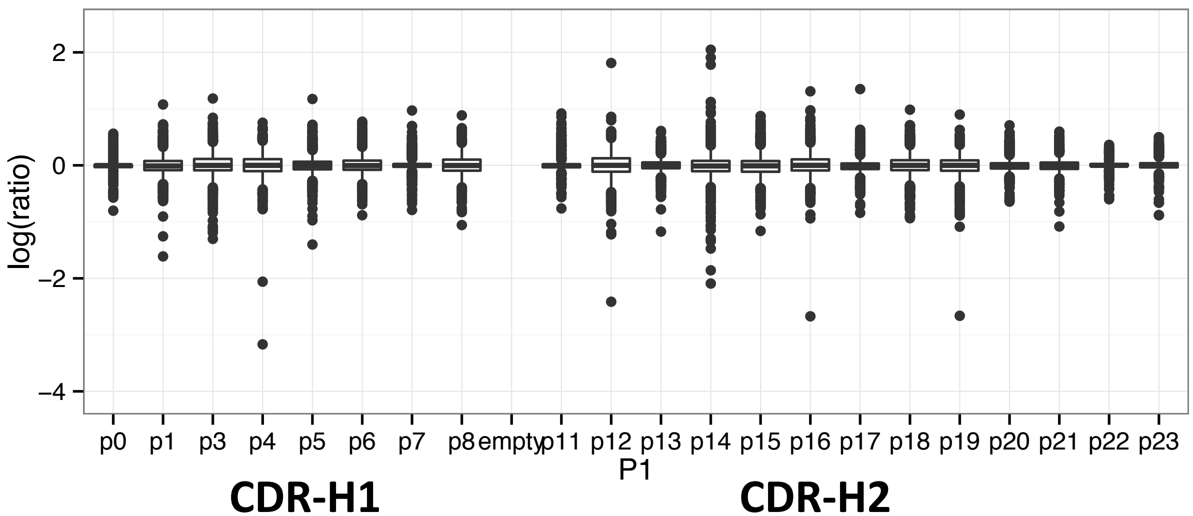




Zhai & Glanville et al, "Synthetic antibodies designed on natural sequence landscapes", Journal of Molecular Biology, 2011

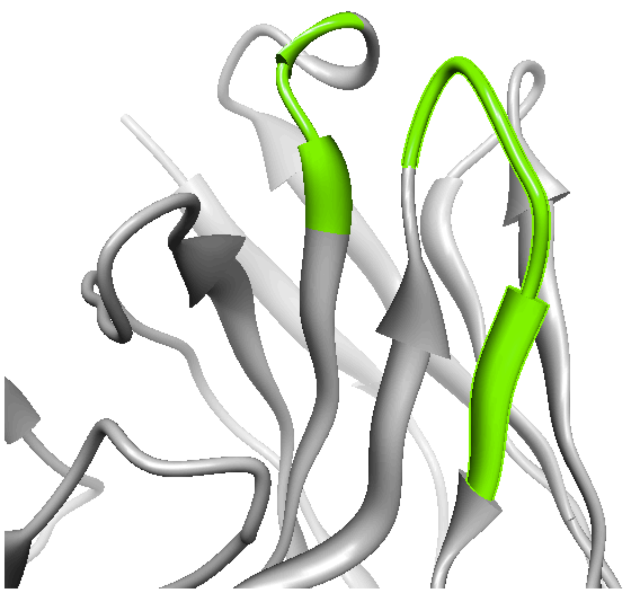

IGHV3−23.cdrs.pwm
Amino Acid Frequencies
0
20
40
60
80
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
H1 H2 and H3 stem Positions
Y
W
V
T
S
R
Q
P
N
M
L
K
I
H
G
F
E
D
C
A
CDR-H1
CDR-H2
CDR-H3 N
CDR-H3 C
Human scaffold IGHV3-23

Observed vs expected paired mutation frequencies

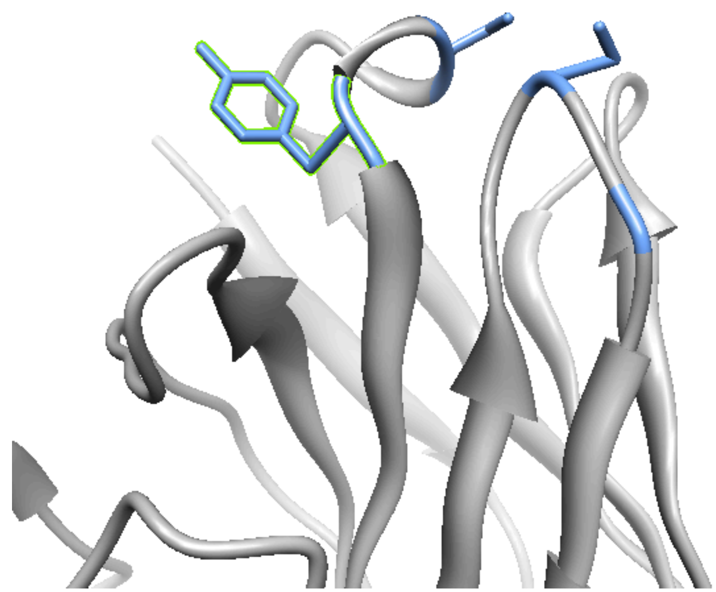


IGHV3-23 positional biases

30	31	N	I	326.5%	
31	33	N	S	4.2%	N-linked glycosylation site
32	29	F	F	323.6%	
51	53	A	Y	8.9%	
51	53	V	Y	611.9%	
53	54	Y	S	774.3%	
53	54	Y	G	594.1%	
53	57	Y	S	674.1%	
54	56	N	S	0.0%	N-linked glycosylation site
55	57	D	V	370.8%	
55	57	N	S	6.9%	N-linked glycosylation site
55	57	N	T	0.0%	N-linked glycosylation site
56	59	S	I	385.8%	
58	60	N	S	7.0%	N-linked glycosylation site

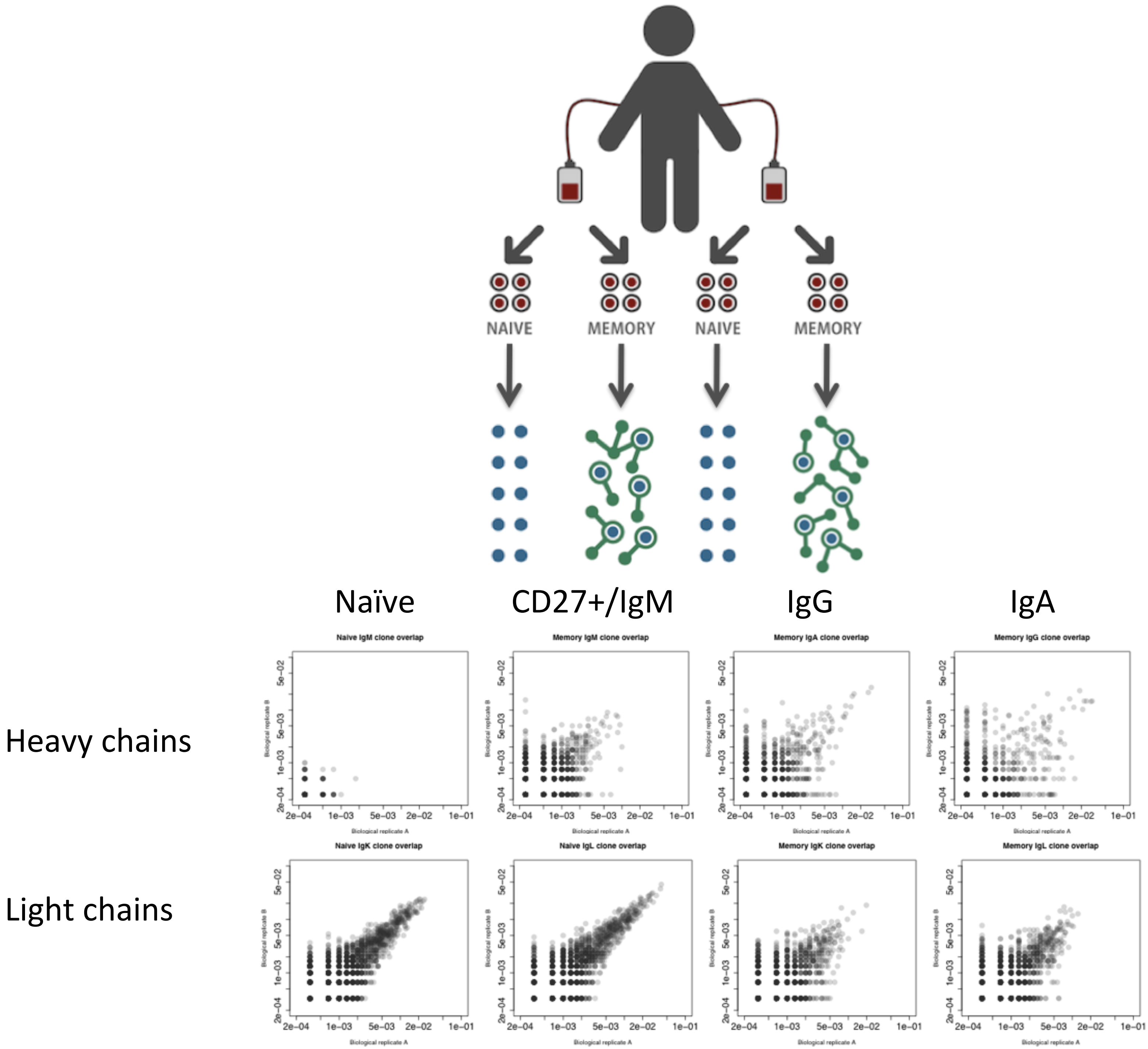


N-linked glycosylation aversion sites

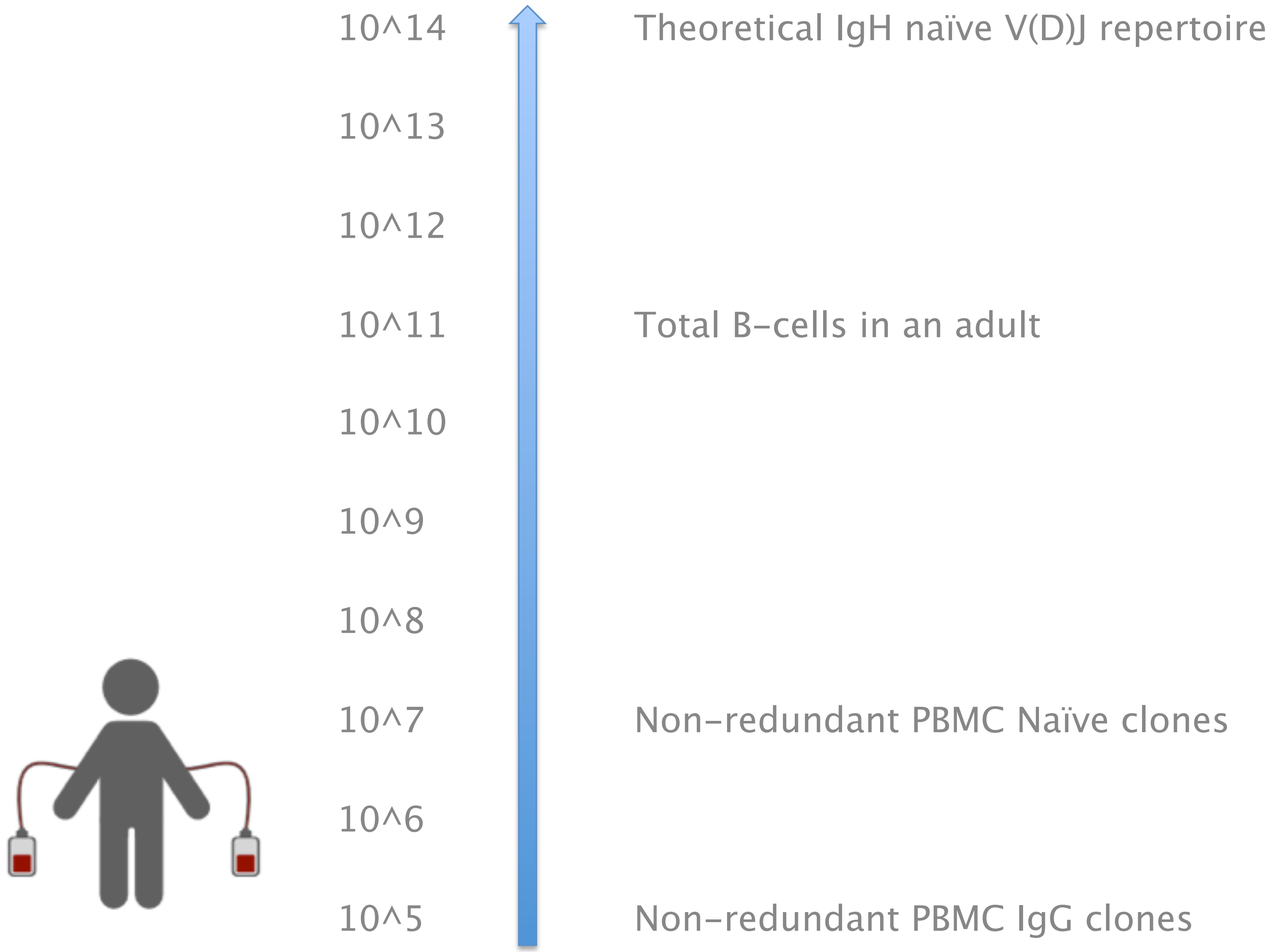


Coordinated somatic hypermutation sites

Could we find such a synthesis technology?

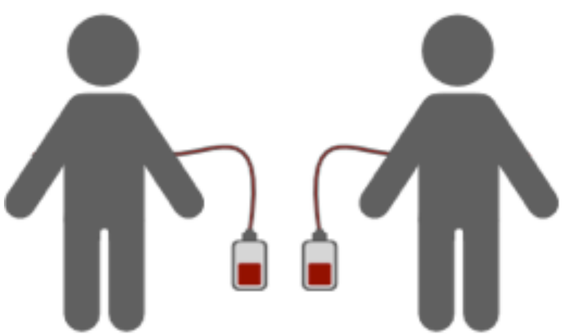

NAIVE
MEMORY
NAIVE
MEMORY
Naïve
CD27+/IgM
IgG
IgA
Heavy chains
Light chains
Naive IgM clone overlap
Memory IgM clone overlap
Memory IgA clone overlap
Memory IgG clone overlap
Naive IgK clone overlap
Naive IgL clone overlap
Memory IgK clone overlap
Memory IgL clone overlap
Biological replicate A
Biological replicate B
2e−04
1e−03
5e−03
2e−02
1e−01
5e−02

Individual adults only sample a small part of the antibody repertoire

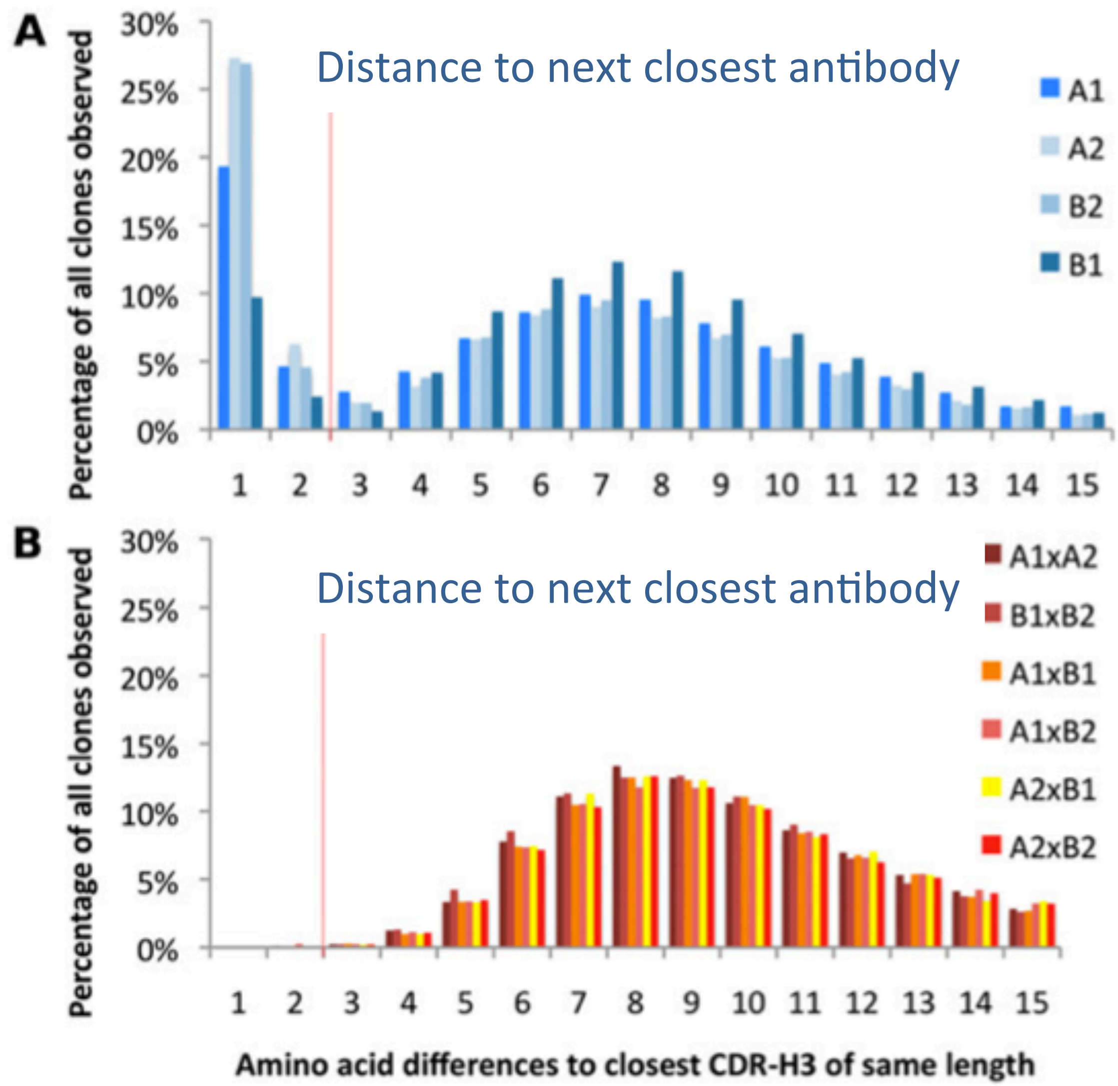


Humans produce dispersed receptor repertoires

WITHIN INDIVIDUAL

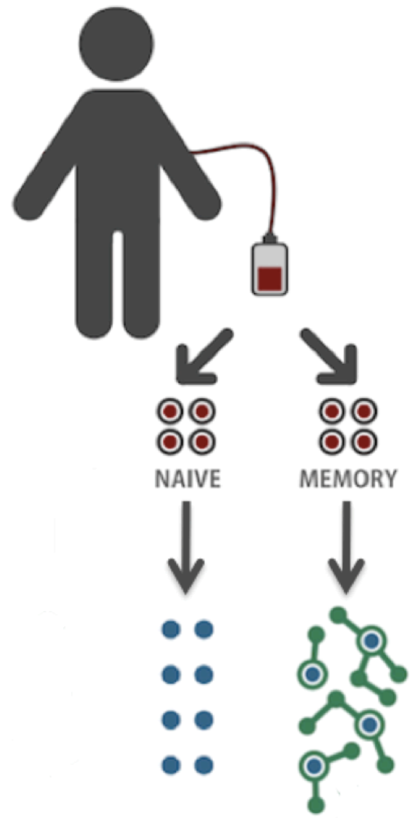


BETWEEN INDIVIDUALS

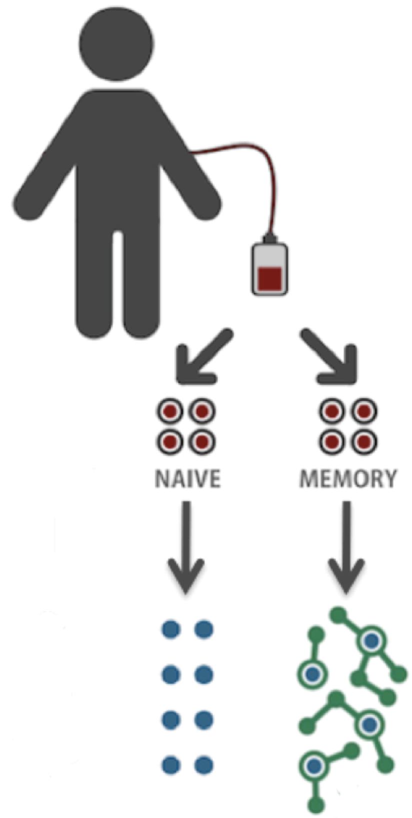


Glanville et al, PNAS 2011

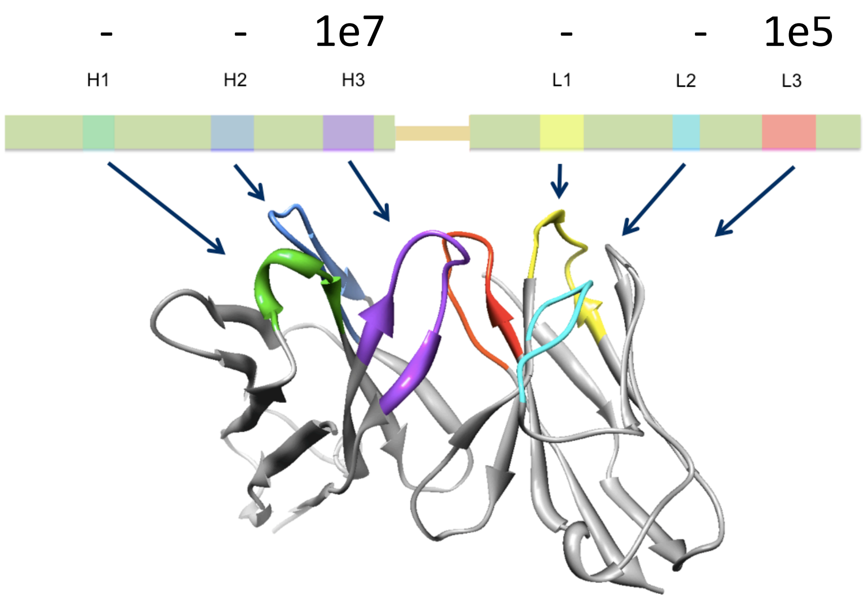
Per person

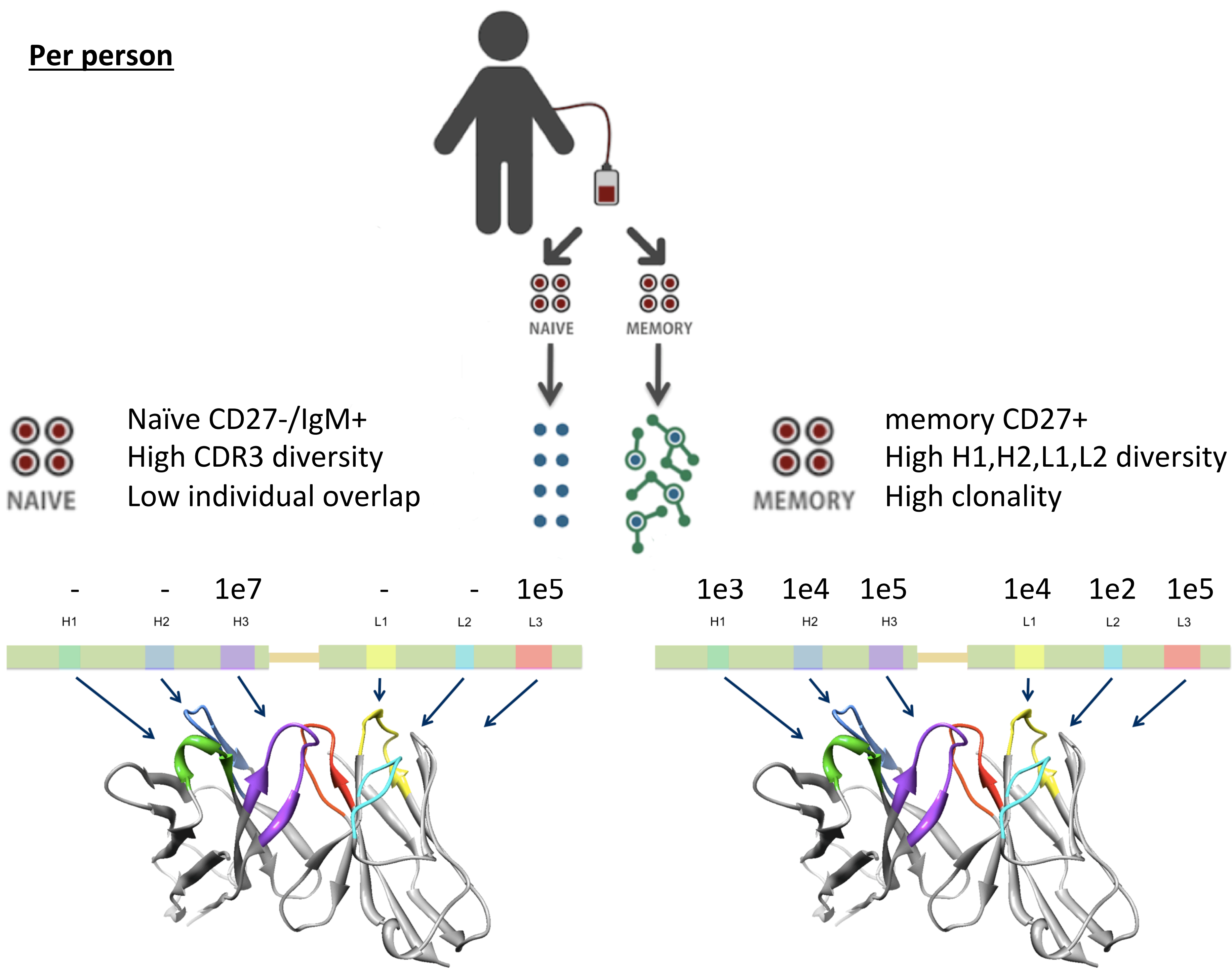


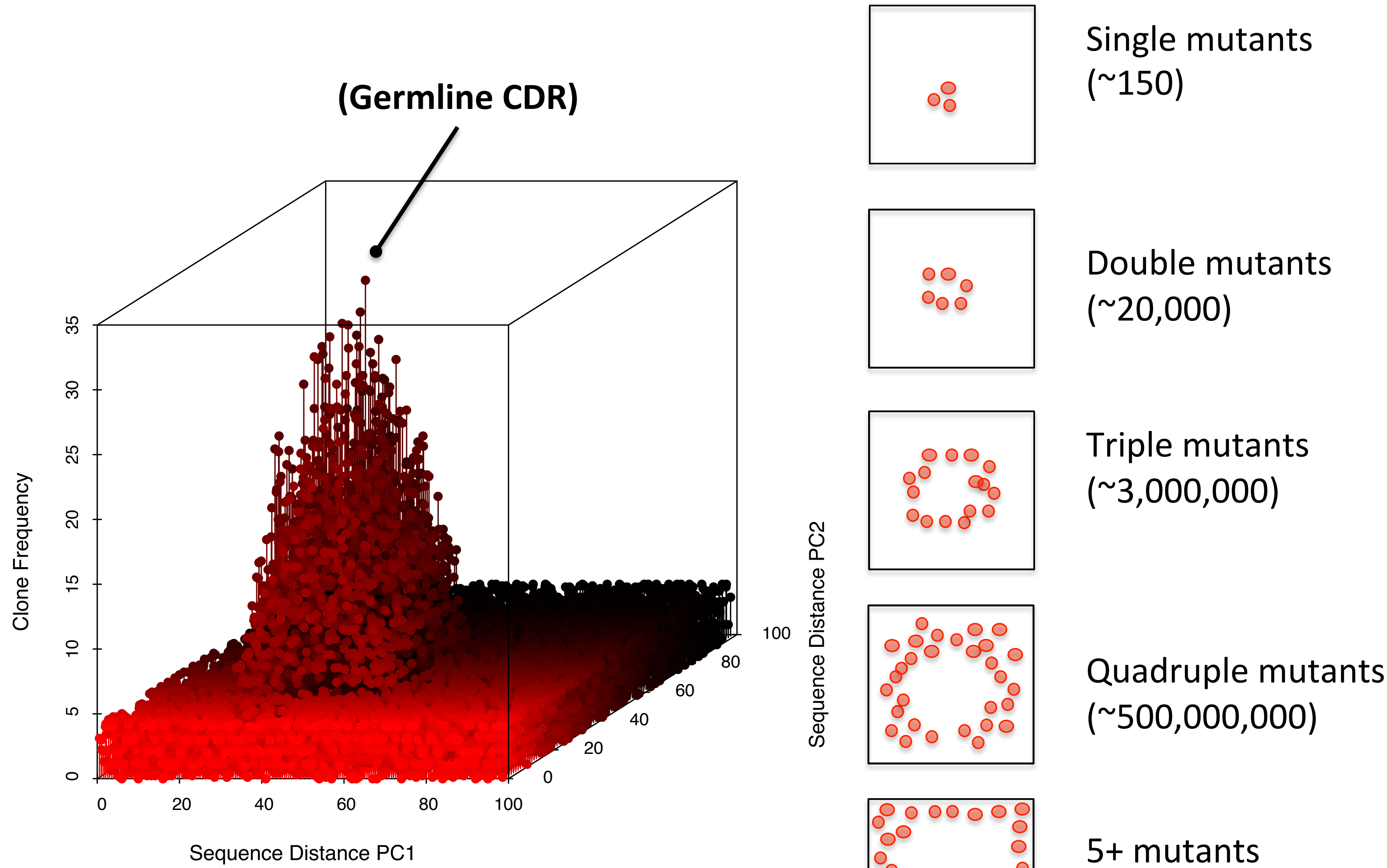
Per person



Naïve CD27-/IgM+
High CDR3 diversity
Low individual overlap



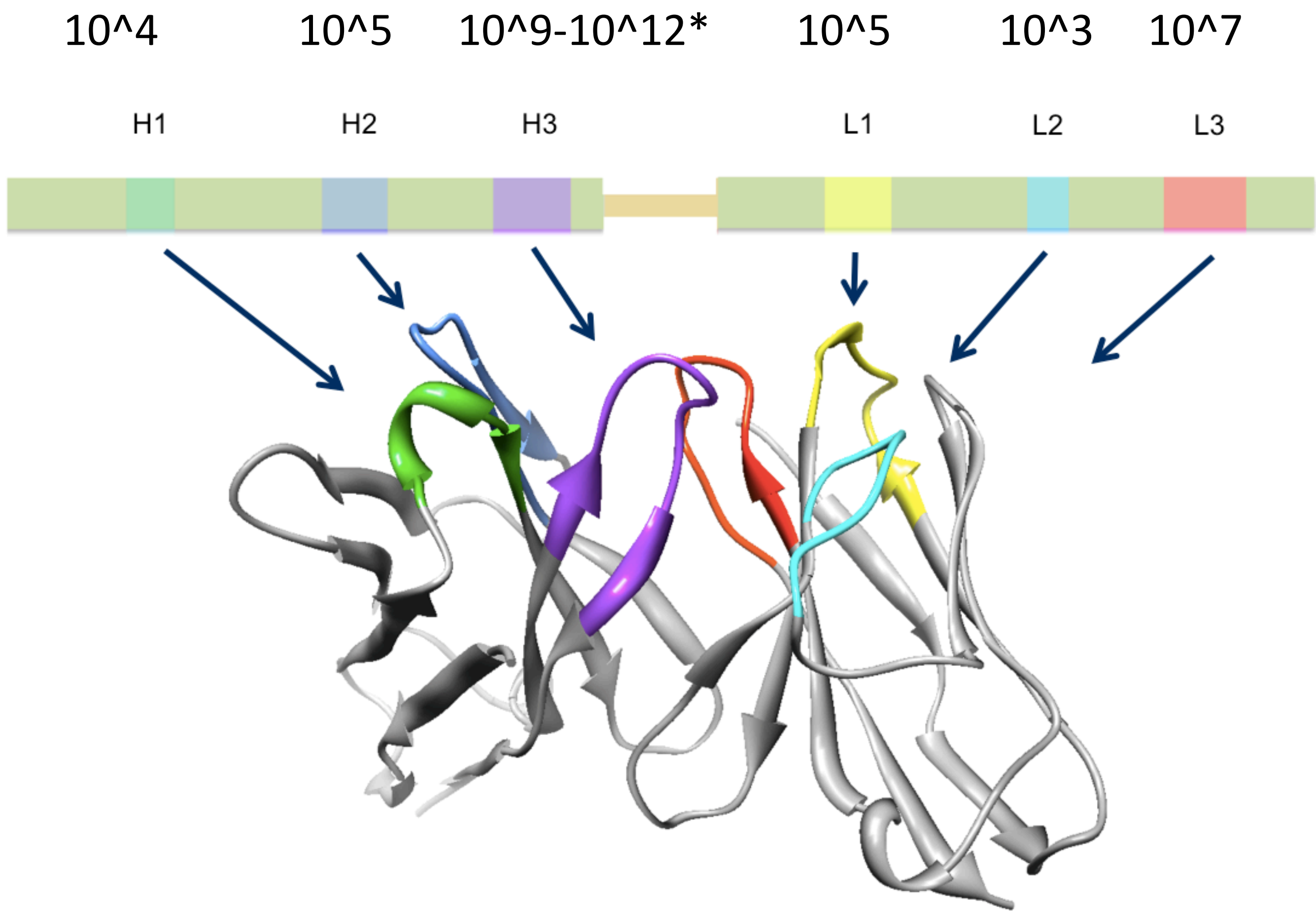

Per person
NAIVE
MEMORY
Naïve CD27-/IgM+
High CDR3 diversity
Low individual overlap
memory CD27+
High H1,H2,L1,L2 diversity
High clonality
- - 1e7 - - 1e5
H1 H2 H3 L1 L2 L3
1e3 1e4 1e5 1e4 1e2 1e5
H1 H2 H3 L1 L2 L3



Comprehensive affinity maturation landscape from 100 people

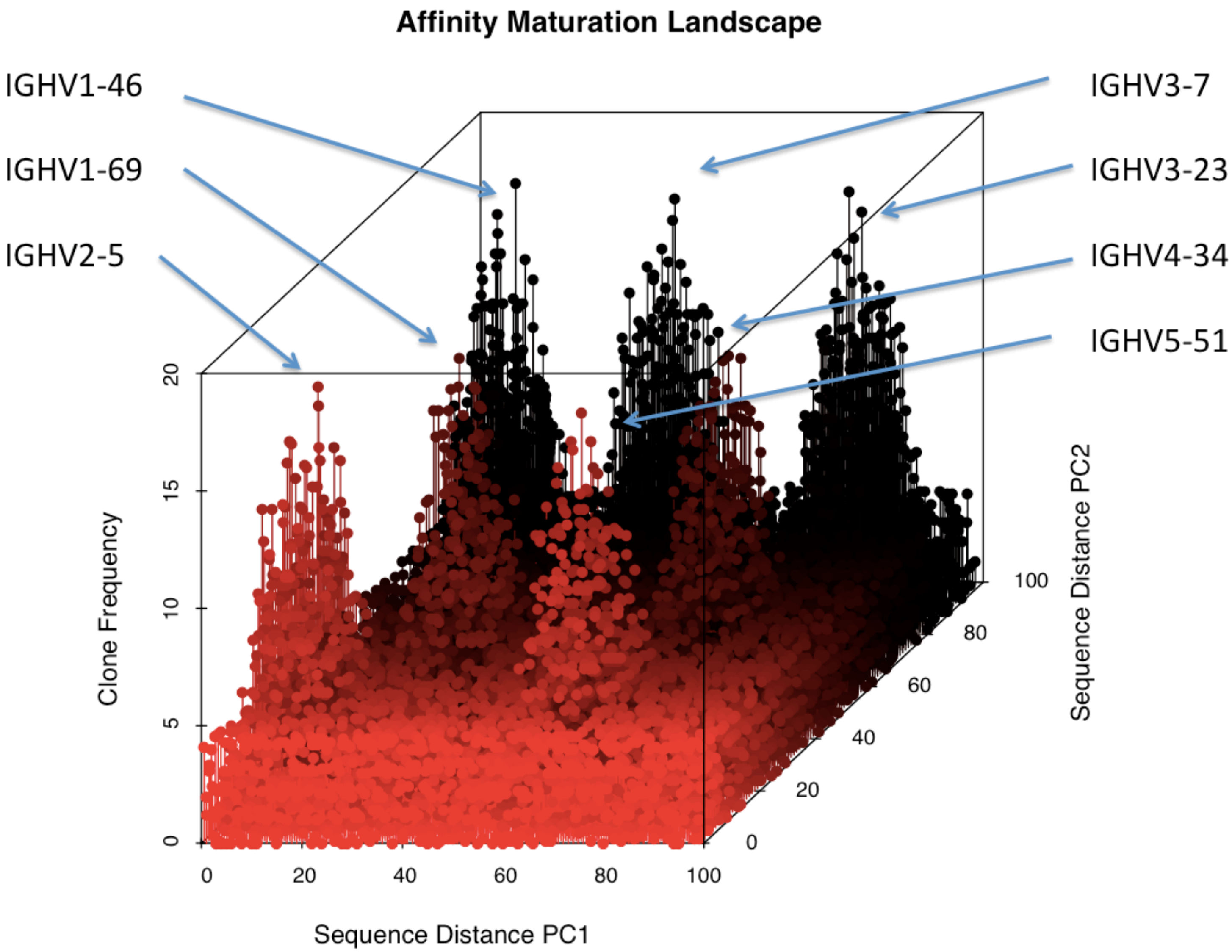
Per CDR, for every framework of interest

Naturally vetted diversity from sorted B-cells from 100 healthy donors

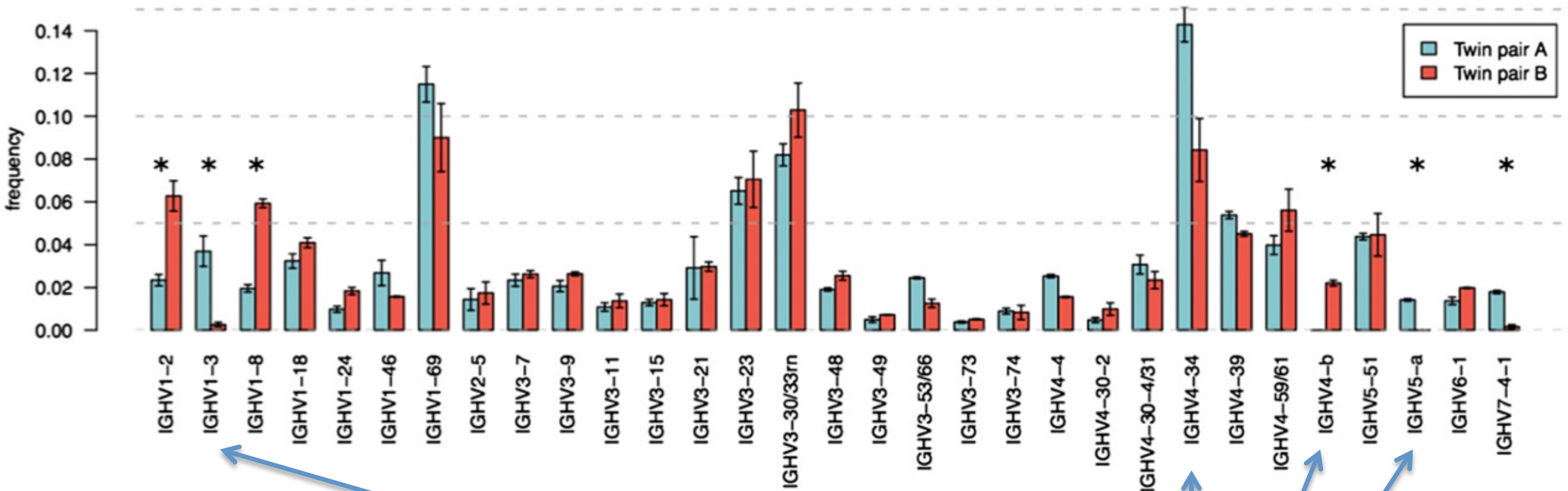


4VH & 4 VK curated scaffolds from the best human druggable V-segments

*10^12 with enzymatic expansion



- Stable
- Used by all people
- Previously used as drugs
- Structurally diverse
- Non-redundant
- Not-aggregation prone

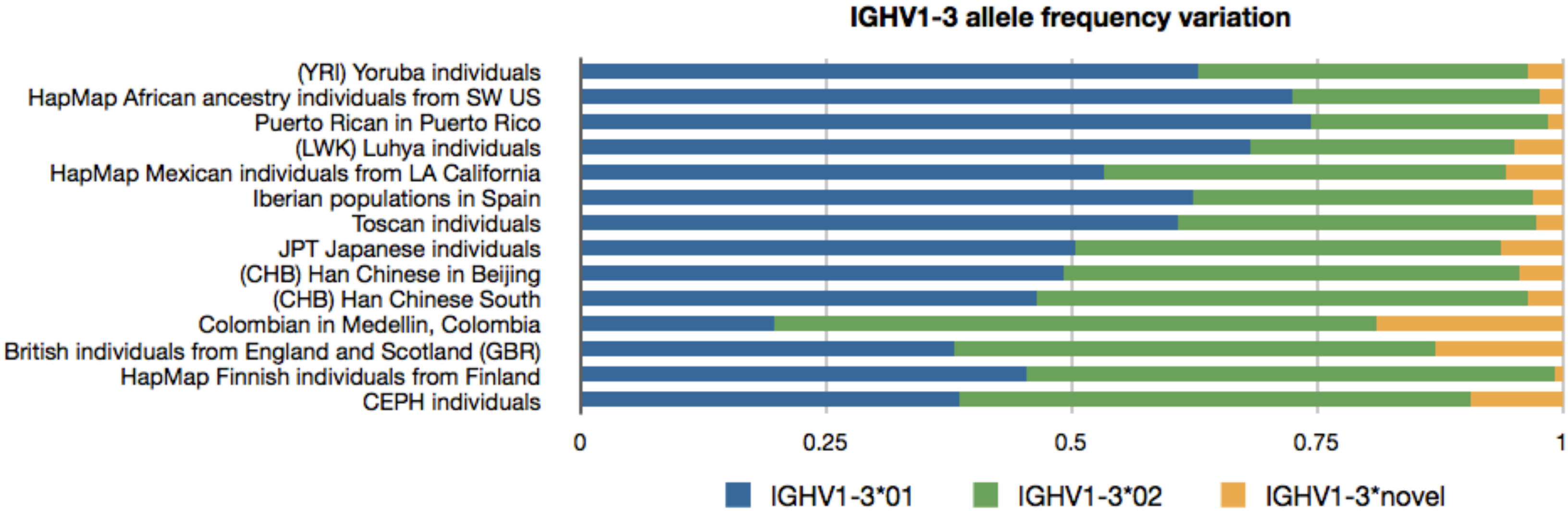


Problem alleles

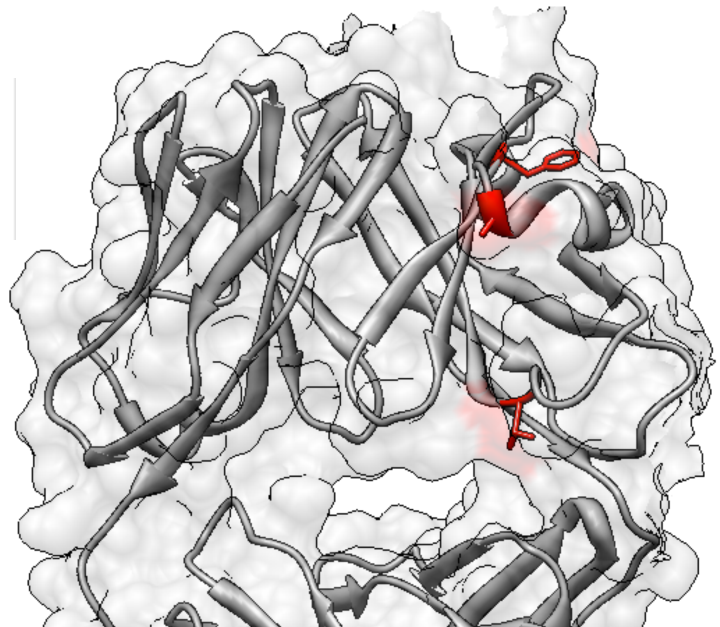
Allele variation in human populations

Missing/autoreactive in some individuals

Should we avoid polymorphic V-genes?



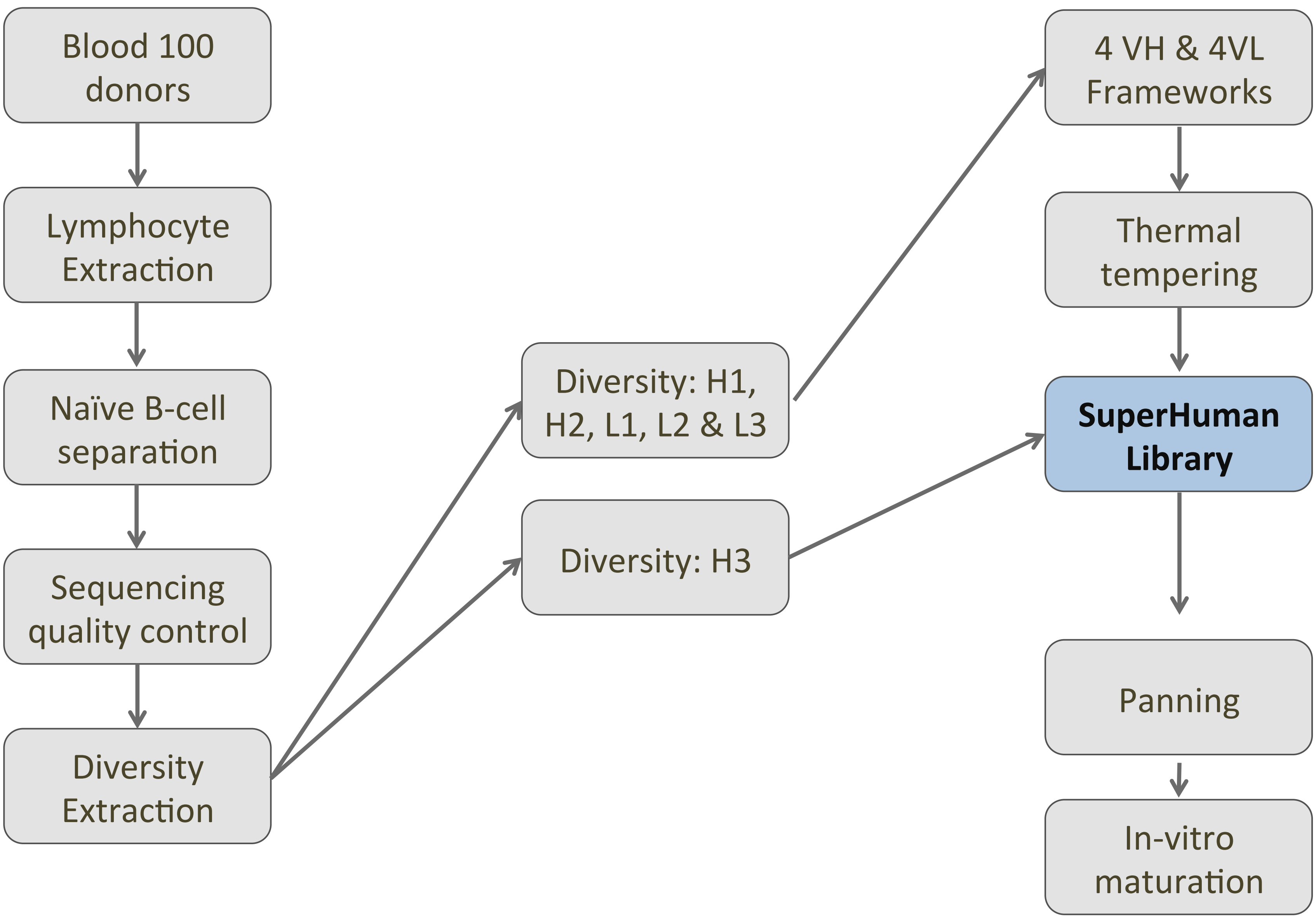

```
IGHV1-3*01/1-98    1 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQAPGQRLEWMG 49
IGHV1-3*02/1-98    1 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQAPGQRLEWMG 49

IGHV1-3*01/1-98   50 WINAGNGNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCAR 98
IGHV1-3*02/1-98   50 WSNAGNGNTKYSQEFQGRVTITRDTSASTAYMELSSLRSEDMAVYYCAR 98
```

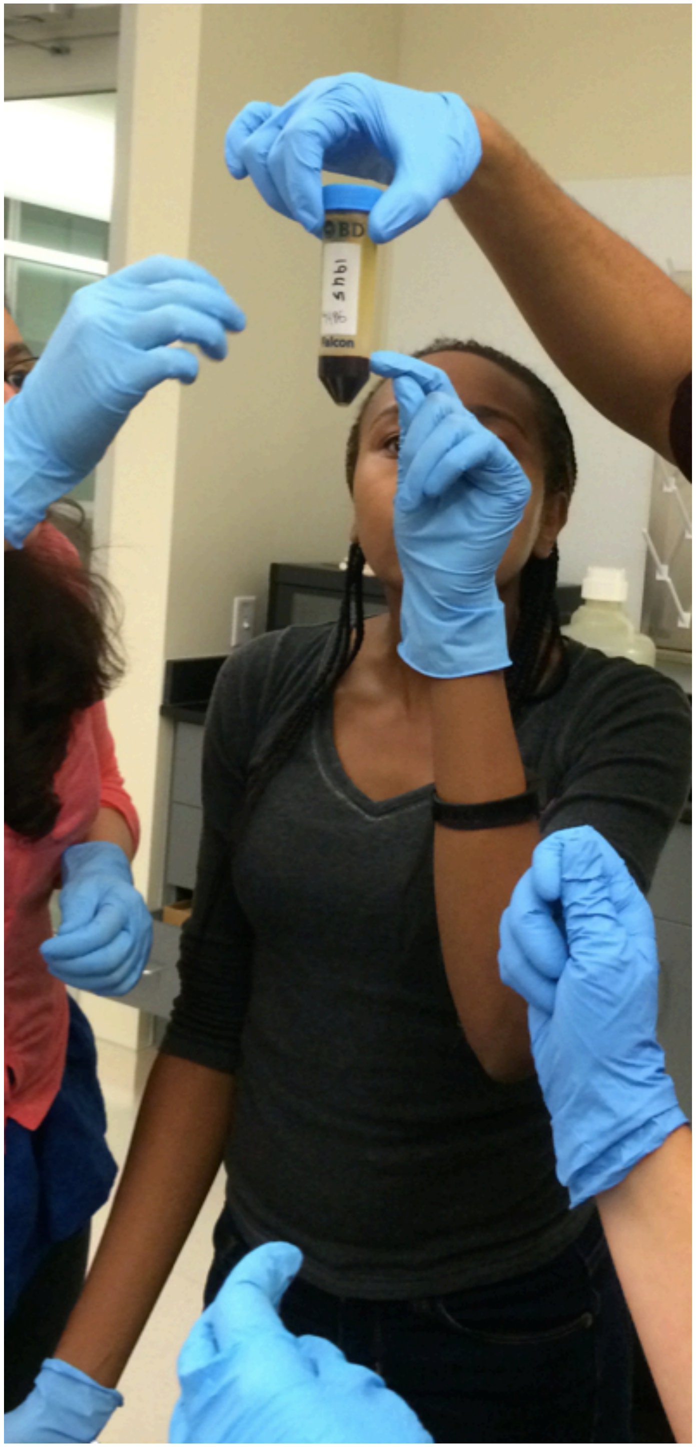


1000 Genomes

A Deep Catalog of Human Genetic Variation

Building and using the SuperHuman library

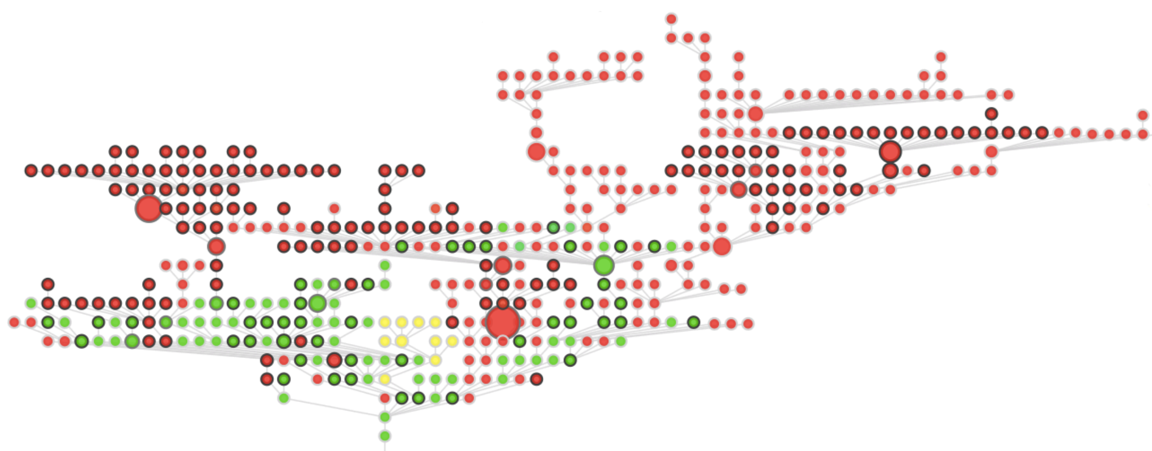




targeted libraries

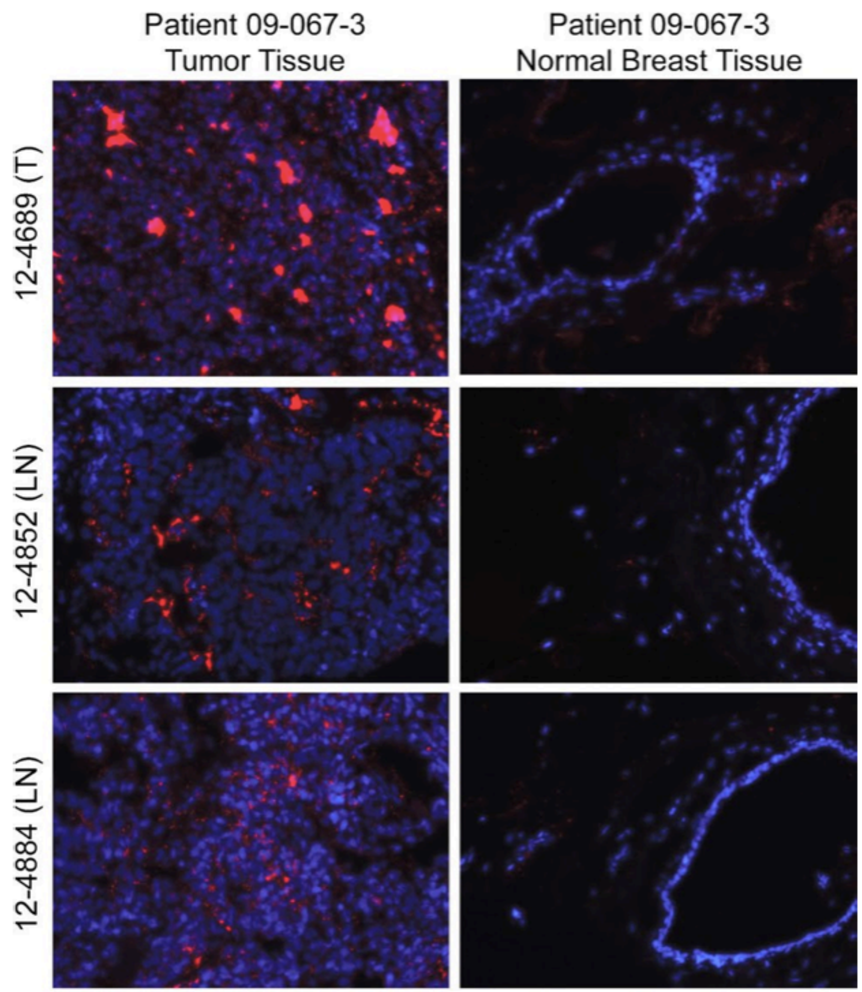
for many targets, a vast library already exists
a reservoir of existing functional, matured antibodies

- Virii
- Bacteria
- Anti-venom
- Cancer



Anti-Ebola antibodies in the blood of survivors

Anti-tumor antibodies in the blood of cancer survivors



Cancer Immunol Immunother (2013) 62:1397–1410
DOI 10.1007/s00262-013-1443-5

ORIGINAL ARTICLE

Intravenous infusion of phage-displayed antibody library in human cancer patients: enrichment and cancer-specificity of tumor-homing phage-antibodies

Girja S. Shukla · David N. Krag · Elena N. Peletskaya · Stephanie C. Pero · Yu-Jing Sun · Chelsea L. Carman · Laurence E. McCahill · Thomas A. Roland

Cancer Immunol Immunother
DOI 10.1007/s00262-014-1612-1

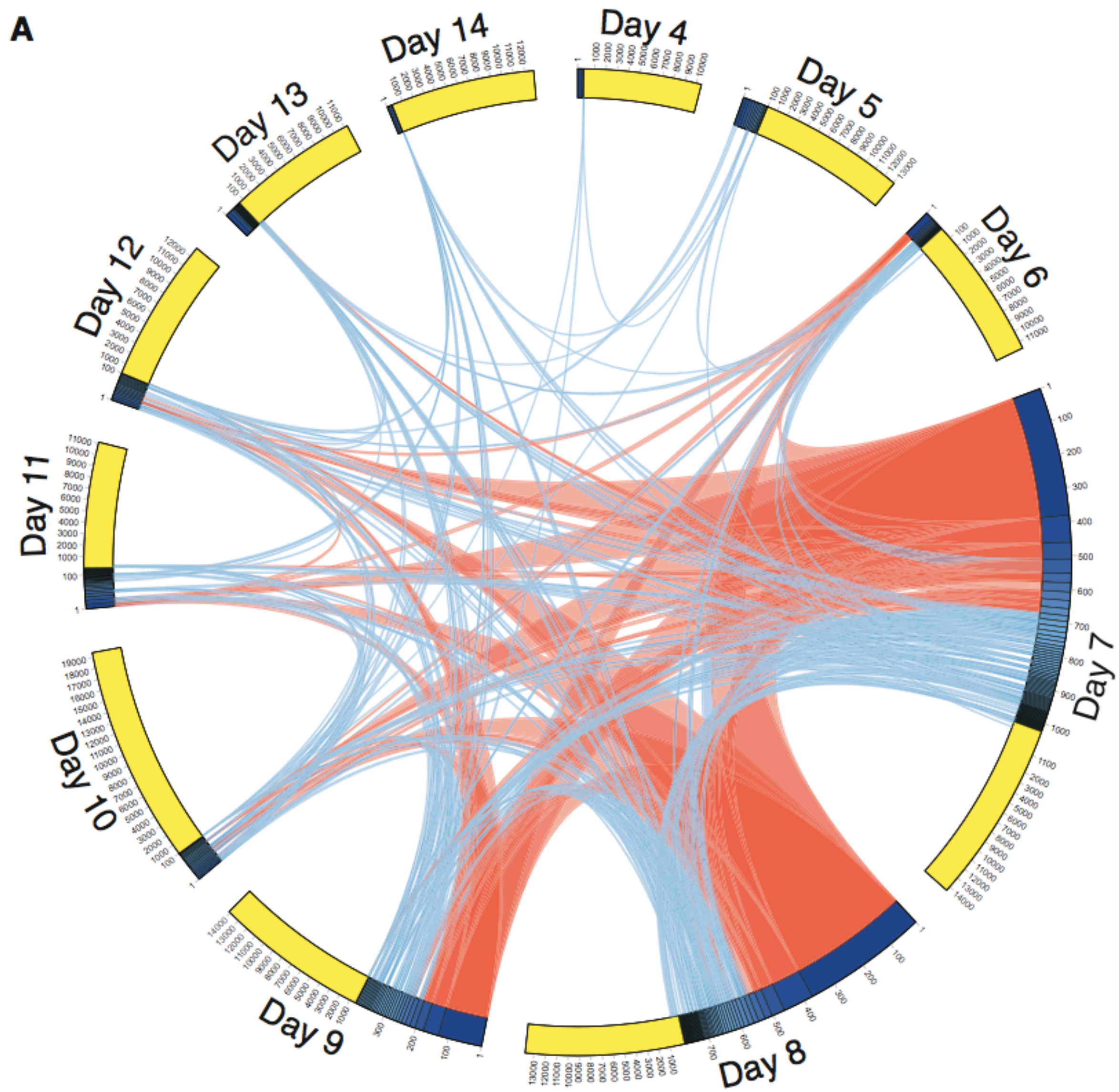
ORIGINAL ARTICLE

Identification of tumor-binding scFv derived from clonally related B cells in tumor and lymph node of a patient with breast cancer

Leah J. Novinger · Takamaru Ashikaga · David N. Krag

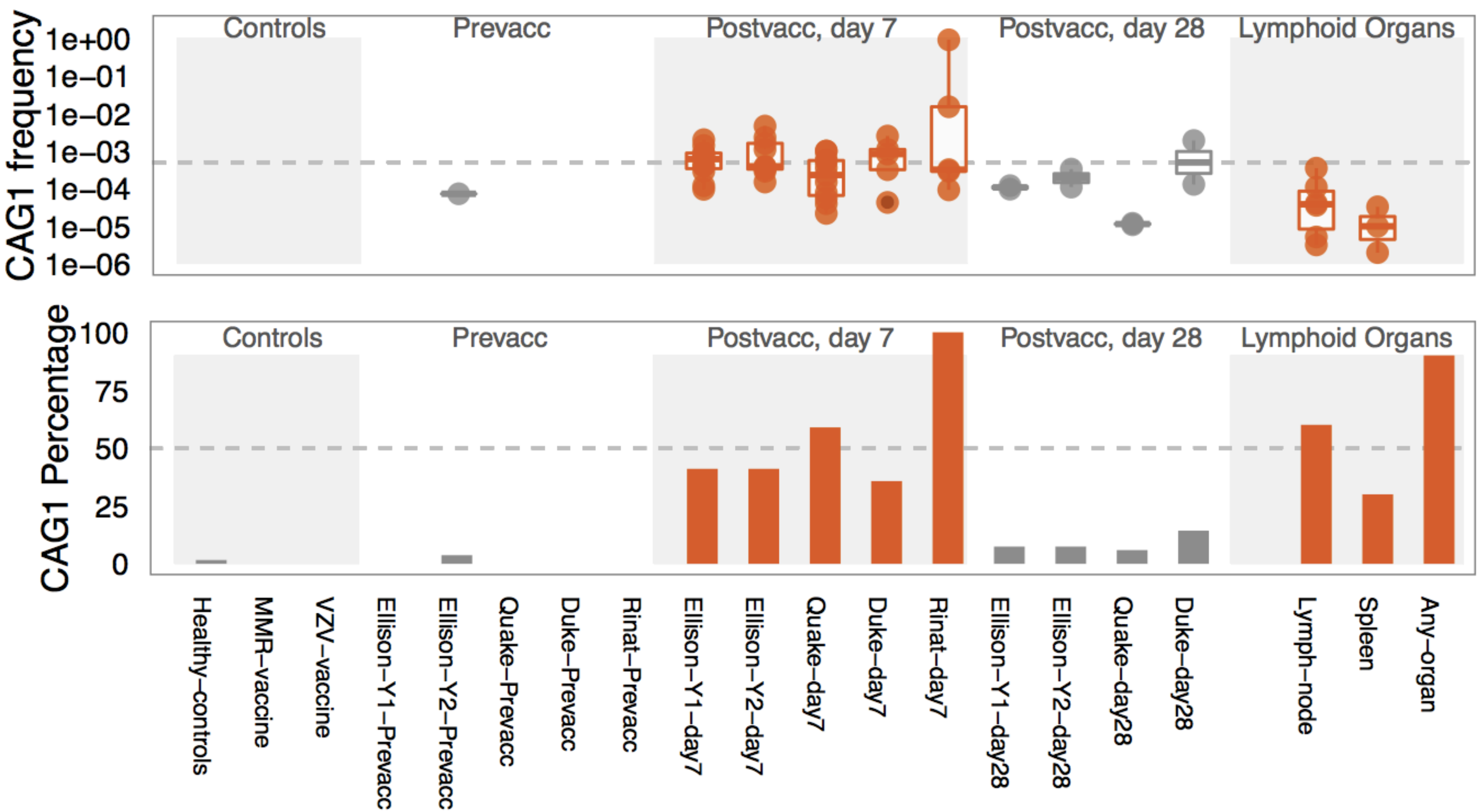
But when to recover them? Chemo damages repertoire, and response may be time sensitive

Vaccination: memory only transiently available in periphery



Jackson, Cell Host & Microbe, 2013

Vaccination: memory stored in secondary lymphoid organs



Who is the right population?

Figure 1

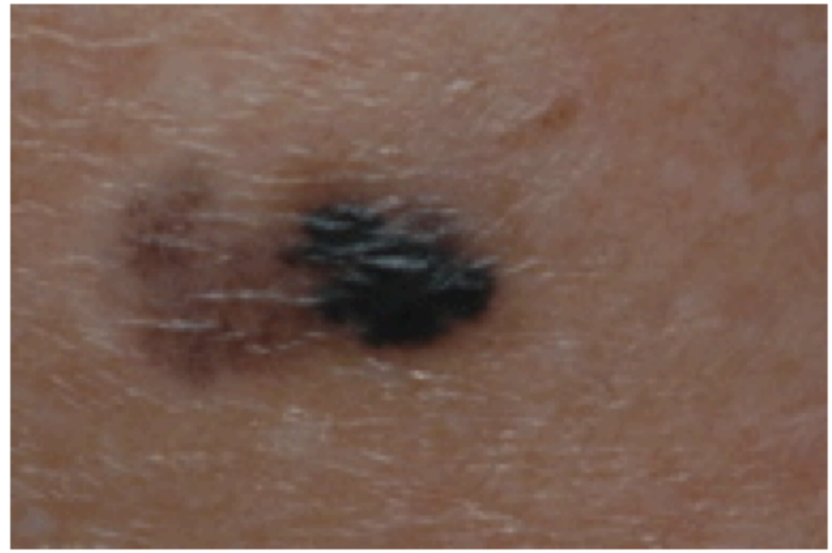


Figure 2

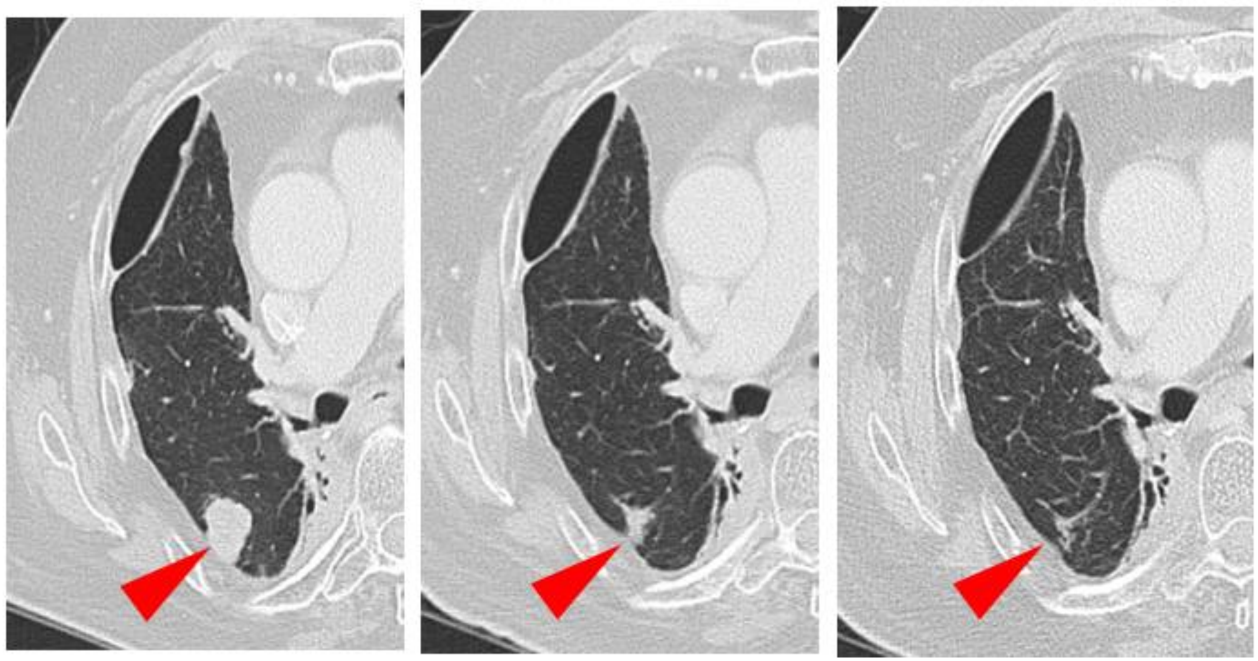
Complete regression of melanoma associated with vitiligo

Enric Piqué-Duran, Santiago Palacios-Llopis, MªSol Martínez-Martín, Juan A Pérez-Cejudo

Dermatology Online Journal 17 (1): 4

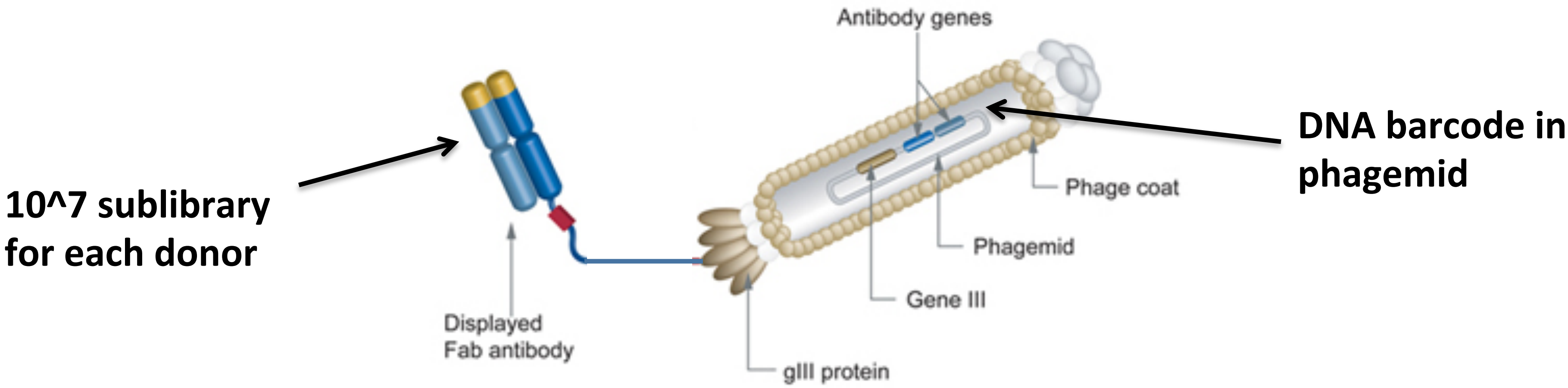
Immune checkpoint blockade & induced remission

Lung cancer response to anti-PD-1 (4 prior treatments)



BMS

The immune memory of 10,000 people can be loaded into a barcoded library



Syndrome X **100 Healthy controls**


We are crowdsourcing recruitment
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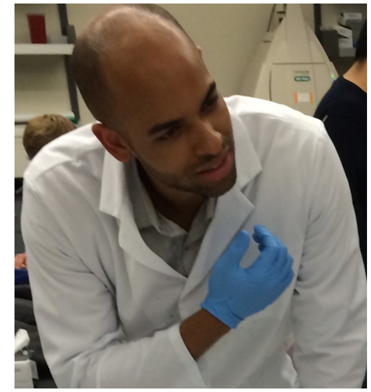
distributed bio

Giles Day
Chris Smith
Amy Geurts
Casey Keyes
David Louis

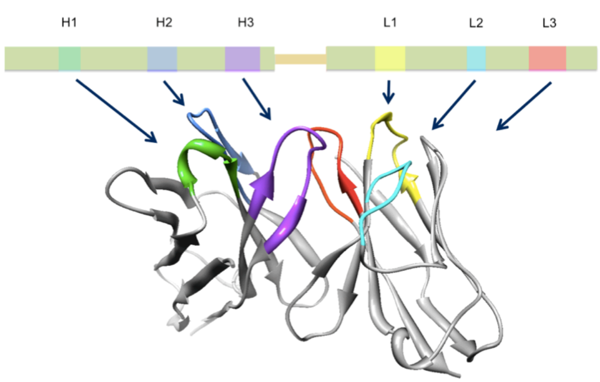
David Maurer
Gayathri Shankar
Maureen Kagimbi
Usha Maloney

Giles Day

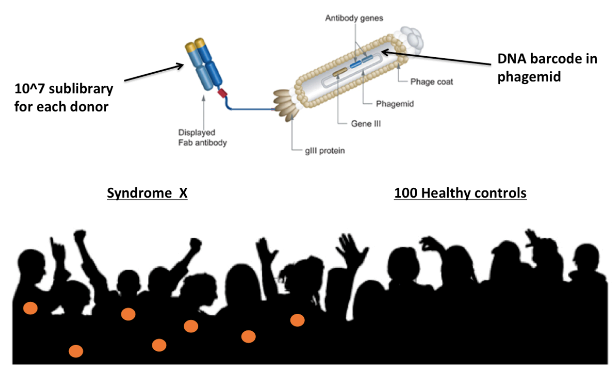


David Louis
(poster session)

jake@distributedbio.com



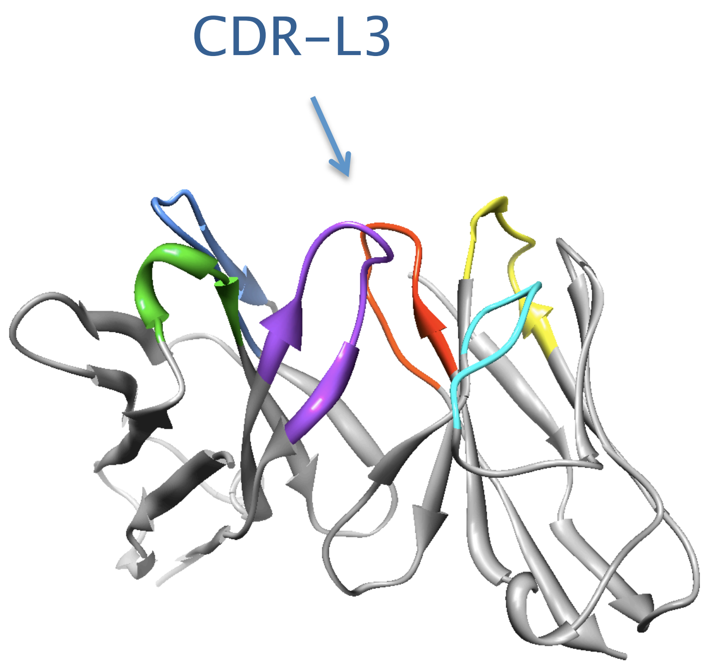
SuperHuman Library

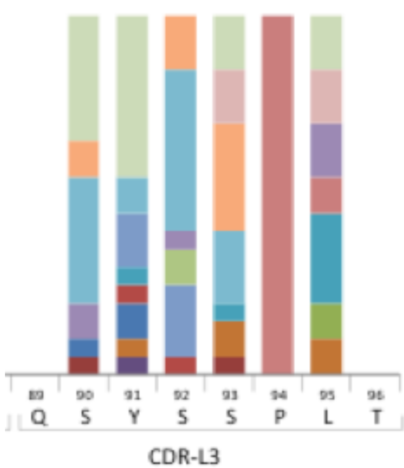


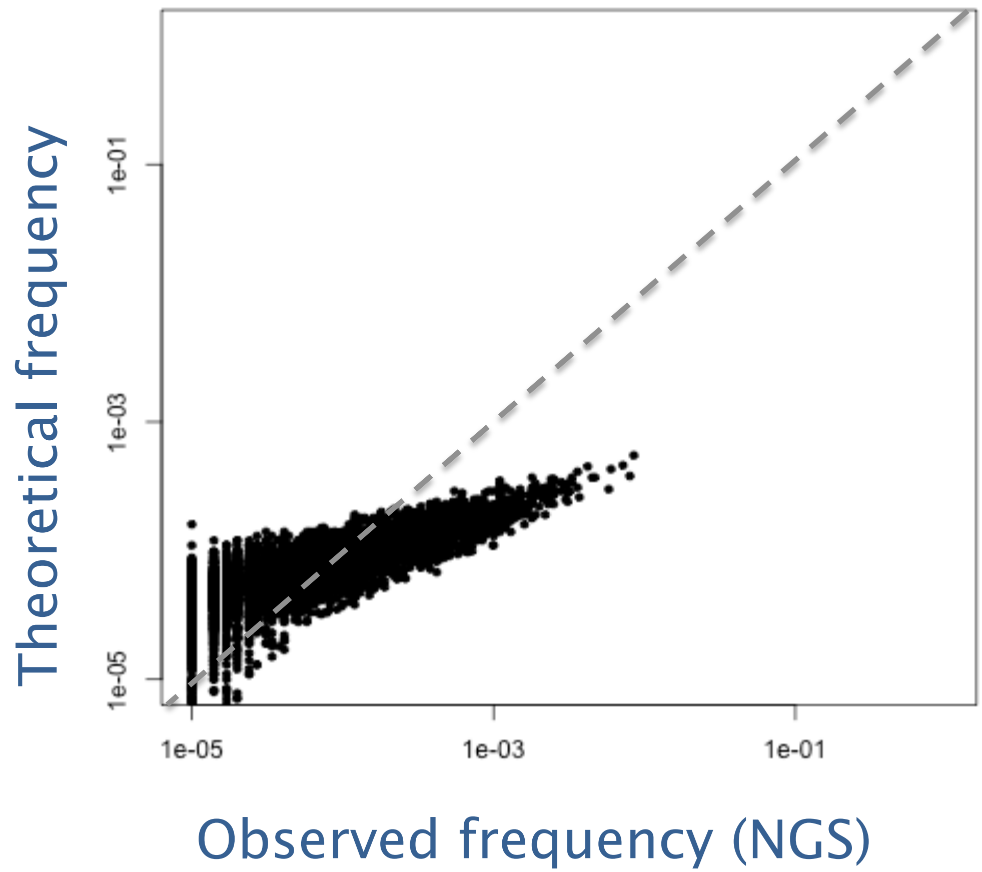
Survivor Library

Supp slides

Synthetic methods introduce hidden diversity bottlenecks



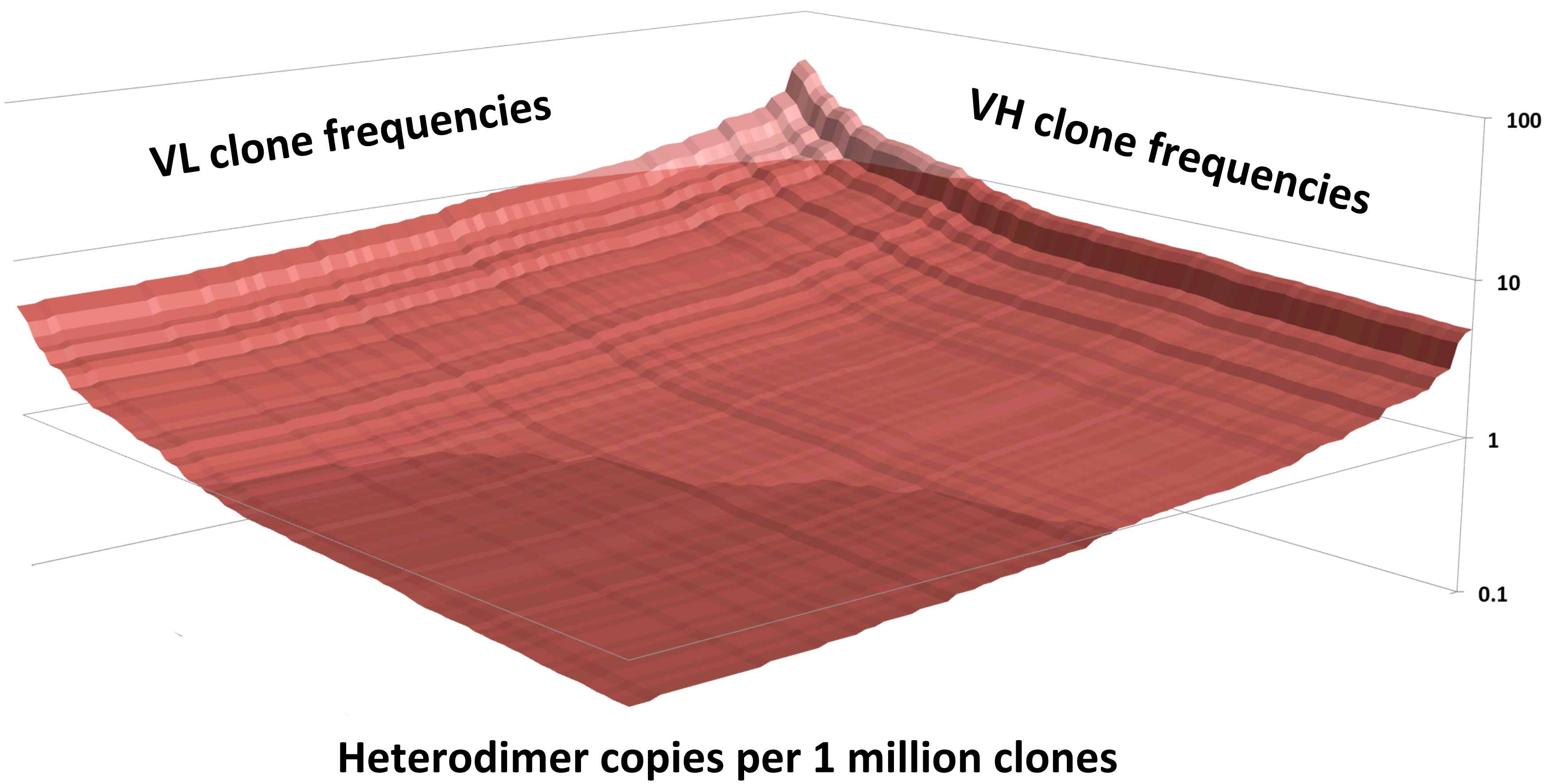


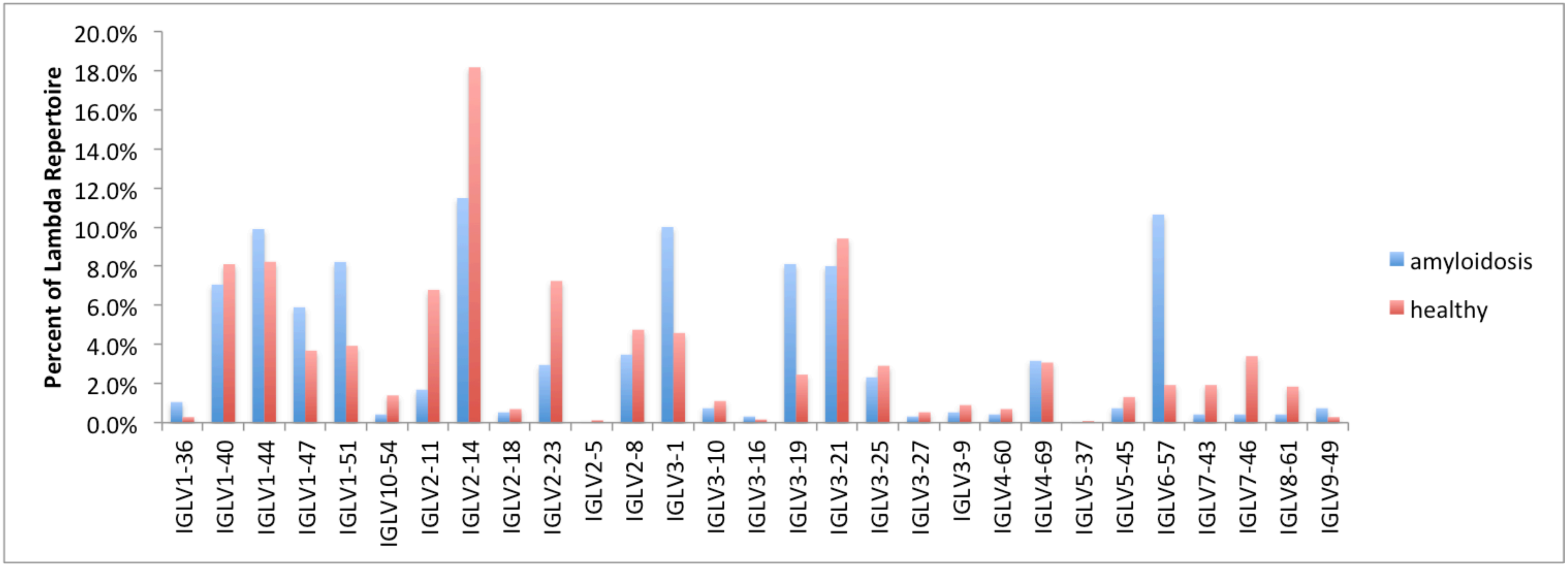


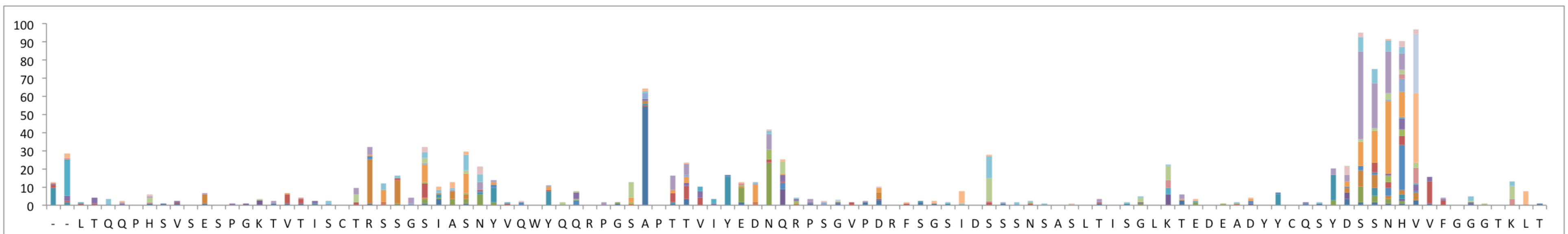
$$f_i = \mathrm{Prob}(i) = \mathrm{Prob}(\lambda_i)^* \prod_{p=1}^{\lambda_i} \mathrm{Prob}\left(\alpha_{i,p} \,|\, \mathrm{PFM}_\lambda\right)$$

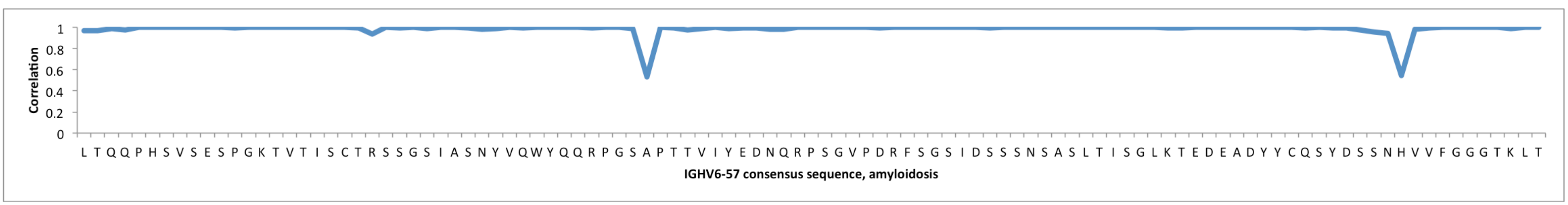
Immune capture is time sensitive

- Exhaustive heterodimer coverage of top 5,000 clones
- Fair heterodimer coverage of top 50,000 clones
- Some heterodimer sampling of top 500,000 clones



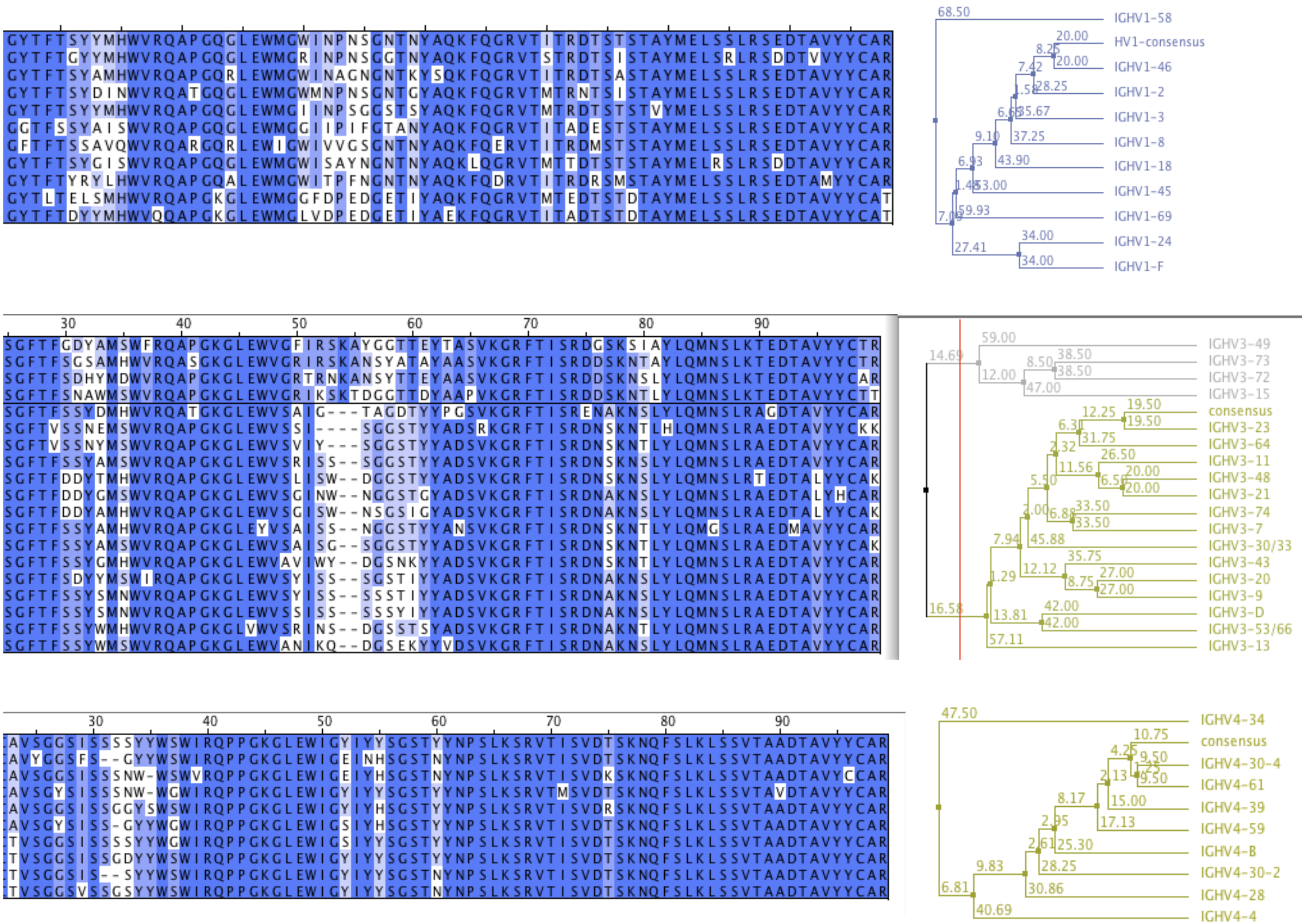






BOSTON UNIVERSITY

AMYLOID LIGHT CHAIN DATABASE


IGHV1-58
HV1-consensus
IGHV1-46
IGHV1-2
IGHV1-3
IGHV1-8
IGHV1-18
IGHV1-45
IGHV1-69
IGHV1-24
IGHV1-F
30 40 50 60 70 80 90
IGHV3-49
IGHV3-73
IGHV3-72
IGHV3-15
consensus
IGHV3-23
IGHV3-64
IGHV3-11
IGHV3-48
IGHV3-21
IGHV3-74
IGHV3-7
IGHV3-30/33
IGHV3-43
IGHV3-20
IGHV3-9
IGHV3-D
IGHV3-53/66
IGHV3-13
30 40 50 60 70 80 90
IGHV4-34
consensus
IGHV4-30-4
IGHV4-61
IGHV4-39
IGHV4-59
IGHV4-B
IGHV4-30-2
IGHV4-28
IGHV4-4