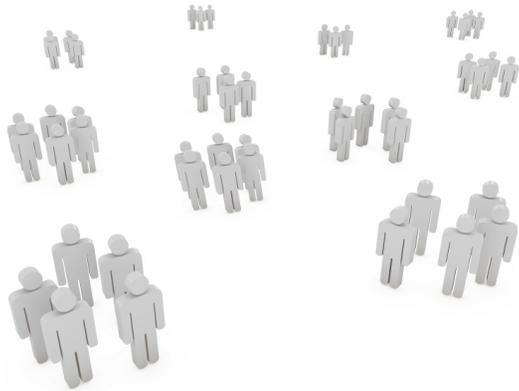


Call of the wild: a new generation of antibody discovery from human populations

Jacob Glanville, CSO
Distributed Bio

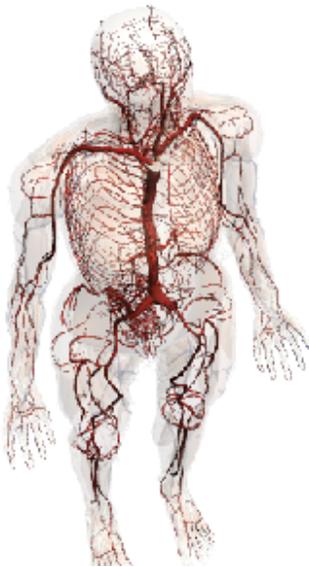
Two main types of libraries, and two main ways to create them



Primary discovery library



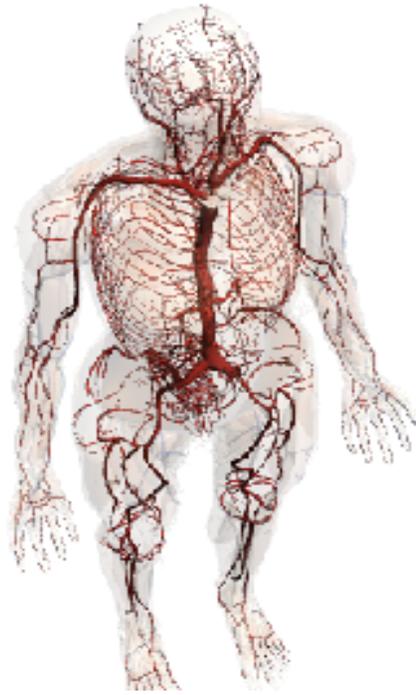
Targeted library



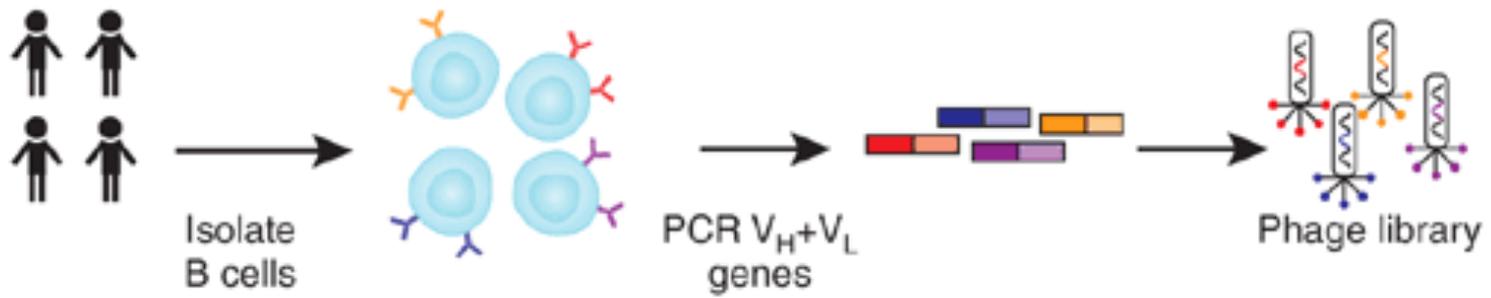
Natural diversity



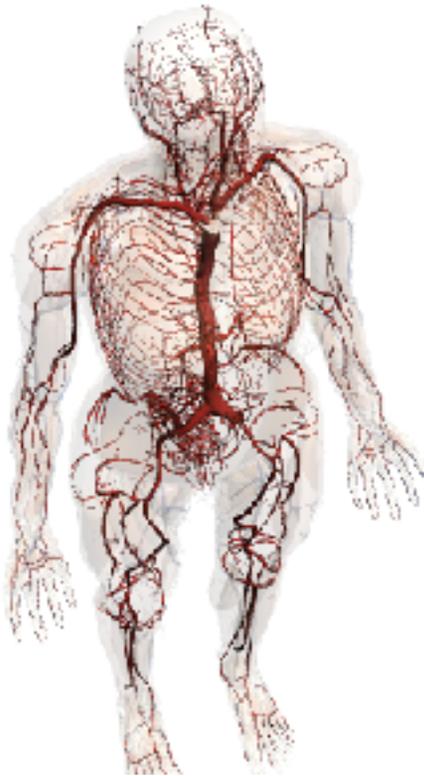
Synthetic diversity



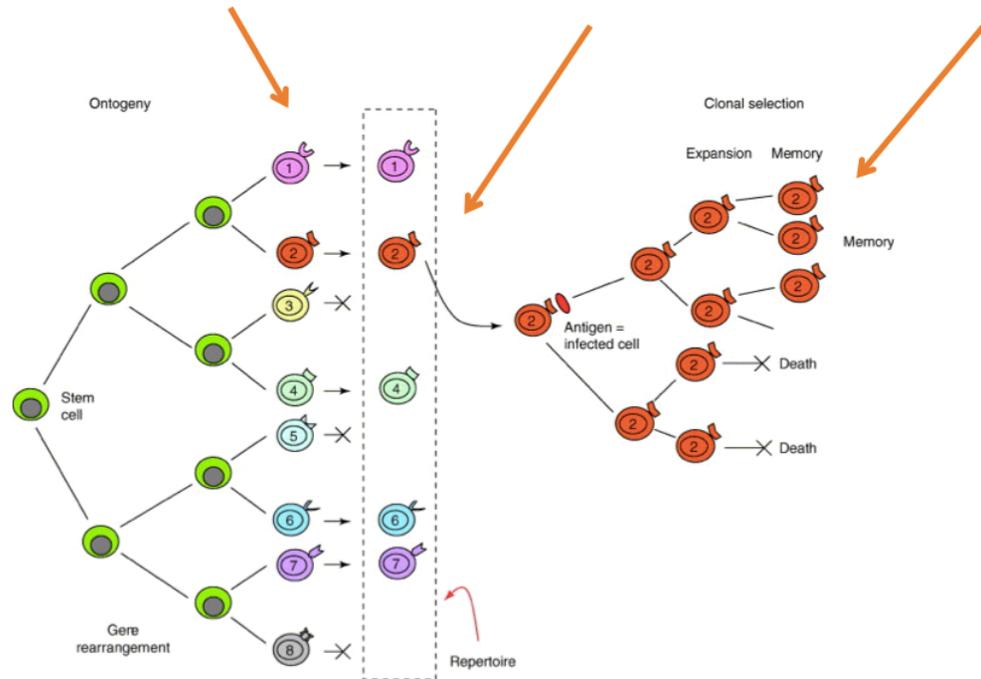
Natural diversity



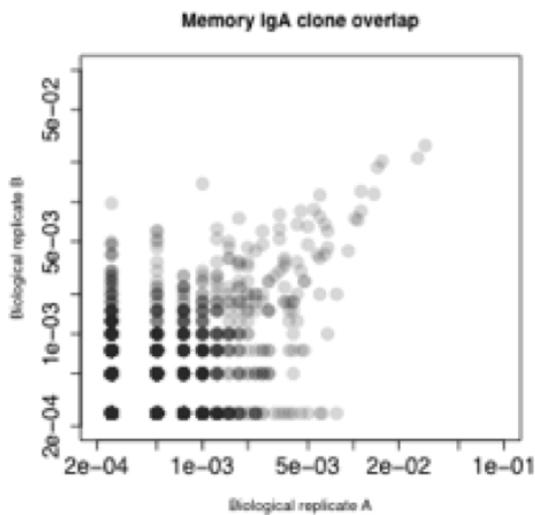
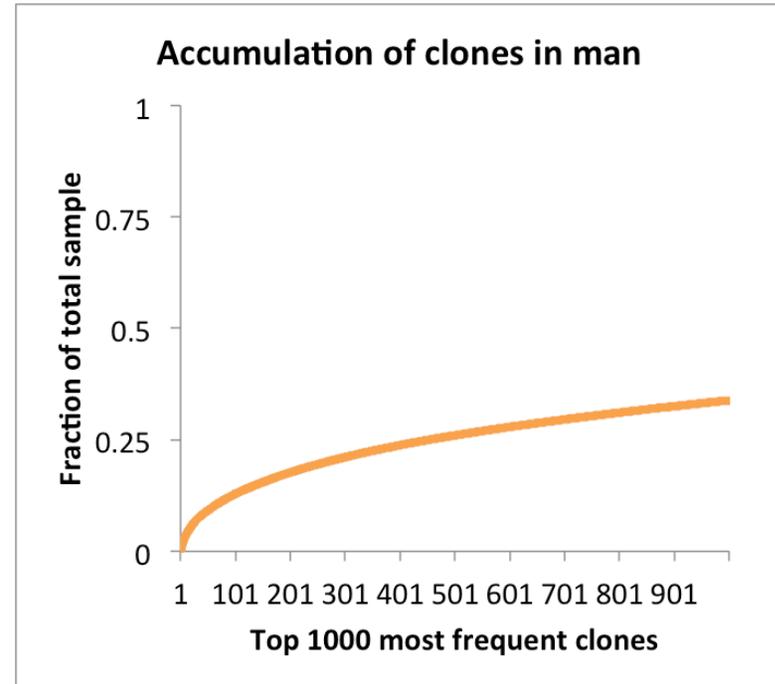
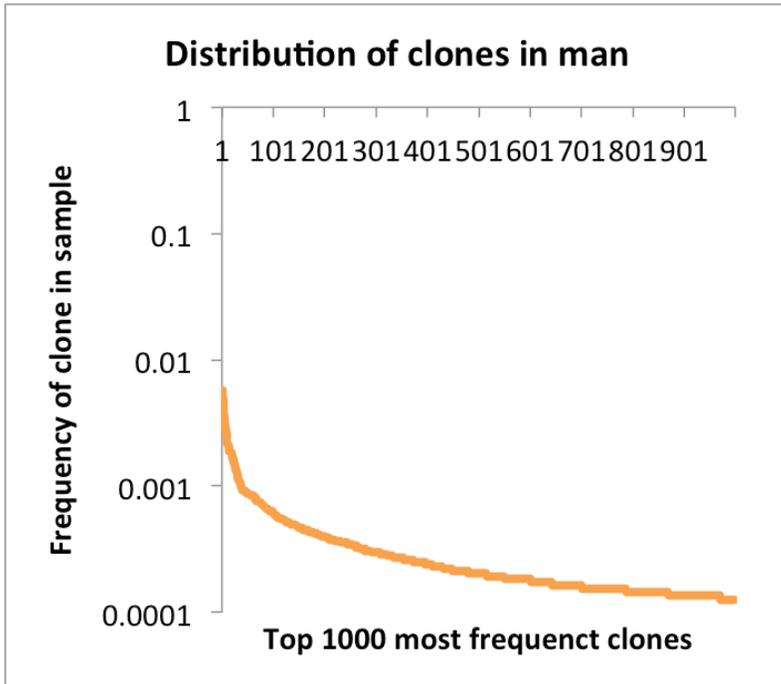
How immunology poisons a natural library



V(D)J recombination antigen selection somatic hypermutation

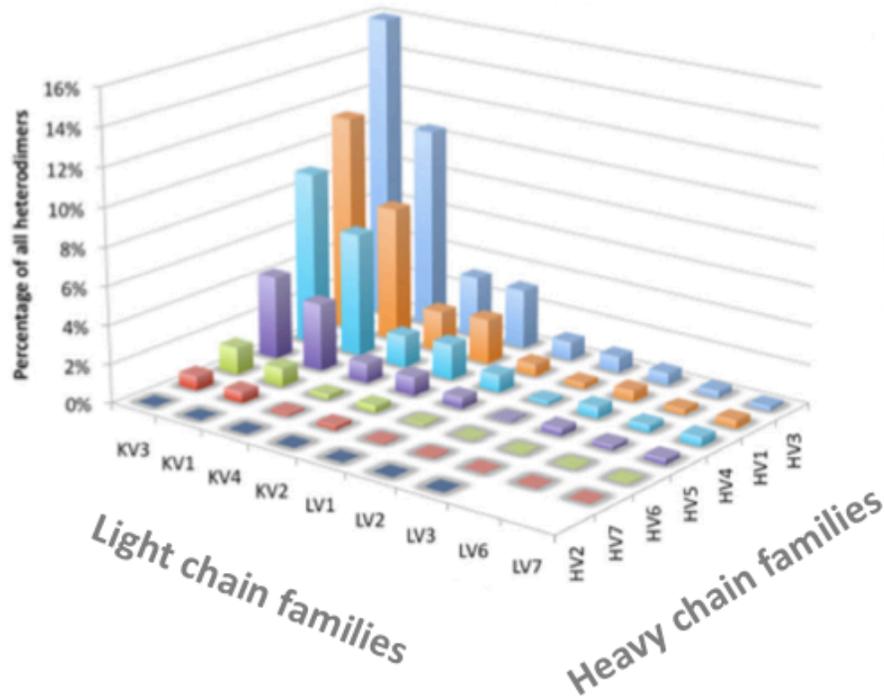


How immunology poisons a natural library

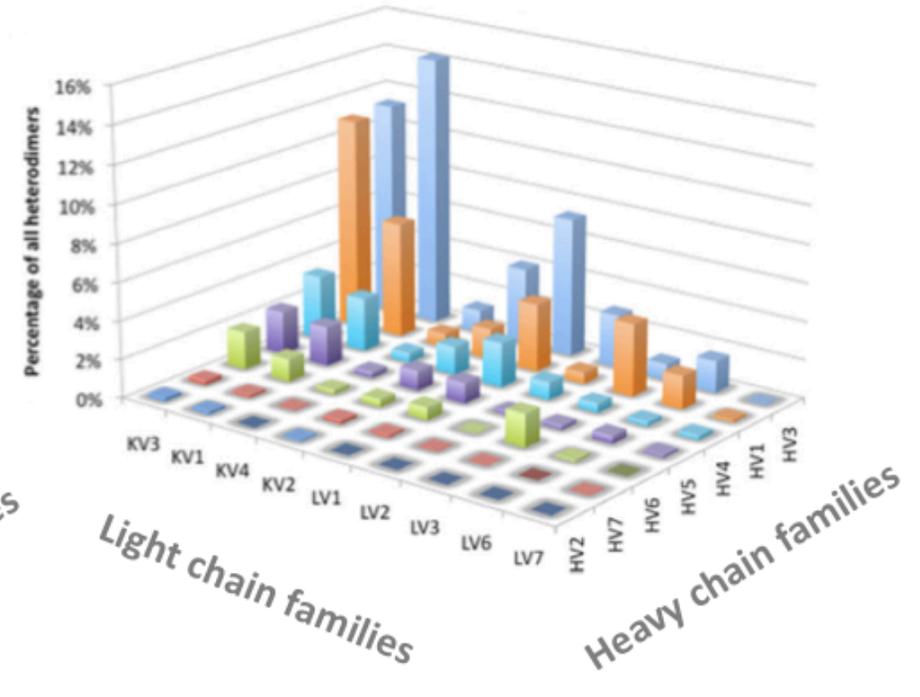


In-vivo immunology translates imperfectly to phage

From NGS sequencing of library



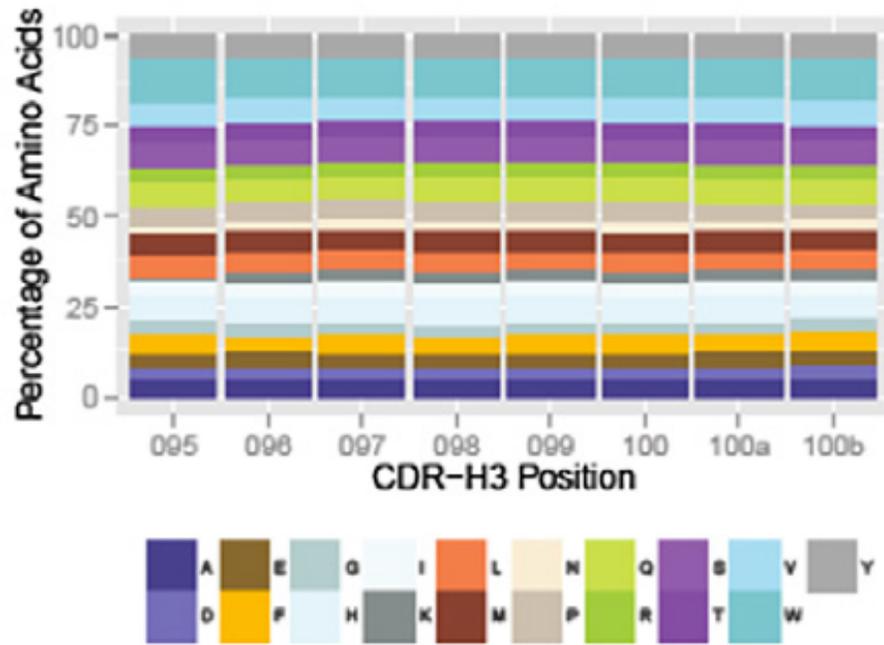
From >20k antigen-specific clones





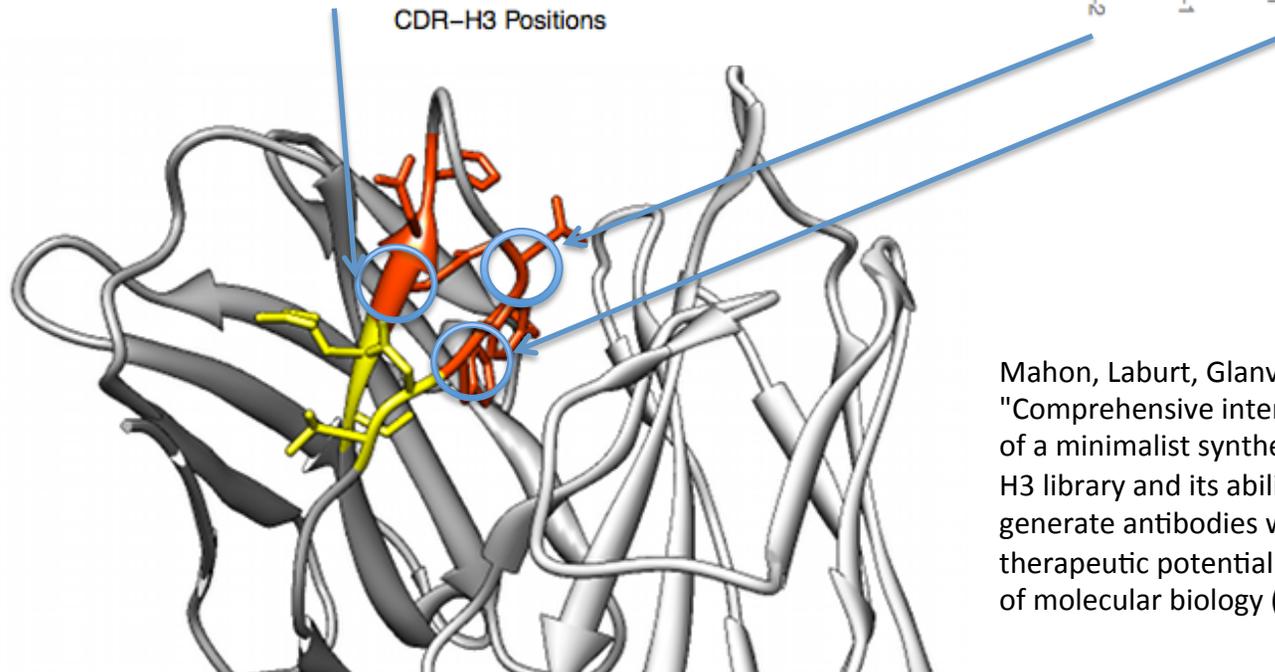
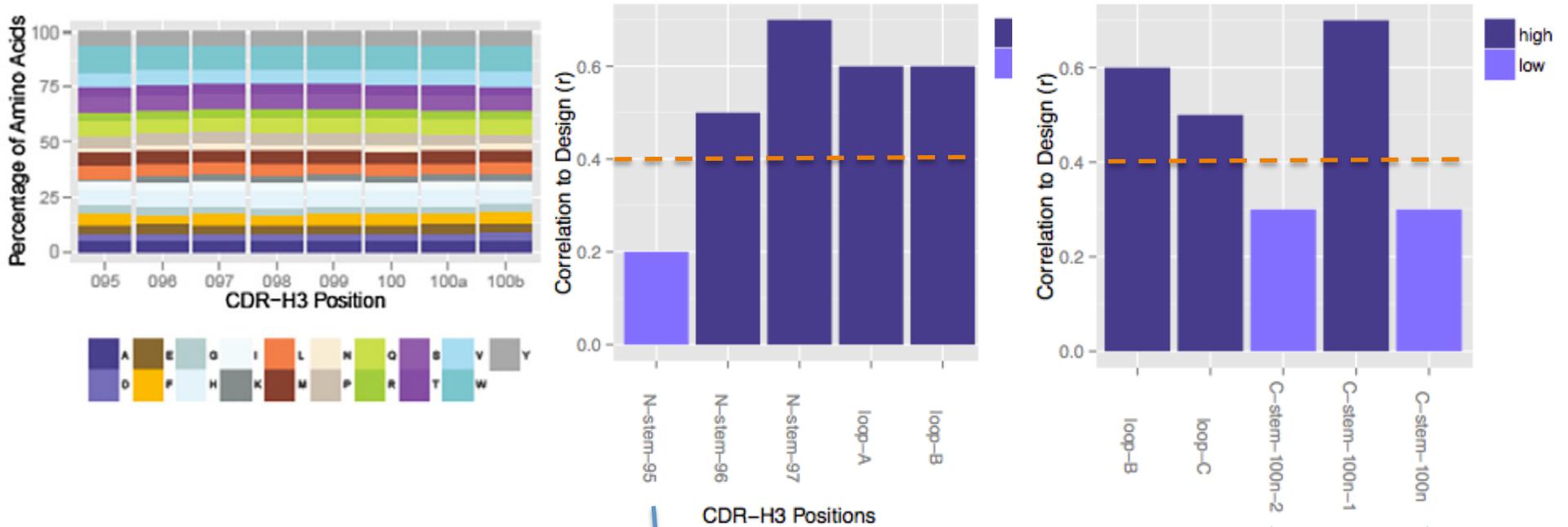
Synthetic diversity

How lack of immunology poisons a synthetic library



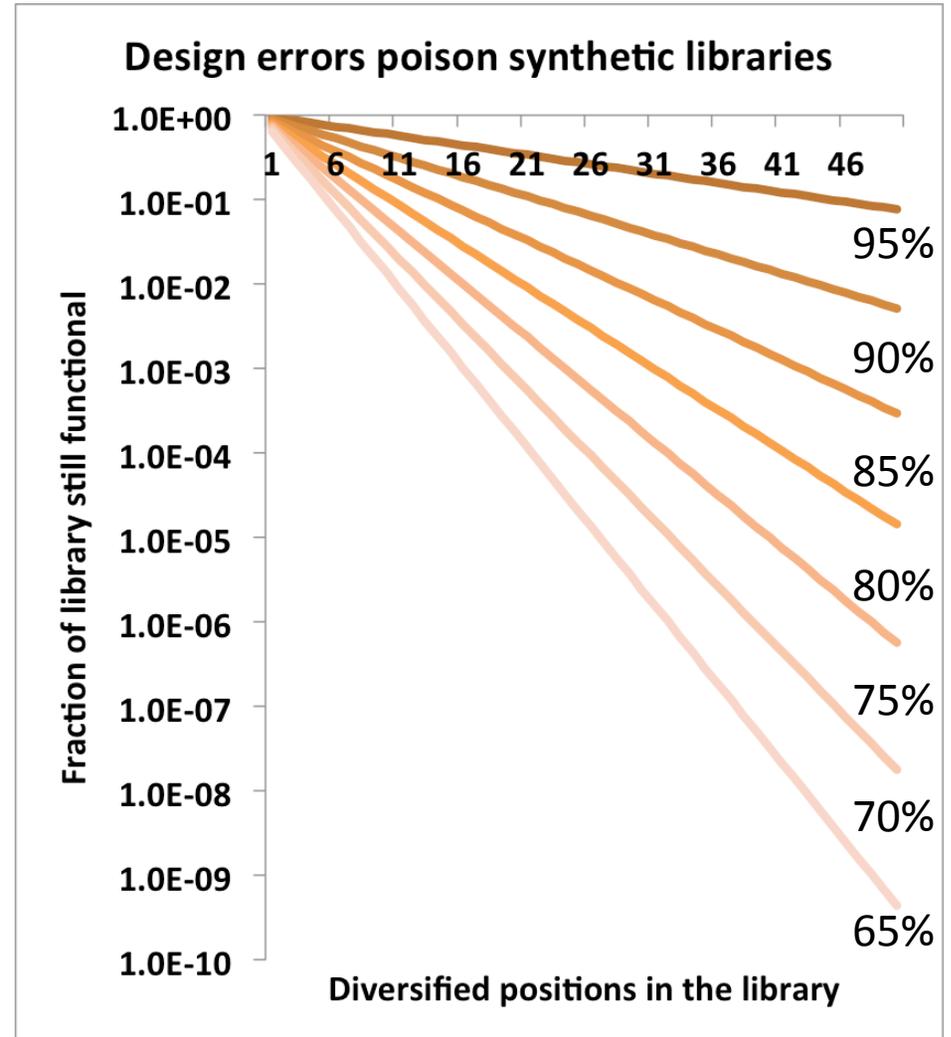
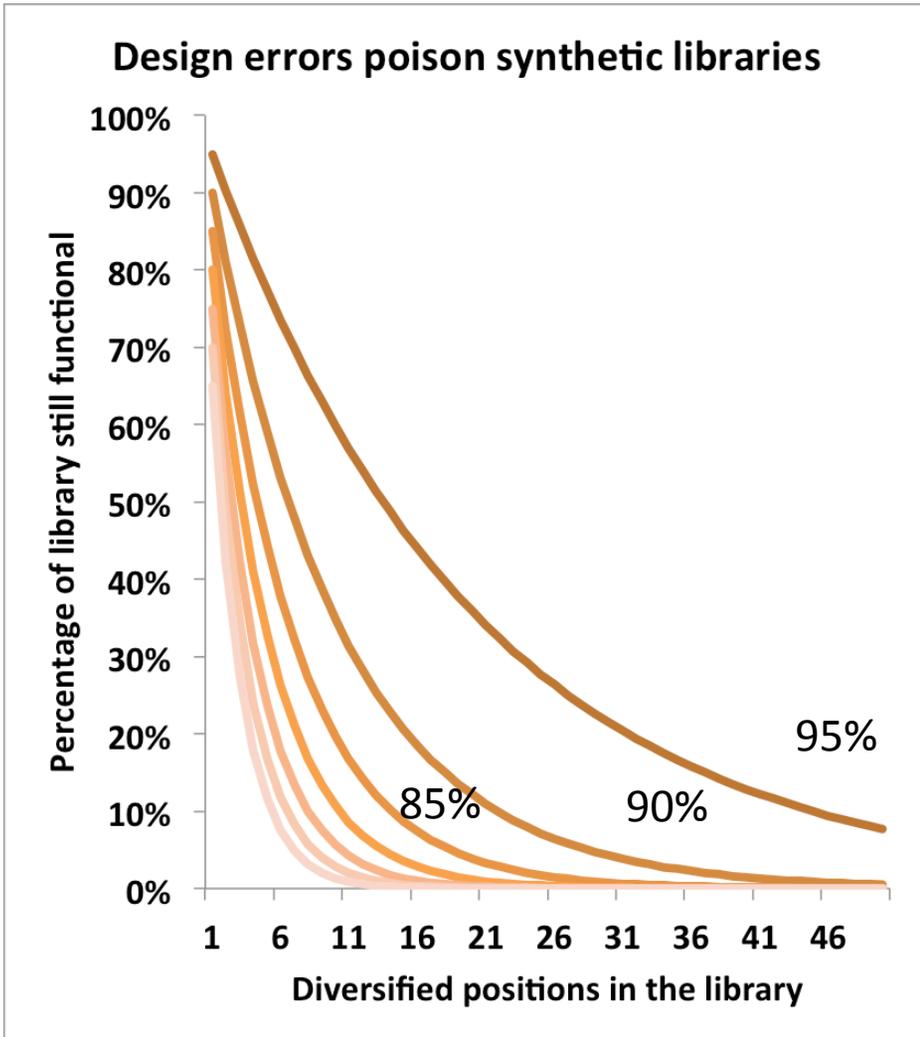
Mahon, Laburt, Glanville, et al. "Comprehensive interrogation of a minimalist synthetic CDR-H3 library and its ability to generate antibodies with therapeutic potential." *Journal of molecular biology* (2013).

Non-natural diversity not tolerated in many positions

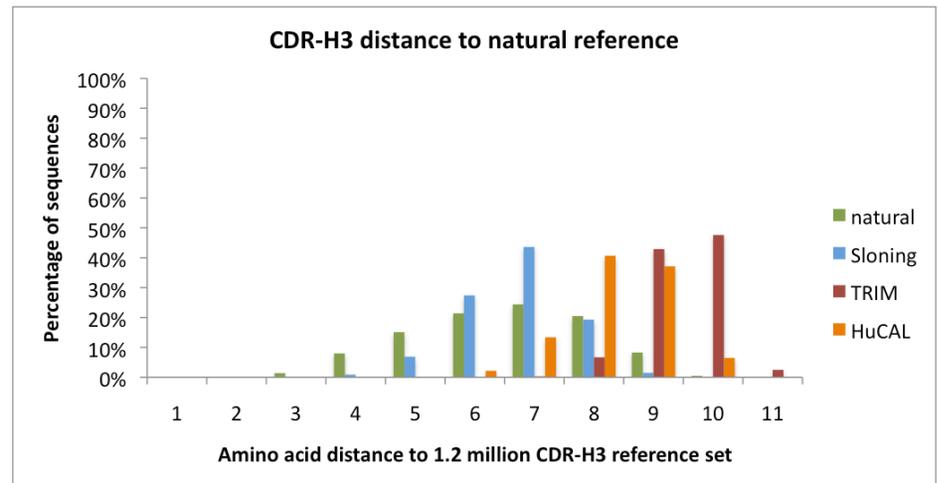
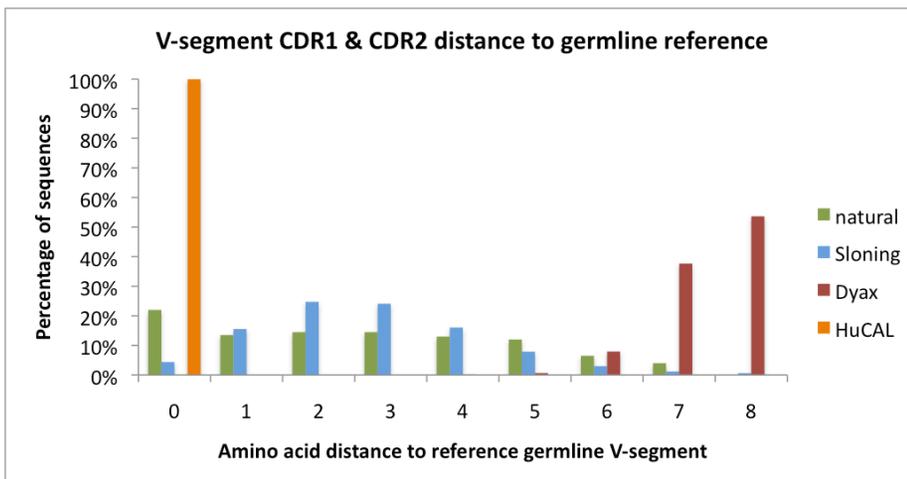
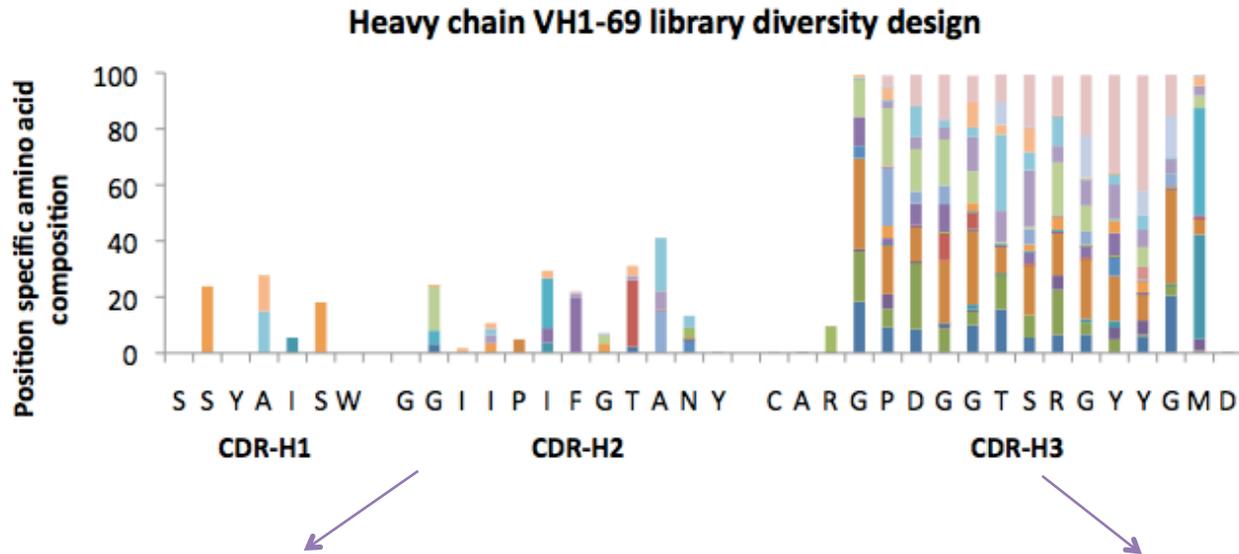


Mahon, Laburt, Glanville, et al. "Comprehensive interrogation of a minimalist synthetic CDR-H3 library and its ability to generate antibodies with therapeutic potential." *Journal of molecular biology* (2013).

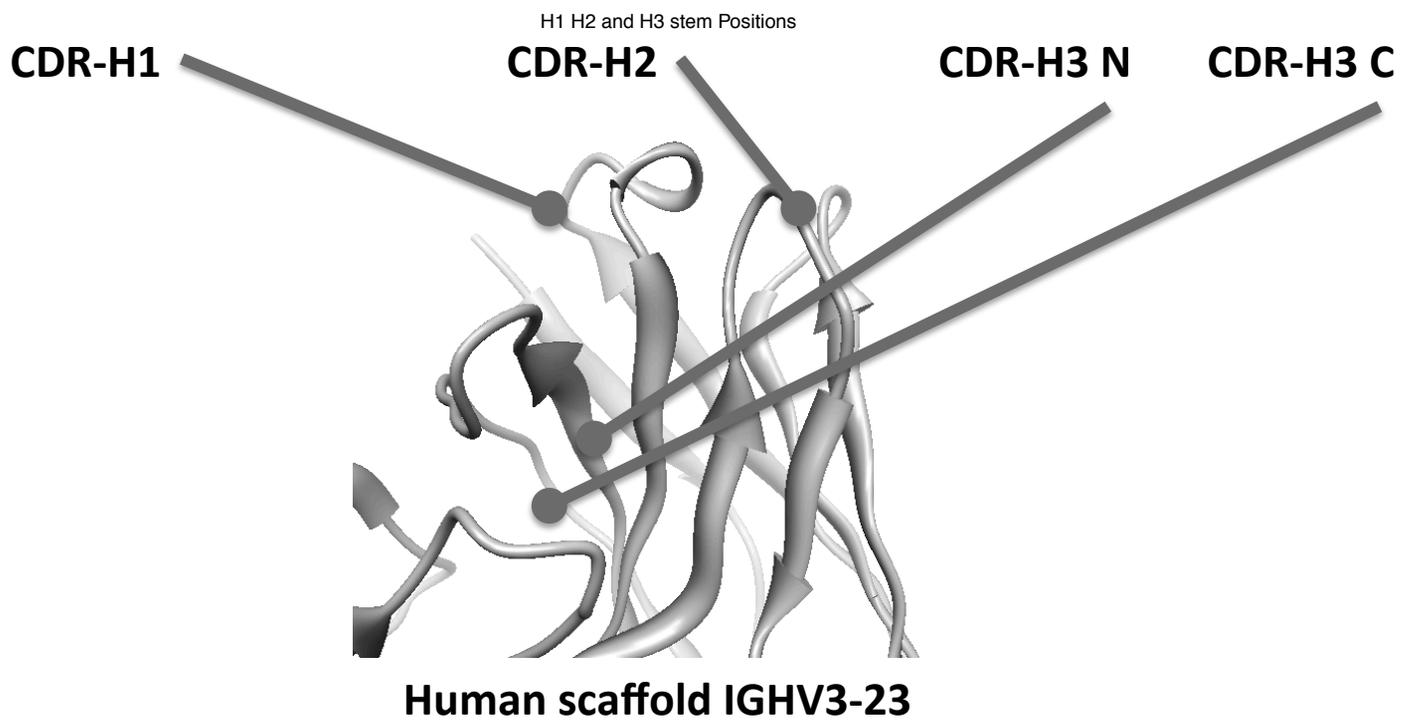
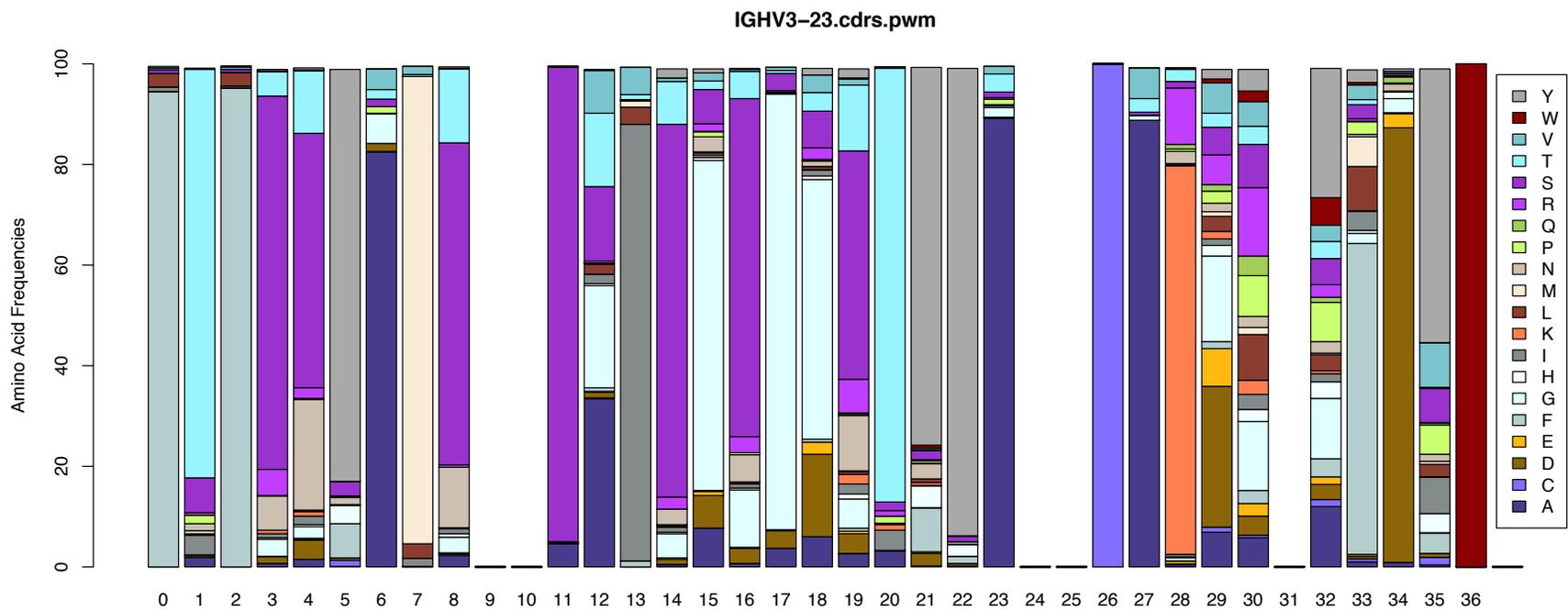
How the accumulation of small errors poisons a synthetic library



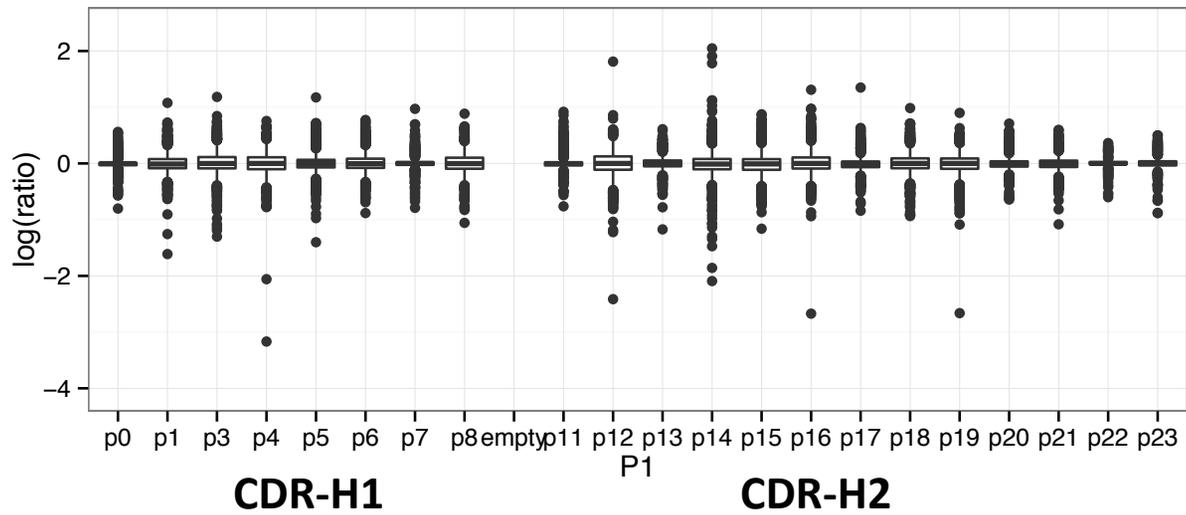
Synthetic libraries designed on natural sequence landscapes



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

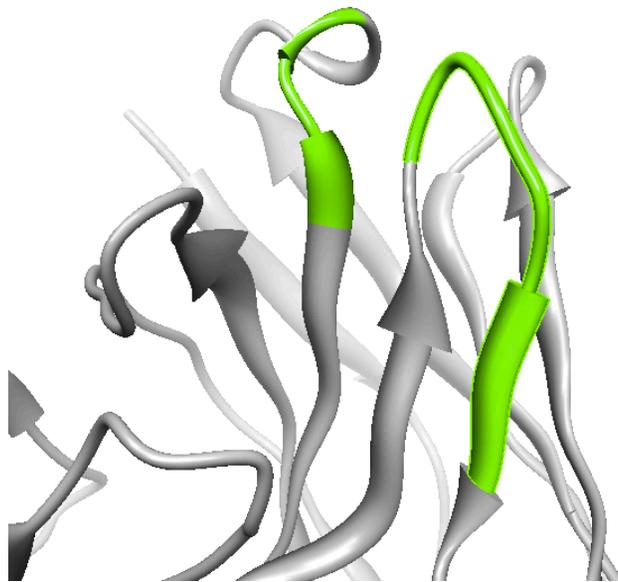


Observed vs expected paired mutation frequencies

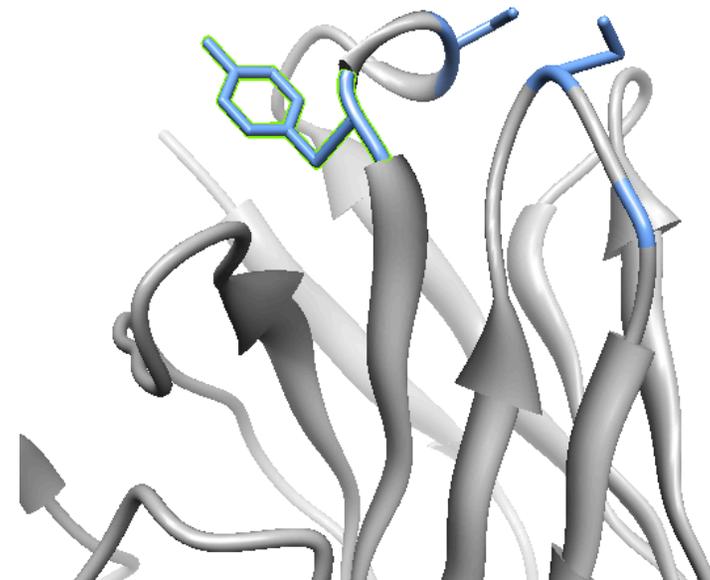


IGHV3-23 positional biases

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

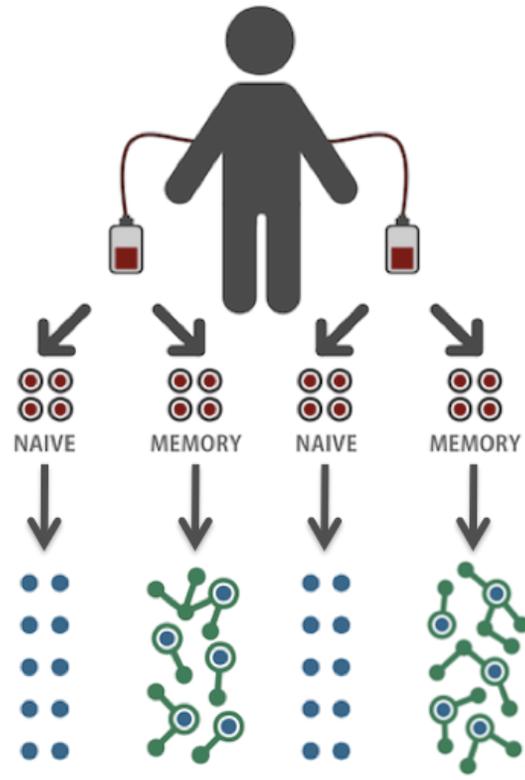


N-linked glycosylation aversion sites



Coordinated somatic hypermutation sites

Could we find such a synthesis technology?

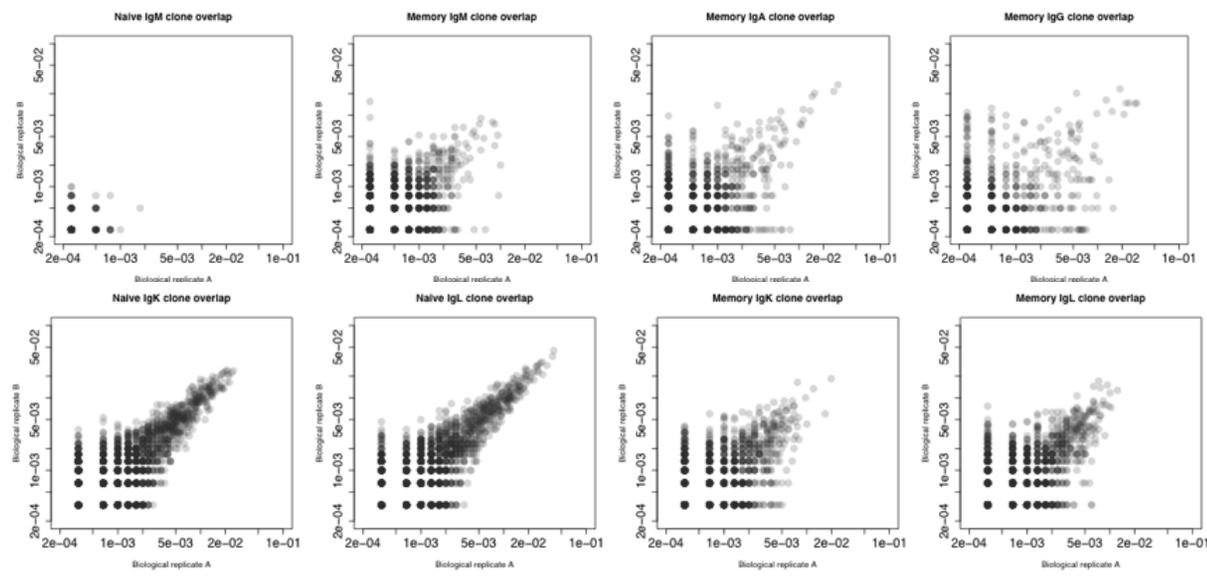


Naive

CD27+/IgM

IgG

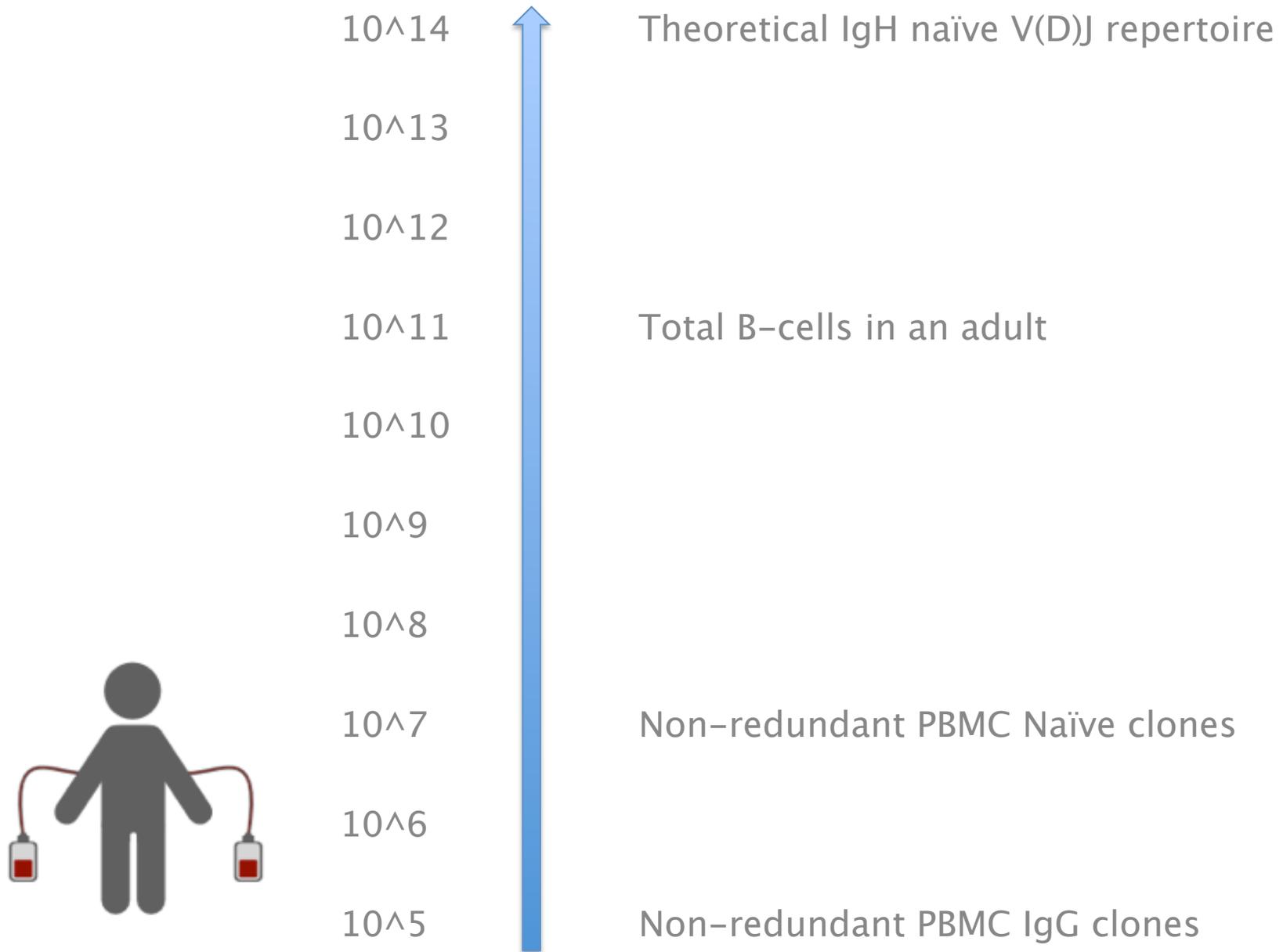
IgA



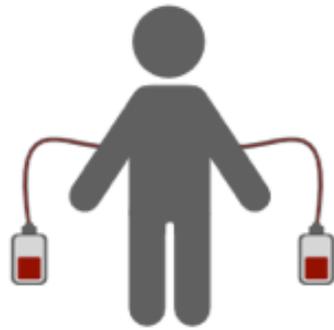
Heavy chains

Light chains

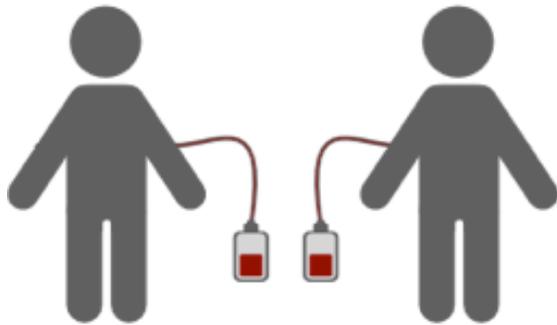
Individual adults only sample a small part of the antibody repertoire



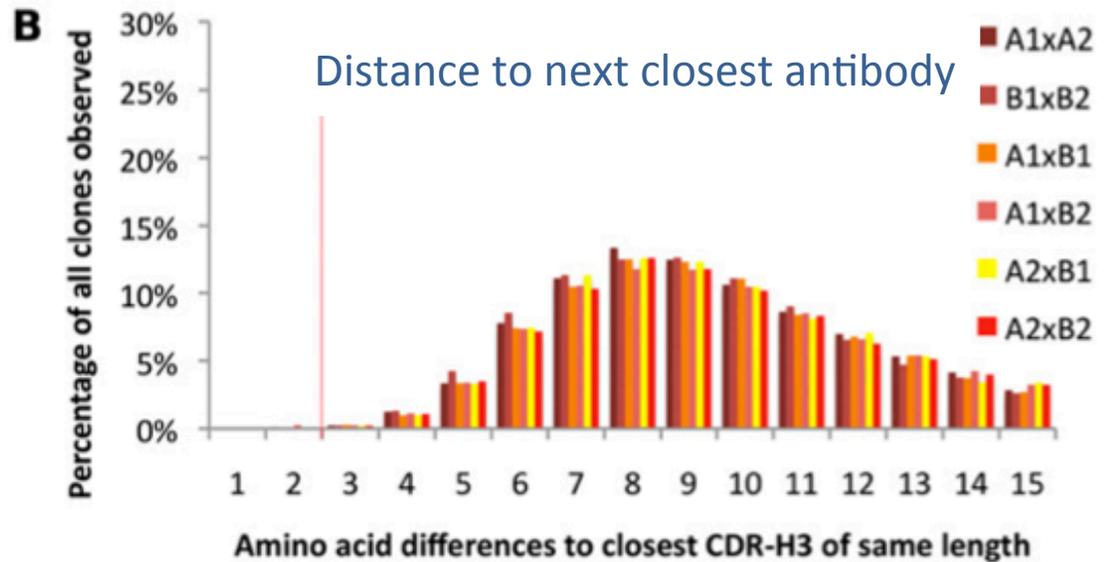
Humans produce dispersed receptor repertoires



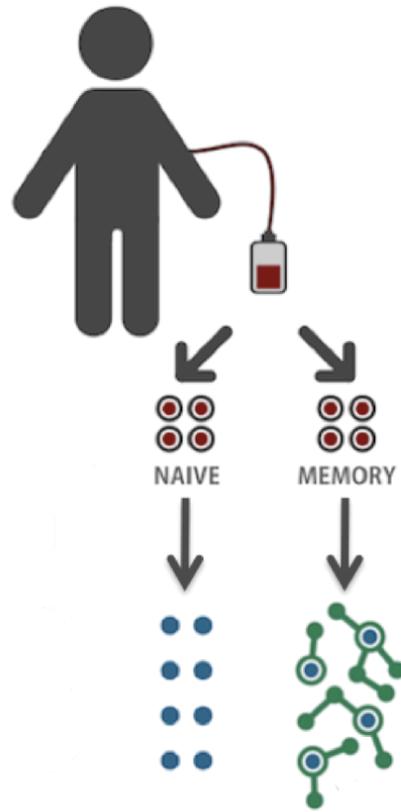
WITHIN
INDIVIDUAL



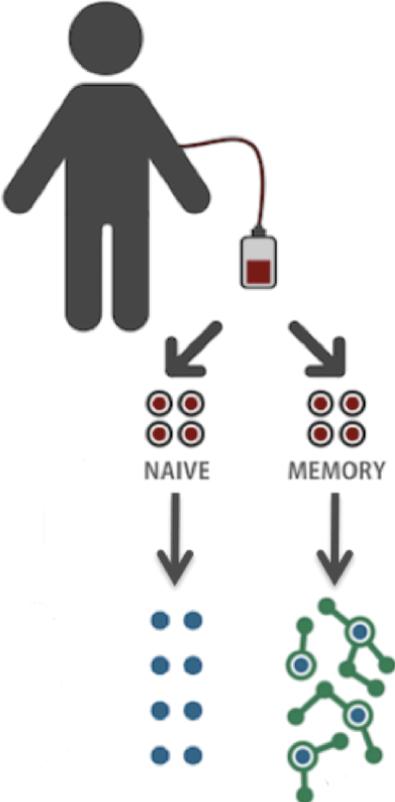
BETWEEN
INDIVIDUALS



Per person

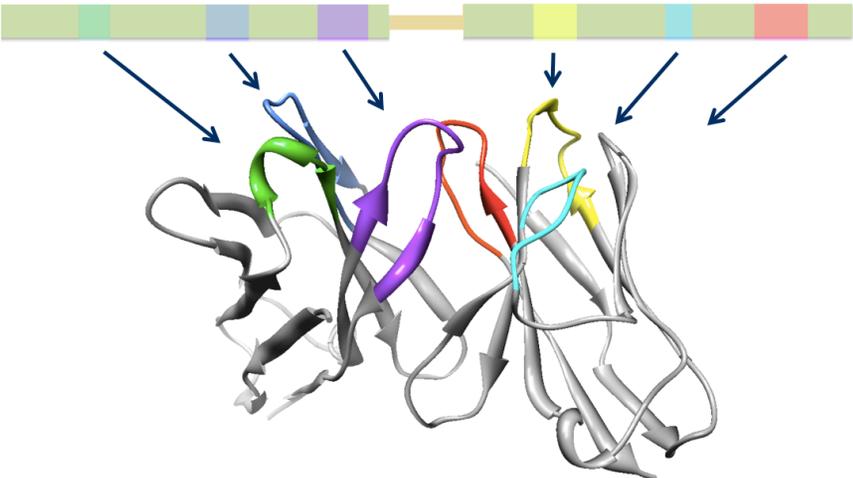


Per person

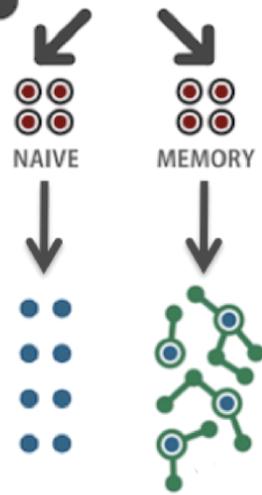


Naive CD27-/IgM+
High CDR3 diversity
Low individual overlap

- - 1e7 - - 1e5
H1 H2 H3 L1 L2 L3



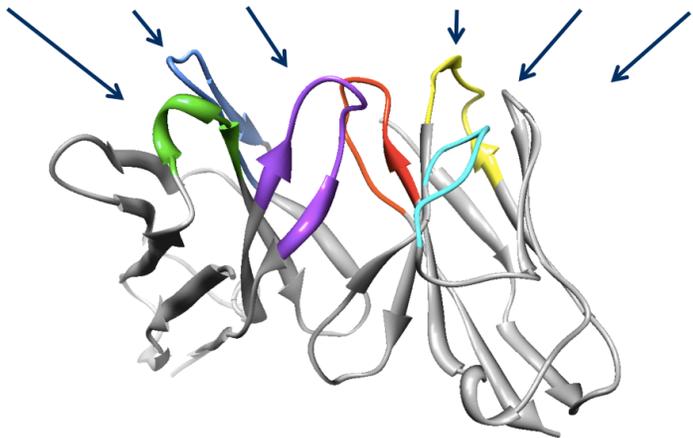
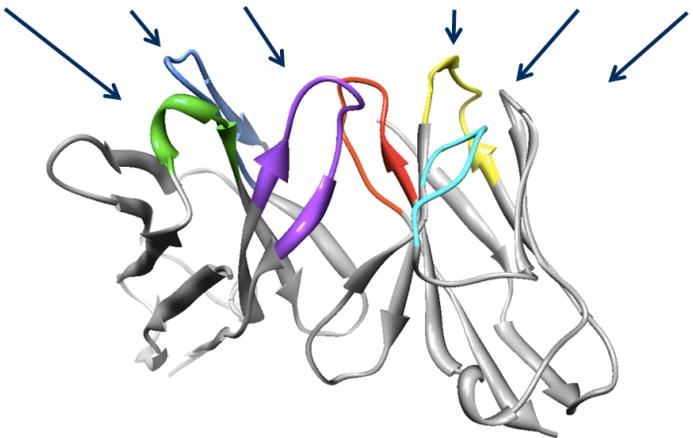
Per person

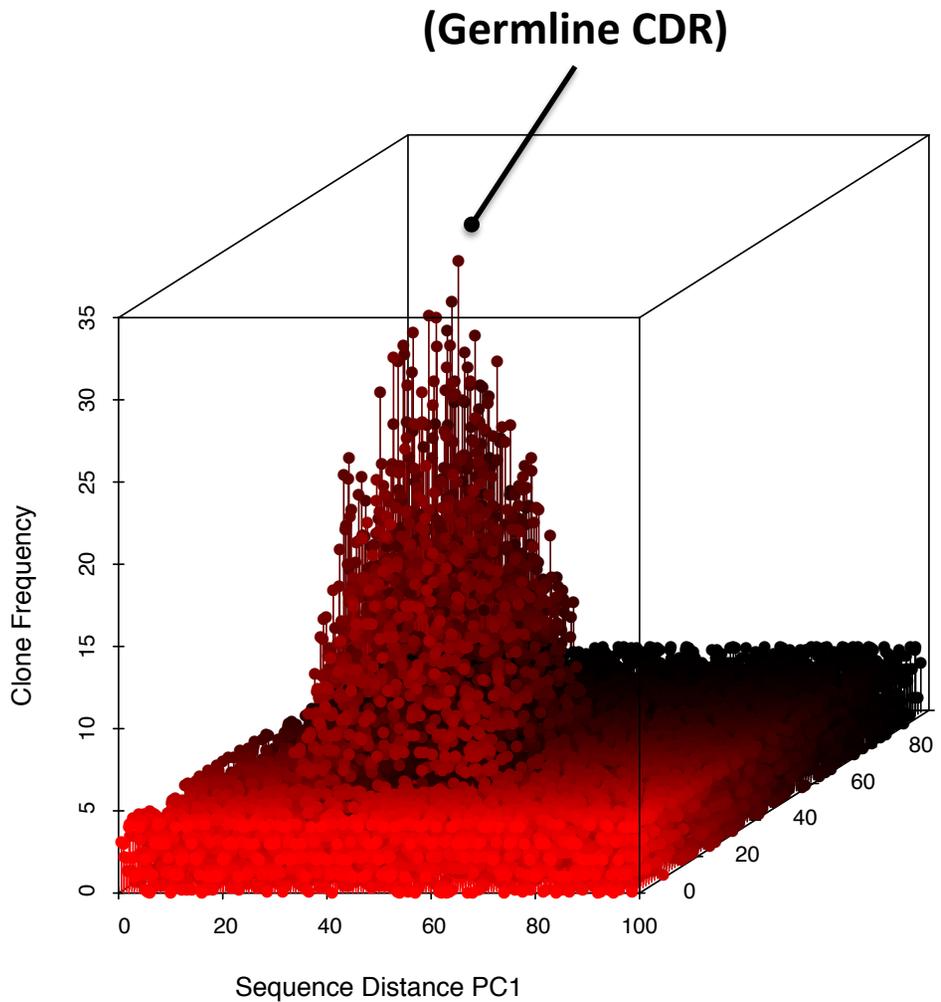


Naive CD27-/IgM+
High CDR3 diversity
Low individual overlap

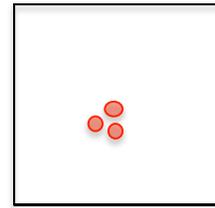


memory CD27+
High H1,H2,L1,L2 diversity
High clonality

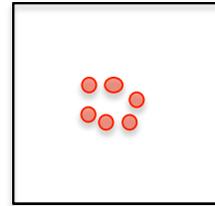




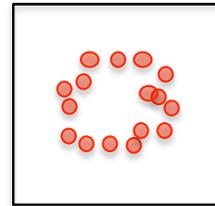
**Comprehensive affinity maturation
landscape from 100 people**



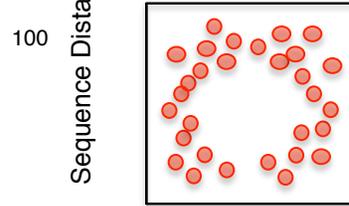
Single mutants
(~150)



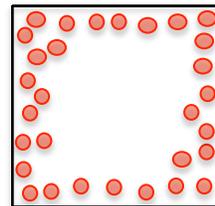
Double mutants
(~20,000)



Triple mutants
(~3,000,000)



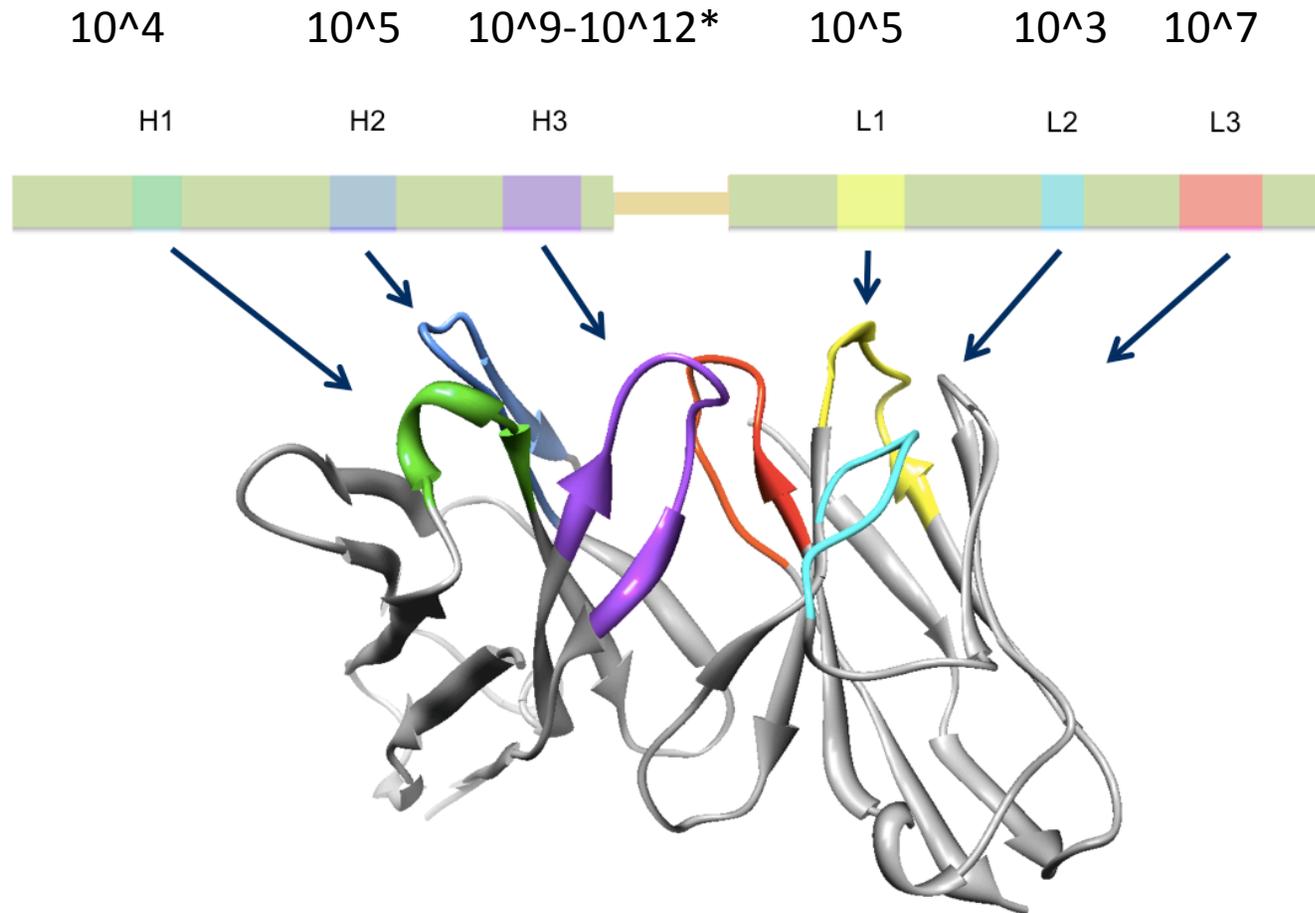
Quadruple mutants
(~500,000,000)



5+ mutants
(~75,000,000,000)

Per CDR, for every framework of interest

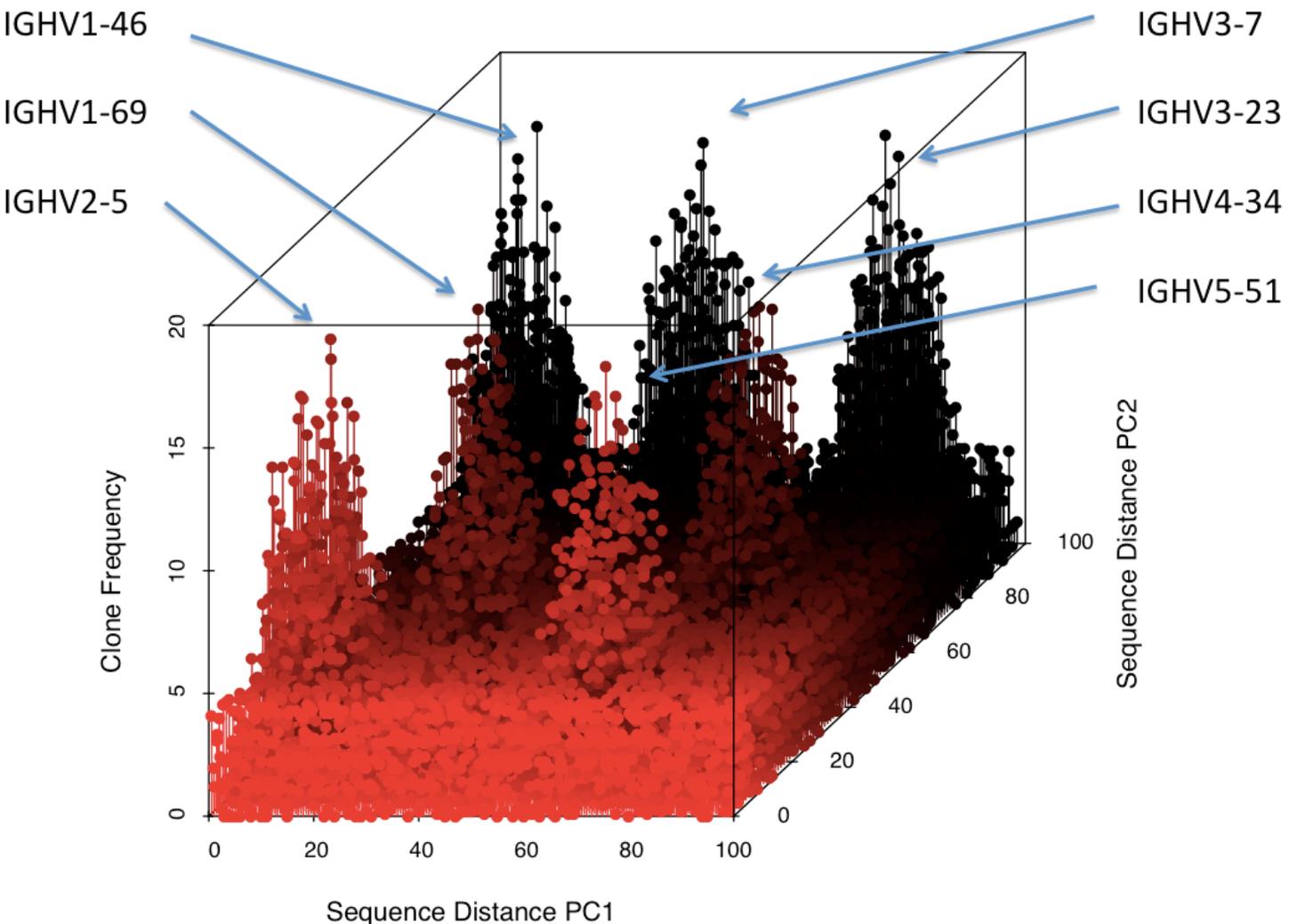
Naturally vetted diversity from sorted B-cells from 100 healthy donors



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

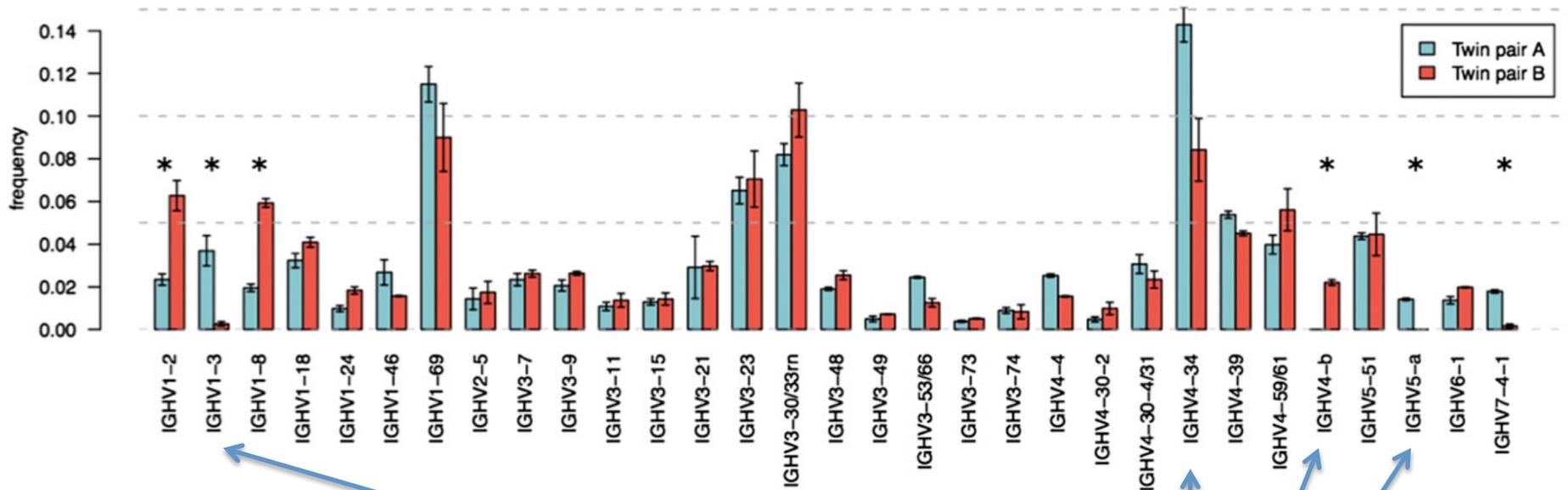
* 10^{12} with enzymatic expansion

Affinity Maturation Landscape



- Stable
- Used by all people
- Previously used as drugs

- Structurally diverse
- Non-redundant
- Not-aggregation prone



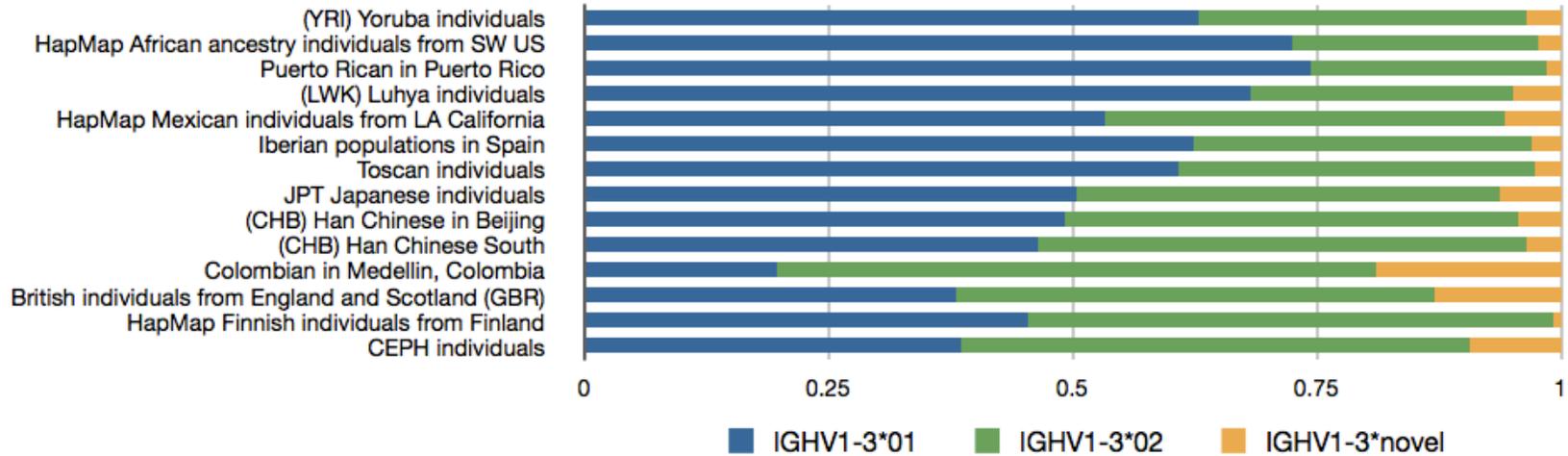
Problem alleles

Allele variation in human populations

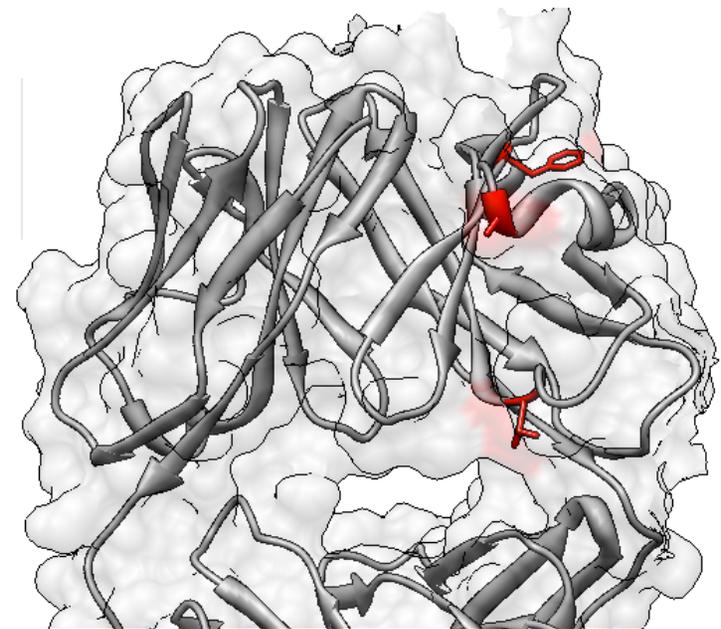
Missing/autoreactive in some individuals

Should we avoid polymorphic V-genes?

IGHV1-3 allele frequency variation

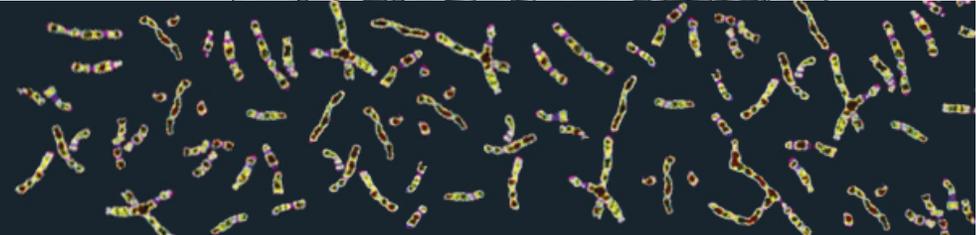


IGHV1-3*01/1-98	1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQAPGQRLWEMG	49
IGHV1-3*02/1-98	1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQAPGQRLWEMG	49
IGHV1-3*01/1-98	50	WLNAGNGNTKYSQK FQGRVTITRDTSASTAYMELSSLRSED TAVYYCAR	98
IGHV1-3*02/1-98	50	WLNAGNGNTKYSQE FQGRVTITRDTSASTAYMELSSLRSED MAVYYCAR	98

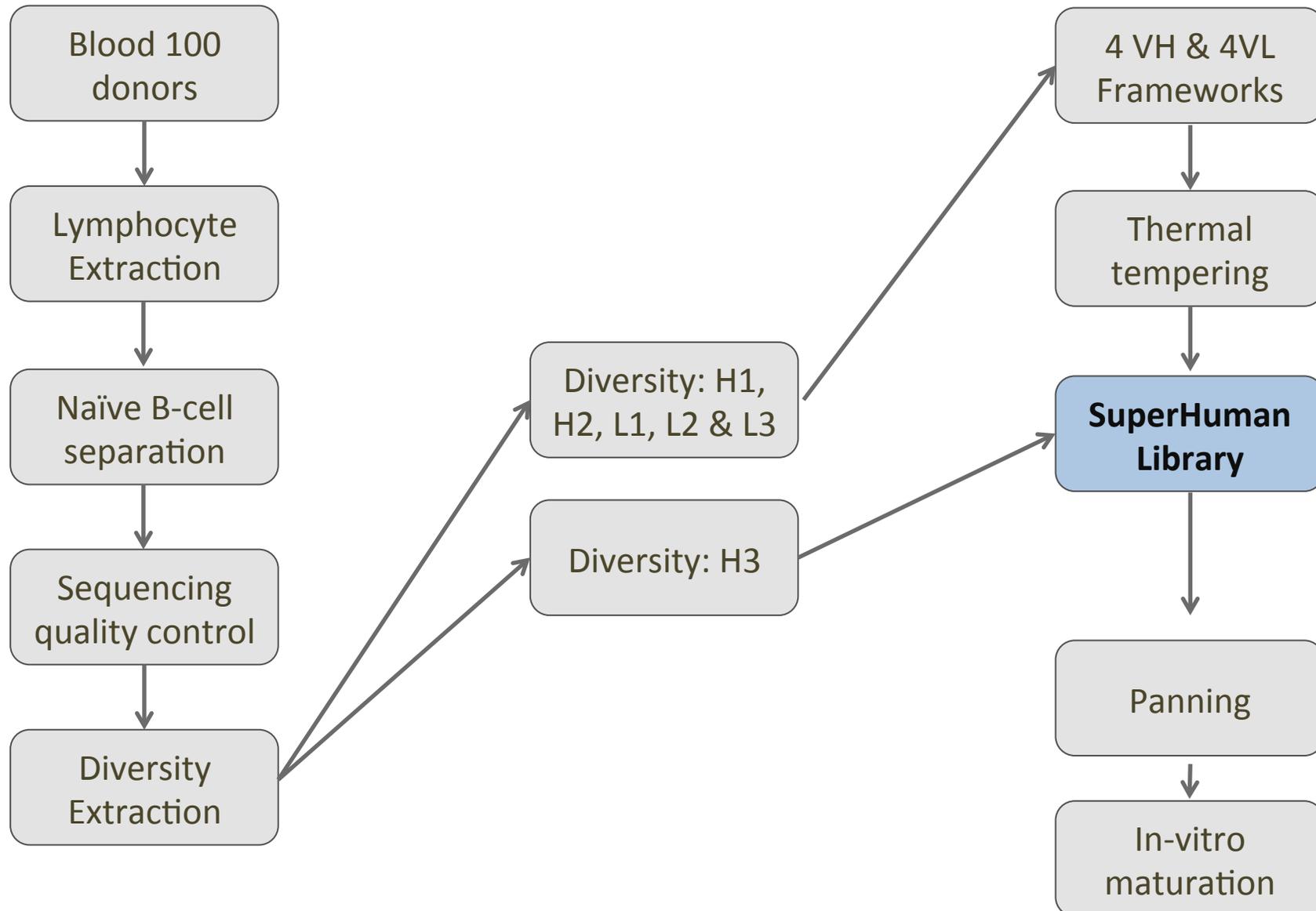


1000 Genomes

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

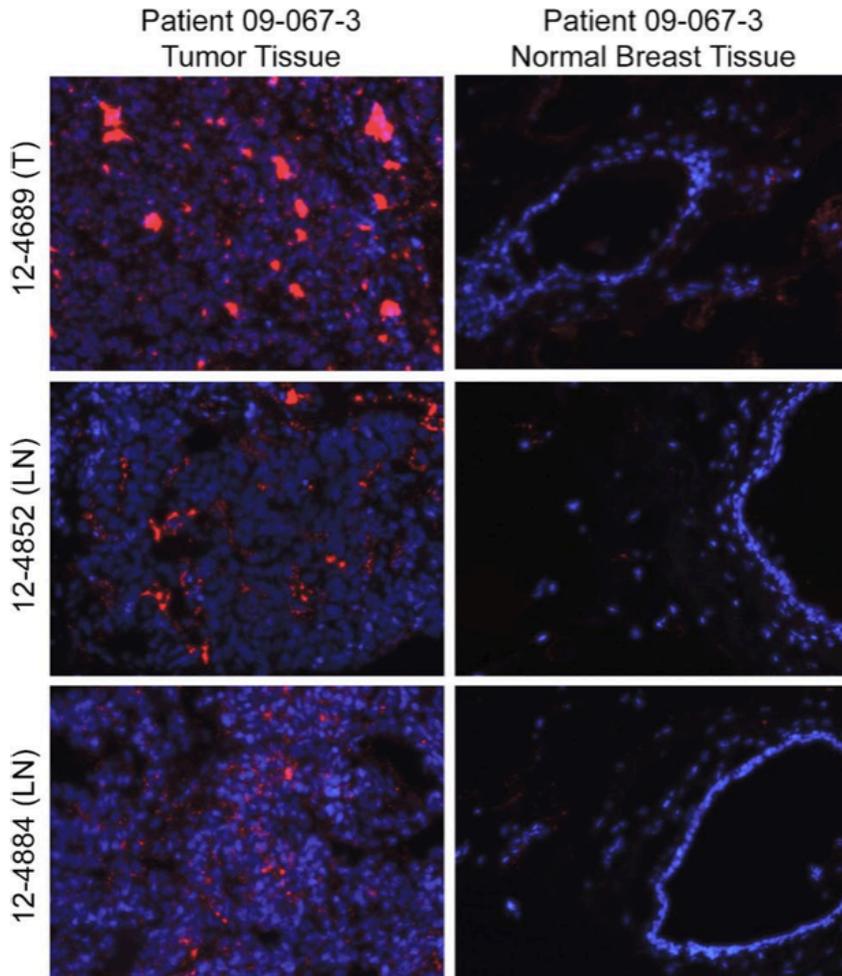
- Virii
- Bacteria
- Anti-venom
- Cancer



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

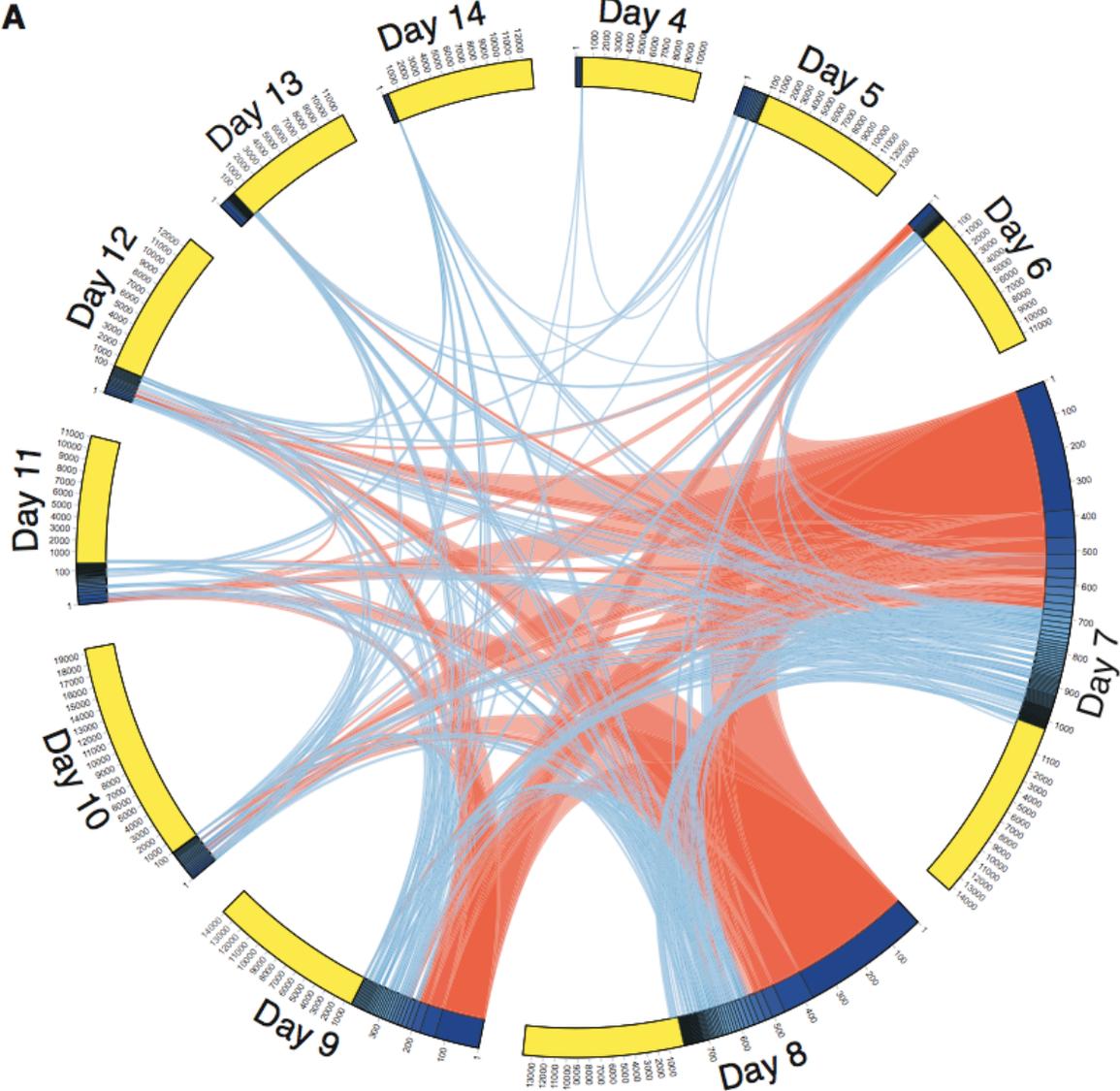
ORIGINAL ARTICLE

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



Vaccination: memory stored in secondary lymphoid organs



Who is the right population?





Figure 1

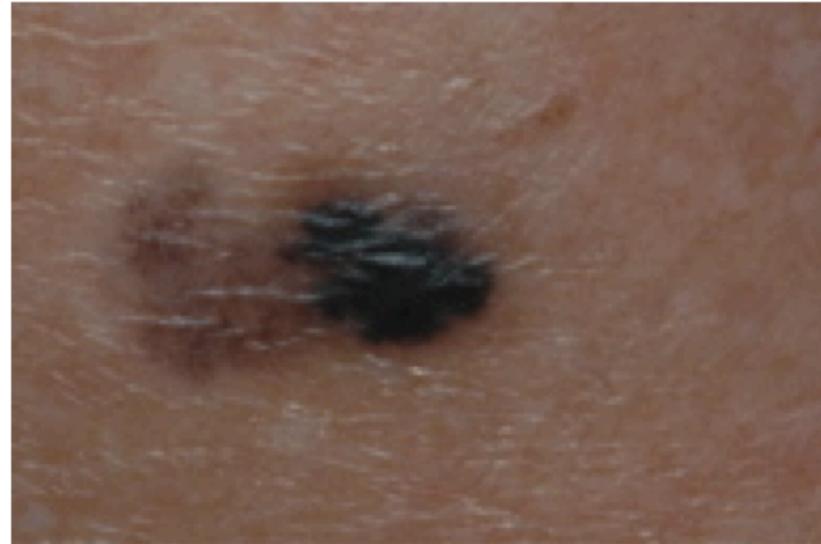


Figure 2

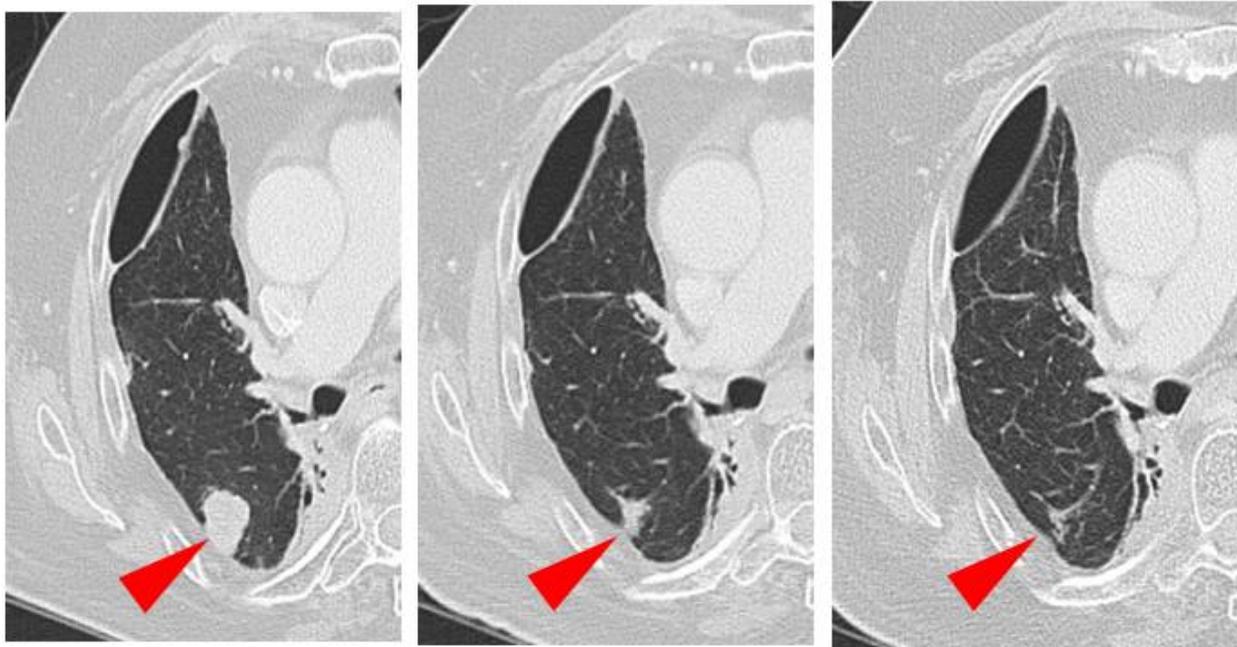
Complete regression of melanoma associated with vitiligo

Enric Piqué-Duran, Santiago Palacios-Llopis, M^aSol Martínez-Martín, Juan A Pérez-Cejudo

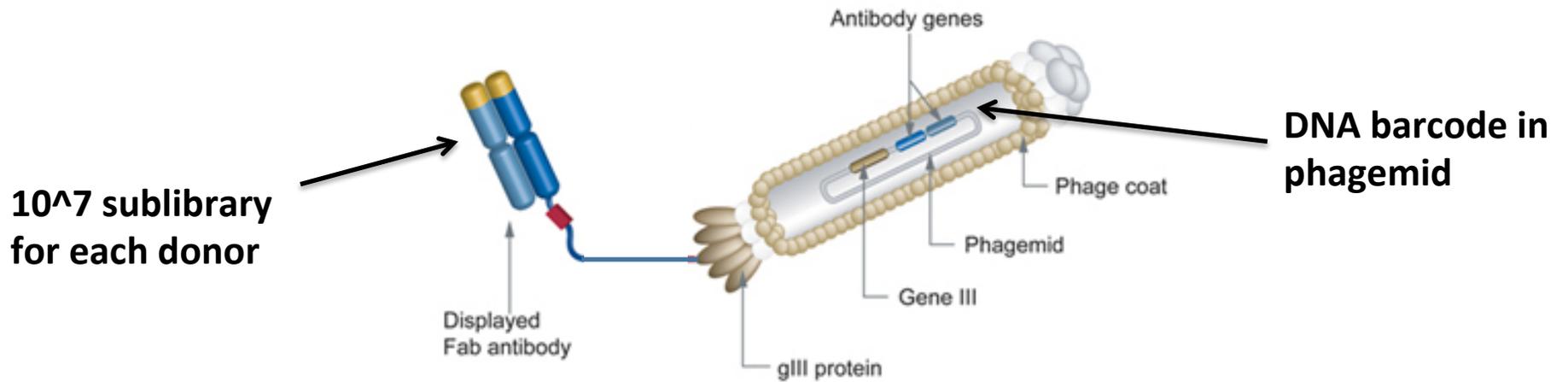
Dermatology Online Journal 17 (1): 4

Immune checkpoint blockade & induced remission

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



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



10^7 sublibrary
for each donor

DNA barcode in
phagemid

Syndrome X

100 Healthy controls





**We are crowdsourcing recruitment
Donate blood
Share the video
Tell your friends**



Giles Day
Chris Smith
Amy Geurts
Casey Keyes
David Louis

David Maurer
Gayathri Shankar
Maureen Kagimbi
Usha Maloney

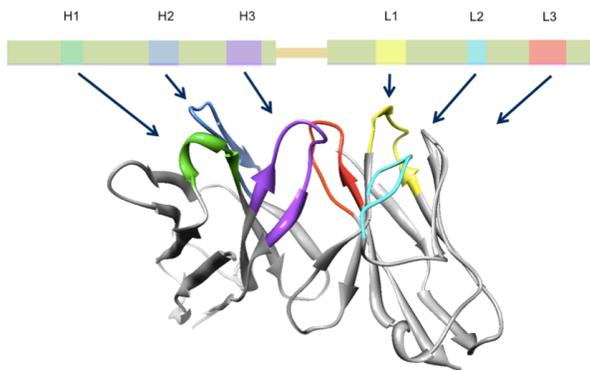


Giles Day

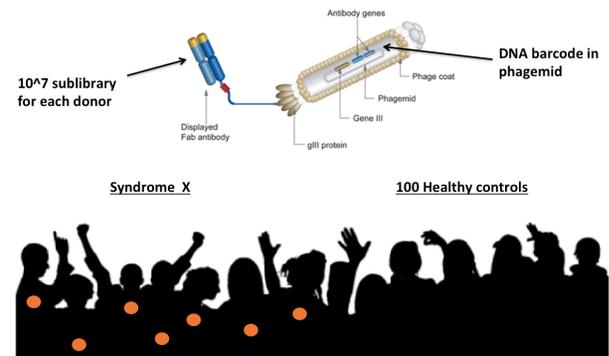


David Louis
(poster session)

jake@distributedbio.com



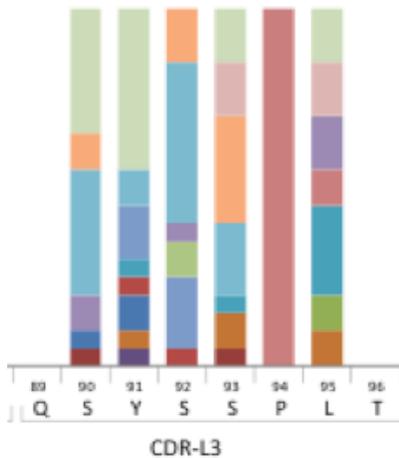
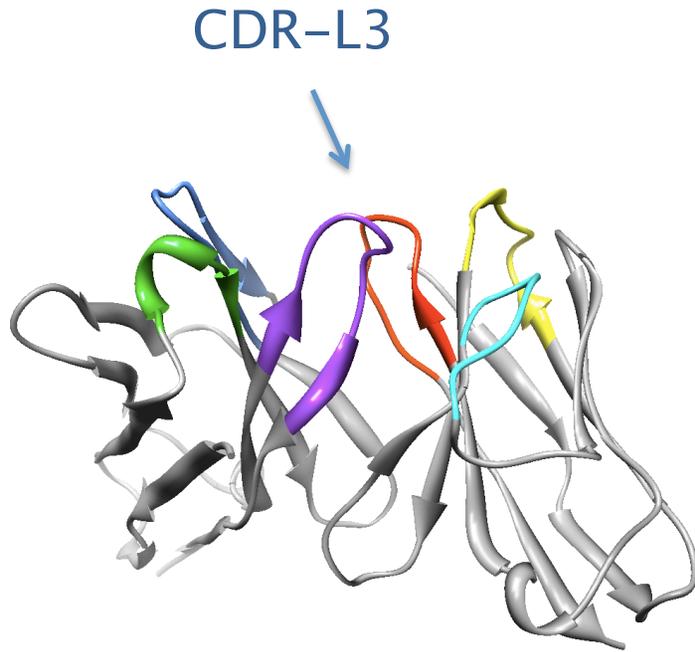
SuperHuman Library



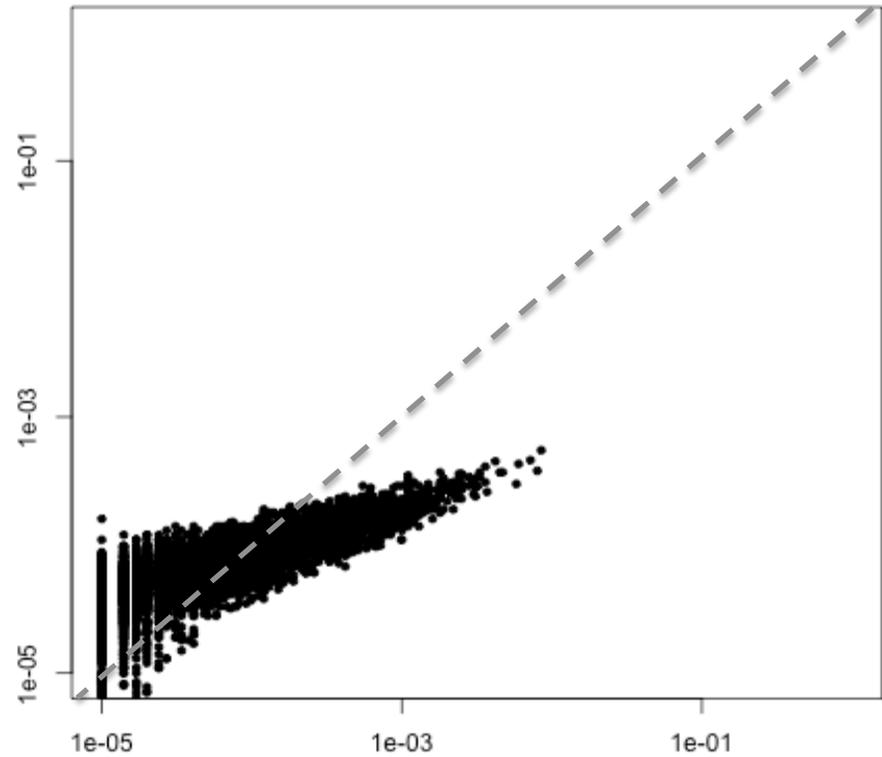
Survivor Library

Supp slides

Synthetic methods introduce hidden diversity bottlenecks



Theoretical frequency

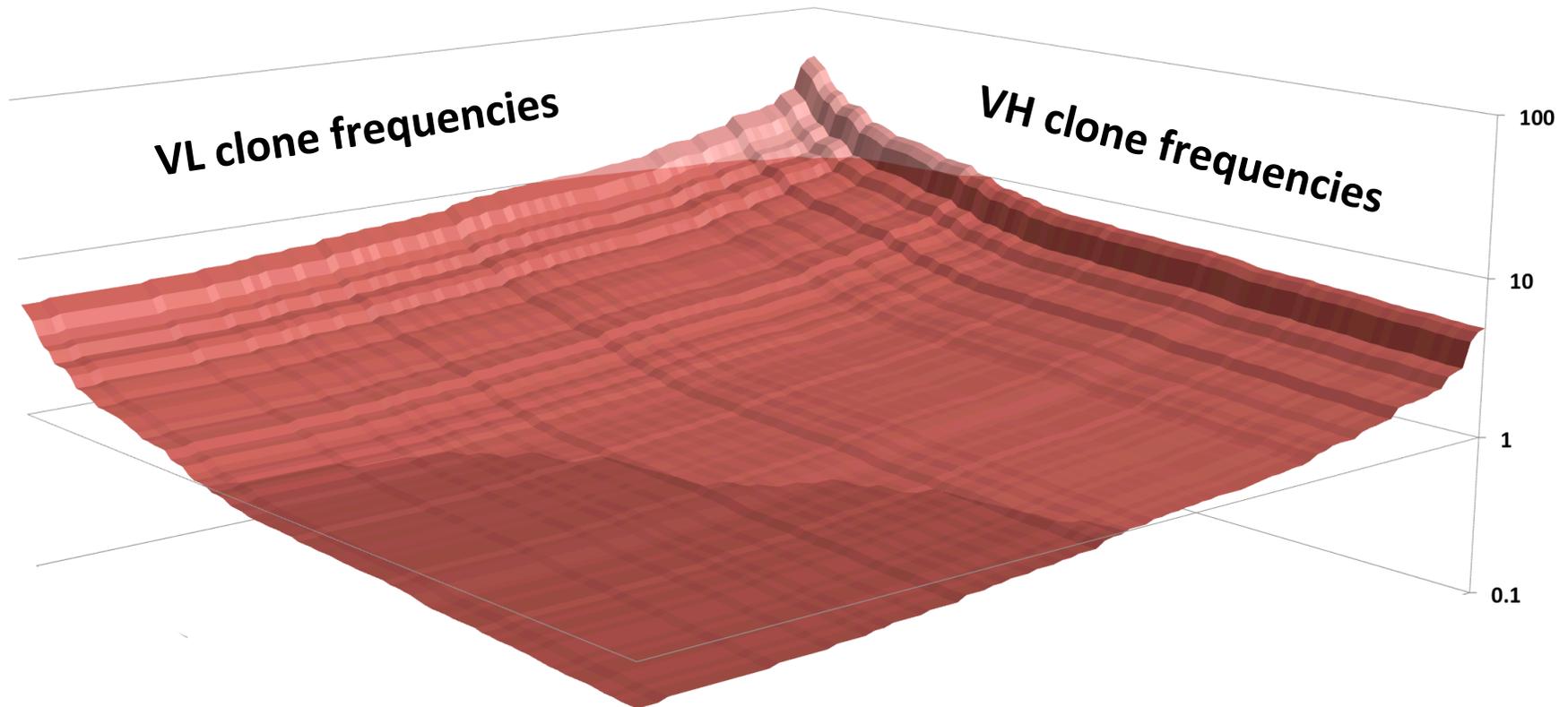


Observed frequency (NGS)

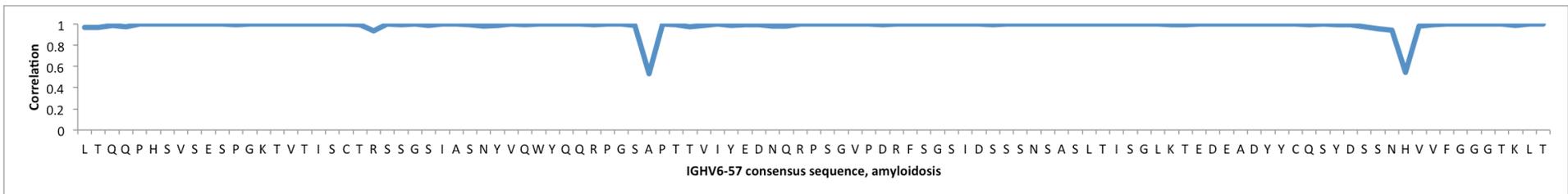
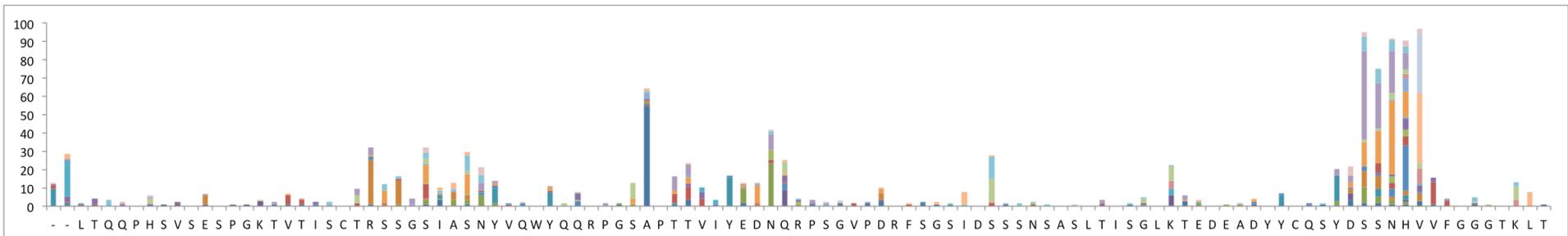
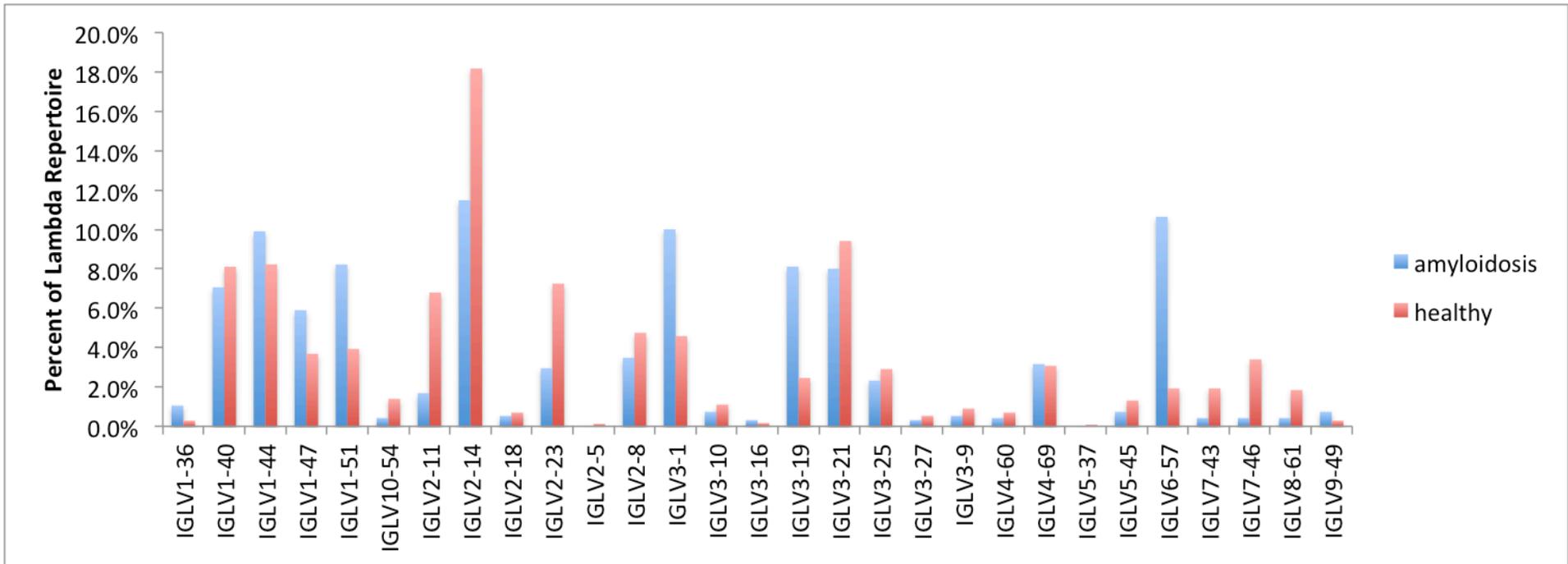
$$f_i = \text{Prob}(i) = \text{Prob}(\lambda_i) * \prod_{p=1}^{\lambda_i} \text{Prob}(\alpha_{i,p} | \text{PFM}_{\lambda})$$

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

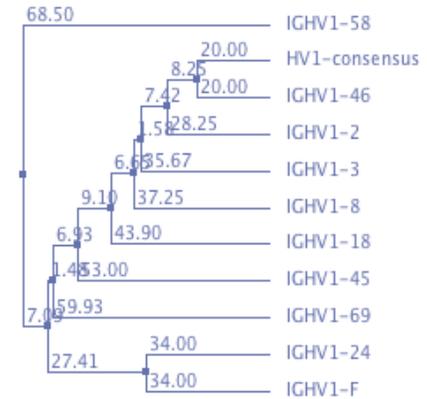


Heterodimer copies per 1 million clones

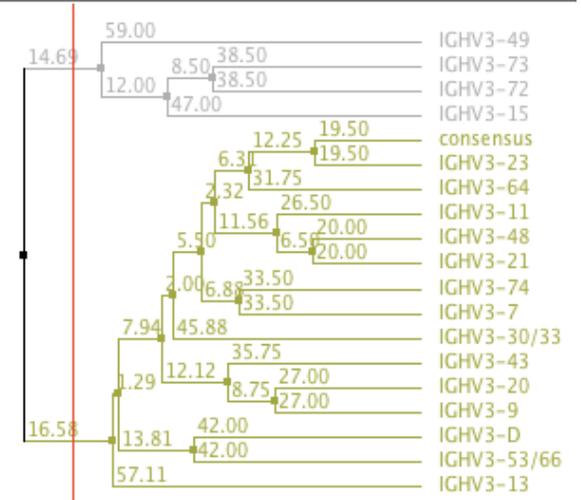


AMYLOID LIGHT CHAIN DATABASE

GY T F T S Y Y M H W V R Q A P G Q G L E W M G W I N P N S G N T N Y A Q K F Q G R V T I T R D T S T S T A Y M E L S S L R S E D T A V Y Y C A R
 G Y T F T G Y Y M H W V R Q A P G Q G L E W M G R I N P N S G G T N Y A Q K F Q G R V T S T R D T S I S T A Y M E L S R L R S D D T V Y Y C A R
 G Y T F T S Y A M H W V R Q A P G Q R L E W M G W I N A G N G N T K Y S Q K F Q G R V T I T R D T S A S T A Y M E L S S L R S E D T A V Y Y C A R
 G Y T F T S Y D I N W V R Q A T G Q G L E W M G W M N P N S G N T G Y A Q K F Q G R V T M T R N T S I S T A Y M E L S S L R S E D T A V Y Y C A R
 G Y T F T S Y Y M H W V R Q A P G Q G L E W M G I I N P S G G S T S Y A Q K F Q G R V T M T R D T S T S T V Y M E L S S L R S E D T A V Y Y C A R
 G G T F S S Y A I S W V R Q A P G Q G L E W M G G I I P I F G T A N Y A Q K F Q G R V T I T A D E S T S T A Y M E L S S L R S E D T A V Y Y C A R
 C F T F T S S A V Q W V R Q A R G Q R L E W I G W I V V G S G N T N Y A Q K F Q E R V T I T R D M S T S T A Y M E L S S L R S E D T A V Y Y C A R
 G Y T F T S Y G I S W V R Q A P G Q G L E W M G W I S A Y N G N T N Y A Q K L Q G R V T M T T D T S T S T A Y M E L R S L R S D D T A V Y Y C A R
 G Y T F T Y R Y L H W V R Q A P G Q A L E W M G W I T P F N G N T N Y A Q K F Q D R V T I T R D R S M S T A Y M E L S S L R S E D T A M Y Y C A R
 G Y T L T E L S M H W V R Q A P G K G L E W M G G F D P E D G E T I Y A Q K F Q G R V T M T E D T S T D T A Y M E L S S L R S E D T A V Y Y C A T
 G Y T F T D Y Y M H W V Q Q A P G K G L E W M G L V D P E D G E T I Y A E K F Q G R V T I T A D T S T D T A Y M E L S S L R S E D T A V Y Y C A T



S G F T F G D Y A M S W F R Q A P G K G L E W V G F I R S K A Y G G T T E Y T A S V K G R F T I S R D G S K S I A Y L Q M N S L K T E D T A V Y Y C T R
 S G F T F S G S A M H W V R Q A S G K G L E W V G R I R S K A N S Y A T A Y A A S V K G R F T I S R D D S K N T A Y L Q M N S L K T E D T A V Y Y C T R
 S G F T F S D H Y M D W V R Q A P G K G L E W V G R T R N K A N S Y T T E Y A A S V K G R F T I S R D D S K N S L Y L Q M N S L K T E D T A V Y Y C A R
 S G F T F S N A W M S W V R Q A P G K G L E W V G R I K S K T D G G T T D Y A A P V K G R F T I S R D D S K N T L Y L Q M N S L K T E D T A V Y Y C T T
 S G F T F S S Y D M H W V R Q A T G K G L E W V S A I G - - - T A G D T Y Y P G S V K G R F T I S R E N A K N S L Y L Q M N S L R A G D T A V Y Y C A R
 S G F T V S S N E M S W V R Q A P G K G L E W V S S I - - - - S G G S T Y Y A D S R K G R F T I S R D N S K N T L H L Q M N S L R A E D T A V Y Y C K K
 S G F T V S S N Y M S W V R Q A P G K G L E W V S V I Y - - - S G G S T Y Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R
 S G F T F S S Y A M S W V R Q A P G K G L E W V S R I S S - - S G G S T Y Y A D S V K G R F T I S R D N S K N S L Y L Q M N S L R A E D T A V Y Y C A R
 S G F T F D D Y T M H W V R Q A P G K G L E W V S L I S W - - D G G S T Y Y A D S V K G R F T I S R D N S K N S L Y L Q M N S L R T E D T A L Y Y C A K
 S G F T F D D Y G M S W V R Q A P G K G L E W V S G I N W - - N G G S T G Y A D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A L Y H C A R
 S G F T F D D Y A M H W V R Q A P G K G L E W V S G I S W - - N S G S I G Y A D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A L Y Y C A K
 S G F T F S S Y A M H W V R Q A P G K G L E Y V S A I S S - - N G G S T Y Y A N S V K G R F T I S R D N S K N T L Y L Q M G S L R A E D T A V Y Y C A R
 S G F T F S S Y A M S W V R Q A P G K G L E W V S A I S G - - S G G S T Y Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K
 S G F T F S S Y G M H W V R Q A P G K G L E W V A V I W Y - - D G S N K Y Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R
 S G F T F S D Y Y M S W I R Q A P G K G L E W V S Y I S S - - S G S T I Y Y A D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A V Y Y C A R
 S G F T F S S Y S M N W V R Q A P G K G L E W V S Y I S S - - S S S T I Y Y A D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A V Y Y C A R
 S G F T F S S Y S M N W V R Q A P G K G L E W V S S I S S - - S S S Y I Y Y A D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A V Y Y C A R
 S G F T F S S Y W M H W V R Q A P G K G L V W V S R I N S - - D G S S T S Y A D S V K G R F T I S R D N A K N T L Y L Q M N S L R A E D T A V Y Y C A R
 S G F T F S S Y W M S W V R Q A P G K G L E W V A N I K Q - - D G S E K Y Y V D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A V Y Y C A R



A V S G G S I S S S Y Y W S W I R Q P P G K G L E W I G Y I Y Y S G S T Y Y N P S L K S R V T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R
 A V Y G G S F S - - G Y Y W S W I R Q P P G K G L E W I G E I N H S G S T N Y N P S L K S R V T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R
 A V S G G S I S S S N W - W S W V R Q P P G K G L E W I G E I Y H S G S T N Y N P S L K S R V T I S V D K S K N Q F S L K L S S V T A A D T A V Y C A R
 A V S G Y S I S S S N W - W G W I R Q P P G K G L E W I G Y I Y Y S G S T Y Y N P S L K S R V T M S V D T S K N Q F S L K L S S V T A V D T A V Y Y C A R
 A V S G G S I S S G G Y S W S W I R Q P P G K G L E W I G Y I Y H S G S T Y Y N P S L K S R V T I S V D R S K N Q F S L K L S S V T A A D T A V Y Y C A R
 A V S G Y S I S S - G Y Y W G W I R Q P P G K G L E W I G S I Y H S G S T Y Y N P S L K S R V T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R
 T V S G G S I S S S Y Y W G W I R Q P P G K G L E W I G S I Y Y S G S T Y Y N P S L K S R V T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R
 T V S G G S I S S G D Y Y W S W I R Q P P G K G L E W I G Y I Y Y S G S T Y Y N P S L K S R V T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R
 T V S G G S I S - - S Y Y W S W I R Q P P G K G L E W I G Y I Y Y S G S T N Y N P S L K S R V T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R
 T V S G G S V S S G S Y Y W S W I R Q P P G K G L E W I G Y I Y Y S G S T N Y N P S L K S R V T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R

