Somatic diversification of rearranged antibody gene segments by genome-wide templated mutagenesis

AIRR Dec 2020

Gordon Dale, PhD
Jacob Lab
Yerkes National Primate Research Center
Emory University
USA

Overview

1) **Introduction**
2) **Microhomology Gene Conversion***
3) **Genome-Wide Gene Conversion**
4) **Summary**

Somatic Hypermutation

- Mechanism by which antigen specific, high affinity antibodies are made during humoral immune response

- Two methods:
  - Untemplated - Canonical somatic hypermutation
    - Mutations are unrelated to preexisting DNA sequences
  - Templated - Gene conversion
    - Mutations are copied via reading of preexisting DNA sequences

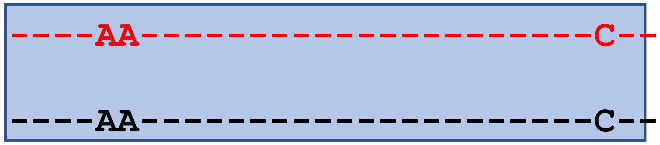
Somatic Hypermutation and Gene Conversion

Somatic Hypermutation

Germline 1	**ATCTGTAATAGCATCAGGGTAGGAGATTTAGACAGA**	
Mutated Sequence	**---G---C----T-------C---------------**	**Untemplated**
Germline 2	**----AA---------------------C---T-T-T**	

Somatic Hypermutation and Gene Conversion

Gene Conversion

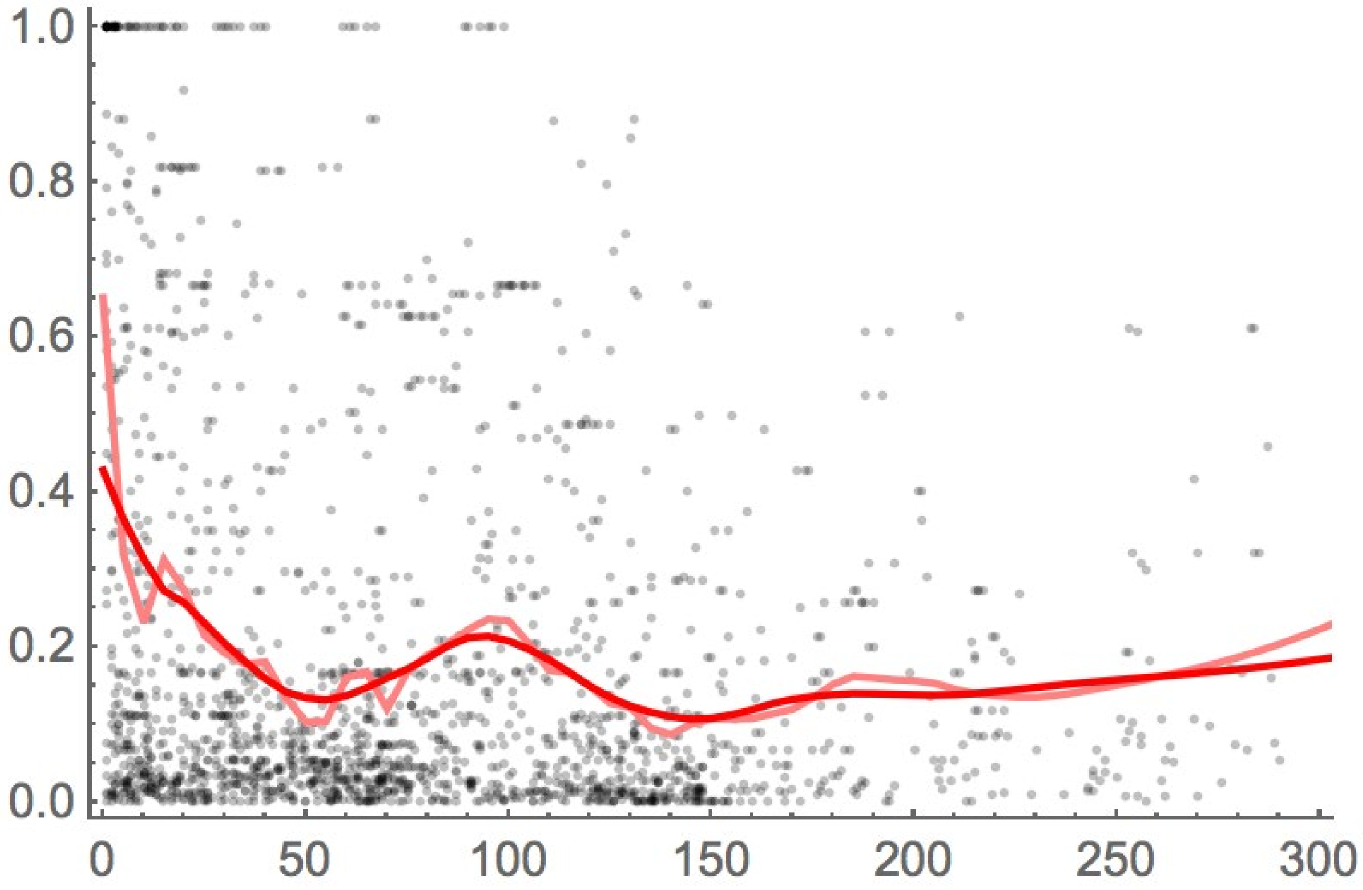


```
Germline 1           ATCTGTAATAGCATCAGGGTAGGAGATTTAGACAGA
Mutated Sequence     ----AA---------------------C--------      Templated
Germline 2           ----AA---------------------C---T-T-T
```

Linkage Disequilibrium

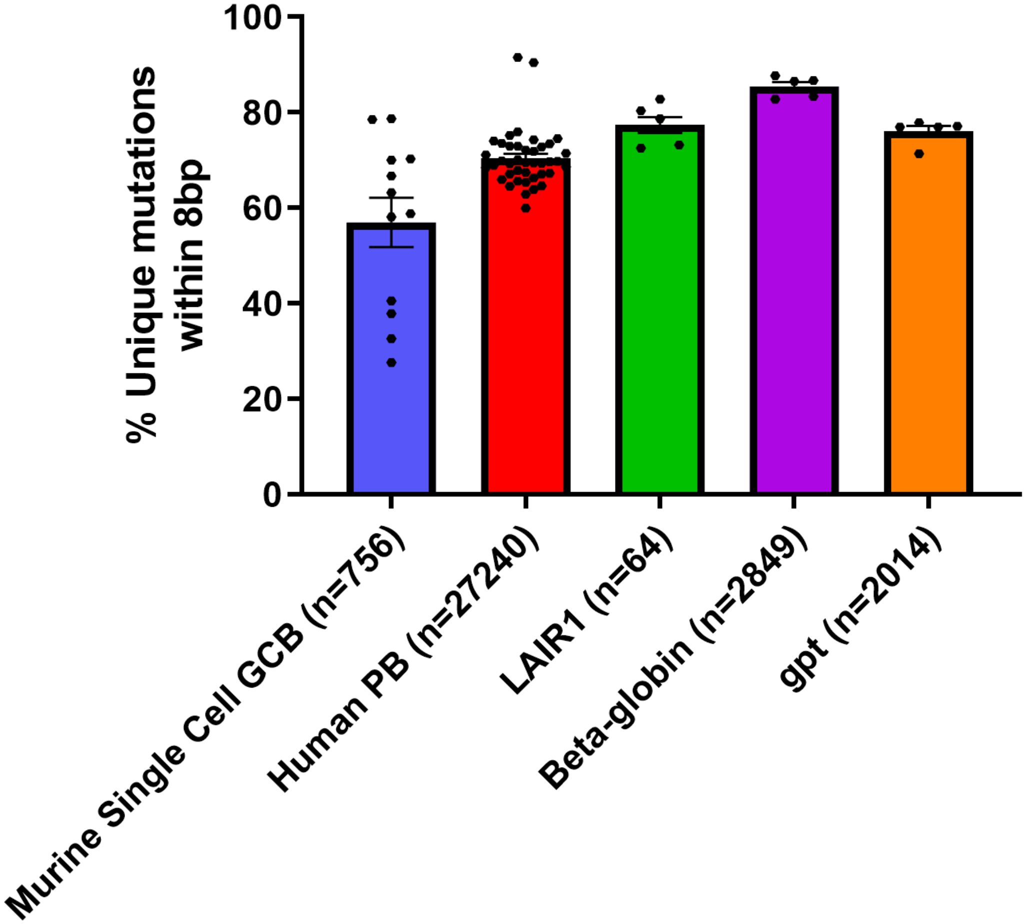
Mouse Germinal Center B

Rabbit Germinal Center B



Cluster of mutations and Gene conversion

- 60-80% of clusters of mutations had a template in the IgHV gene segments
- LAIR1 → human exogenous gene segment at the IgH locus
- Gpt/β-globin → murine transgenes at the passenger IgH locus
  - No selection



Cluster of mutations and Gene conversion

- Utilized SHM substitution tables to determine if clusters were significantly matching IgHV gene segments

- Monte Carlo approach to simulate somatic hypermutation activity at sites of mutation

Human

From\To	A	C	G	T
A	■	23.2	53.3	23.5
C	15	■	25.9	59.1
G	50.1	36.1	■	13.8
T	23.2	56.5	20.2	■

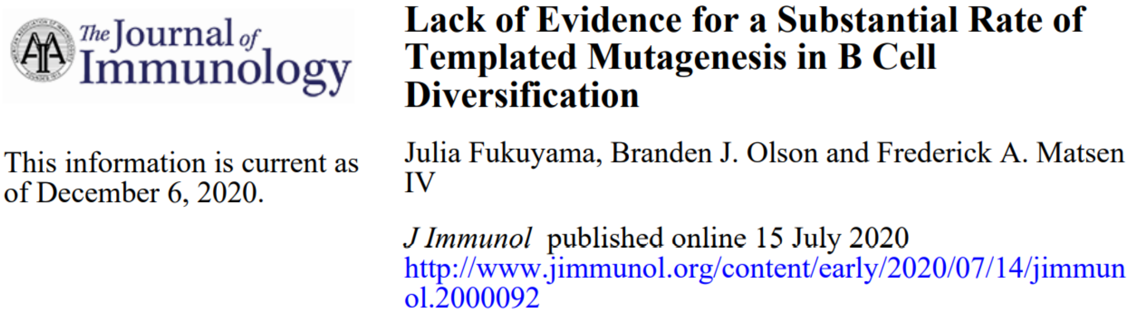
Mouse

From\To	A	C	G	T
A	■	21.2	48.5	30.3
C	20	■	16	64
G	58.3	25	■	16.7
T	27.8	55.6	16.7	■

Conclusions I

- Microhomology-mediated gene conversion introduces micro-clusters of mutations at the IgH locus
- PolyMotifFinder allows unbiased inferential analysis and detection of gene conversion
- Both IgHV genes and non-Ig genes utilize the same templates to diversify
- 60-80% of micro-clusters of mutations match germline motifs in the IgHV repertoire

Limitations



The Journal of Immunology

This information is current as of December 6, 2020.

Lack of Evidence for a Substantial Rate of Templated Mutagenesis in B Cell Diversification

Julia Fukuyama, Branden J. Olson and Frederick A. Matsen IV

J Immunol published online 15 July 2020
http://www.jimmunol.org/content/early/2020/07/14/jimmunol.2000092

- PolyMotifFinder (reimplemented as PyMotifFinder) suffers from a high false positive rate

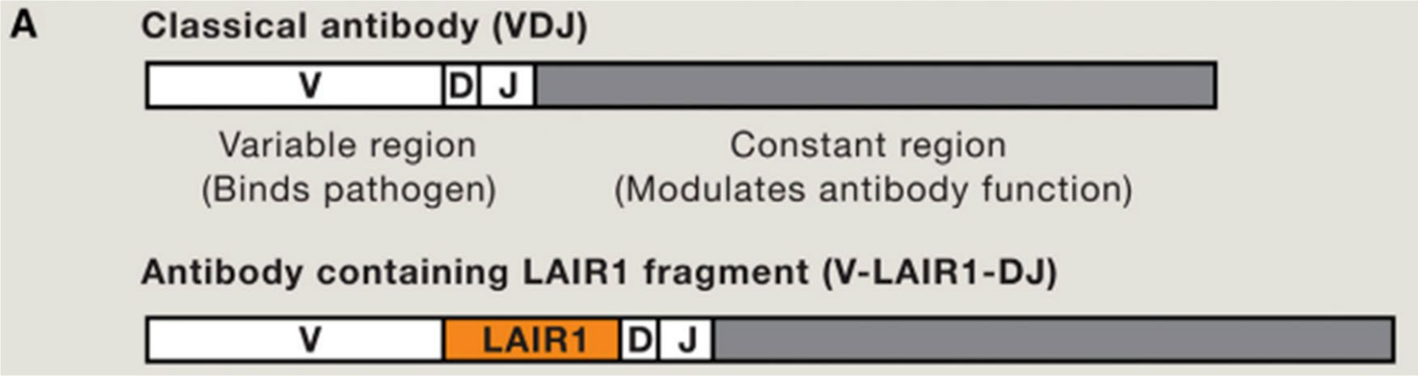
- Stouffer's Z method allows for sensitive detection of differences between the hypothesis and the null but does not describe the effect size.

Is templated mutagenesis real?

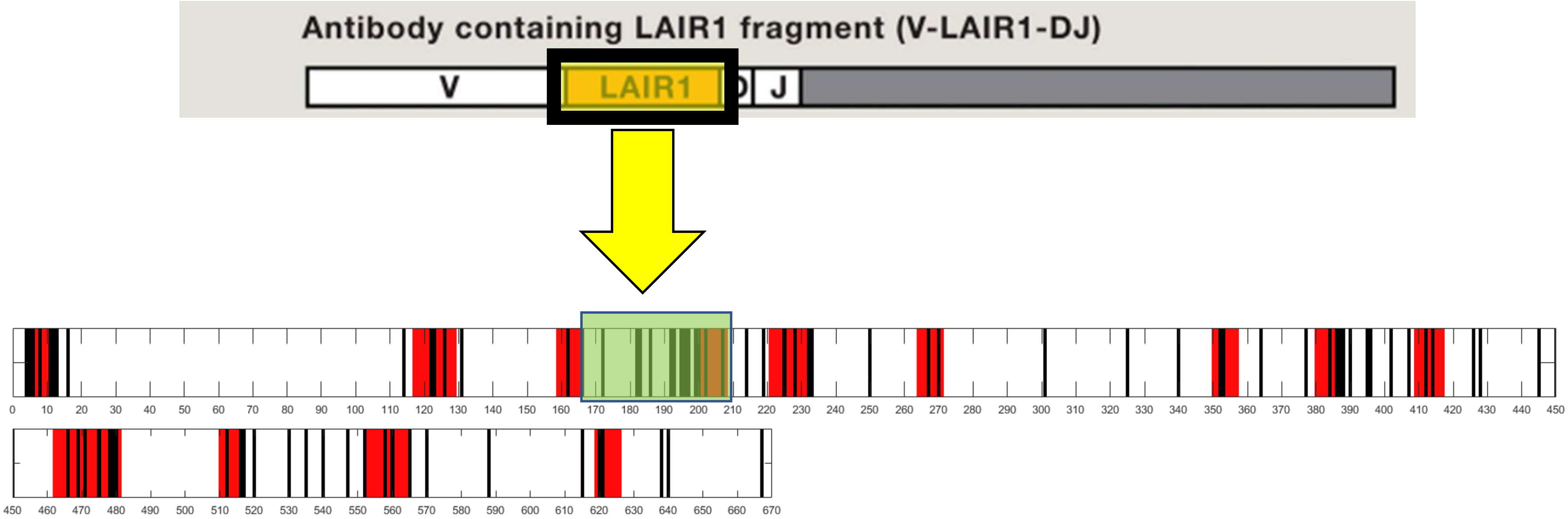
LAIR1-containing antibodies

Tan, J., Pieper, K., Piccoli, L., Abdi, A., Foglierini, M., Geiger, R., . . . Lanzavecchia, A. (2015). A LAIR1 insertion generates broadly reactive antibodies against malaria variant antigens. *Nature*, *529*(7584), 105-109. doi:10.1038/nature16450

- Large insertion of LAIR1 gene into VDJ rearrangement.
- Broadly neutralizing anti-malarial antibodies



IgHV-based Gene Conversion in LAIR1



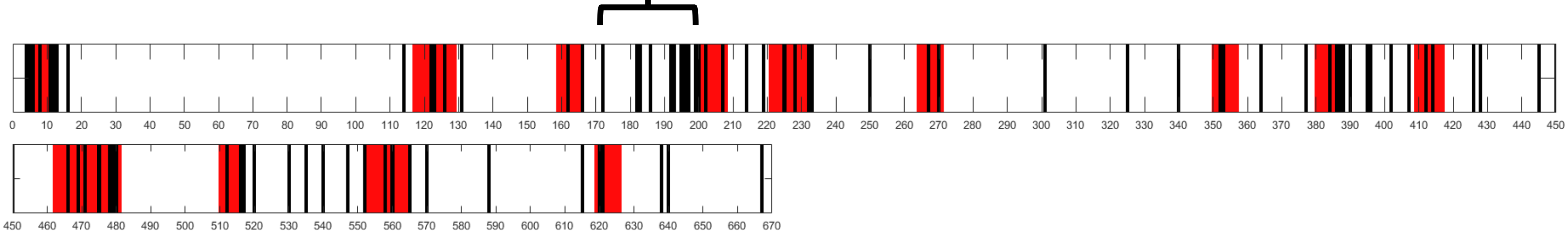
■ Mutations

🟥 Potential GC Tracts

IgHV-based Gene Conversion in LAIR1

E-Value 0.13
P-value 0.122

```
Germline (LAIR1) AAATTGCTAAAATCCCTGAATTGTGAT
MGE9             ---C---------GT--T-----CT-A
Chr3(NC_108914.2) ---C---------AT--TT----CT-A
  139,324,492 - 139,324,518
```



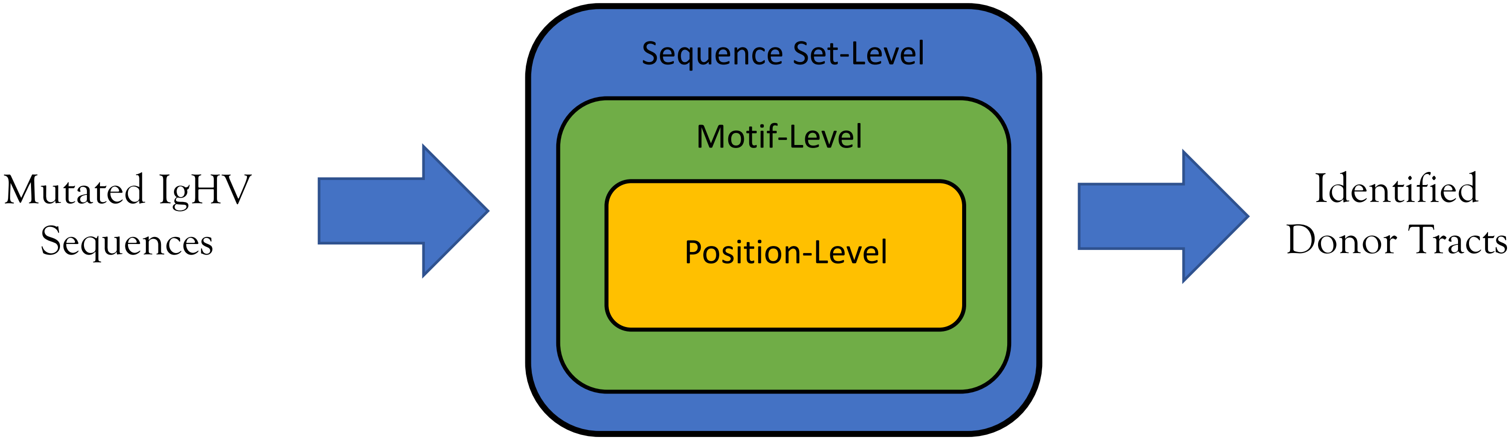
■ Mutations

🟥 Potential GC Tracts

Is it significant?

TRACE

- Template Recognition via Analysis of monte Carlo Experiments
  - Conducts nested Monte Carlo experiments analyzing effects of mutation position, mutation identity, and general background
  - Harnesses BLASTn to identify targets
  - Can only identify regions if significantly mutated (≥8 mutations over 38 bp)


Mutated IgHV Sequences

Sequence Set-Level

Motif-Level

Position-Level

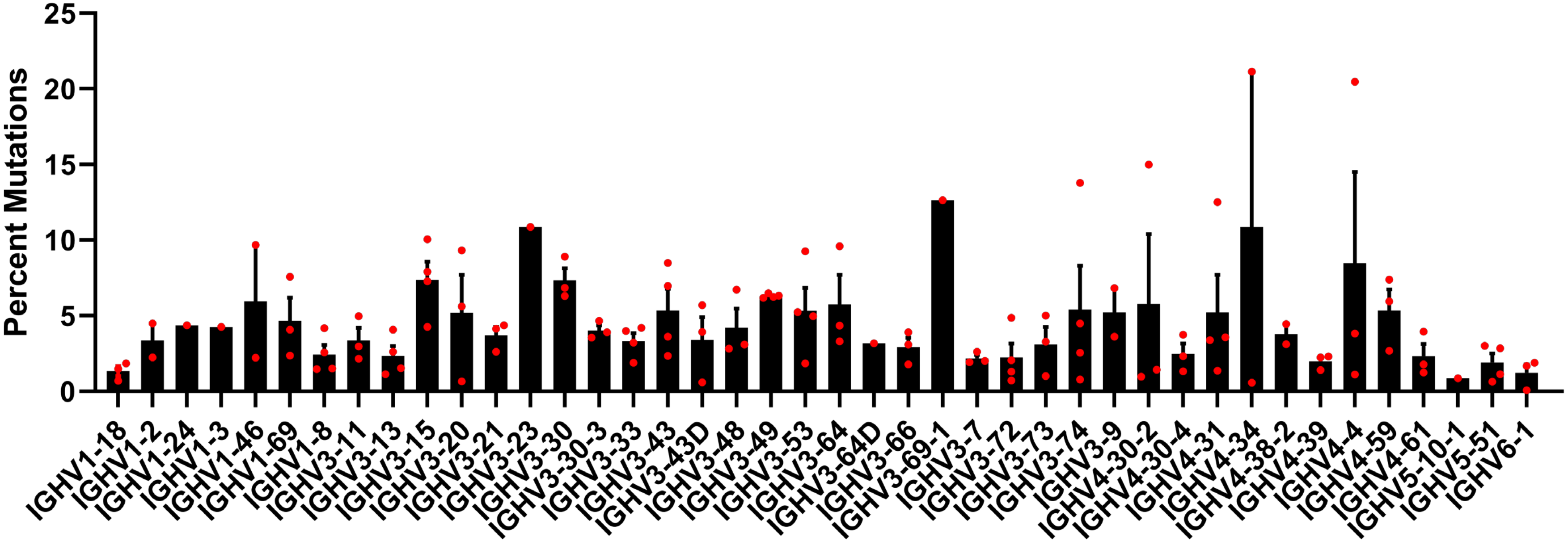
Identified Donor Tracts

Do genome-wide templates contribute to humoral immunity?

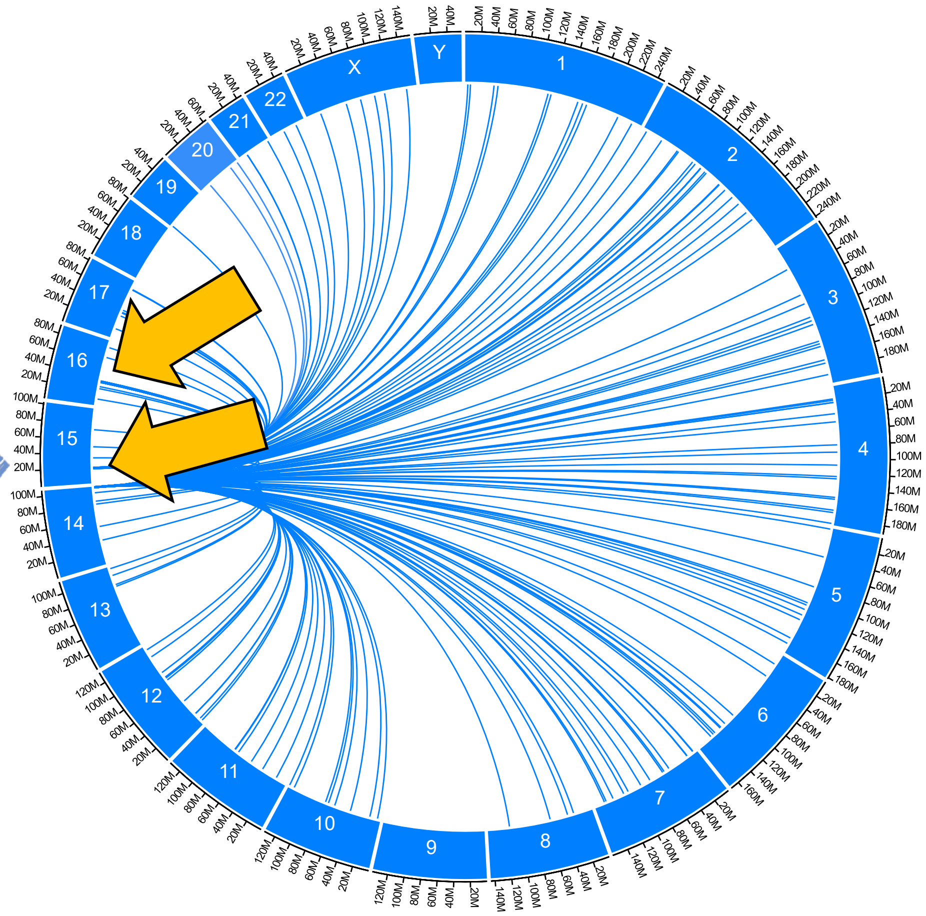
Experimental Approach

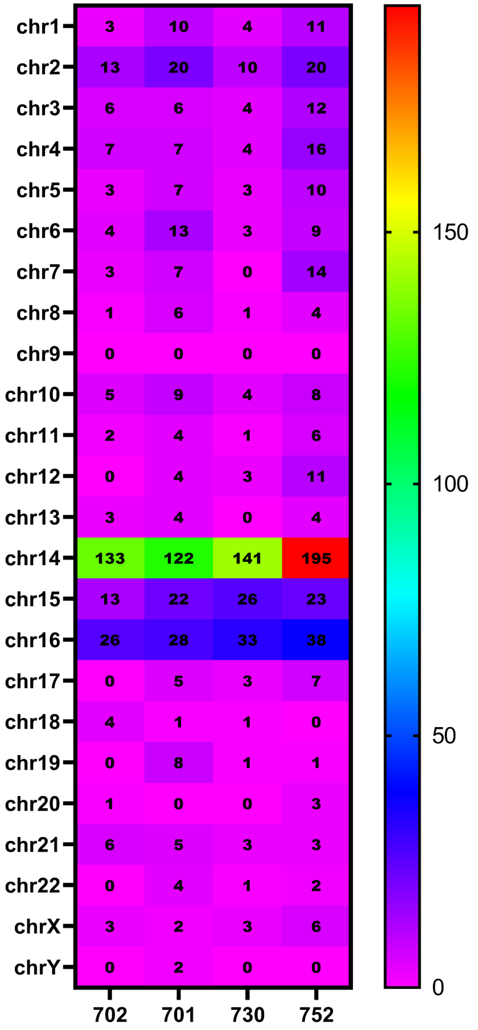
- 3 healthy subjects, 1 SLE patient
- $CD19^{+}IgD^{-}CD27^{+}$ B cells from PBMCs (Switched Memory B Cells)
- IgHV sequences from multiple rearrangements passed through TRACE

Gene Conversion in Memory B cells



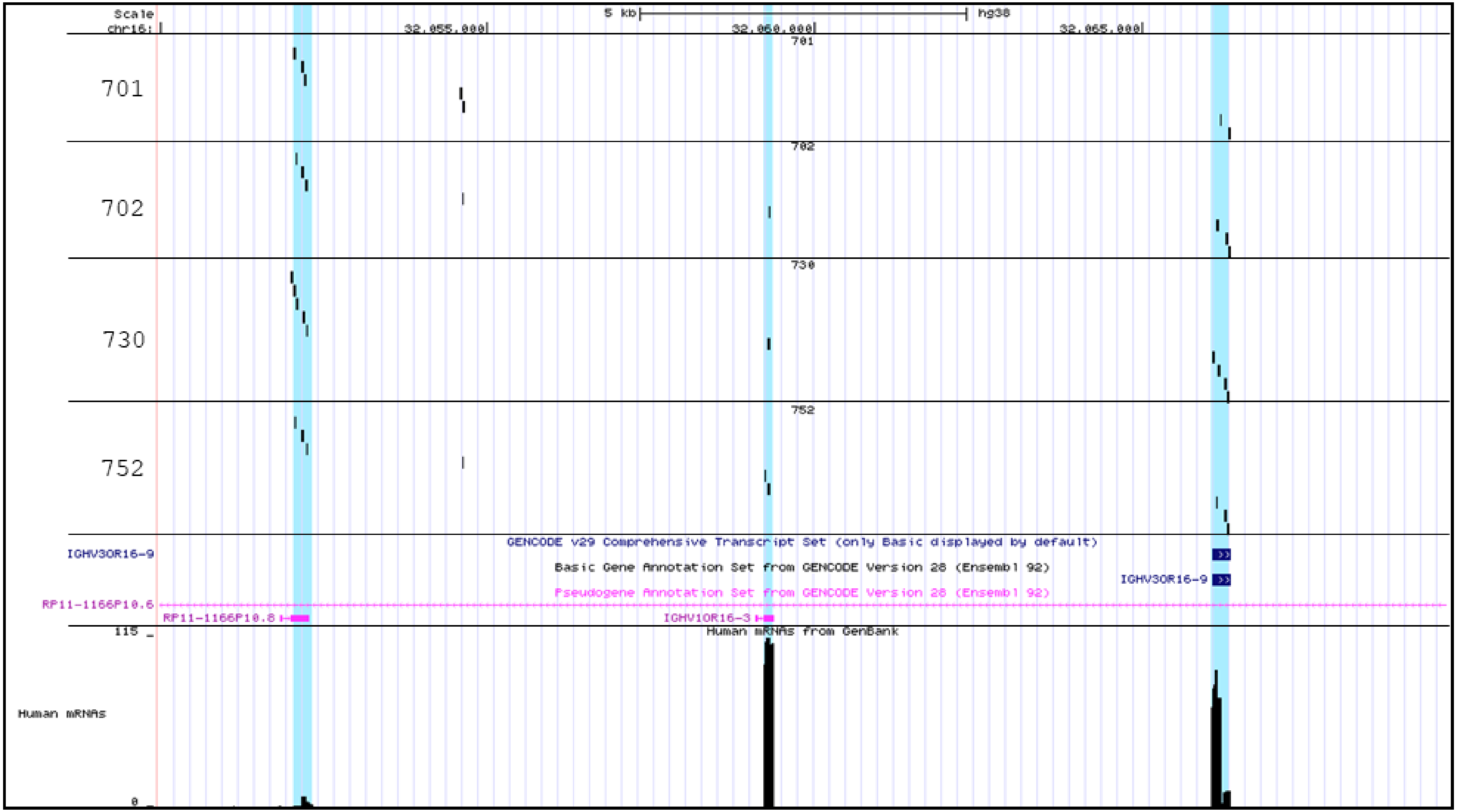
Gene Conversion in Memory B cells



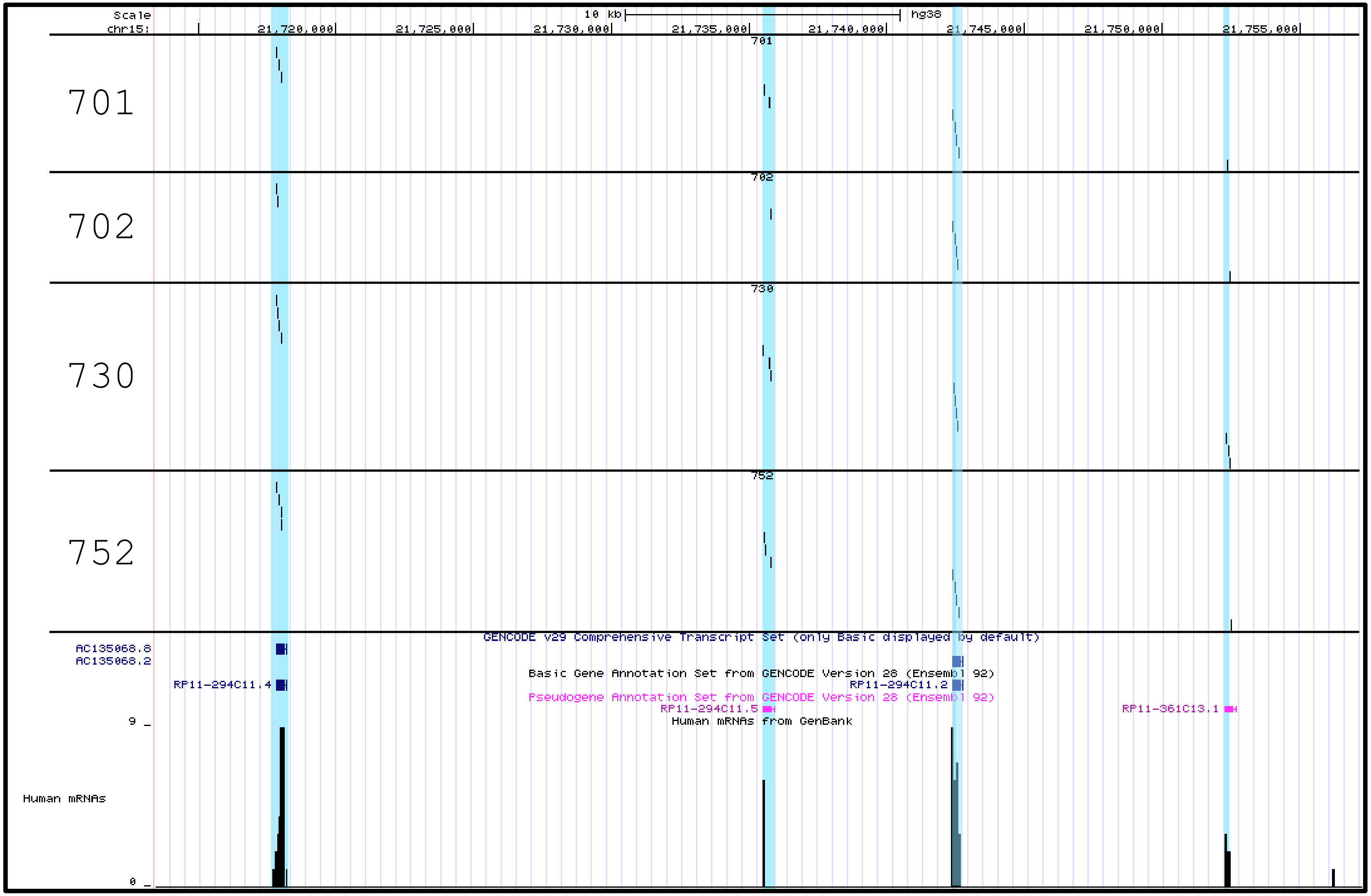


	702	701	730	752
chr1	3	10	4	11
chr2	13	20	10	20
chr3	6	6	4	12
chr4	7	7	4	16
chr5	3	7	3	10
chr6	4	13	3	9
chr7	3	7	0	14
chr8	1	6	1	4
chr9	0	0	0	0
chr10	5	9	4	8
chr11	2	4	1	6
chr12	0	4	3	11
chr13	3	4	0	4
chr14	133	122	141	195
chr15	13	22	26	23
chr16	26	28	33	38
chr17	0	5	3	7
chr18	4	1	1	0
chr19	0	8	1	1
chr20	1	0	0	3
chr21	6	5	3	3
chr22	0	4	1	2
chrX	3	2	3	6
chrY	0	2	0	0

Gene Conversion in Memory B cells

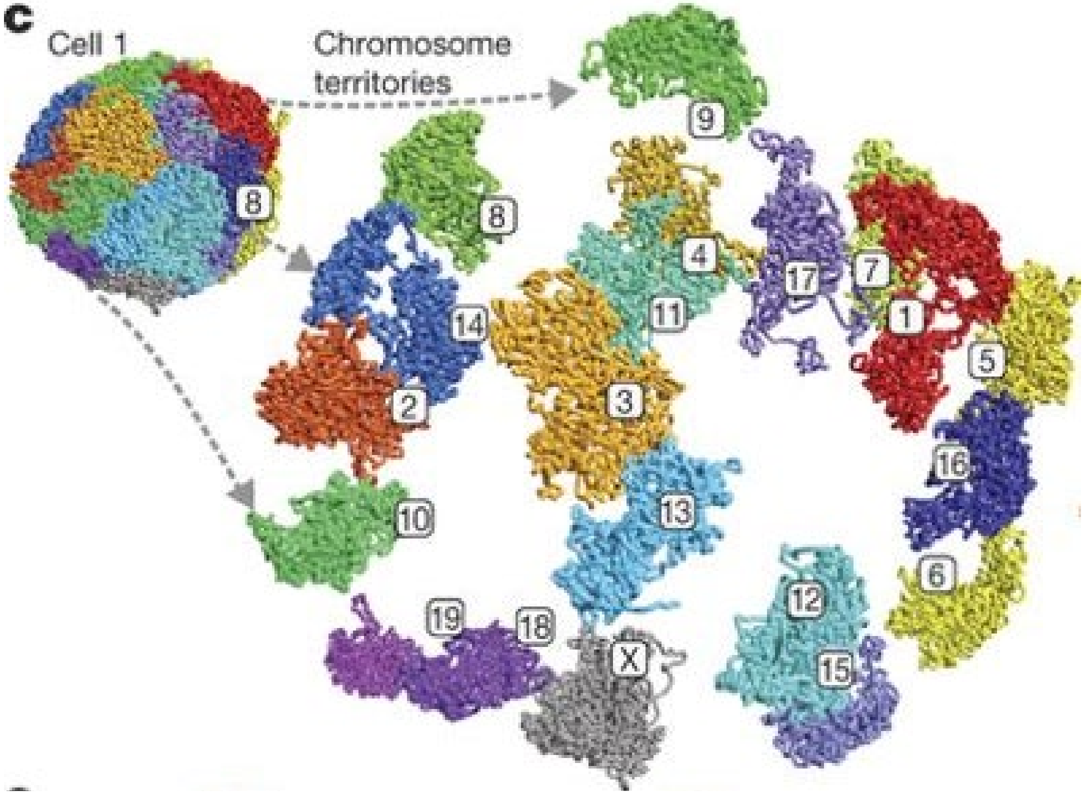


Gene Conversion in Memory B cells



Nuclear organization

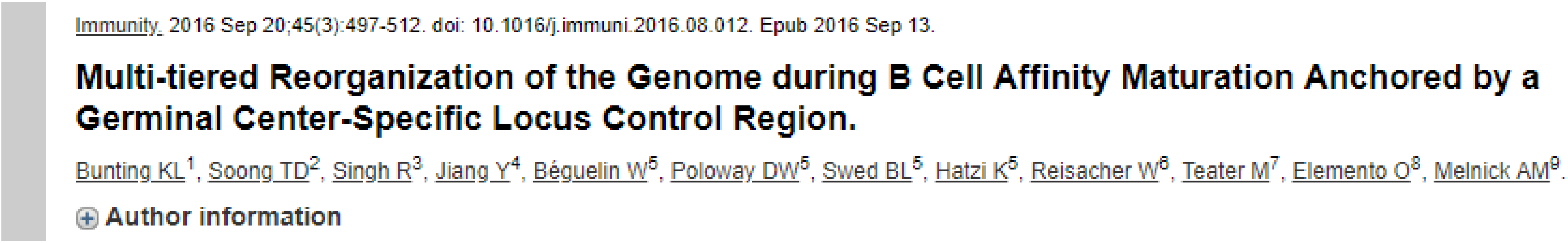
- Chromosomes are organized within the nucleus in distinct “territories.”



Stevens, T. J., Lando, D., Basu, S., Atkinson, L. P., Cao, Y., Lee, S. F., … Laue, E. D. (2017). 3D structures of individual mammalian genomes studied by single-cell Hi-C. Nature, 544(7648), 59–64. doi: 10.1038/nature21429

Nuclear organization

- Human germinal center B cell/naïve B cell HI-C data obtained from:



Immunity. 2016 Sep 20;45(3):497-512. doi: 10.1016/j.immuni.2016.08.012. Epub 2016 Sep 13.

Multi-tiered Reorganization of the Genome during B Cell Affinity Maturation Anchored by a Germinal Center-Specific Locus Control Region.

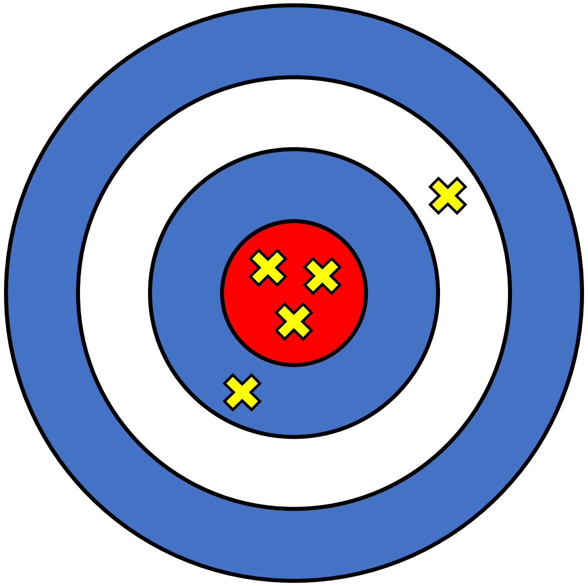
Bunting KL[1], Soong TD[2], Singh R[3], Jiang Y[4], Béguelin W[5], Poloway DW[5], Swed BL[5], Hatzi K[5], Reisacher W[6], Teater M[7], Elemento O[8], Melnick AM[9].

⊕ **Author information**

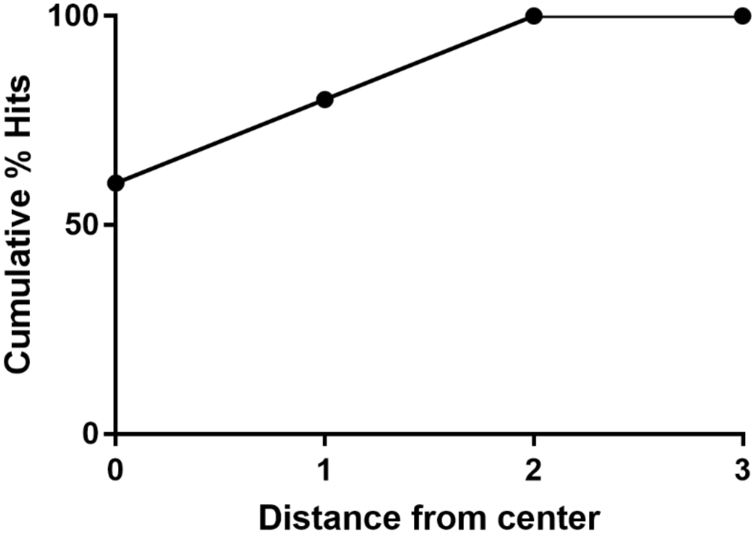
- Compared TRACE predictions and their distance from intrachromosomal contacts with the IgH locus

- Compared against an equal number of random genomic sites

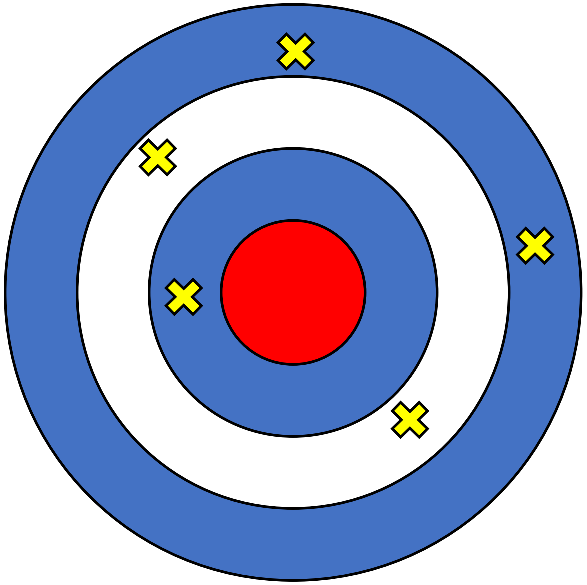
Cumulative Frequency and HI-C



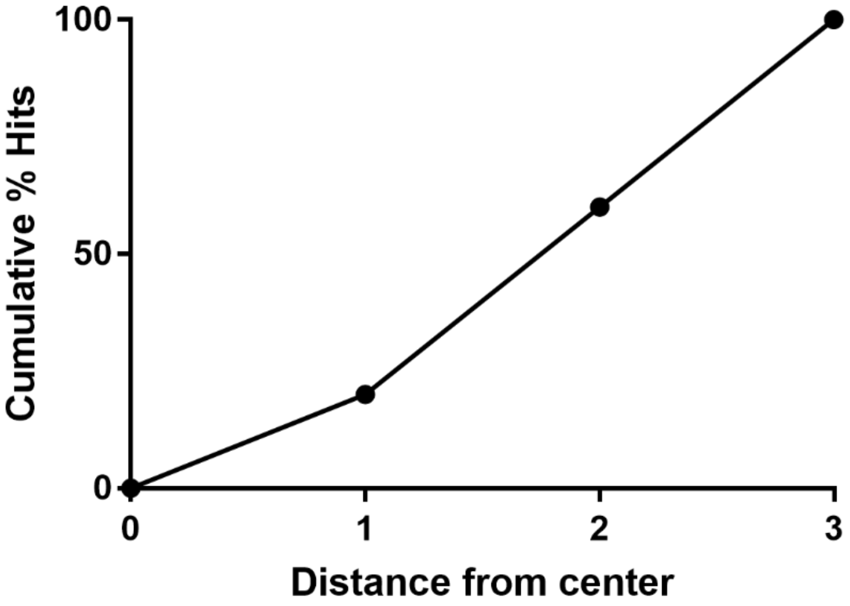
Original



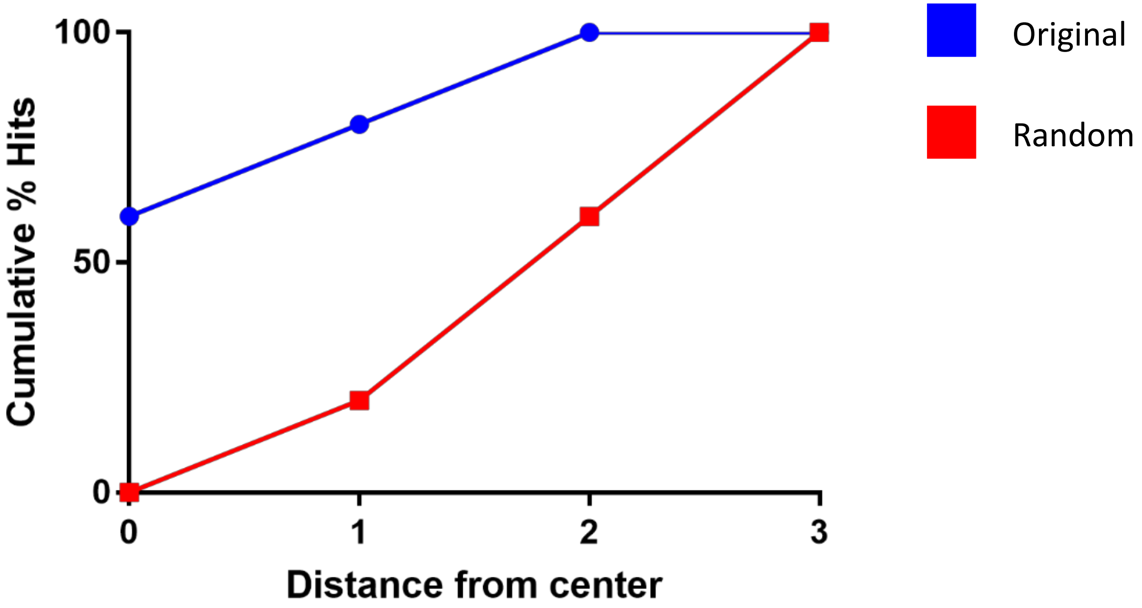
Cumulative Frequency and HI-C



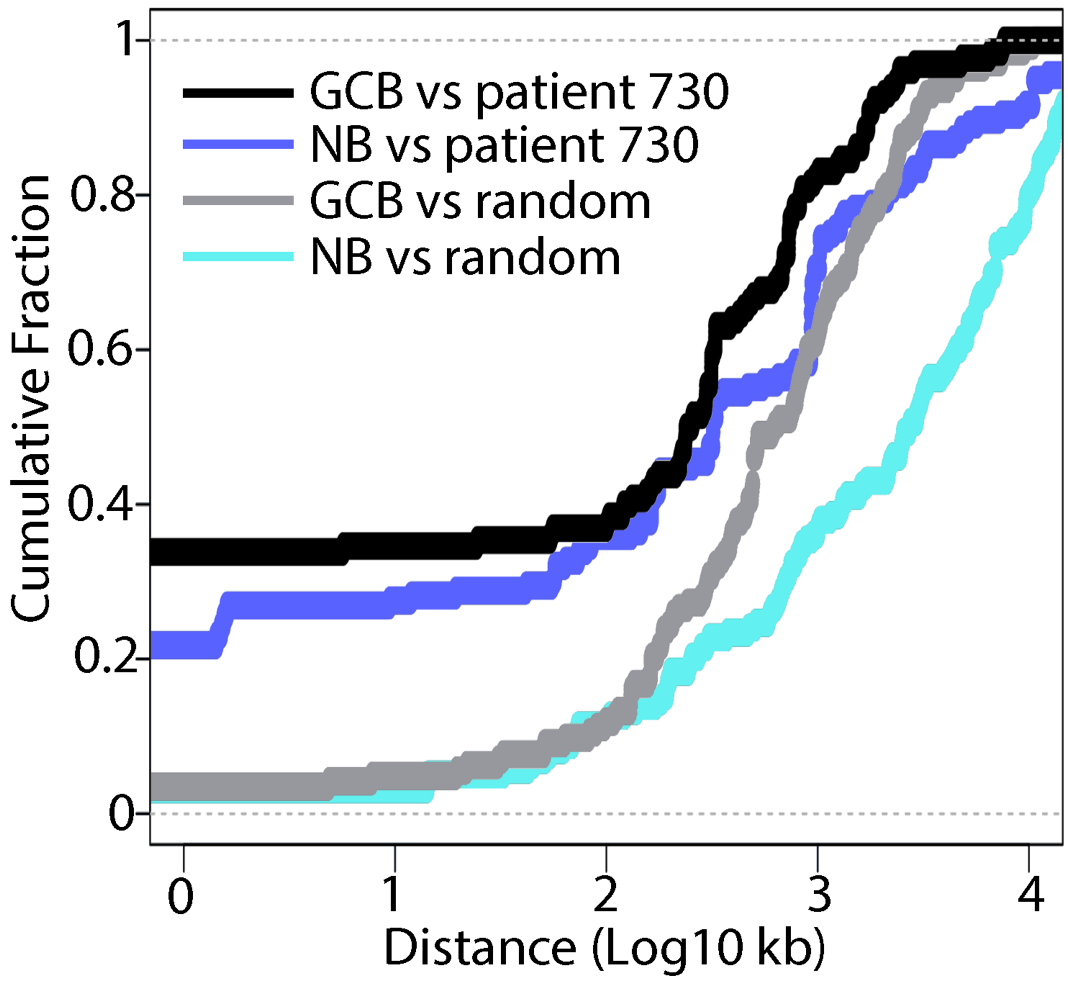
Random



Cumulative Frequency and HI-C



TRACE hits are closer to the IgH locus in the germinal center



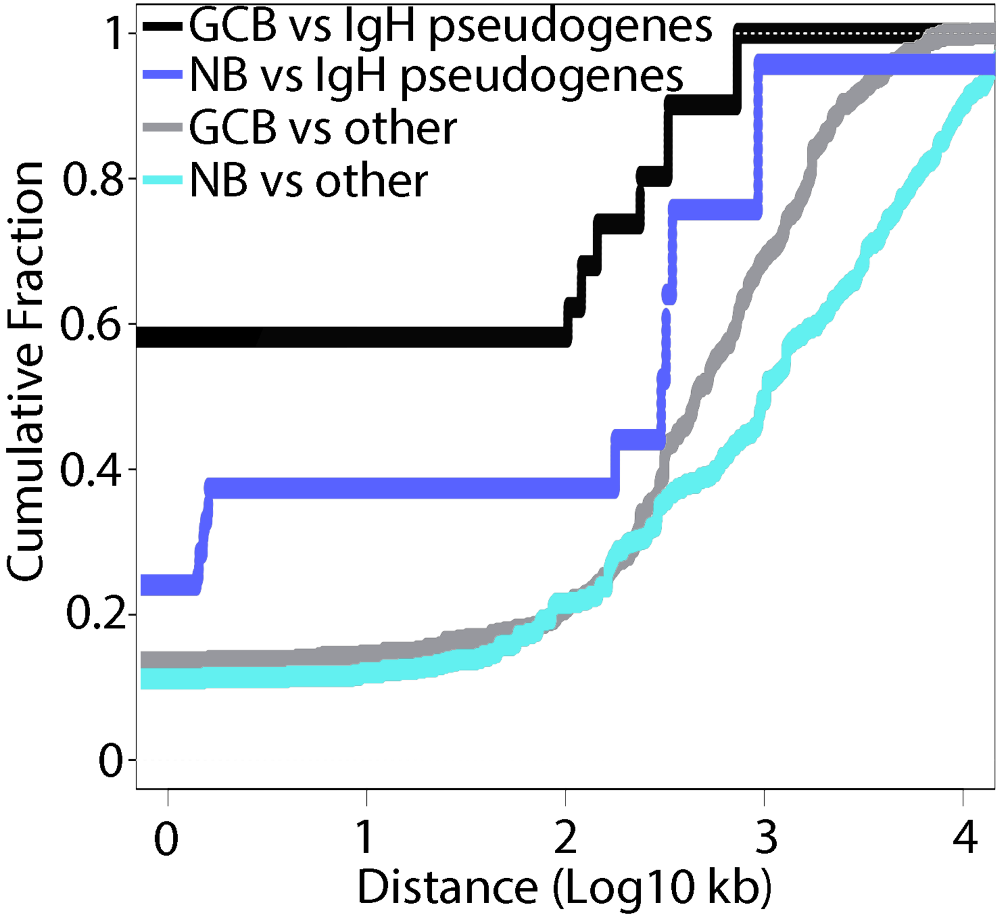
KS Test Statistics

<u>GCB vs NB</u>
p=1.17e-3

<u>GCB vs random</u>
p=1.448e-5

<u>NB vs random</u>
p=9.113e-7

Pseudogene TRACE hits are closer to the IgH locus in the germinal center



KS Test Statistics

<u>GCB vs NB</u>
p= 7.32e-9

<u>GCB vs random</u>
p<2.2e-16

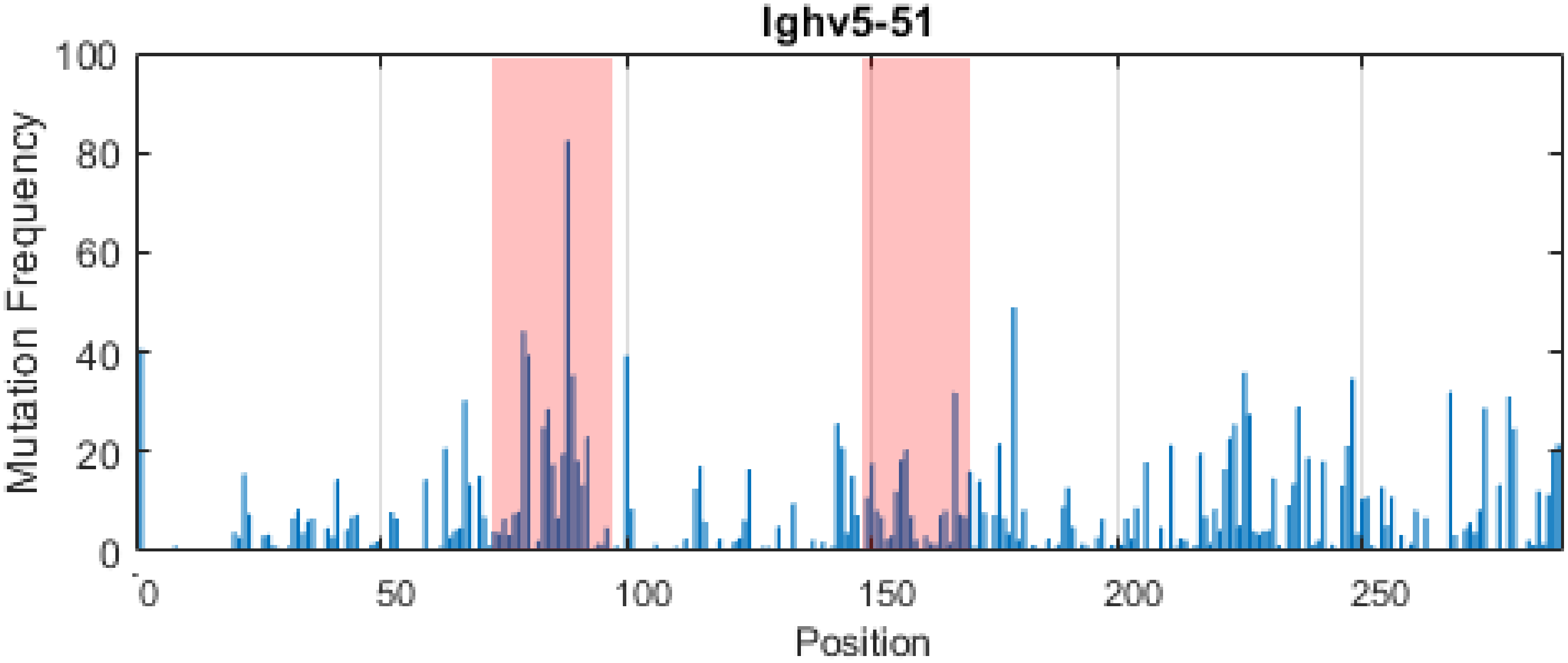
<u>NB vs random</u>
p<2.2e-16

Where in the IgHV sequence are these mutational tracts located?

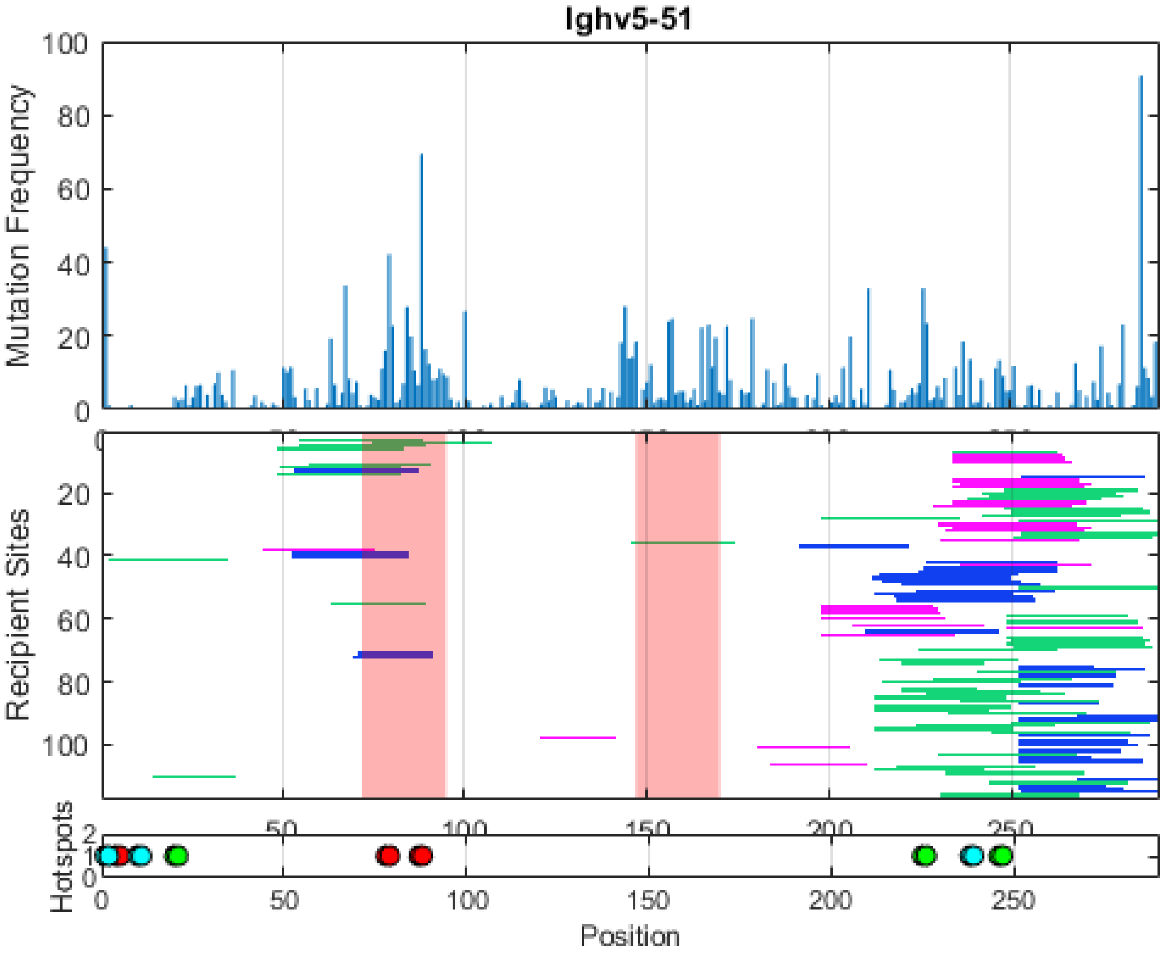
Overlapping AID hotspots

- Composed of overlapping canonical AID hotspots 5’ WRC 3’
- Enriched in class switch regions → promote DSB formation
- Demonstrated to affect mutability of the IgHV gene segment
  - Wei, L., Chahwan, R., Wang, S., Wang, X., Pham, P. T., Goodman, M. F., … Maccarthy, T. (2015). Overlapping hotspots in CDRs are critical sites for V region diversification. Proceedings of the National Academy of Sciences, 112(7). doi: 10.1073/pnas.1500788112
- DSB implicated in gene conversion
  - Bastianello, G., & Arakawa, H. (2016). A double-strand break can trigger immunoglobulin gene conversion. Nucleic Acids Research, 45(1), 231–243. doi: 10.1093/nar/gkw887

TRACE clusters



TRACE clusters



<u>TRACE Clusters</u>

Blue: IgHV genes

Magenta: Intrachromosomal

Green: Interchromosomal

<u>Overlapping AID Hotspots:</u>

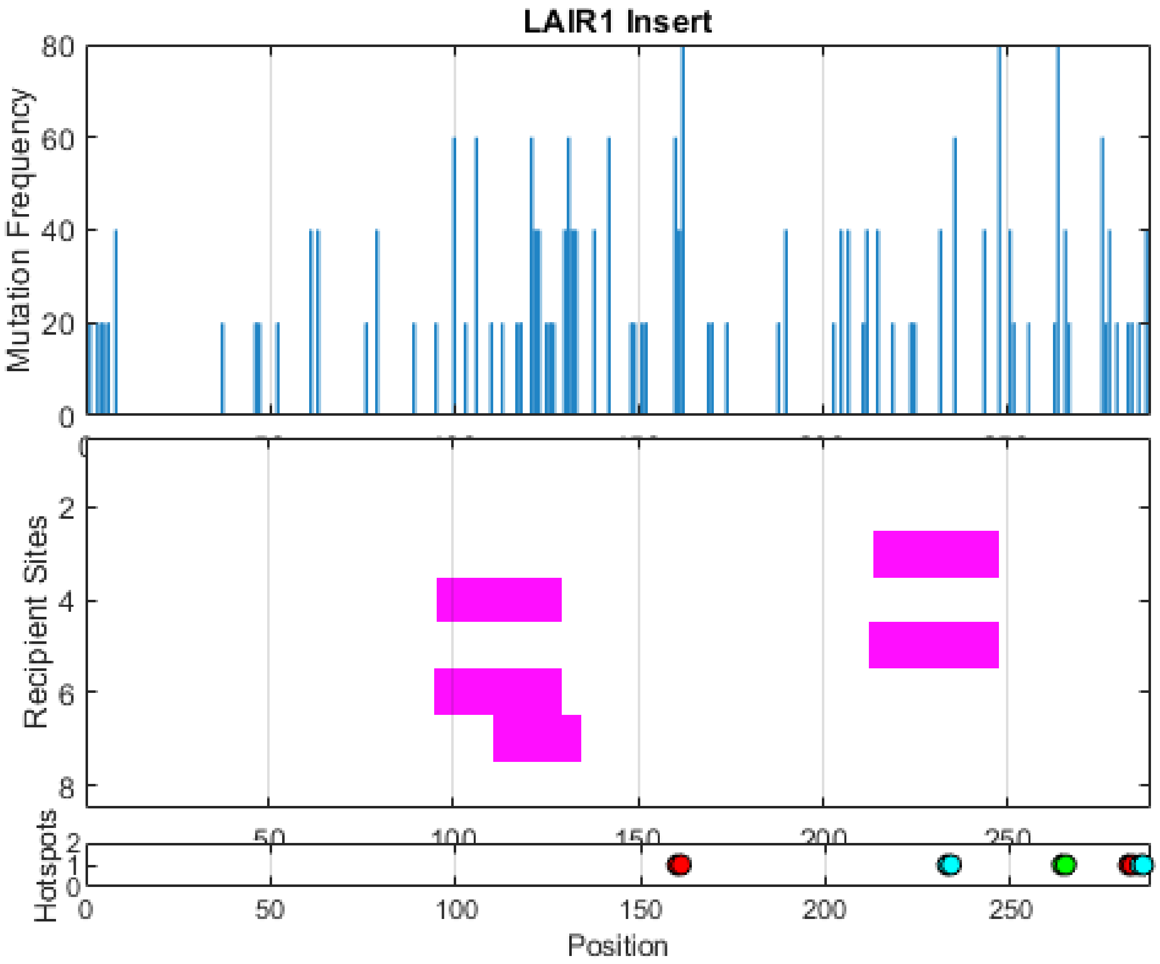
AGCT

AGCA

TGCA

TGCT

TRACE clusters in LAIR1



TRACE Clusters
Magenta: Intrachromosomal
Green: Interchromosomal

Overlapping AID Hotspots:
AGCT
AGCA
TGCA
TGCT

Conclusions II

- Intra- and inter-chromosomal gene conversion contributes to somatically mutated genes
- IgHV genes and IgHV pseudogenes contribute mutations to the somatically mutated genes
- Donor choice is homology-mediated
- Donor templates are physically closer to the IgH locus during the germinal center than in naïve B
- Donor templates cluster between individuals, are in proximity to open chromatin, and are preferentially from genes upregulated in the germinal center reaction
- Gene conversion donations are centered around overlapping AID hotspots, which are DSB prone.

What's Next

- Transgenic mouse model - similar to NP_{lo}, but with overlapping hotspots removed with silent mutations
- Modified CHIP assay to capture/label DNA heteroduplex interactions in vivo
- High resolution microscopy for recruitment of homologous repair factors to the IgH loci after DSB

Acknowledgements

Jacob Lab

- Karla Navarrete
- Troy von Beck
- Nikita Mullick
- Leela Thomas
- Joshy Jacob

Sanz Lab

- Deepak Tomar
- Chris Tipton
- Jennifer Hom
- Matthew Woodruff
- Inaki Sanz

Bedford Lab

- Trevor Bedford

Boss Lab

- Chris Scharer
- Jerry Boss

Hunter Lab

- Daniel Wilkins
- Dario Dilernia

Corces Lab

- Jordan Rowley
- Victor Corces

Antia Lab

- Rustom Antia

Flow/Sorting

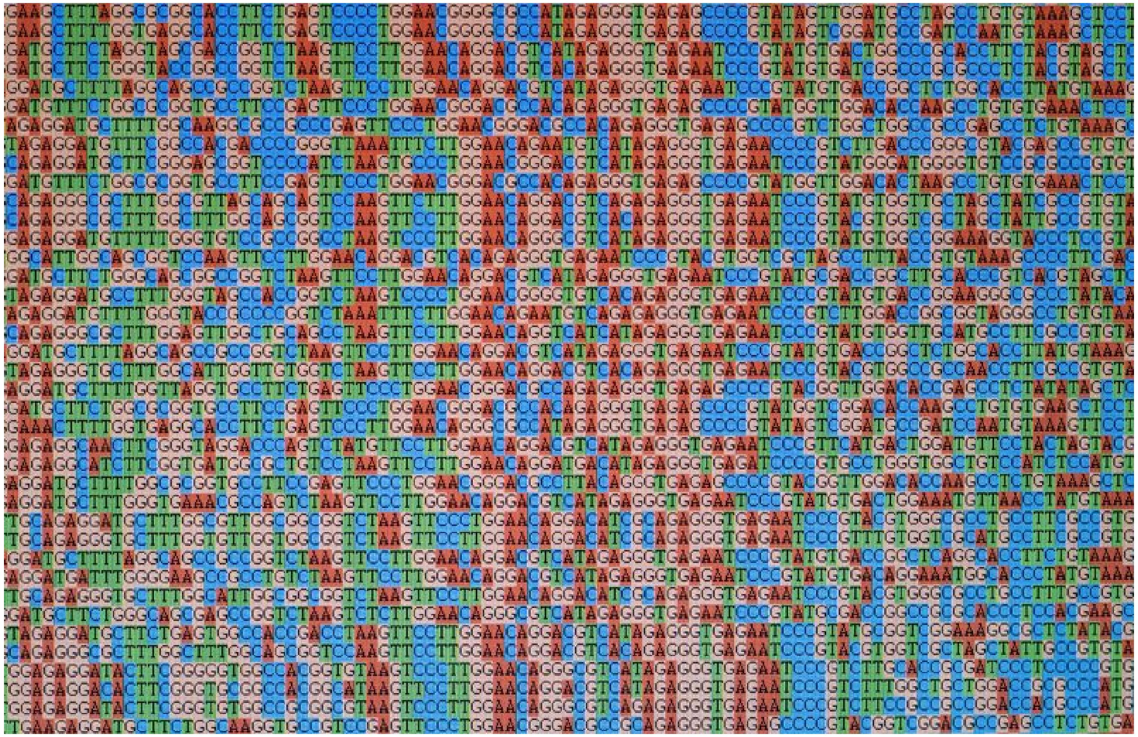
- Kiran Gill
- Barbara Cervasi

Software

- Matlab
- Bioedit
- BLAST

Funding

- T32 AI7610-15
- F30 AI123568



Questions?

Berek and Milstein (1987)

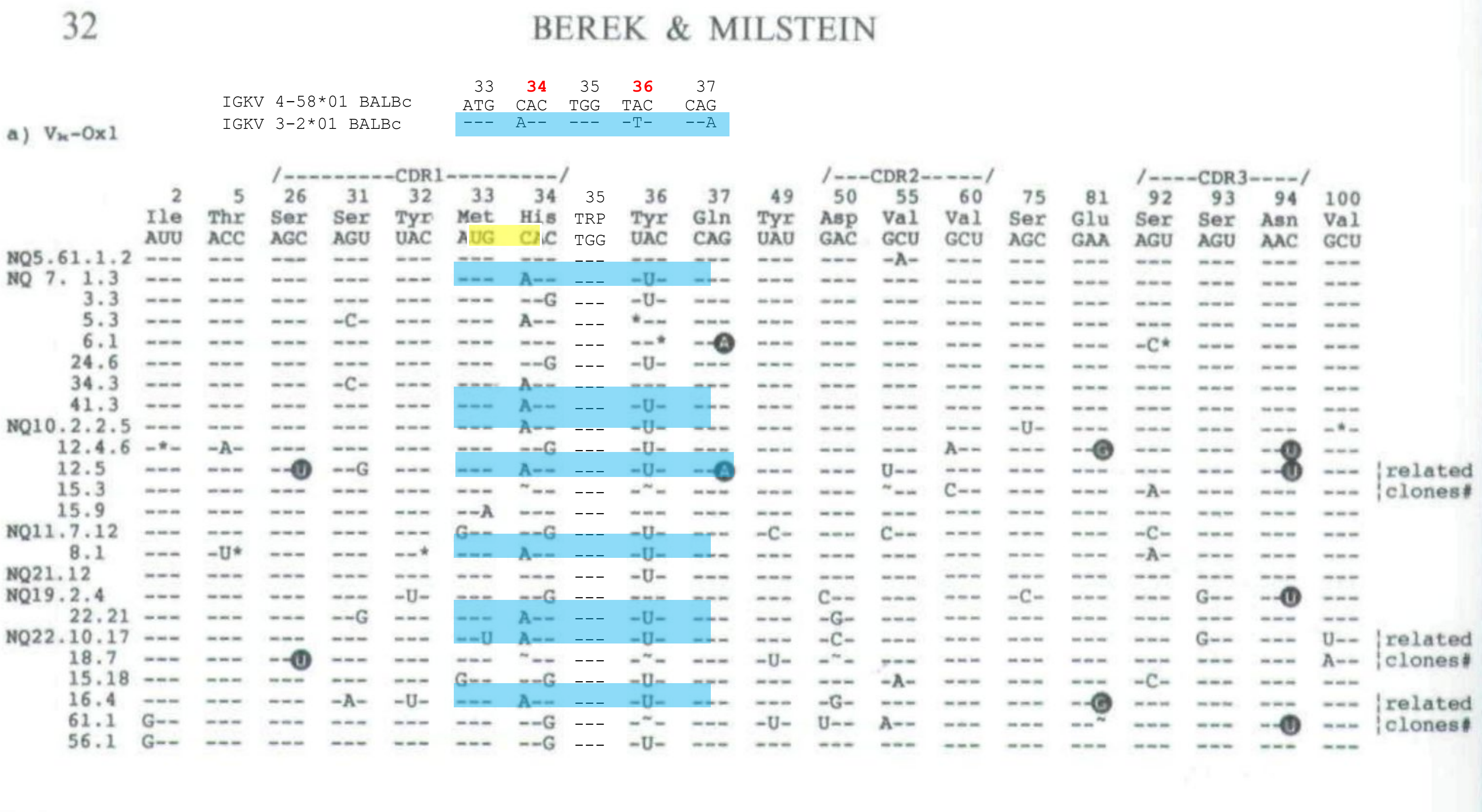
Repeated immunizations with Oxazalone induced accumulating mutations in the CDRs of V_K-Oxl and V_H-Oxl gene segments of BALB/cJ mice

Found in 17/21 independent clones were mutated at codon 34 (10x increased affinity) in V_K

Of these, 14/17 have a second mutation at codon 36 (no increase in affinity)

Berek, C. and Milstein, C. (1987), Mutation Drift and Repertoire Shift in the Maturation of the Immune Response. Immunological Reviews, 96: 23–41.

Berek and Milstein (1987)



32 BEREK & MILSTEIN

	33	34	35	36	37
IGKV 4-58*01 BALBc	ATG	CAC	TGG	TAC	CAG
IGKV 3-2*01 BALBc	---	A--	---	-T-	--A

a) Vκ-Ox1

			/---------CDR1---------/									/---CDR2-----/					/----CDR3----/				
	2	5	26	31	32	33	34	35	36	37	49	50	55	60	75	81	92	93	94	100	
	Ile	Thr	Ser	Ser	Tyr	Met	His	TRP	Tyr	Gln	Tyr	Asp	Val	Val	Ser	Glu	Ser	Ser	Asn	Val	
	AUU	ACC	AGC	AGU	UAC	AUG	CAC	TGG	UAC	CAG	UAU	GAC	GCU	GCU	AGC	GAA	AGU	AGU	AAC	GCU	
NQ5.61.1.2	---	---	---	---	---	---	---	---	---	---	---	---	-A-	---	---	---	---	---	---	---	
NQ 7. 1.3	---	---	---	---	---	---	A--	---	-U-	---	---	---	---	---	---	---	---	---	---	---	
3.3	---	---	---	---	---	---	--G	---	-U-	---	---	---	---	---	---	---	---	---	---	---	
5.3	---	---	---	-C-	---	---	A--	---	*--	---	---	---	---	---	---	---	---	---	---	---	
6.1	---	---	---	---	---	---	---	---	--*	--A	---	---	---	---	---	---	-C*	---	---	---	
24.6	---	---	---	---	---	---	--G	---	-U-	---	---	---	---	---	---	---	---	---	---	---	
34.3	---	---	---	-C-	---	---	A--	---	---	---	---	---	---	---	---	---	---	---	---	---	
41.3	---	---	---	---	---	---	A--	---	-U-	---	---	---	---	---	---	---	---	---	---	---	
NQ10.2.2.5	---	---	---	---	---	---	A--	---	-U-	---	---	---	---	---	-U-	---	---	---	---	-*-	
12.4.6	-*-	-A-	---	---	---	---	--G	---	-U-	---	---	---	---	A--	---	--G	---	---	--U	---	
12.5	---	---	--U	--G	---	---	A--	---	-U-	--A	---	---	U--	---	---	---	---	---	--U	---	related clones#
15.3	---	---	---	---	---	---	~--	---	-~-	---	---	---	~--	C--	---	---	-A-	---	---	---	
15.9	---	---	---	---	---	--A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
NQ11.7.12	---	---	---	---	---	G--	--G	---	-U-	---	-C-	---	C--	---	---	---	-C-	---	---	---	
8.1	---	-U*	---	---	--*	---	A--	---	-U-	---	---	---	---	---	---	---	-A-	---	---	---	
NQ21.12	---	---	---	---	---	---	---	---	-U-	---	---	---	---	---	---	---	---	---	---	---	
NQ19.2.4	---	---	---	---	-U-	---	--G	---	---	---	---	C--	---	---	-C-	---	---	G--	--U	---	
22.21	---	---	---	--G	---	---	A--	---	-U-	---	---	-G-	---	---	---	---	---	---	---	---	
NQ22.10.17	---	---	---	---	---	--U	A--	---	-U-	---	---	-C-	---	---	---	---	---	G--	---	U--	related clones#
18.7	---	---	--U	---	---	---	~--	---	-~-	---	-U-	-~-	---	---	---	---	---	---	---	A--	
15.18	---	---	---	---	---	G--	--G	---	-U-	---	---	---	-A-	---	---	---	-C-	---	---	---	
16.4	---	---	---	-A-	-U-	---	A--	---	-U-	---	---	-G-	---	---	---	--G	---	---	---	---	related clones#
61.1	G--	---	---	---	---	---	--G	---	-~-	---	-U-	U--	A--	---	---	--~	---	---	--U	---	
56.1	G--	---	---	---	---	---	--G	---	-U-	---	---	---	---	---	---	---	---	---	---	---	

Berek, C. and Milstein, C. (1987), Mutation Drift and Repertoire Shift in the Maturation of the Immune Response. Immunological Reviews, 96: 23–41