

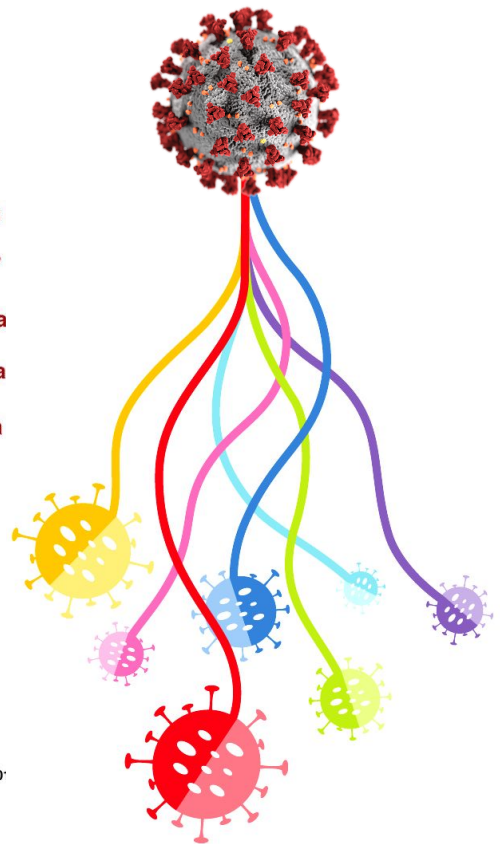
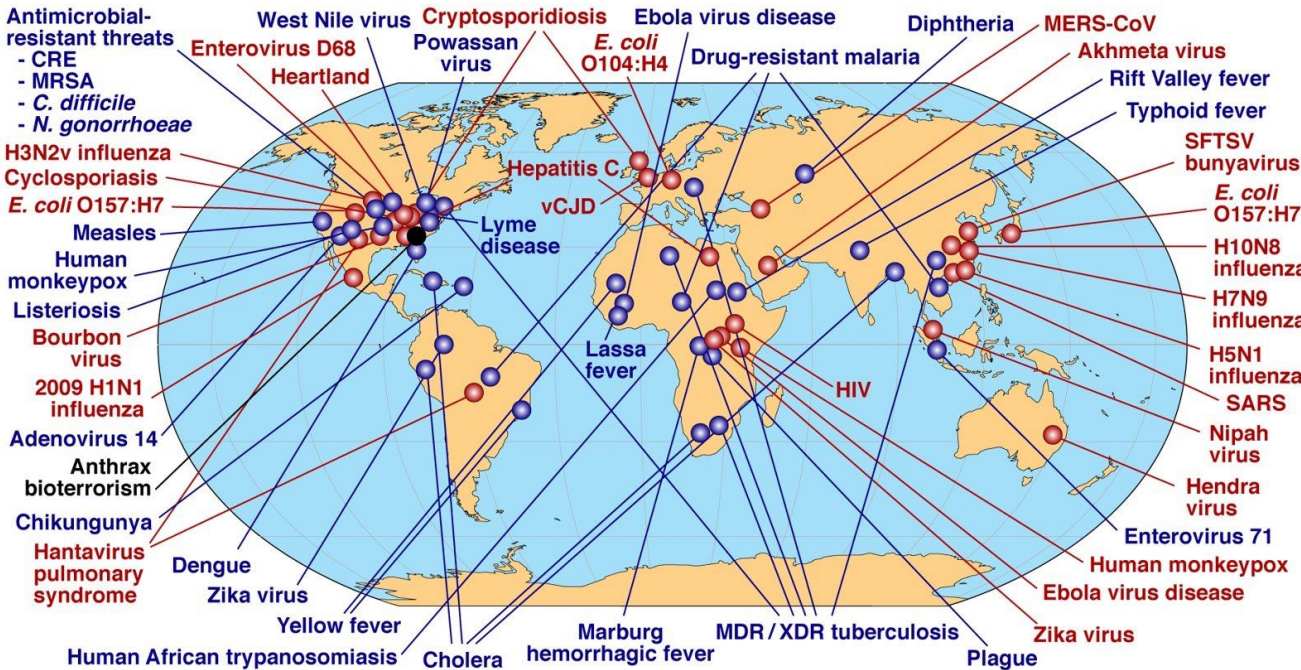


Profiling of antibody repertoires and immunoglobulin loci enables large-scale analysis of adaptive immune responses

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Johns Hopkins University**

Newly emerging and re-emerging diseases



● Newly emerging ● Re-emerging/resurging ● “Deliberately emerging”

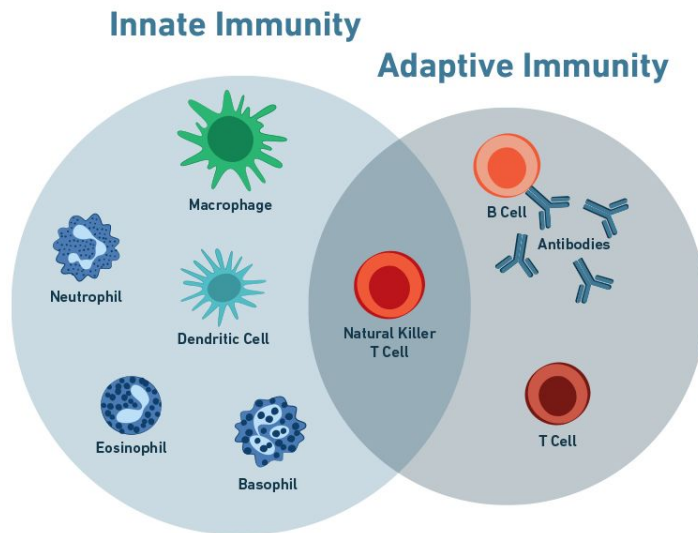
September 20

https://en.wikipedia.org/wiki/Emerging_infectious_disease

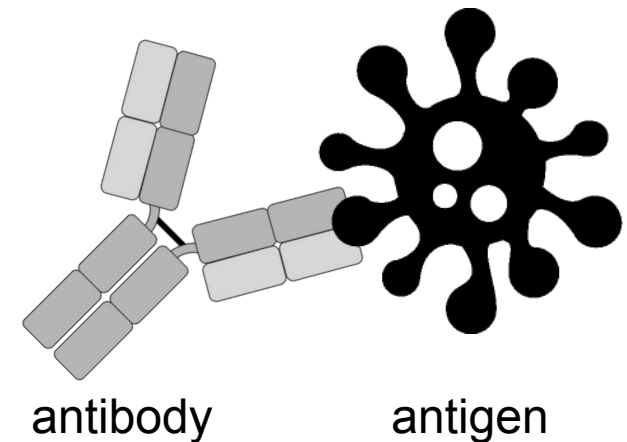
Studying immune responses

- Variety of threats to human body is huge and unpredictable
- **Genome is too small to encode defences against all these threats**

Immune system has an ability to adapt to various threats (antigens) using agents (e.g., antibodies) that **are not encoded** in the genome



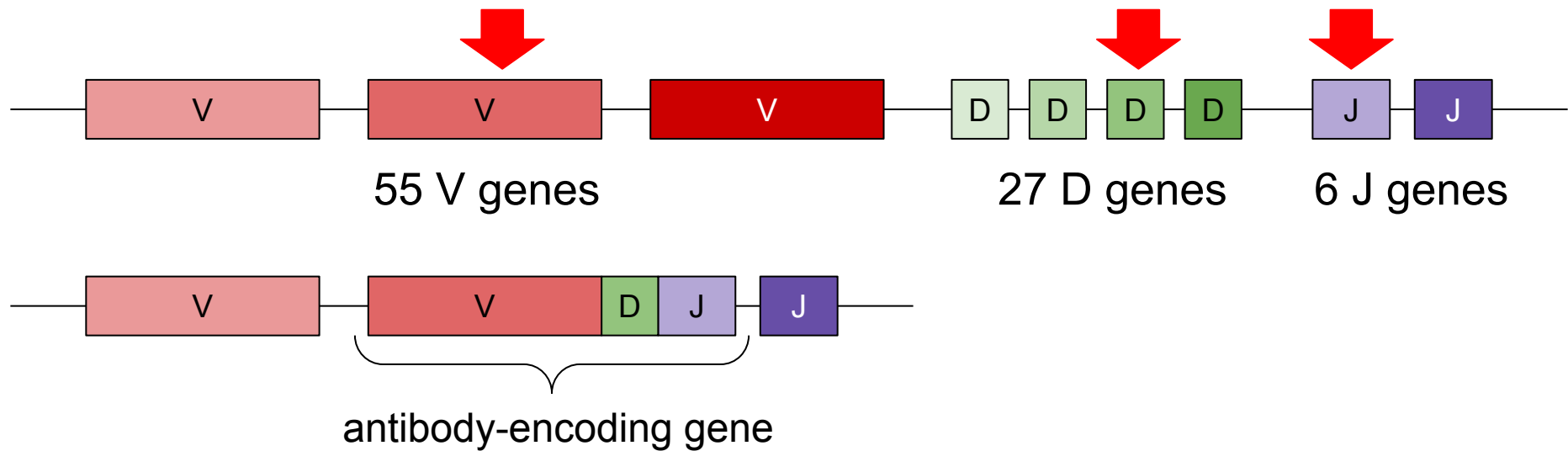
Immune system = innate (inherited) + adaptive (acquired) immune systems



Specificity rule: one antibody recognizes one antigen

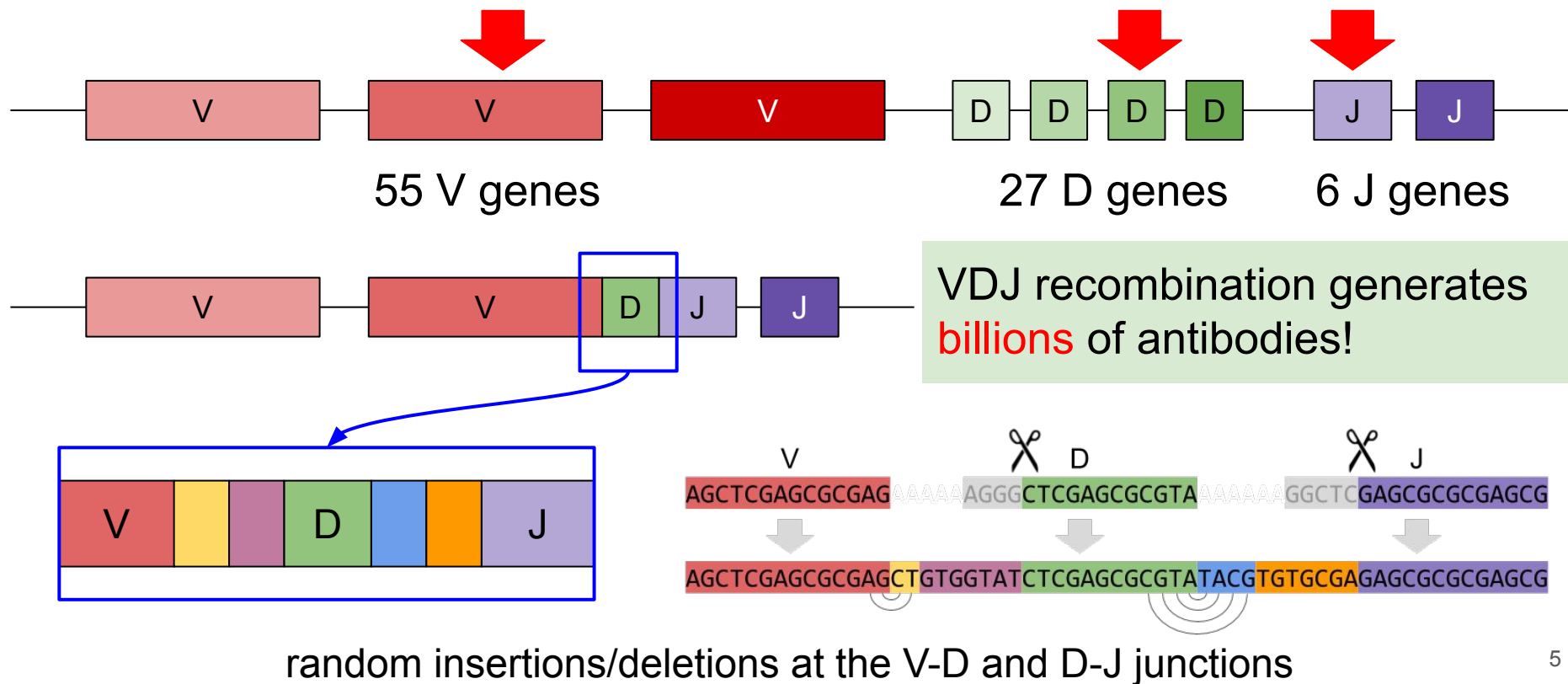
VDJ recombination

Antibodies are not directly encoded in the genome – they are encoded in **B-cells** that result from somatic recombinations of the **immunoglobulin loci**



VDJ recombination generates billions of antibodies

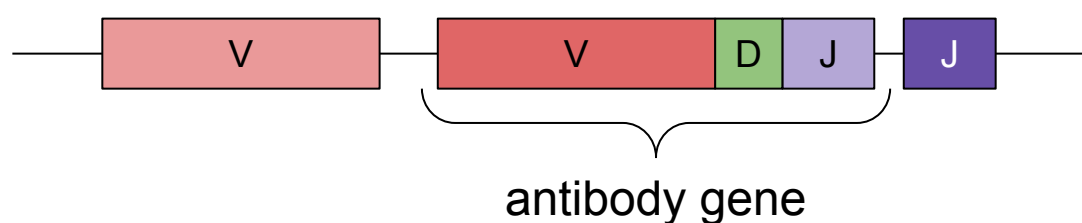
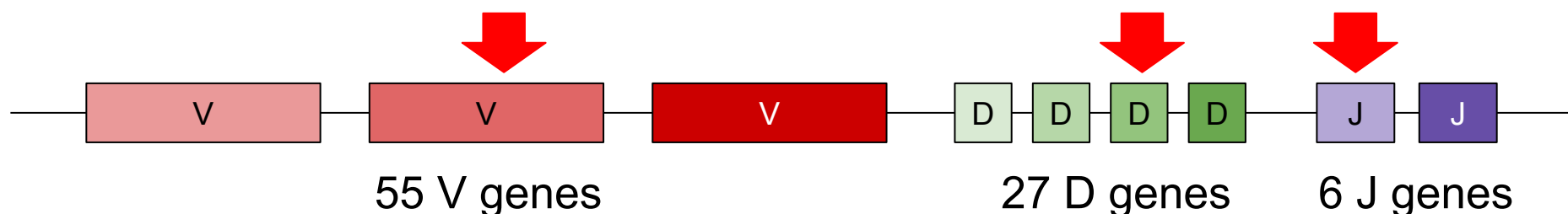
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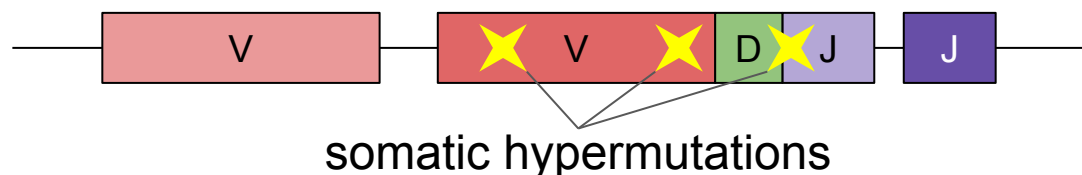
Somatic hypermutations (SHMs)



Antibodies are not directly encoded in the genome – they are encoded in **B-cells** that result from somatic recombinations of the **immunoglobulin loci**

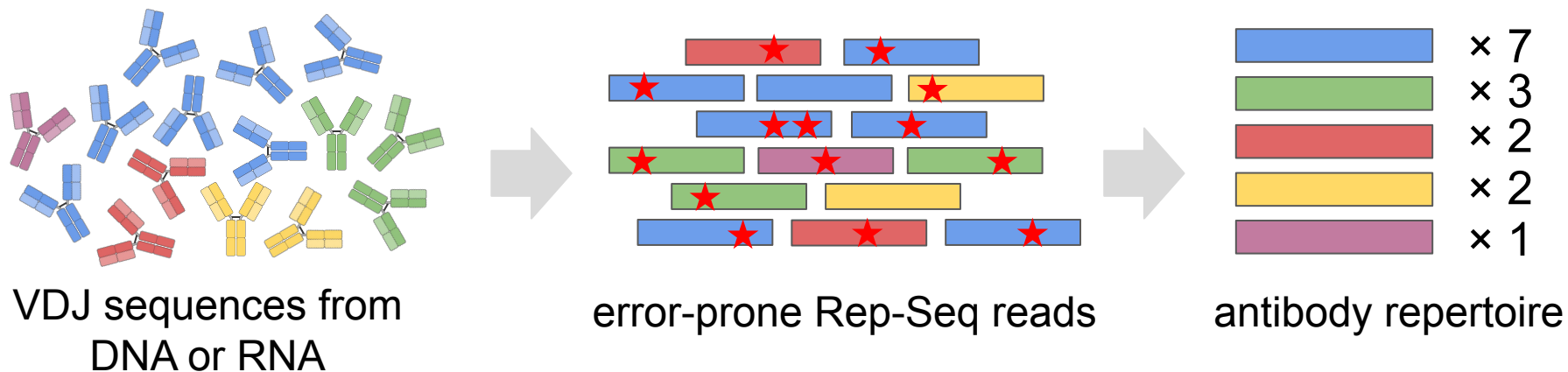


VDJ recombination generates billions of antibodies!

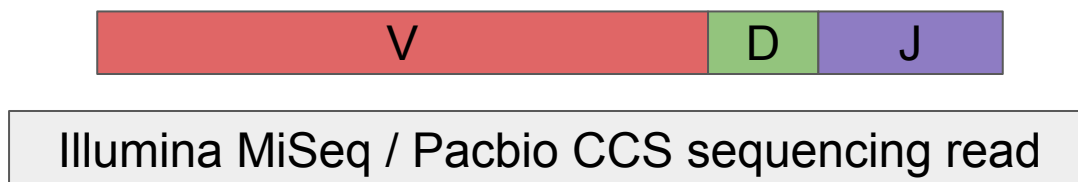


Randomly generated **somatic hypermutations** optimize the binding affinity of antibodies in an evolution-like process

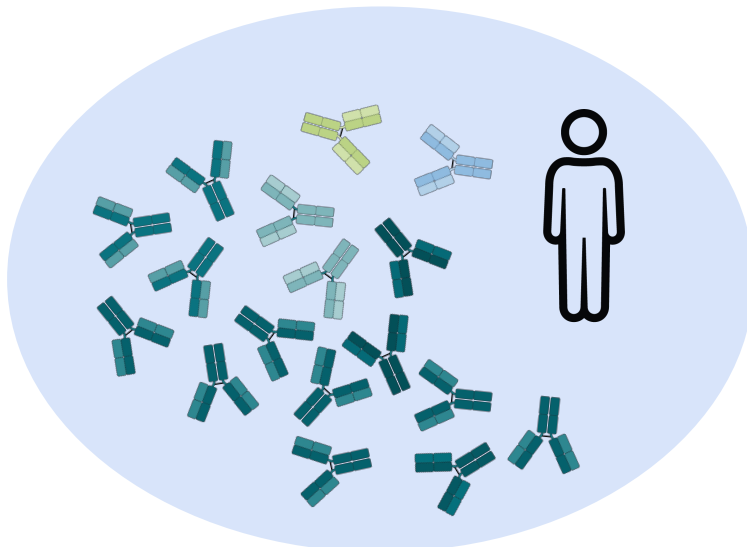
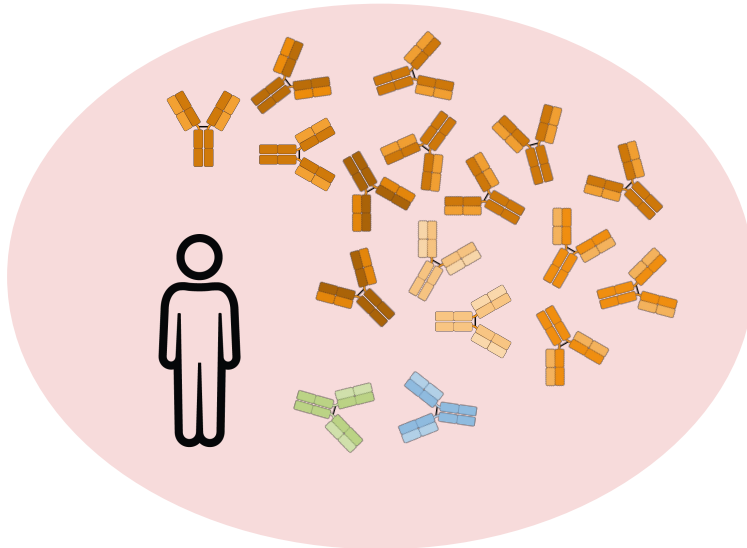
Repertoire sequencing data



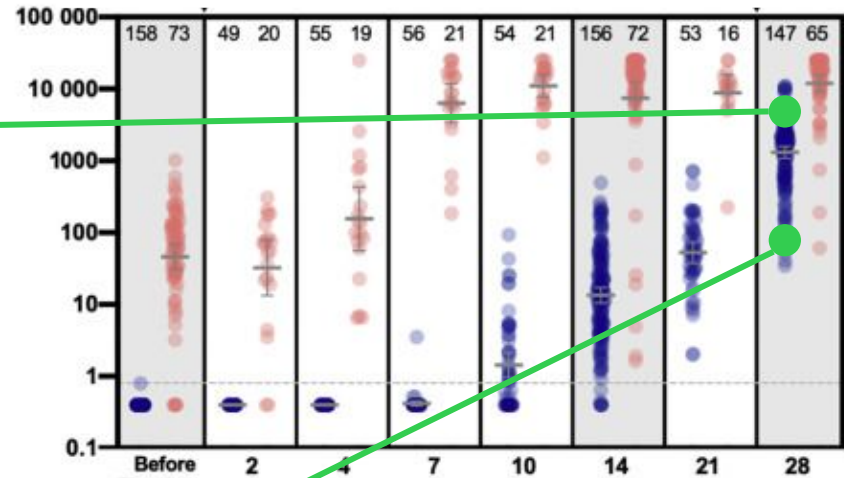
- The first Rep-Seq dataset was sequenced in 2009
- 10,000s Rep-seq datasets are available today



Antibody repertoires and responses



Antibody responses to SARS-CoV-2

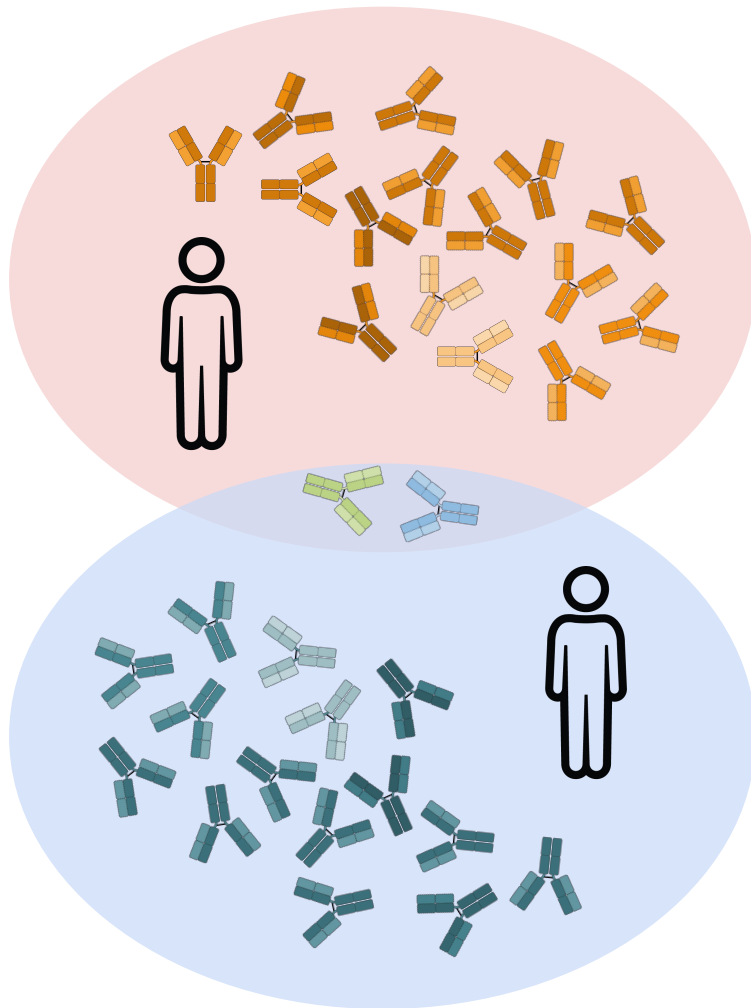


adapted from Favresse et al., Clinical Microbiology and Infection, 2021

red / blue - exposed / naive donors

Can we use Rep-Seq technologies to explain the variance in antibody responses?

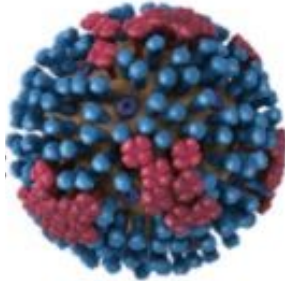
Antibody repertoire is unique for an individual



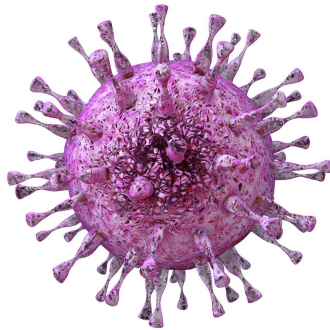
- Antibody repertoires of different individuals barely overlap
- Overlapping antibodies typically represent frequent VDJ recombinations rather than functional antibodies
- **Immunogenomics needs new computational methods**

Variations in IG genes and diseases

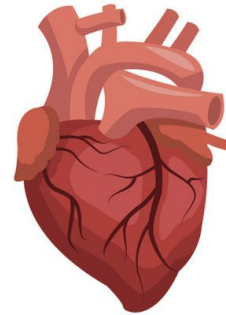
Influenza + IGHV1-69
Lingwood et al., 2012
Avnir et al., 2016



Kawasaki disease +
IGHV3-66
Johnson et al., 2020



Cytomegalovirus +
IGHV3-30, IGKV3-11
Thomson et al., 2008

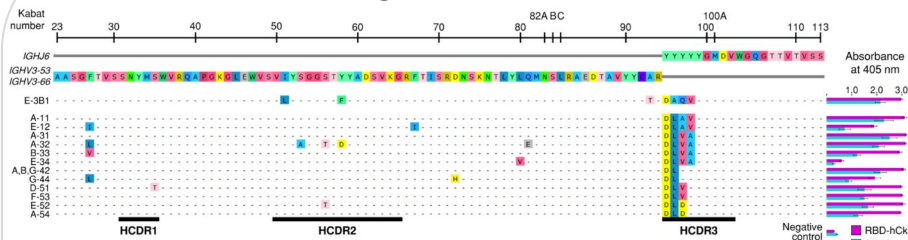


Rheumatic heart
disease + IGHV4-61
Parks et al., 2017

- The immune system often favors specific IG genes to fight specific diseases
- Variations in these genes are associated with susceptibility to diseases and failures/successes of the immune response

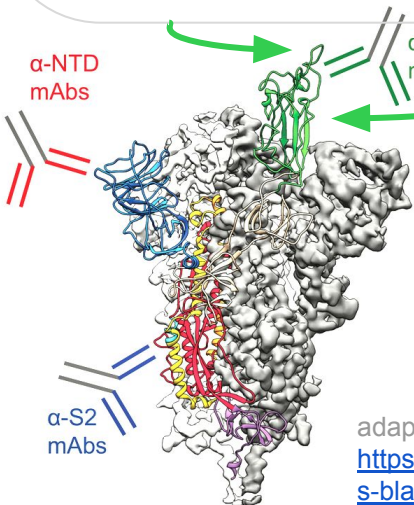
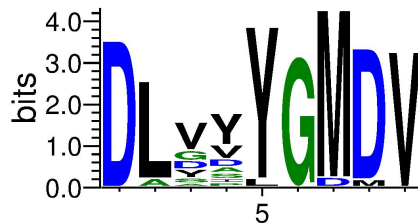
Dissecting antibody responses to COVID-19

Stereotypic antibodies



Kim et al., Sci Trans Med, 2021

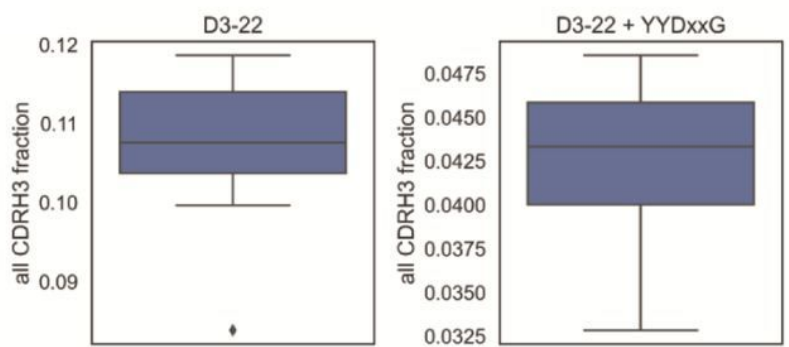
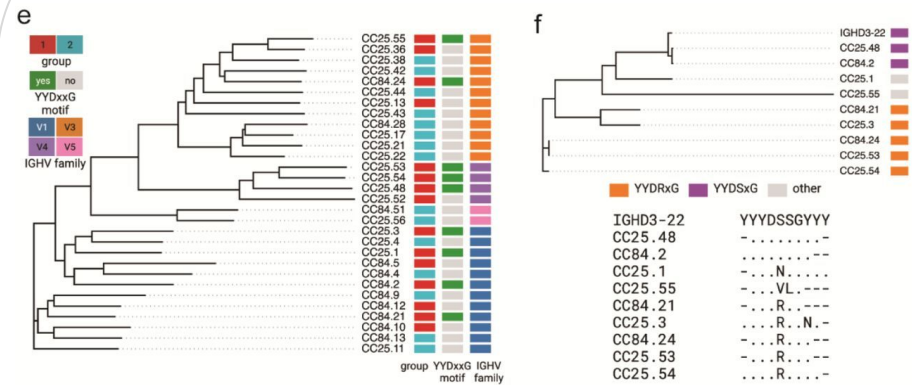
IGHV3-53 /
IGHV3-66 +



YYDxxG antibodies target a more conservative part of the RBD

adapted from <https://news.utexas.edu/2021/05/04/our-immune-system-s-blanket-the-sars-cov-2-spike-protein-with-antibodies/>

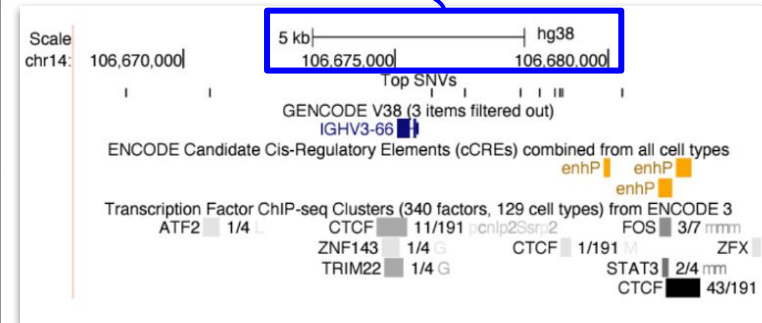
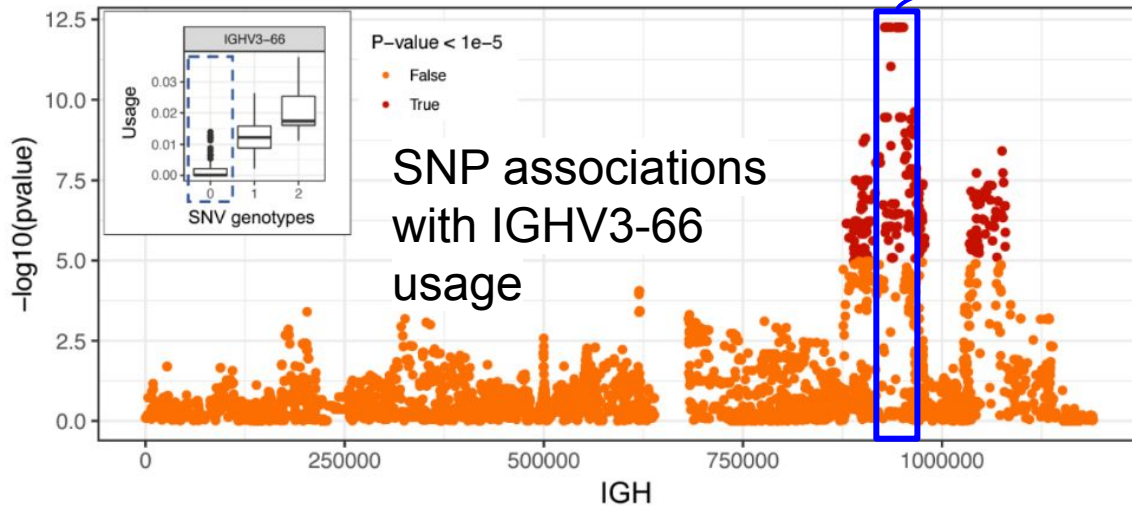
YYDxxG antibodies



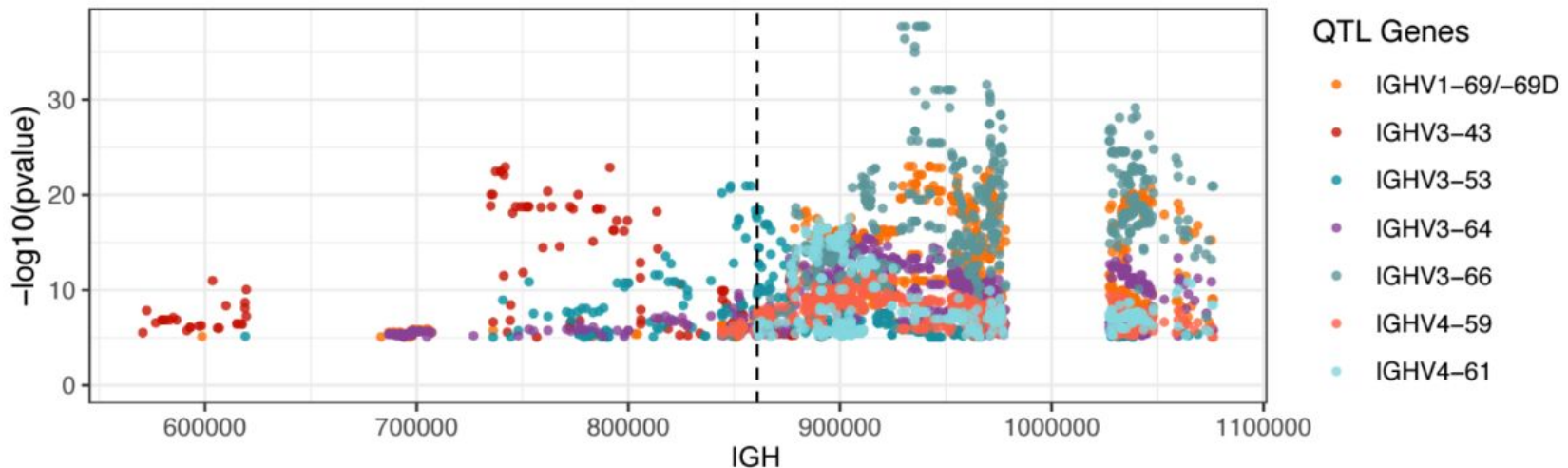
~4% of Abs are derived from IGHV3-22 and have YYDxxG

He et al., Nat Immunol, 2022

Gene usage QTLs of human IGH locus



The region containing top SNPs contain regulatory elements



Long CDR3s (≥ 72 nt) of human Abs are efficient against HIV

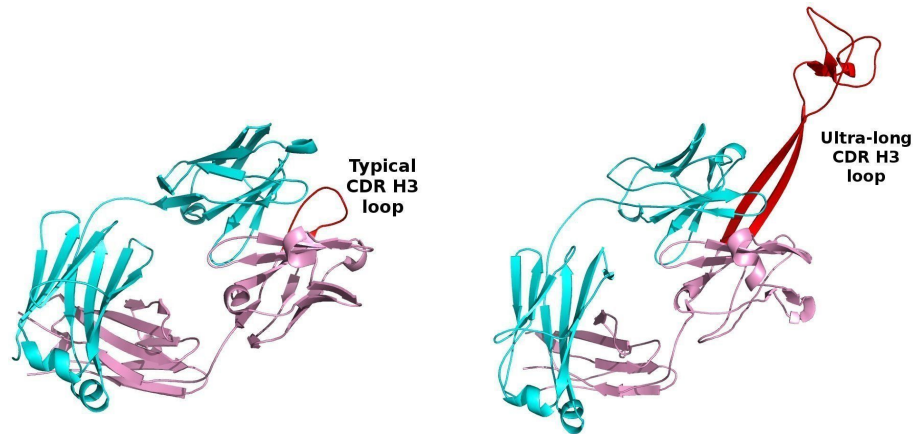
Unusual **VDDJ** recombinations were hypothesized by Tonegawa and discovered by Meek in 1989

Tandem D-D fusions often double CDR3 length and result in **ultralong antibodies**

Many broadly neutralizing antibodies against HIV result from tandem D-D fusions



Prof. Susumu Tonegawa, USA -1987 Nobel Prize in Physiology or Medicine

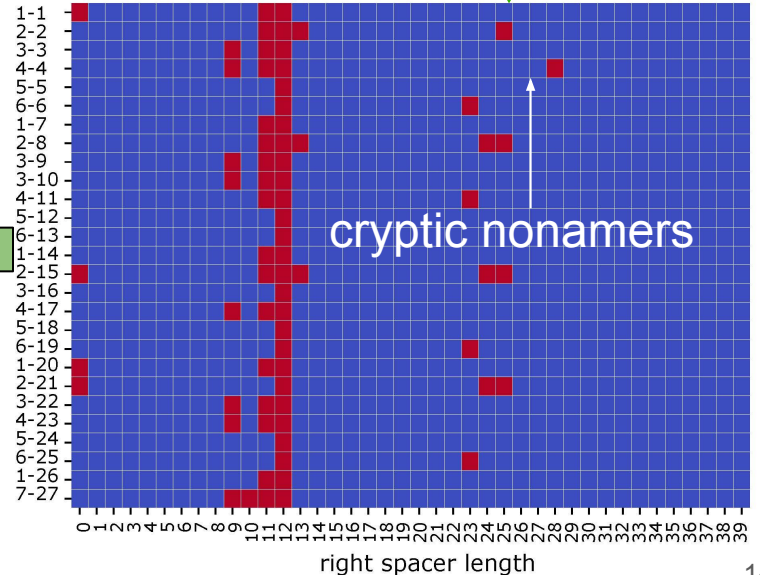
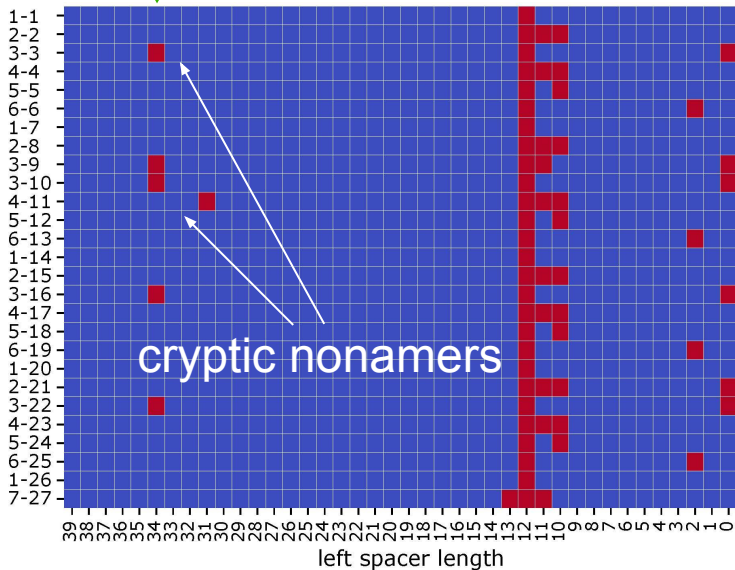
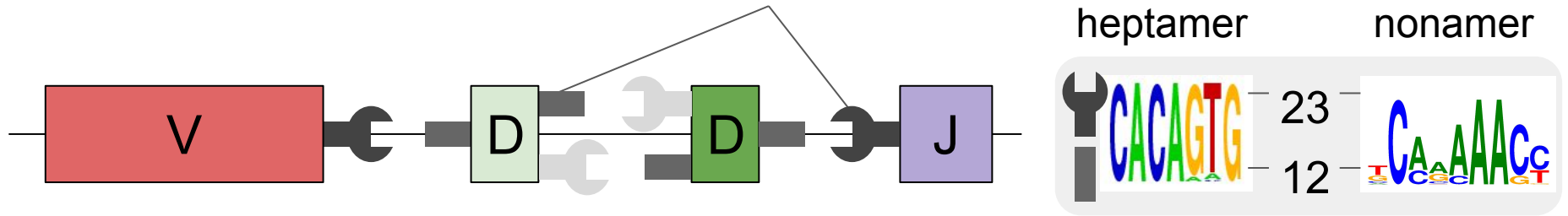


Sok et al., *Nature*, 2017

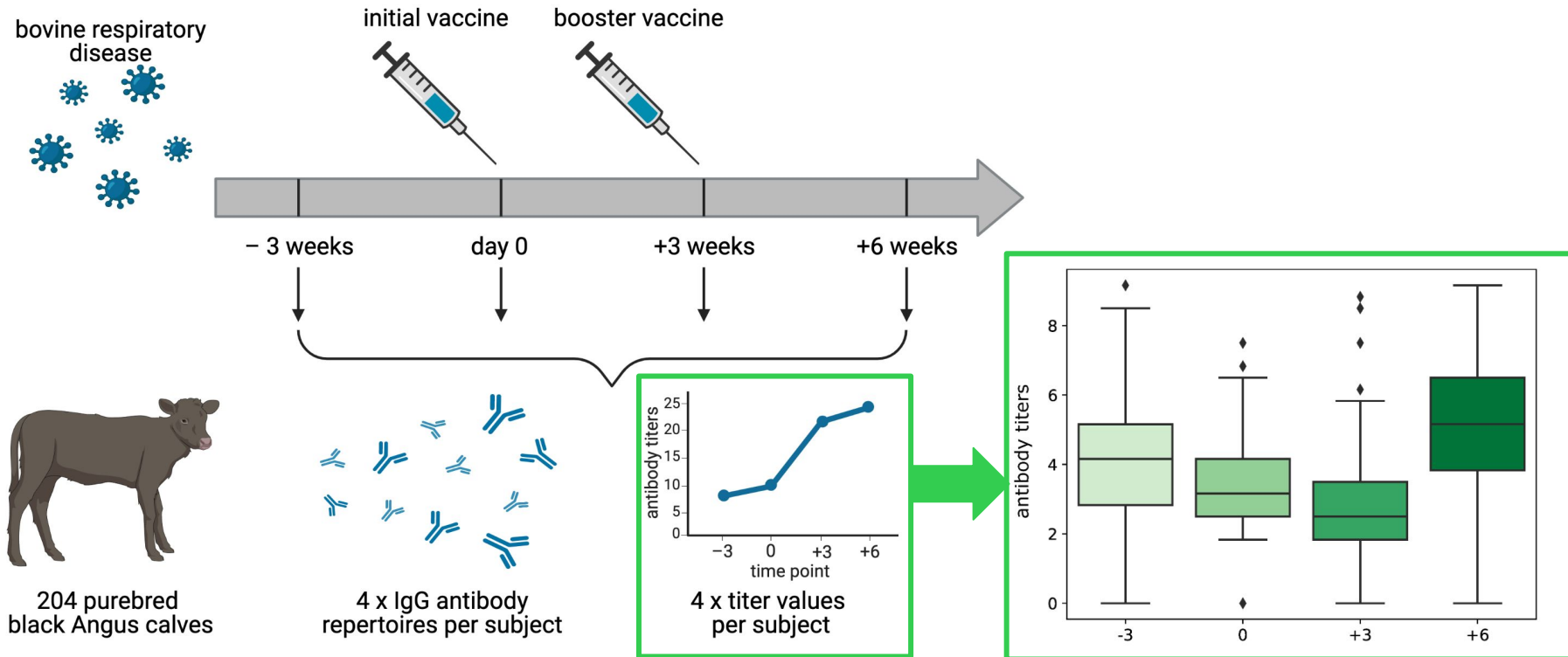


The Cryptic RSS Hypothesis explains 95% of tandem D-D fusions

Recombination signal sequences

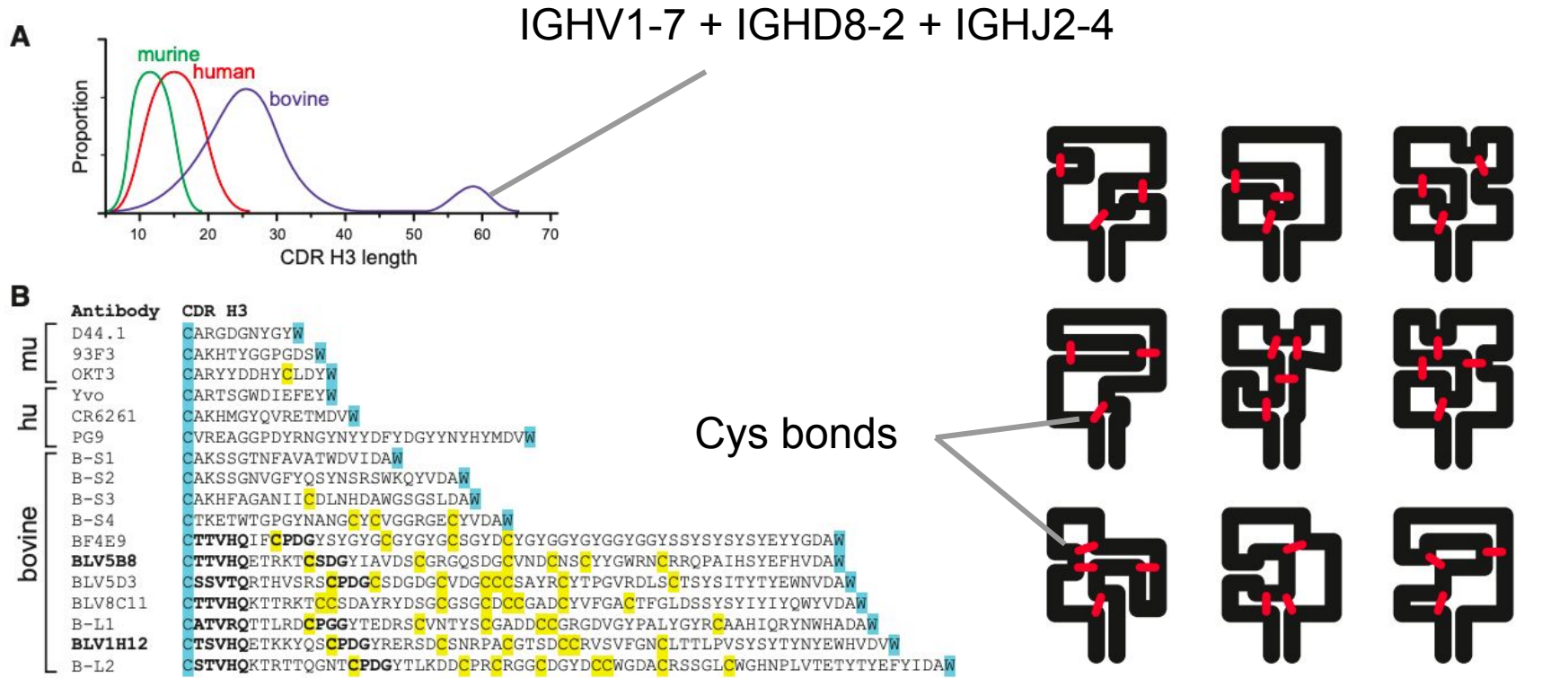


Cattle antibody responses to the BRD vaccine



- The Bovine Respiratory Disease (BRD) is a major cause of economic losses in cattle agriculture
- BRD is associated with four viruses, no treatment is available
- The only way to fight BRD is to prevent it using a vaccine

10% of cattle antibodies have ultra-long CDR3s

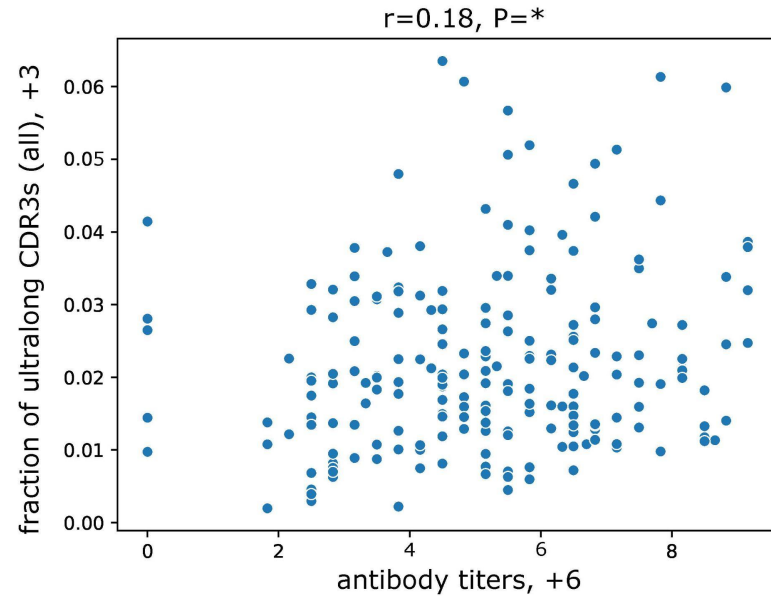
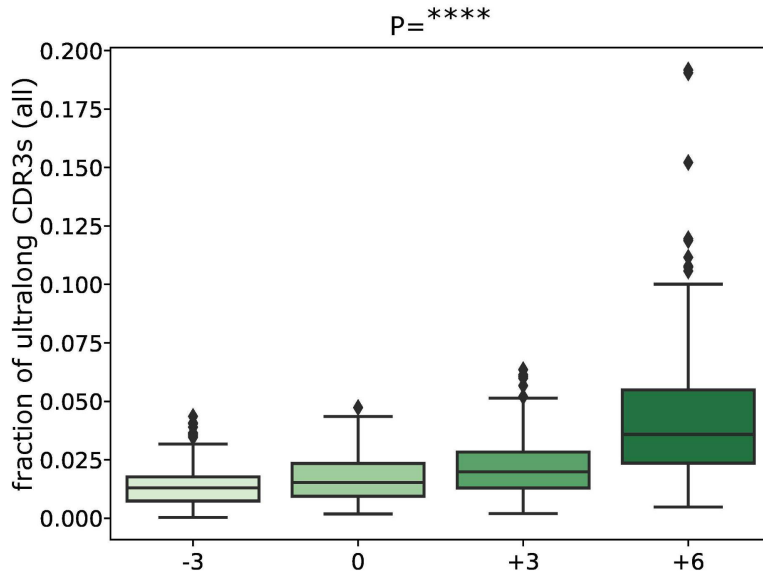


IGHD8-2

GTAGT TGT CCTGAT GGT TAT AGT TAT GGT TAT GGT TGT GGT TAT GGT TAT GG
 TTGT AGT GGT TAT GAT TGT TAT GGT TAT GGT GGT TAT GGT GGT TAT GGT GGT
 TAT GGT TAT AGT AGT TAT AGT TAT AGT TATACT TAC GAATATAC

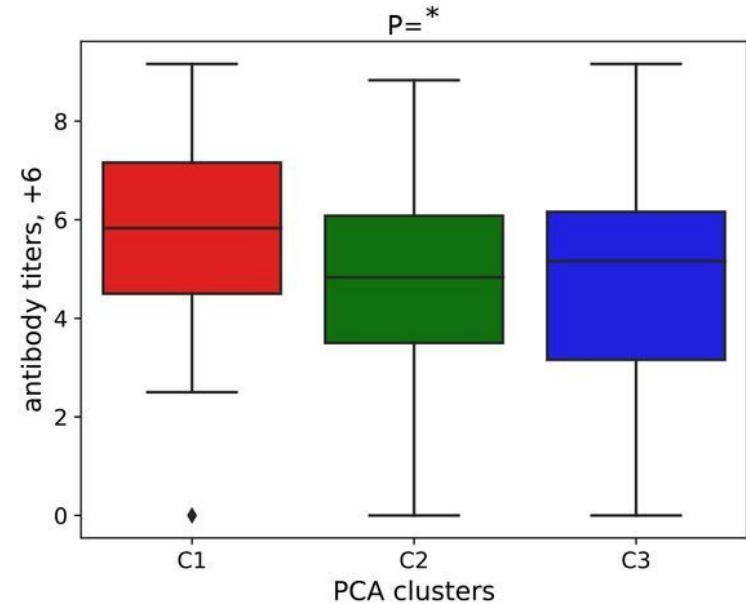
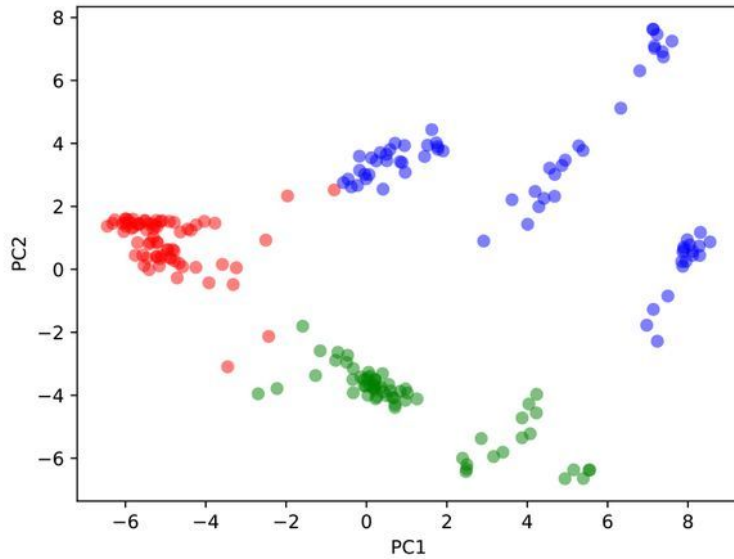
C: TGT, TGC
 G: GGT
 S: AGT
 Y: TAT, TAC

Vaccination triggers production of ultralong CDR3s

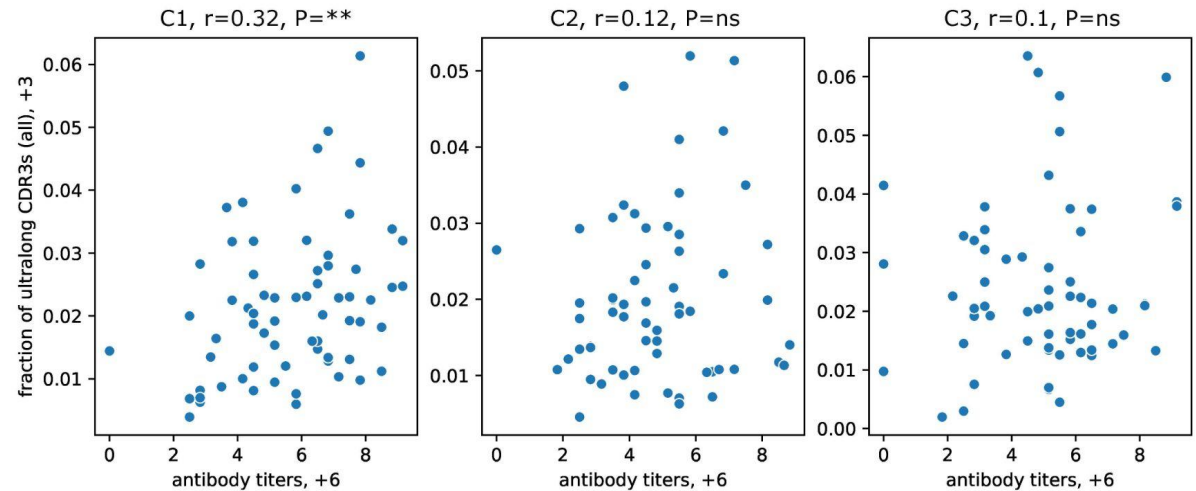


- Vaccination boosts the global fraction of ultralong CDR3s
- The fraction of ultralong CDR3s correlates (albeit weakly) with the final titers

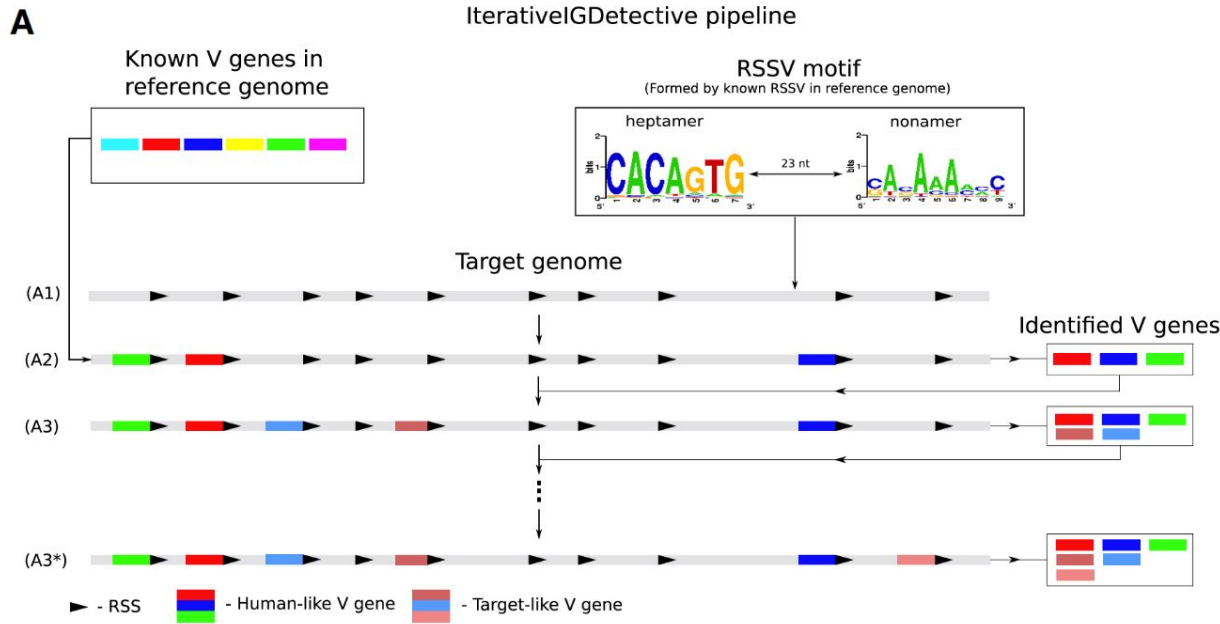
Genotypes of cattle V genes shape Ab responses



- 3 common genotypes are associated with the final titers
- C1 has both high titers and a good correlation with ultralong CDR3s

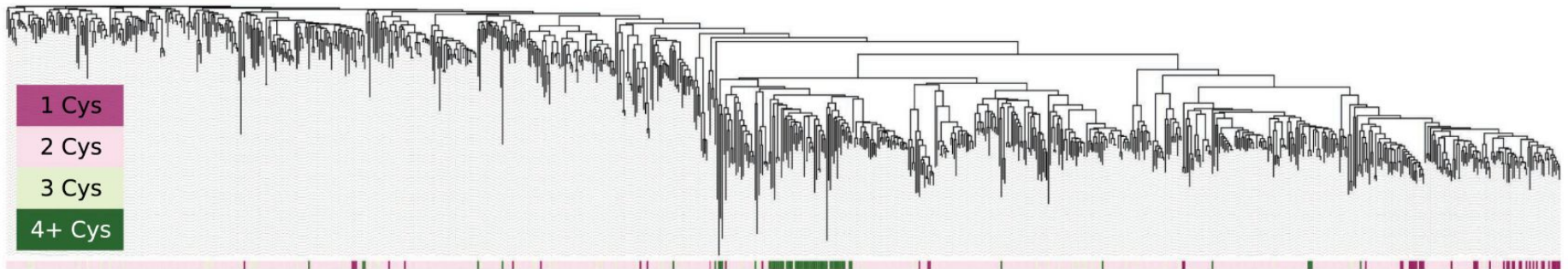


Discovery of novel immunoglobulin genes

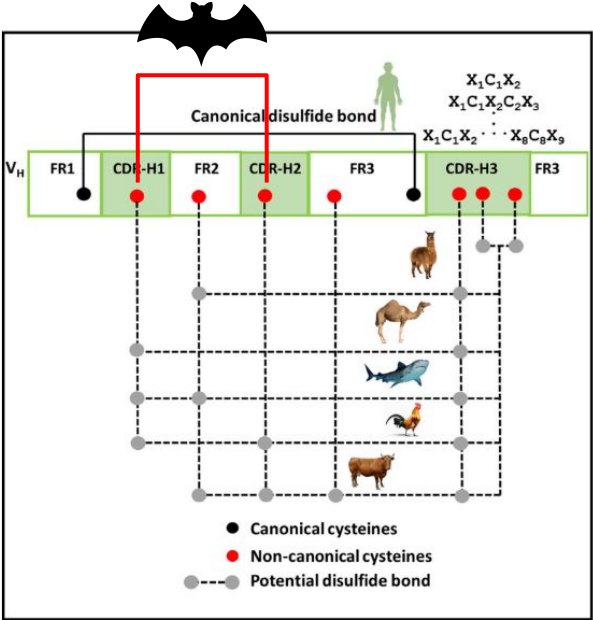


IgDetective *de novo* detects immunoglobulin genes in mammalian assemblies through search of RSSs and then IG genes

1000+ V genes from 20 mammalian species from Vertebrate Species Project

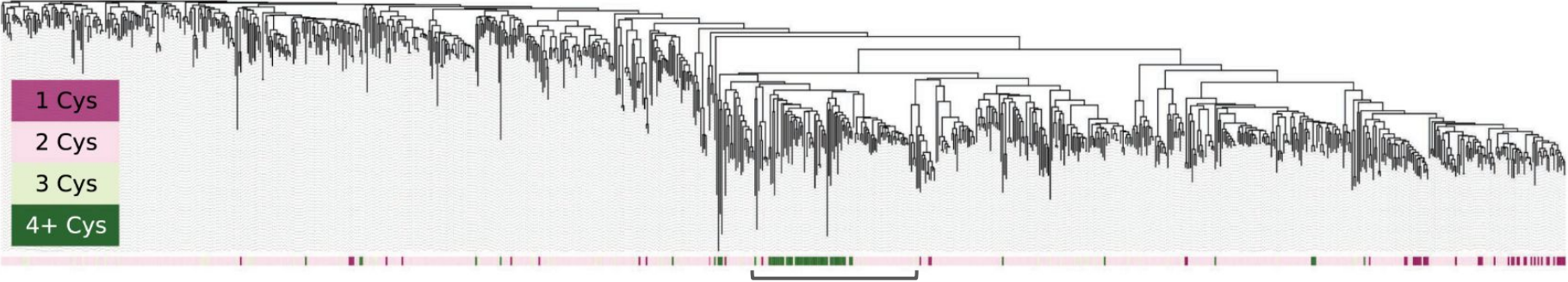


New family of bat immunoglobulin V genes



QVQLQESGPGGLVKPSQTL^CSLTCAVSGFSITTSGY^CWH
 WIRQLPGKGLDWIAIIC^CYDGSTAYNPALKSRSSISR
 TSKNQFSLQLKSVTTEDTAVYY^CAR

Prabakaran and Chowdhury, *Cell Rep*, 2020



Sirupurapu, Safonova, Pevzner, *Genome Res*, 2022

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