UC San Diego

PDMI COMPUTER SCIENCE CLUB

UNIVERSITY OF LOUISVILLE

Computer Science meets Immunology:
how computational analyses help to study diseases

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University of California San Diego
University of Louisville School of Medicine

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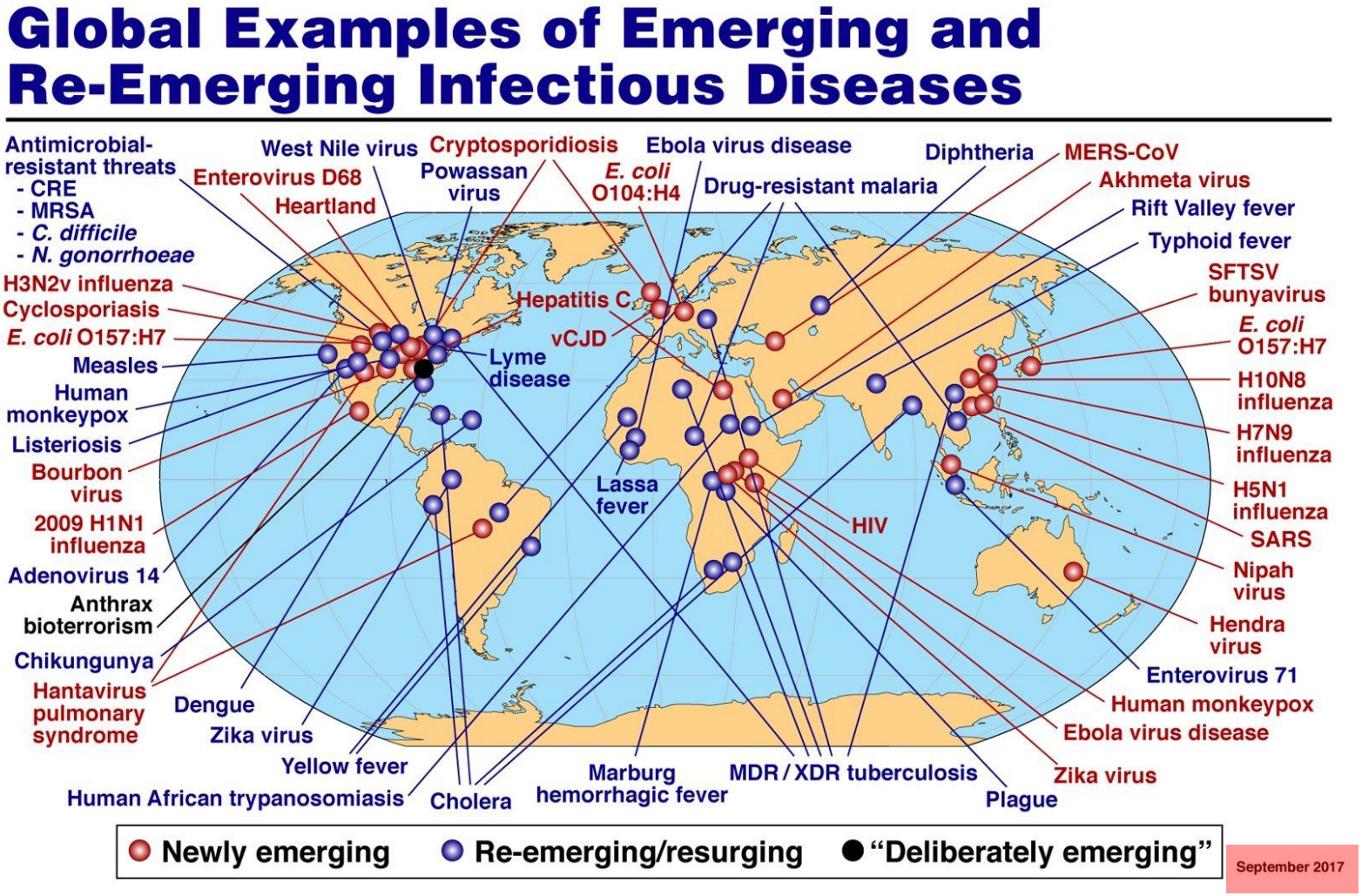
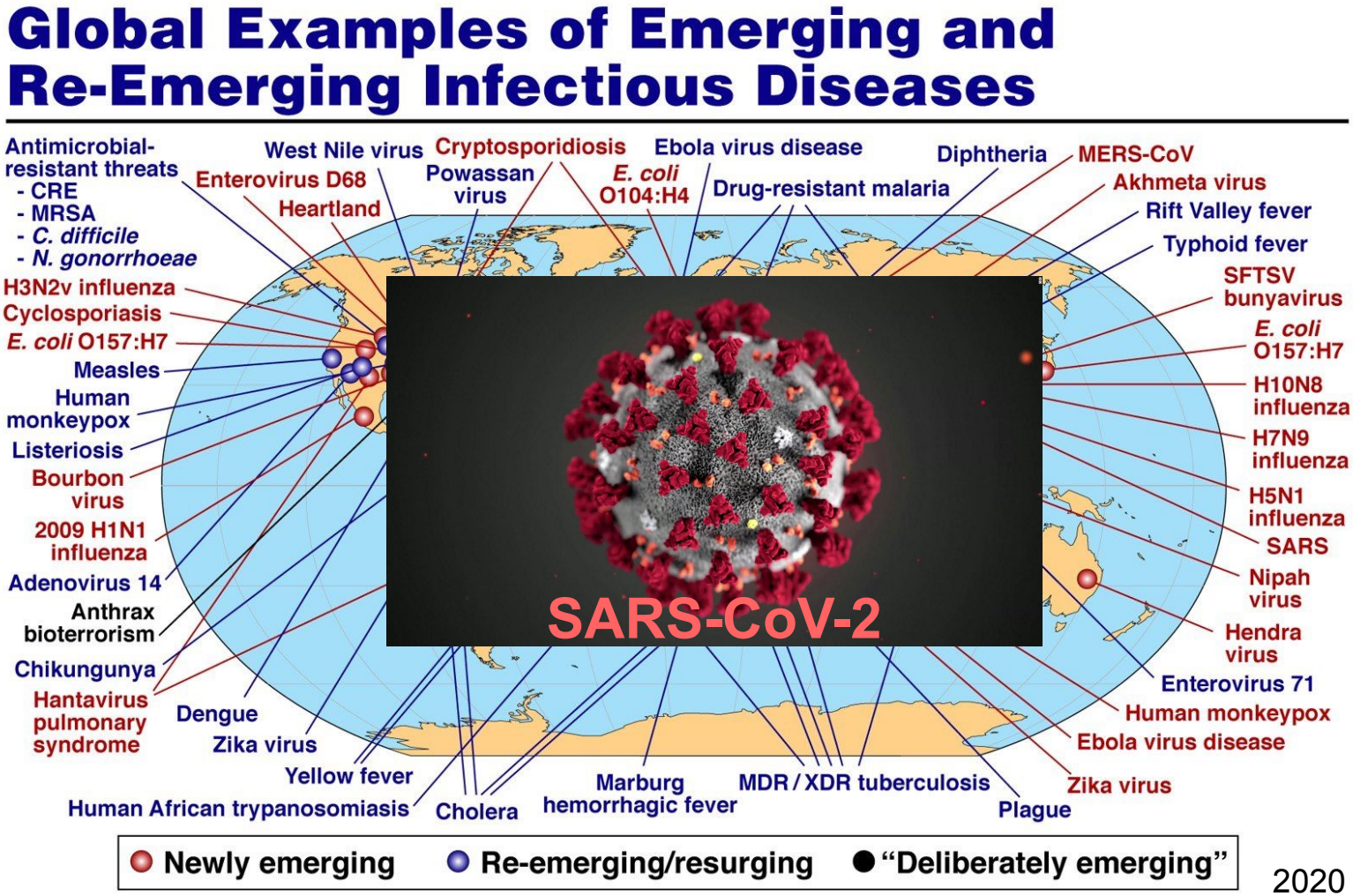
Newly emerging and re-emerging diseases

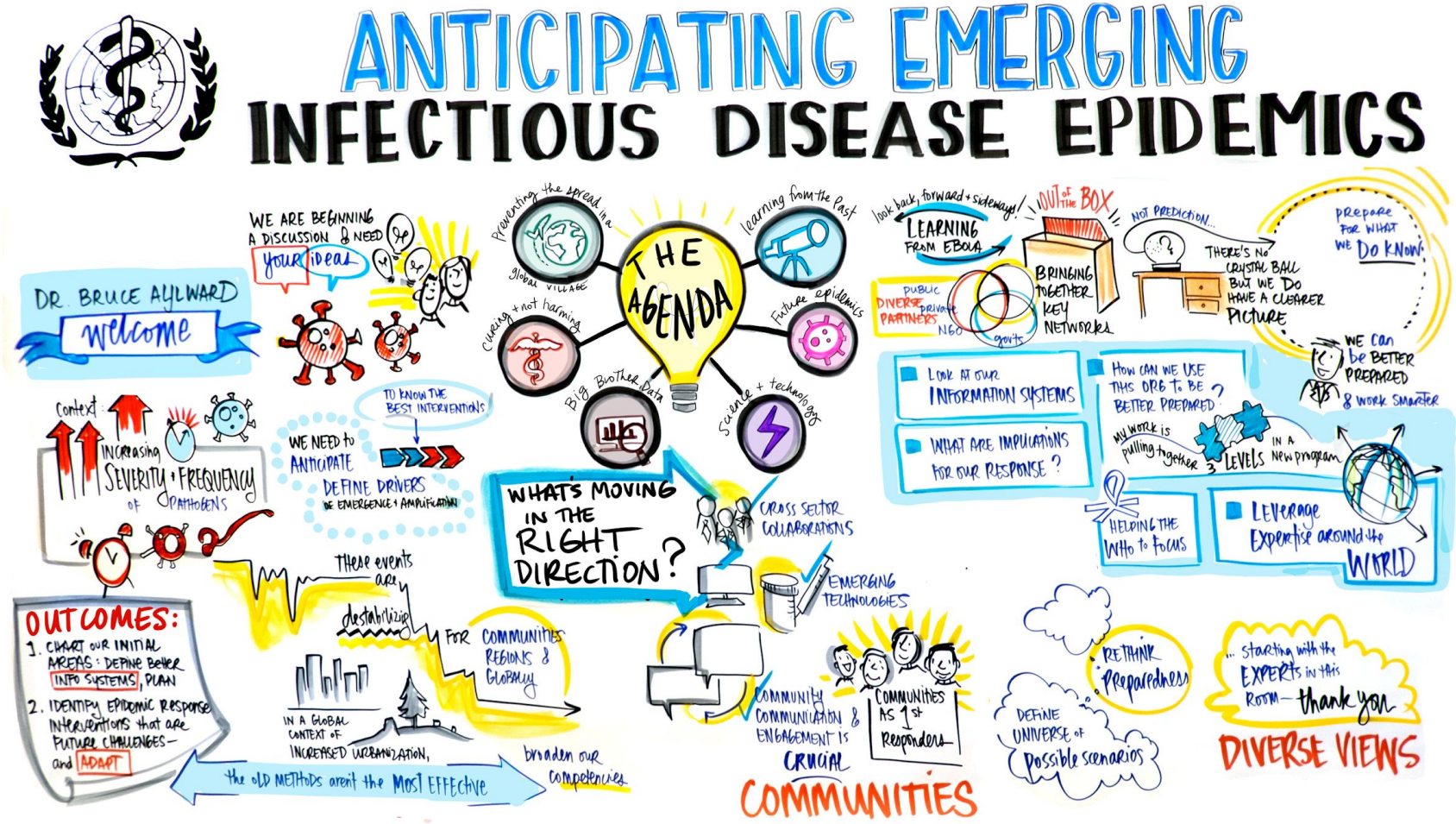


https://en.wikipedia.org/wiki/Emerging_infectious_disease

Newly emerging and re-emerging diseases

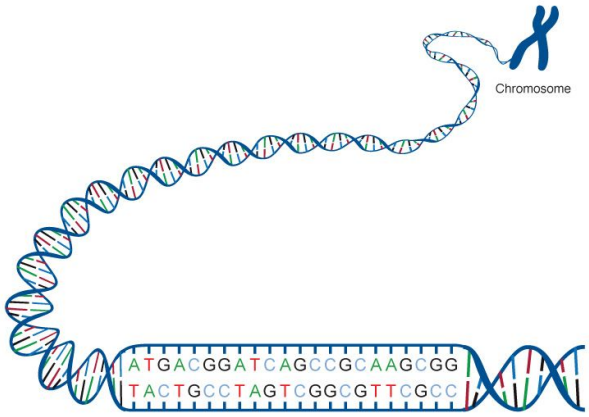


Response to (re)emerging diseases



Response to (re)emerging diseases

Introduction to molecular biology



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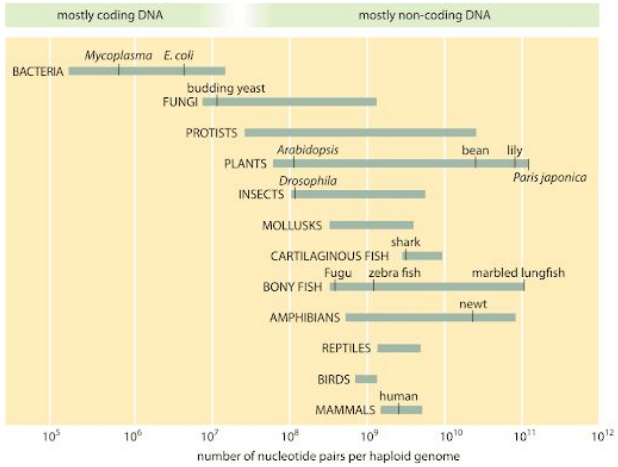
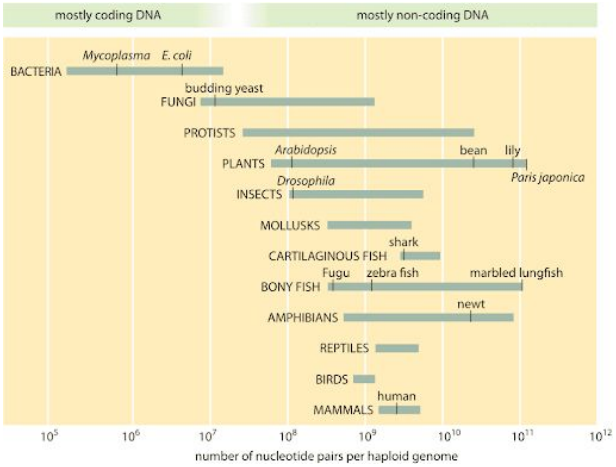


Human genome ~ 3Gbp

Introduction to molecular biology



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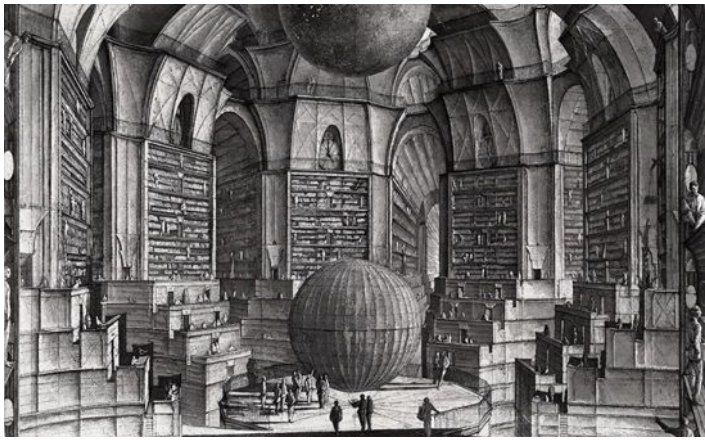
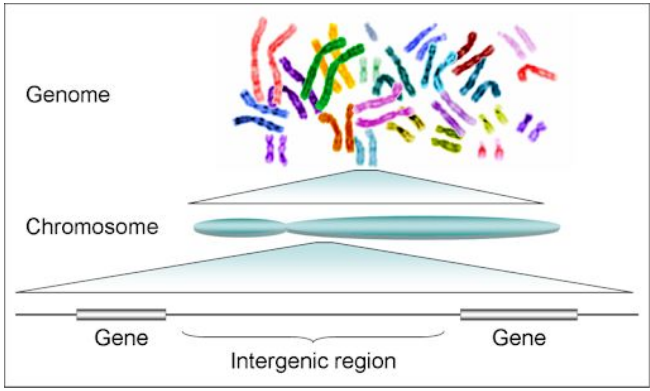
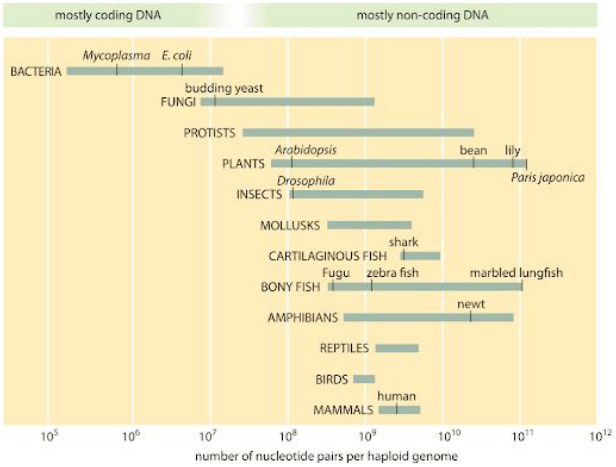


The Library of Babel (H. L. Borges) contains all possible combinations of symbols, mostly meaningless ones



Genes in human genome take 1-2% of its length

Introduction to molecular biology



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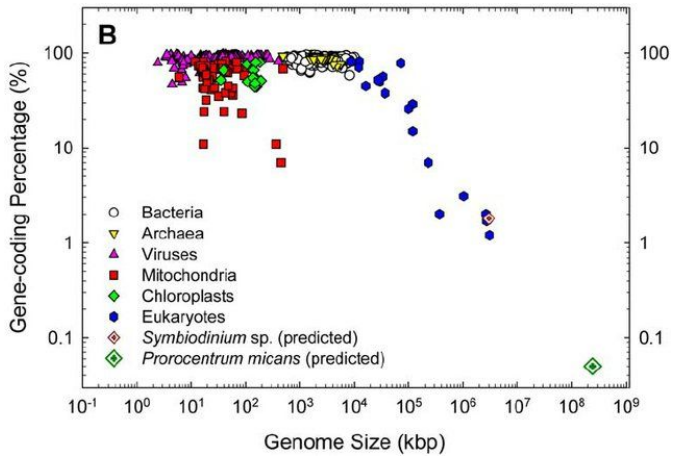
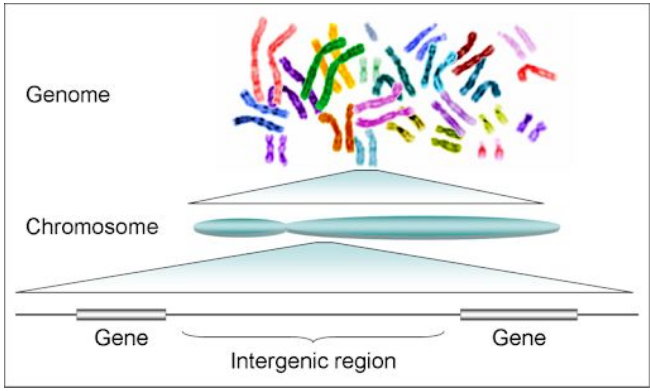


Hou and Lin, PLoS ONE, 2009



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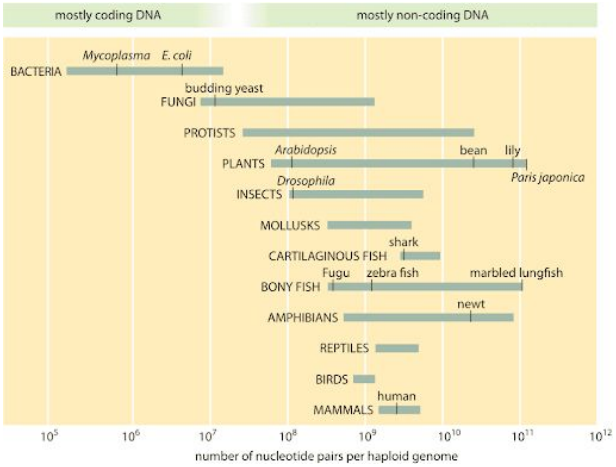

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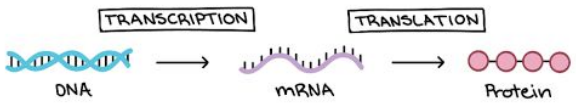
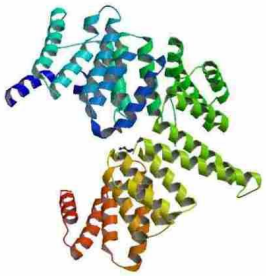
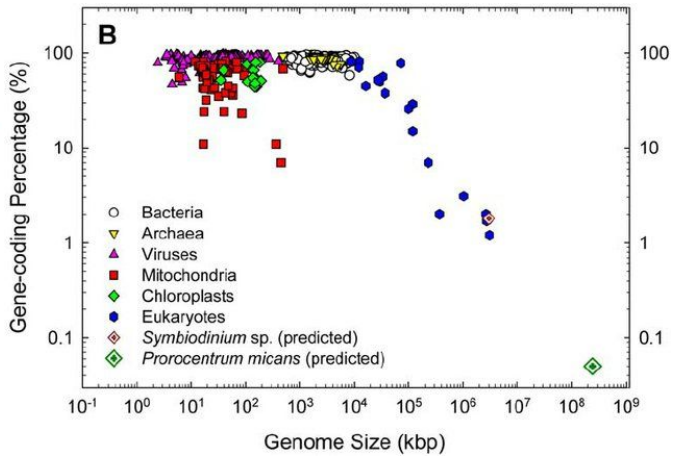
The central dogma of biology







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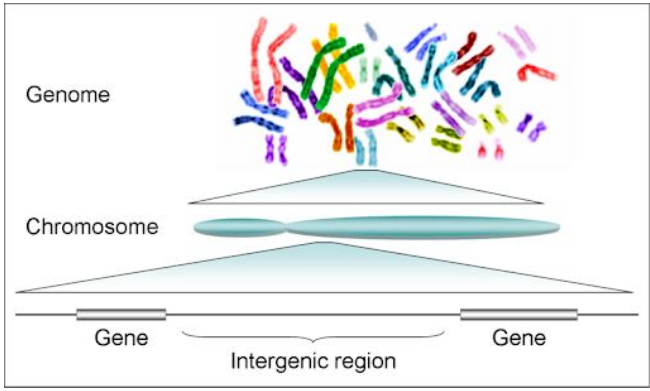
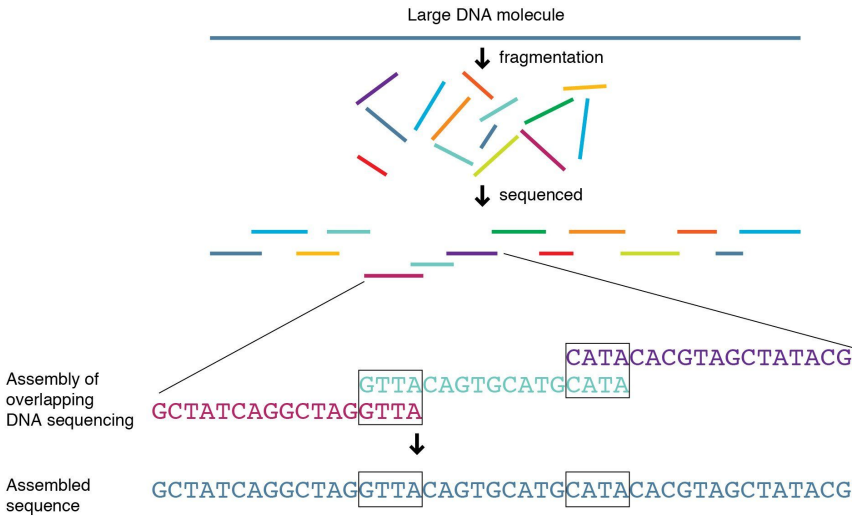
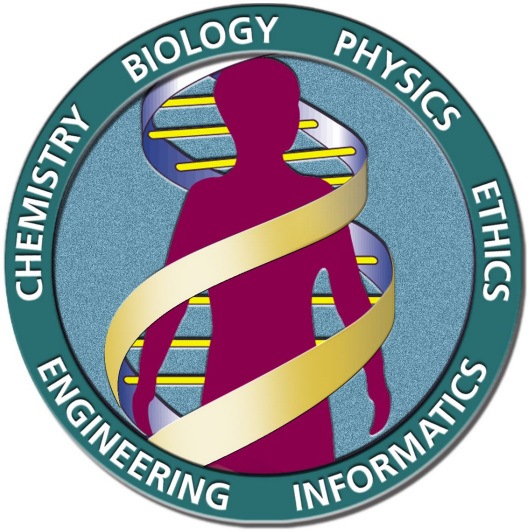


Genes in human genome take 1-2% of its length

The birth of bioinformatics

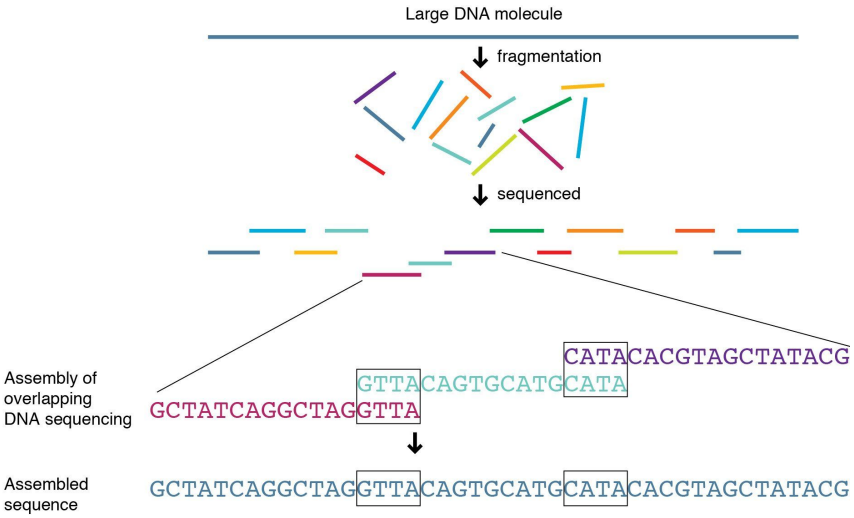


Genome assembly



Human Genome Project
1990 – 2003

The birth of bioinformatics



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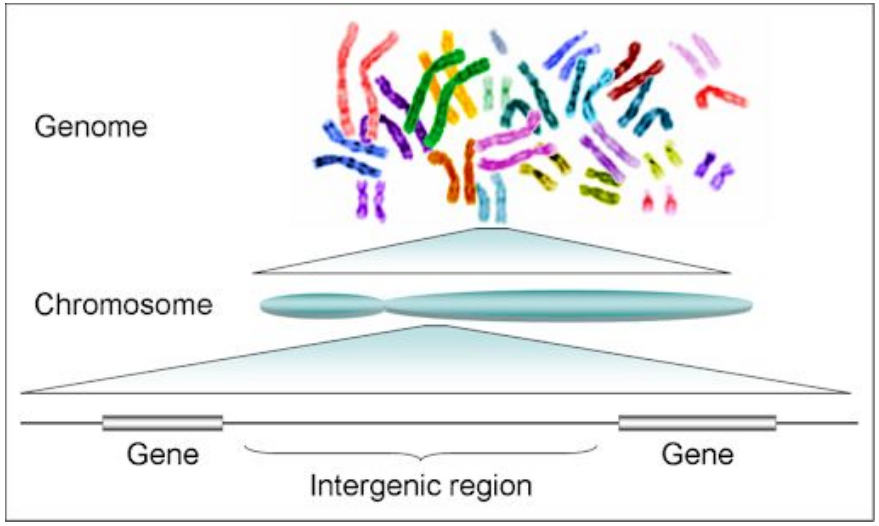
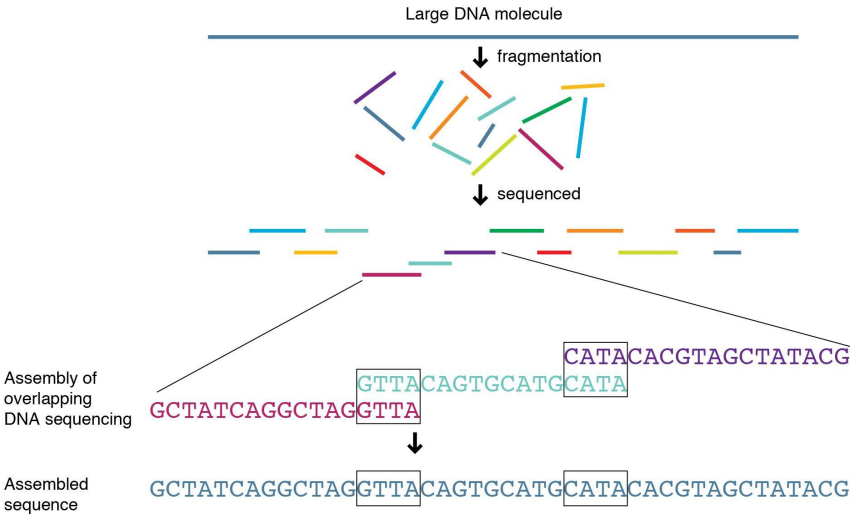


Gene prediction

The birth of bioinformatics



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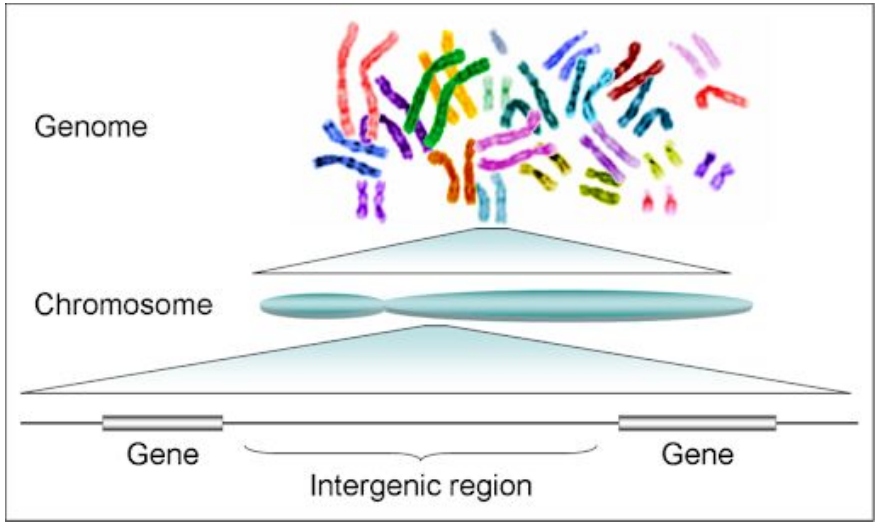
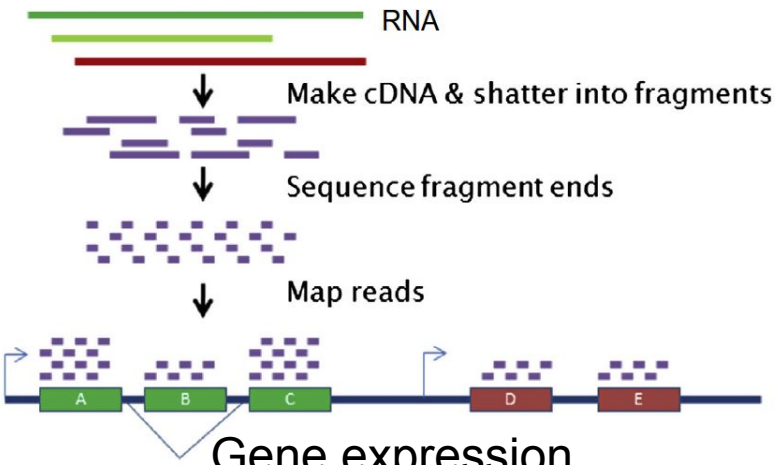
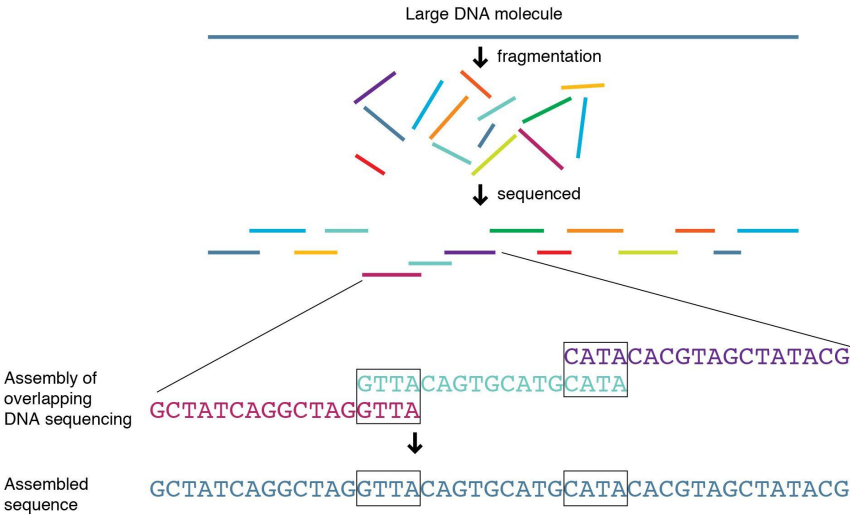


Gene prediction



Gene expression

The birth of bioinformatics



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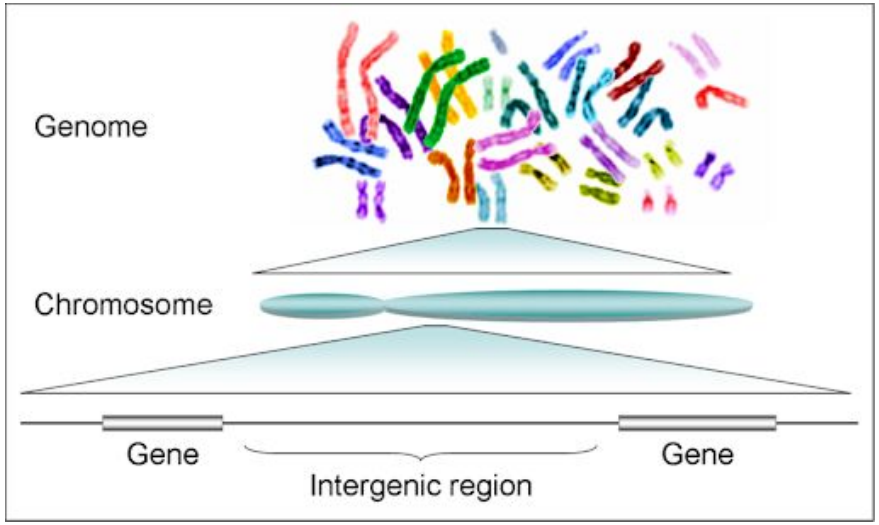

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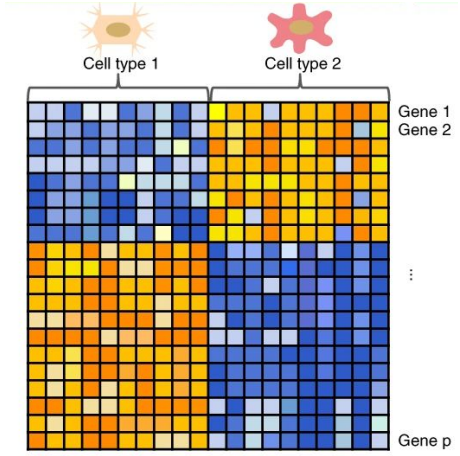
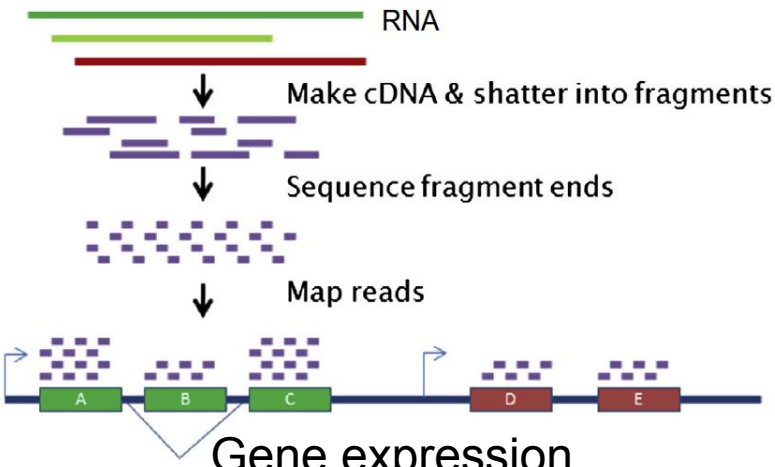
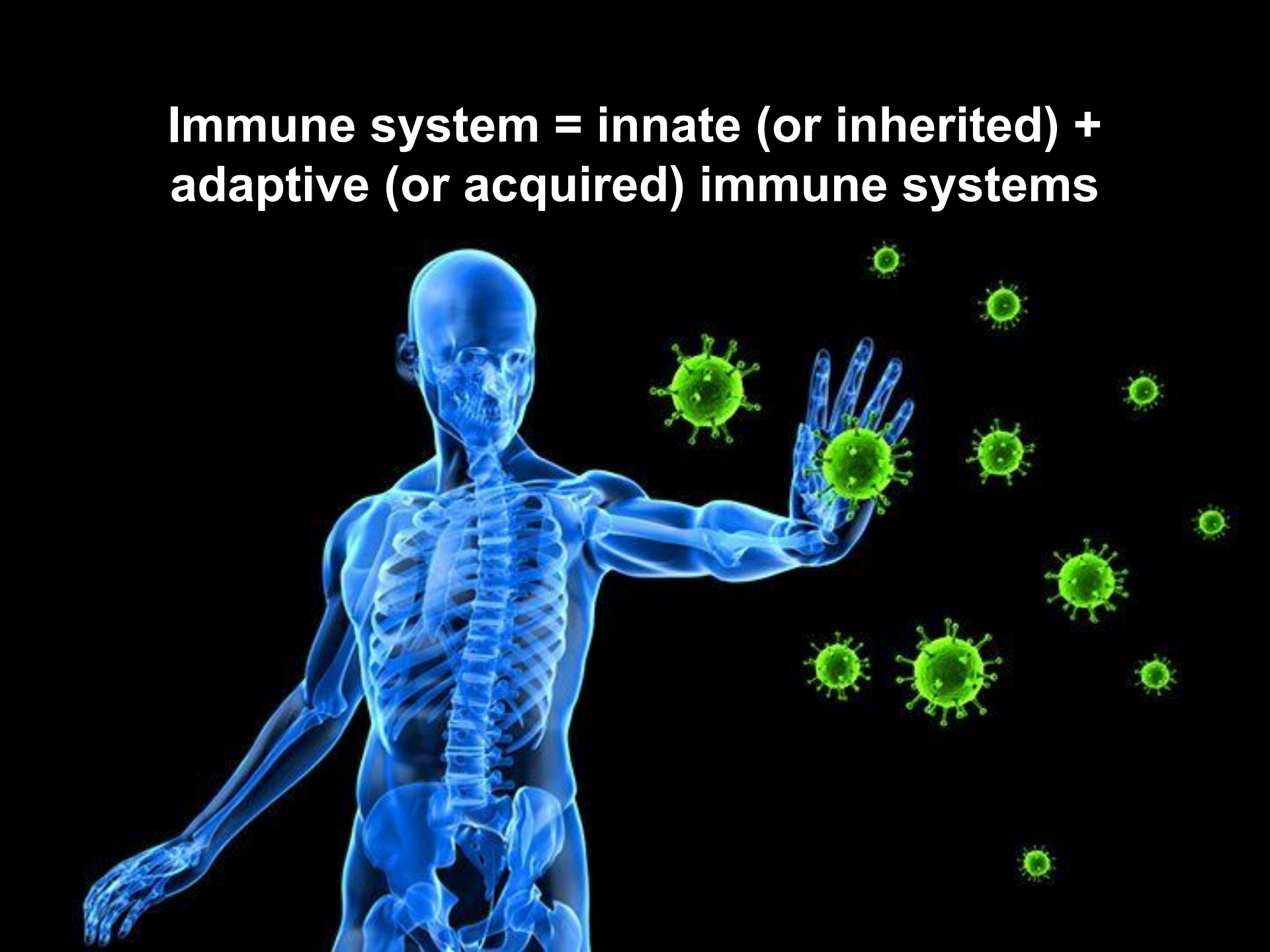


Differential gene expression



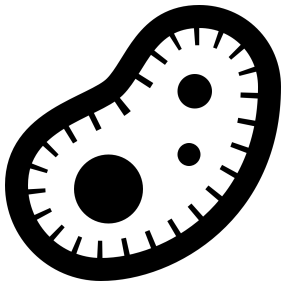
Gene expression

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

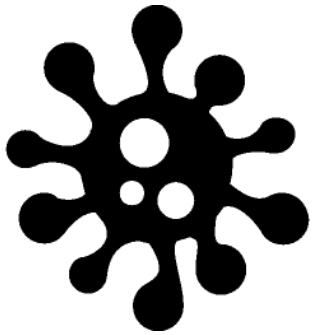


Adaptive immune system

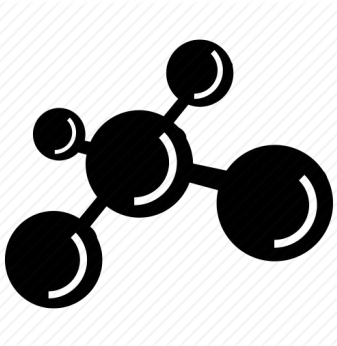
- Variety of threats to human body is huge and unpredictable



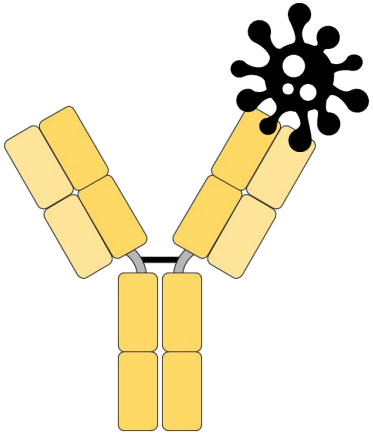
- Genome is too small to encode defences against all these threats



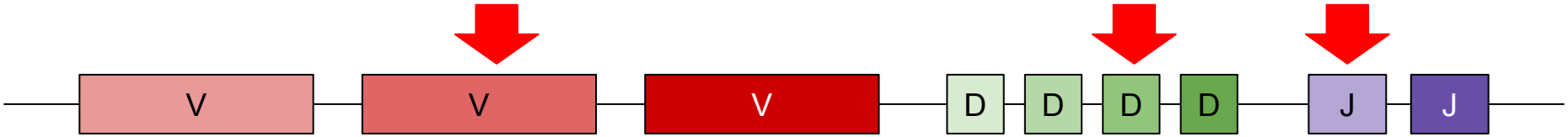
- Immune system has an ability to adapt to various threats using agents (e.g., antibodies) that **are not encoded** in the genome.



Antibodies are agents of the adaptive immune system

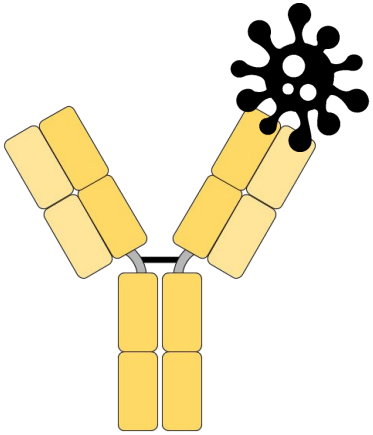


- Antibodies are proteins that bind to an antigen and cause its neutralization
- Antibodies are not encoded in the genome directly, but present a result of somatic genomic recombination



chr 14 (human genome)

Antibodies are agents of the adaptive immune system



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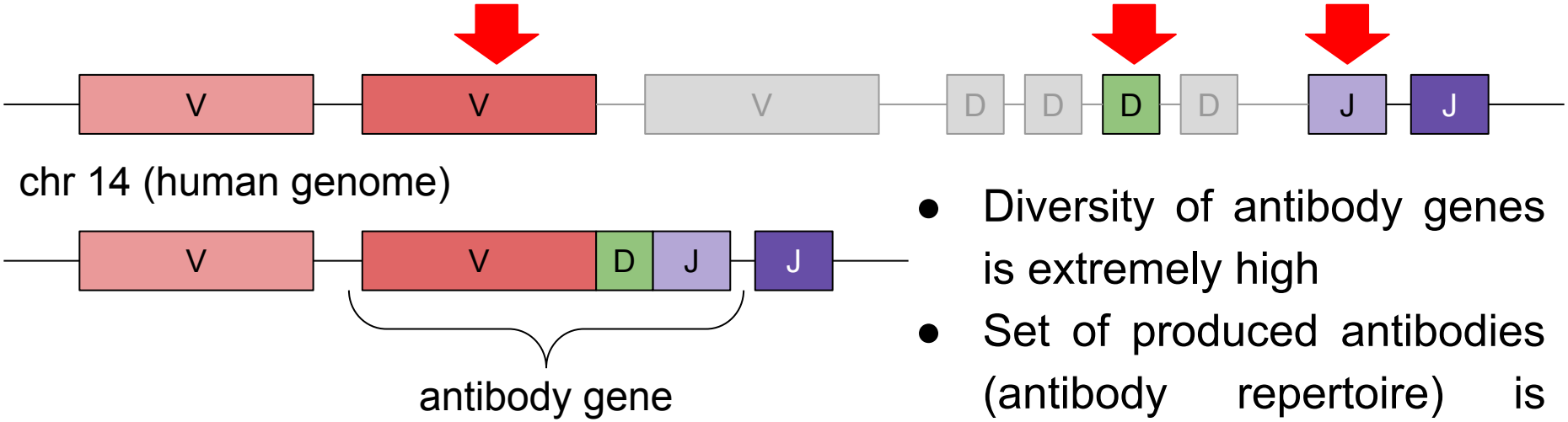
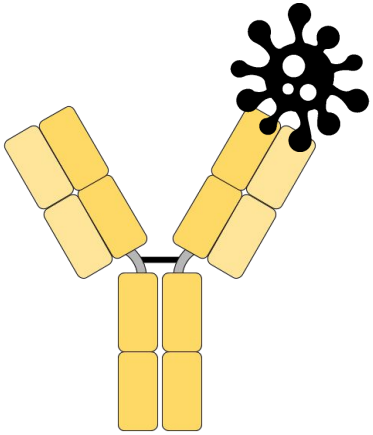


- Diversity of antibody genes is extremely high
- Set of produced antibodies (antibody repertoire) is unique for an individual

Antibodies are agents of the adaptive immune system



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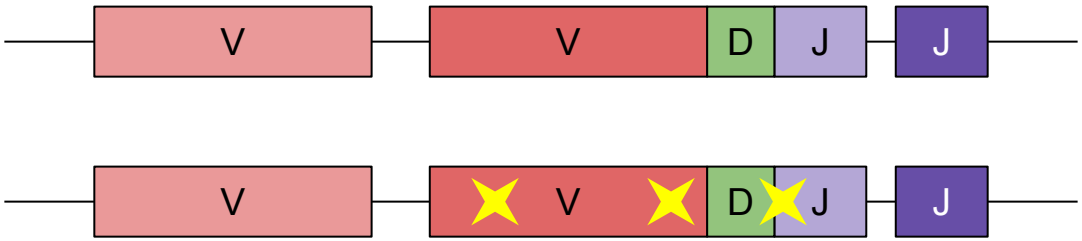

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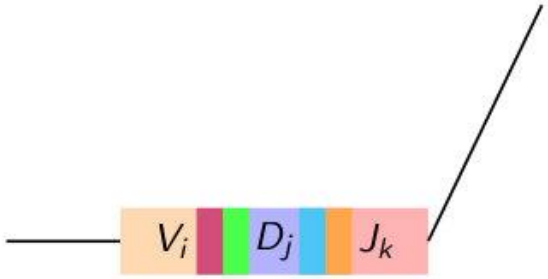
Why are antibodies so versatile if there are only 55×23×6 VDJ recombinations?



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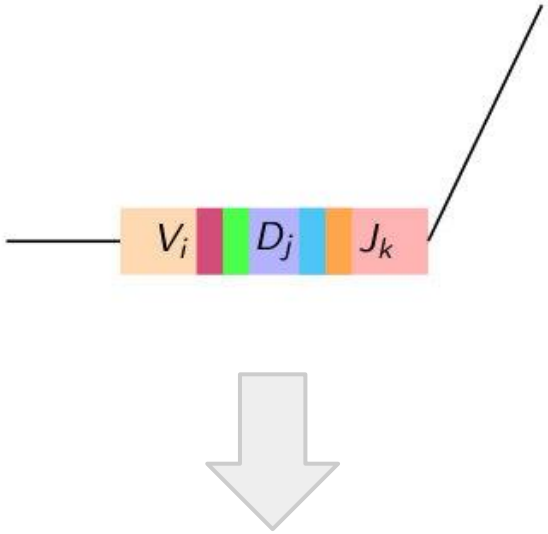
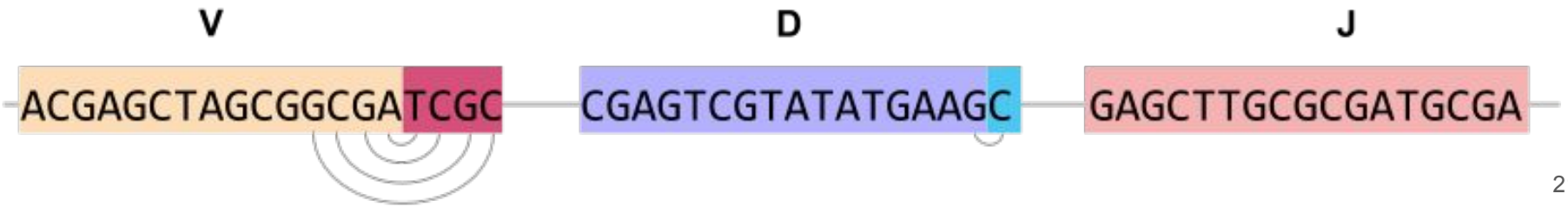


Recombination process is imperfect and includes many random processes:

- **Palindromic insertions**



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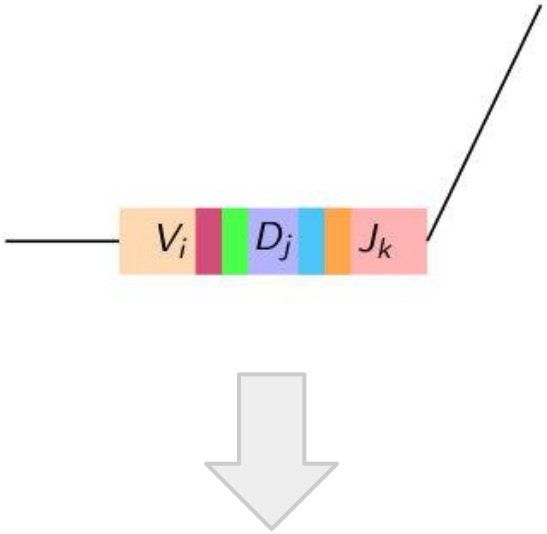

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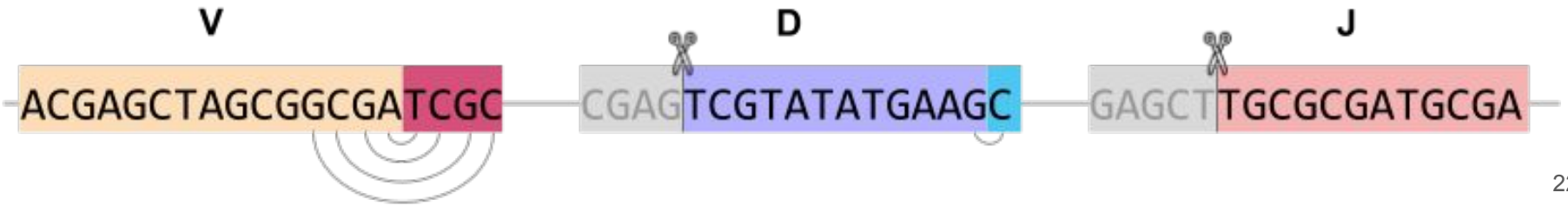
- Palindromic insertions
- **Segment cleavage**



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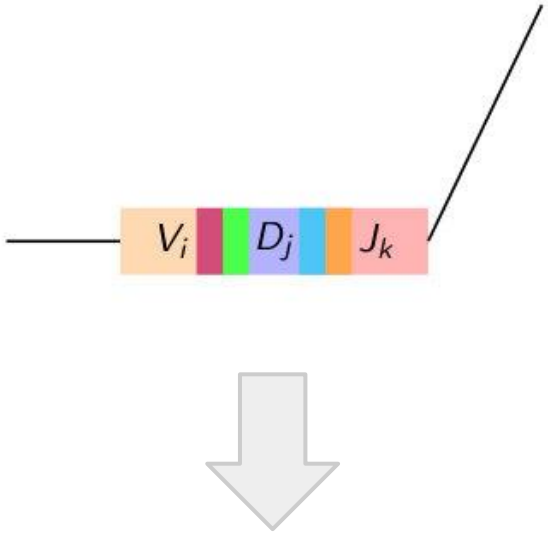

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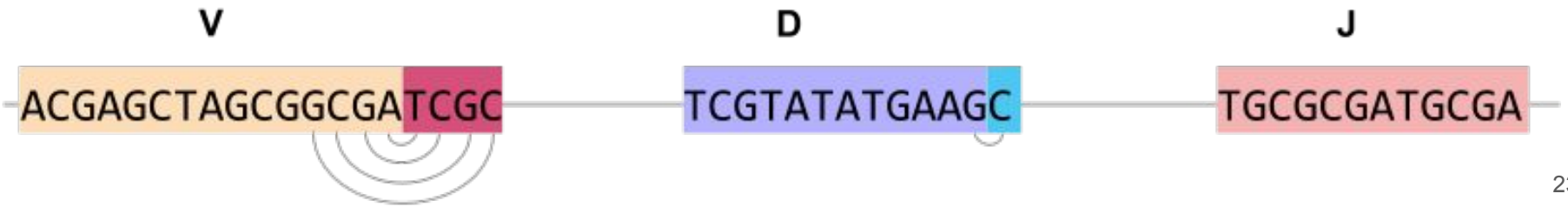

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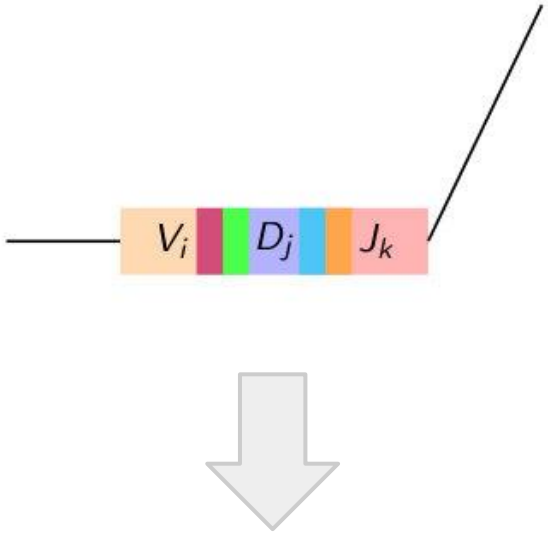
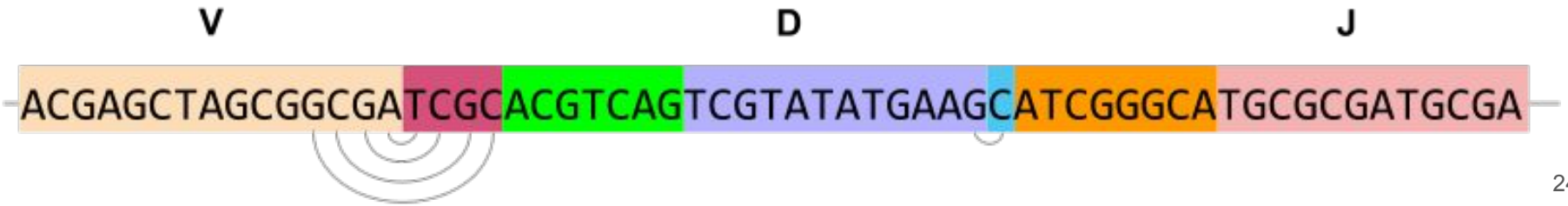


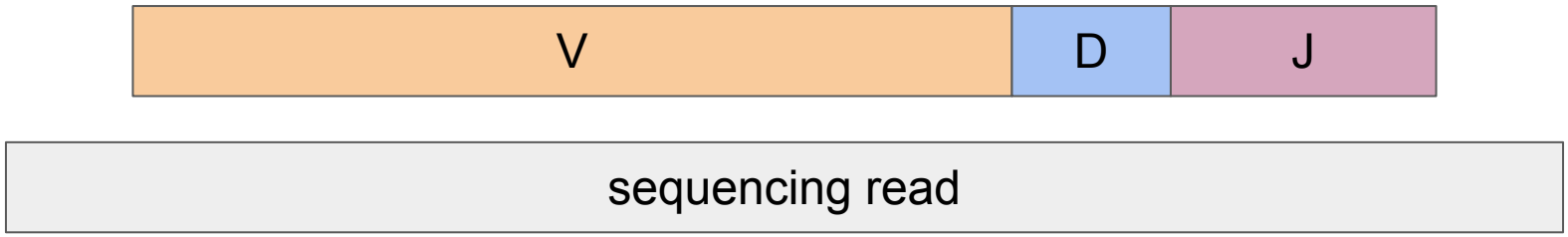
Recombination process is imperfect and includes many random processes:

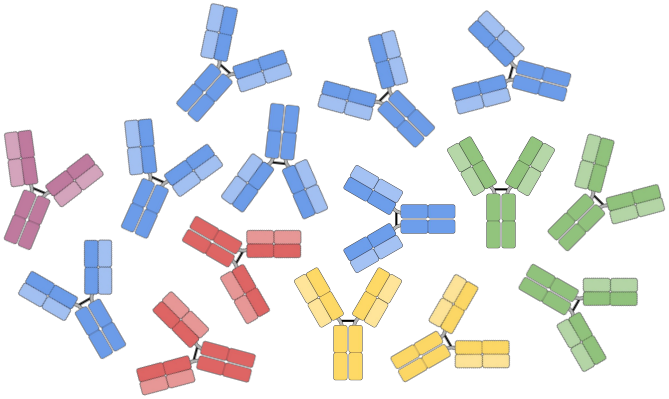
- Palindromic insertions
- Segment cleavage
- **Non-genomic insertions**



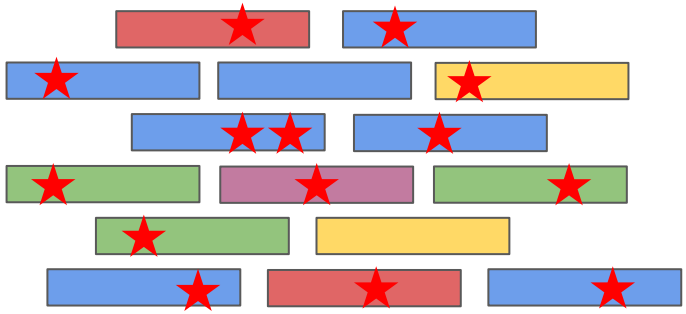
Antibody repertoire sequencing (Rep-seq)

Length: ~360 nt



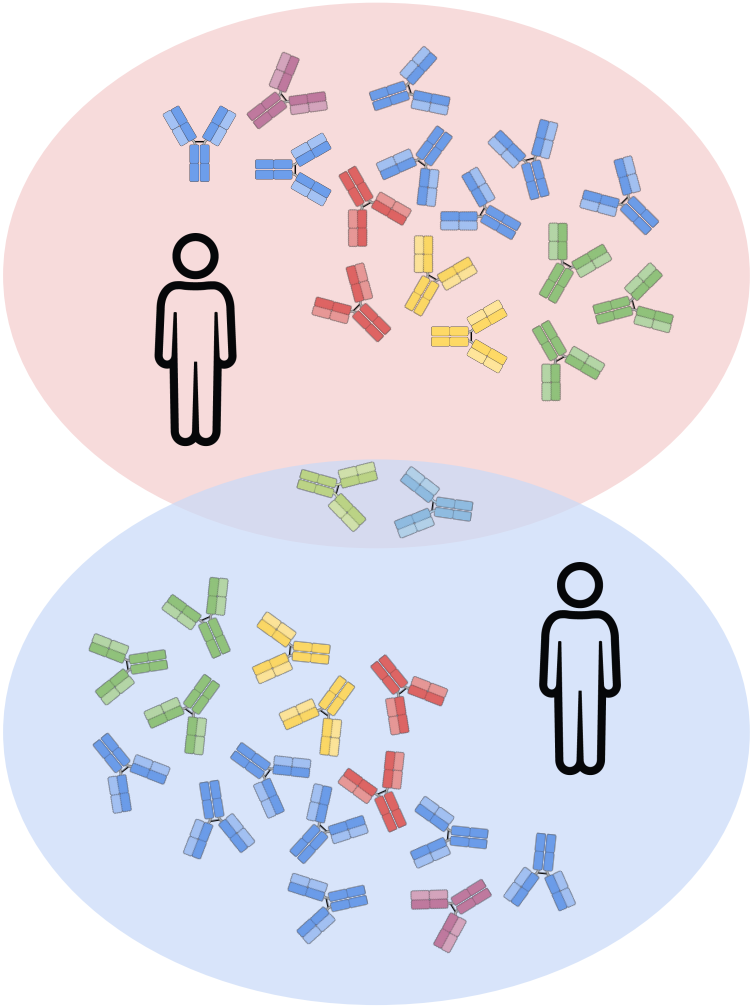


VDJ from DNA or RNA



Error-prone immunosequencing reads

Antibody repertoire is unique for an individual



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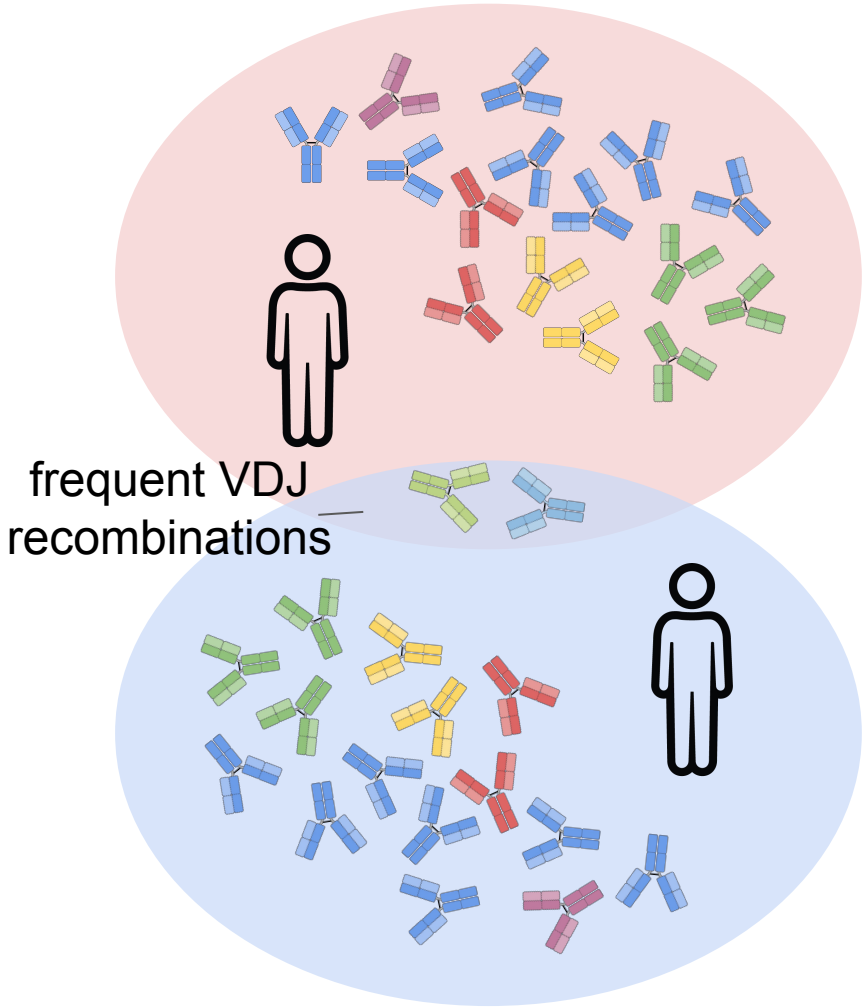
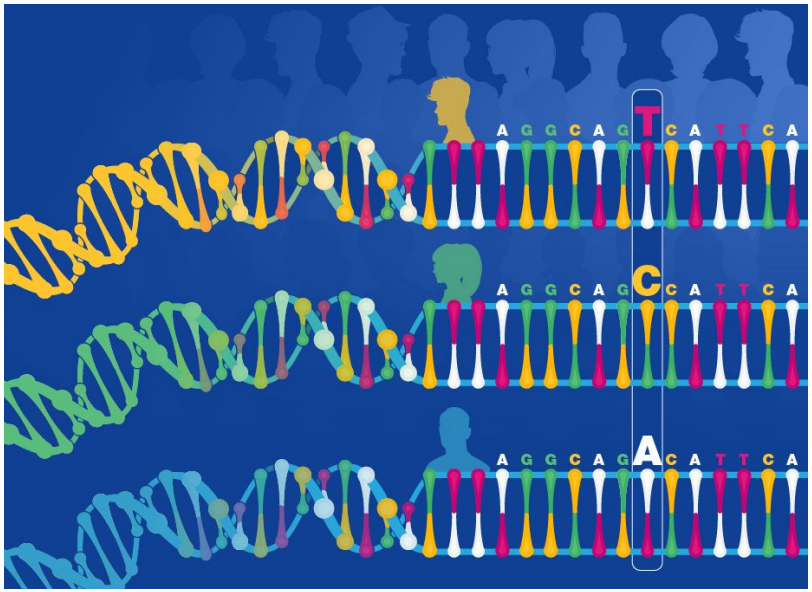
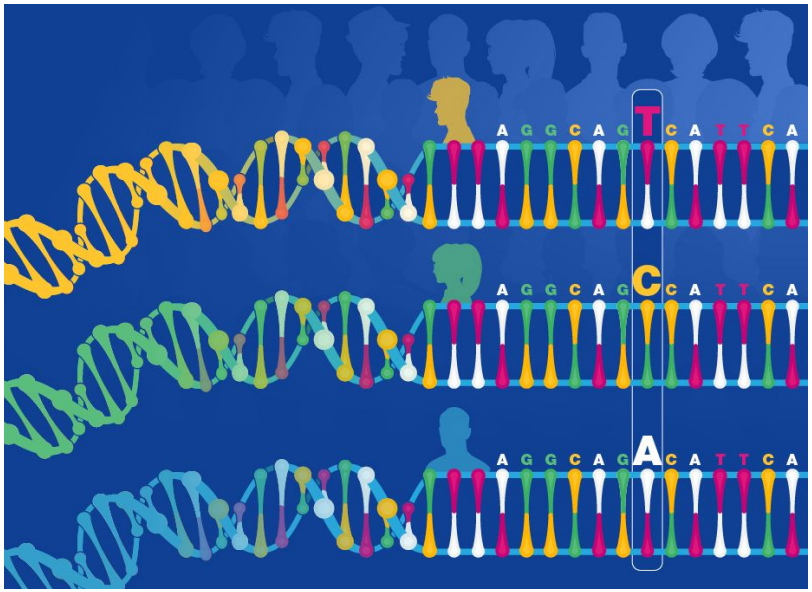


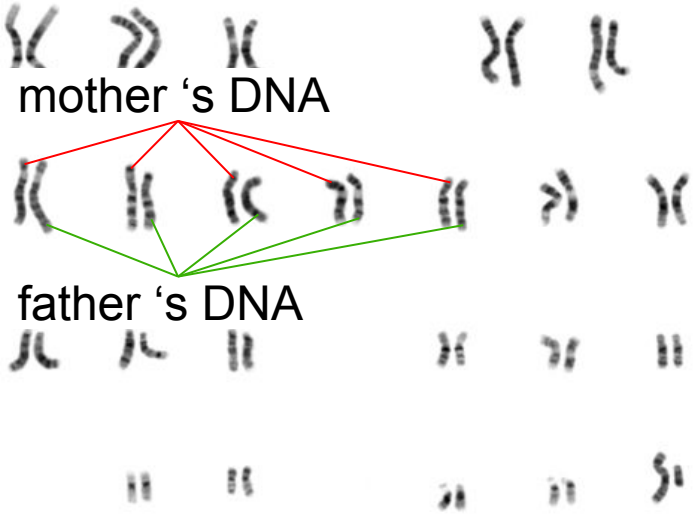
- VDJ sequences are extremely diverse
- If a VDJ sequence is shared between two individuals, it is a likely a frequent recombination rather a functionally important sequence
- **We cannot study antibody responses just comparing VDJ sequences**

Genomic variations

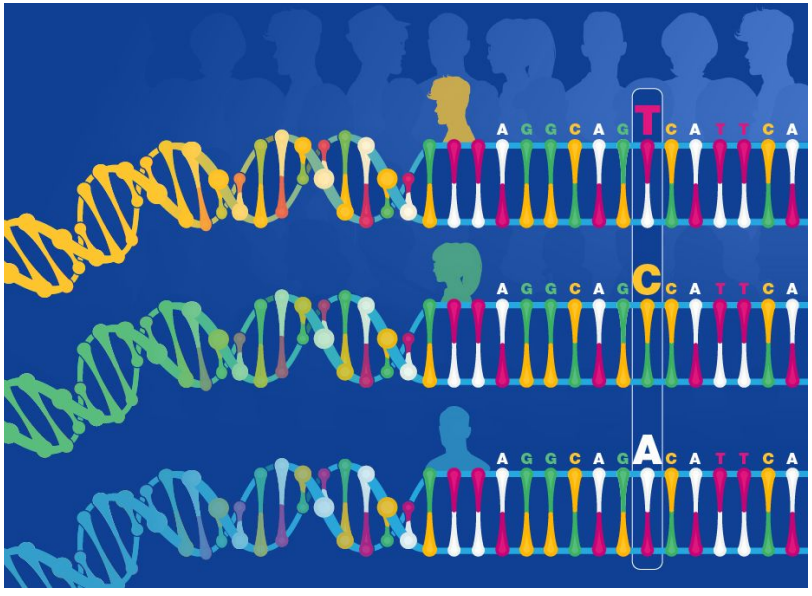


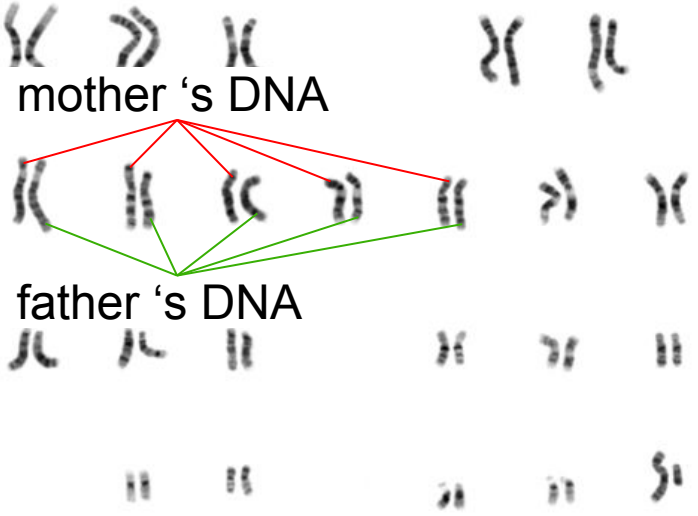
Genomic variations





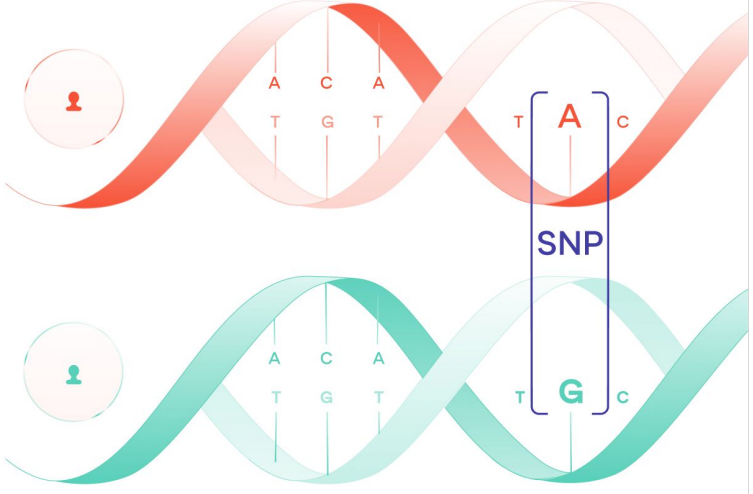
Genomic variations



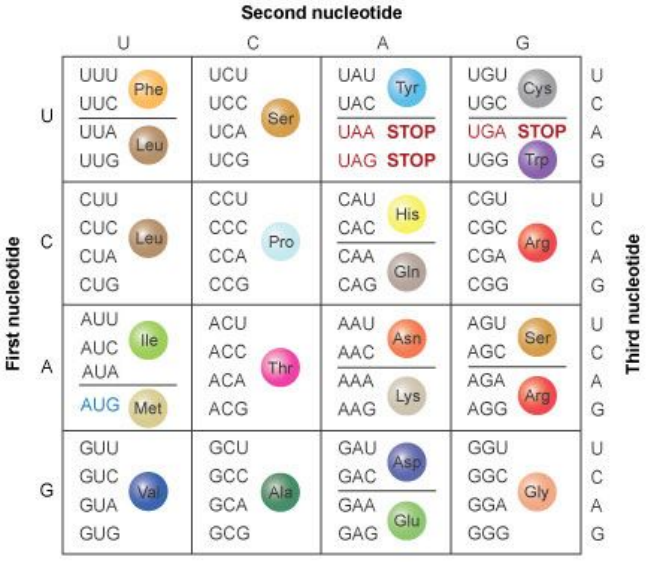


Single Nucleotide Polymorphism (SNP) is associated with a position in the genome:

- A – both chromosomes have A
- A / G – one chromosome has A, another one has G
- G – both chromosomes have G



Altered genes produce altered proteins



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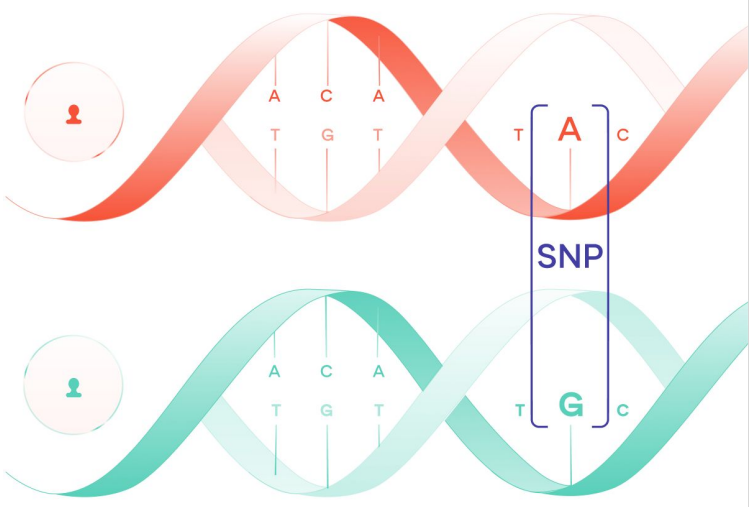

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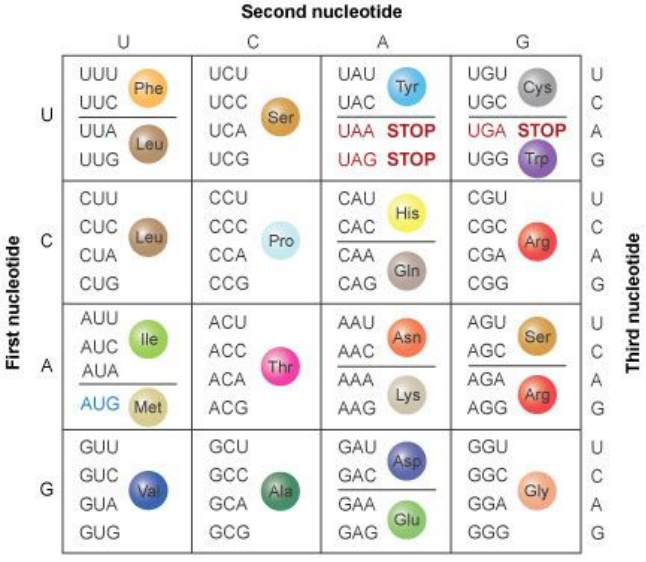

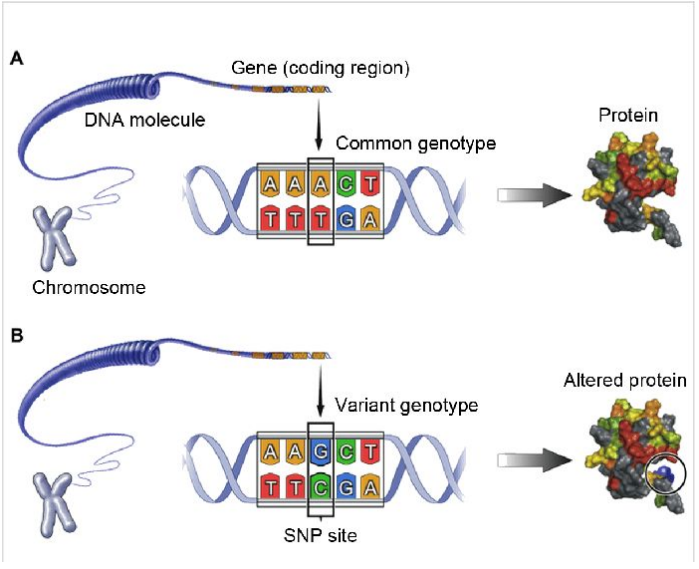

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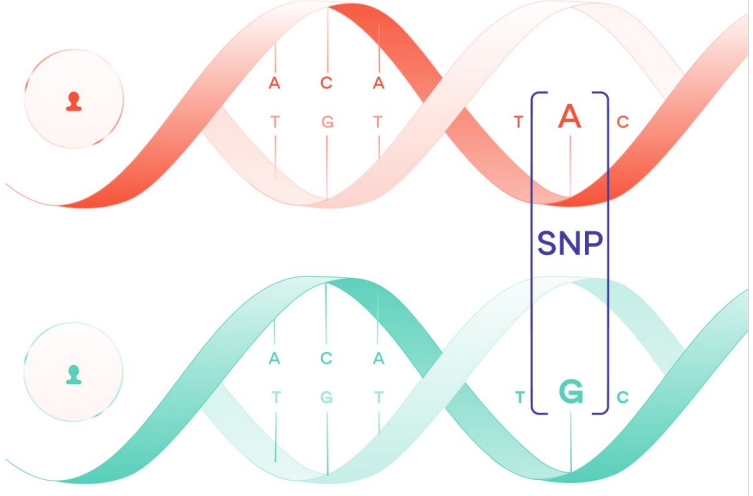
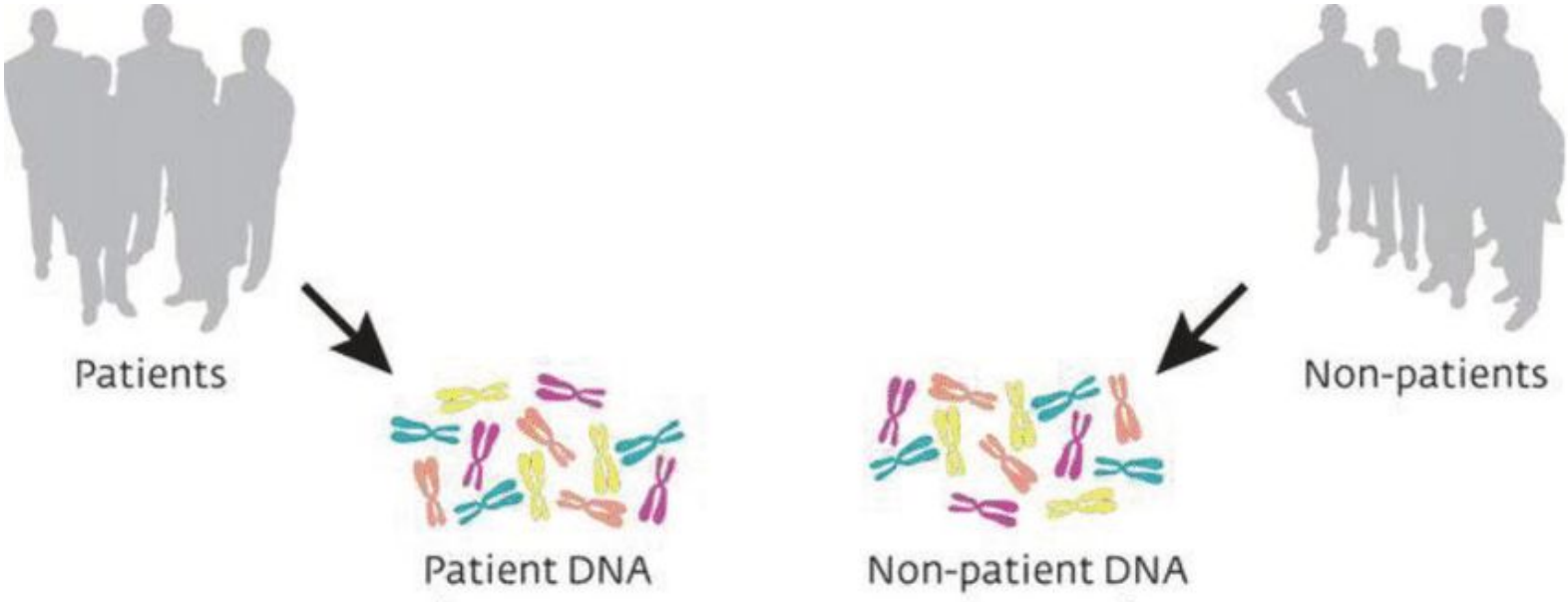


Genome-wide association studies (GWAS)

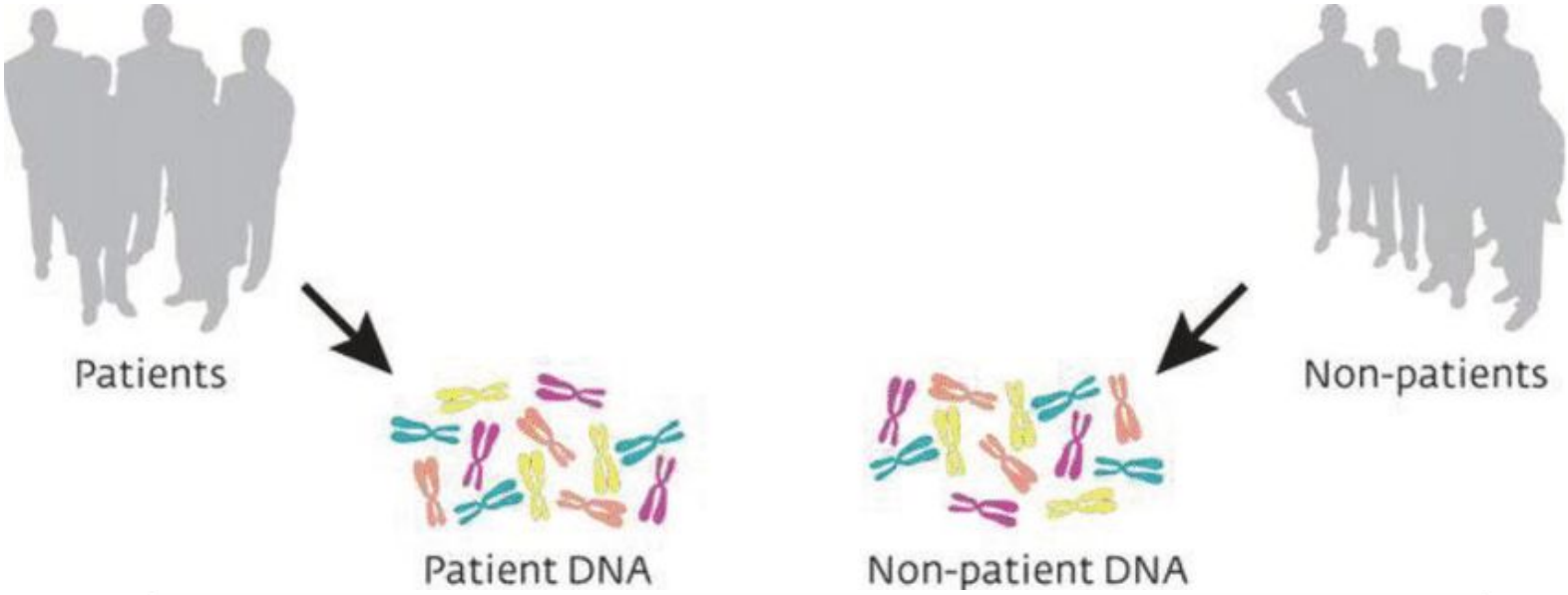


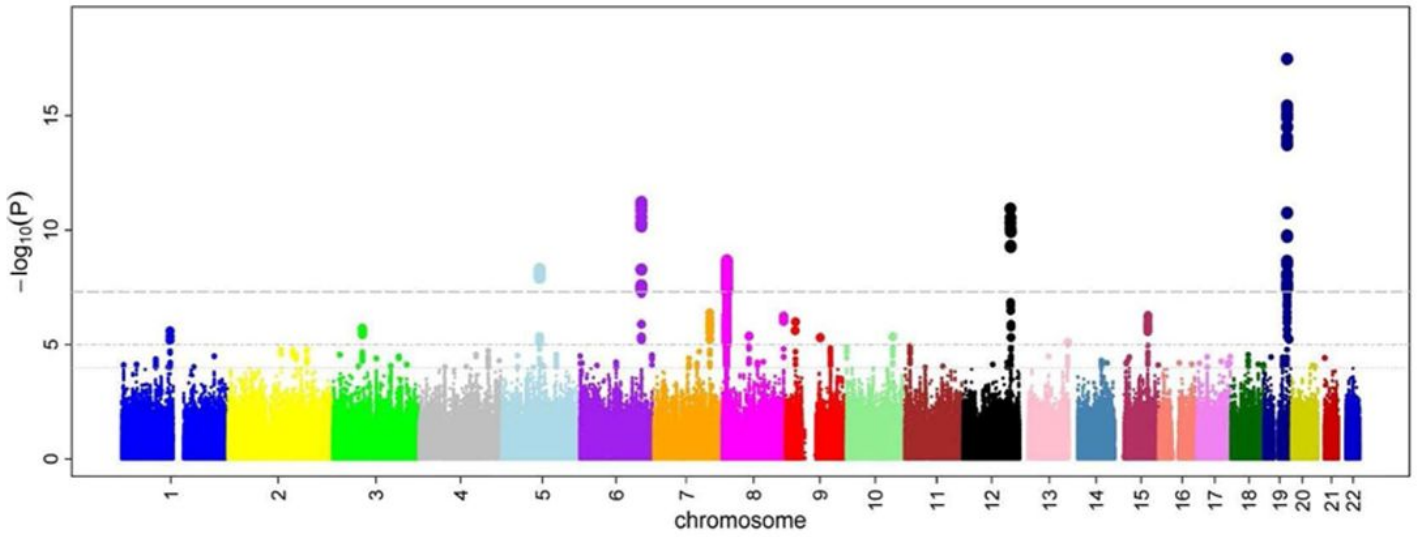
	A	A/G	G
Patients	15	10	8
Controls	2	6	25

a single SNP

P-value (Fisher exact probability test) = 0.000026

Genome-wide association studies (GWAS)





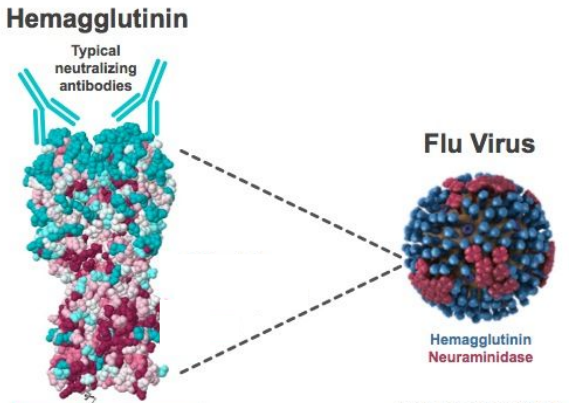
Variants of IGHV1-69 shape Ab response to flu

V	D	J

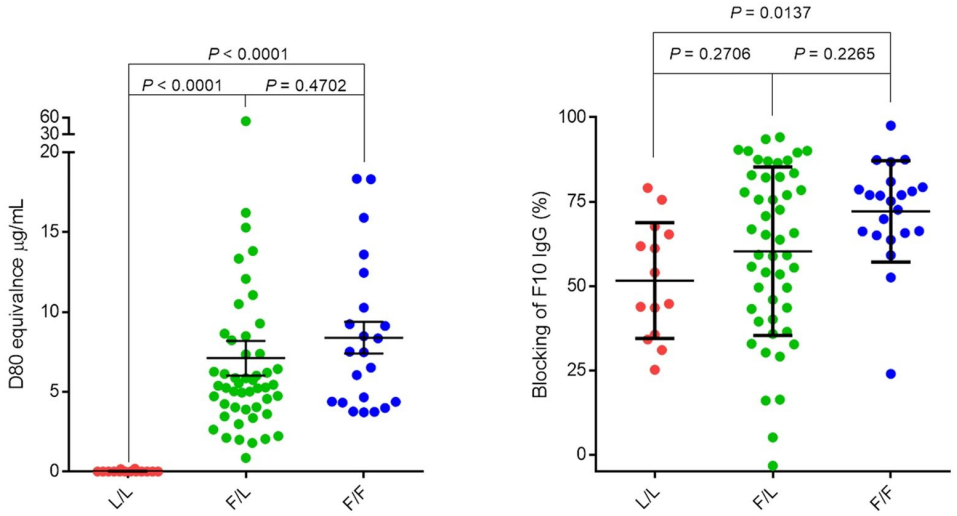
IGHV1-69

IGHV1-69*01	54: F
IGHV1-69*02	54: L
IGHV1-69*03	54: F
IGHV1-69*04	54: L
...	
IGHV1-69*14	54: F

Successful binding to hemagglutinin
Loss of binding properties

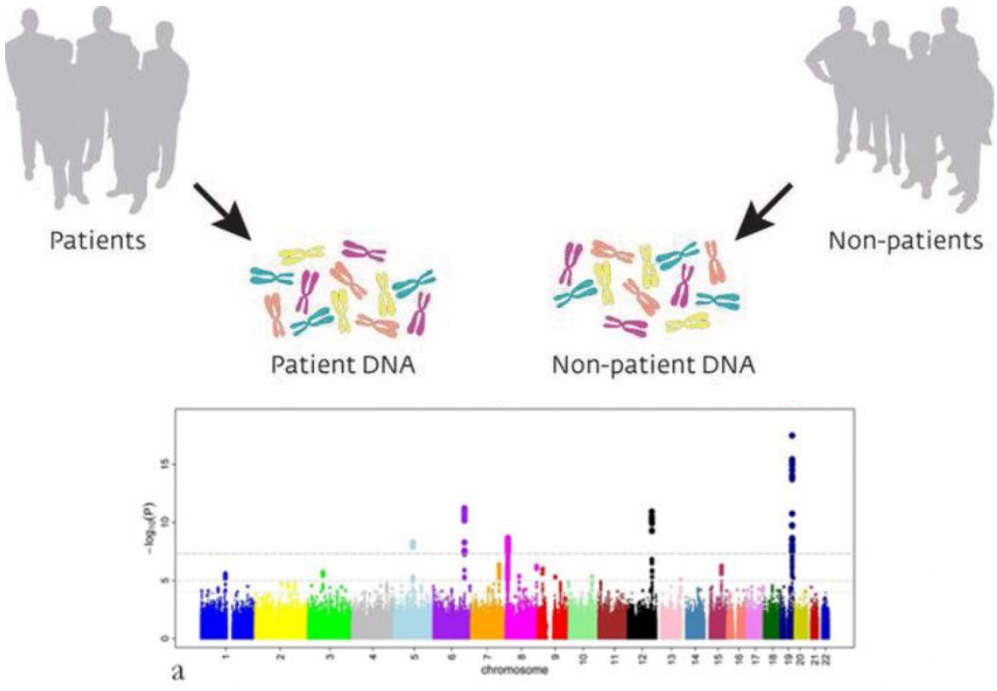


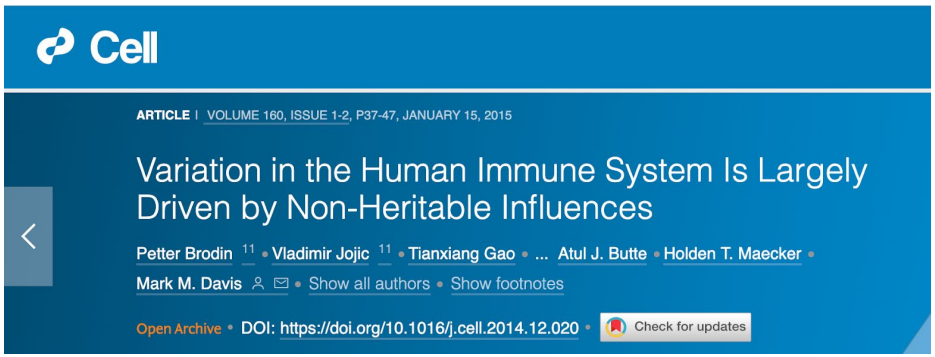
Titers (= antibody counts) before and after immunization



Avnir et al., *Sci Rep*, 2016

GWAS of antibody responses

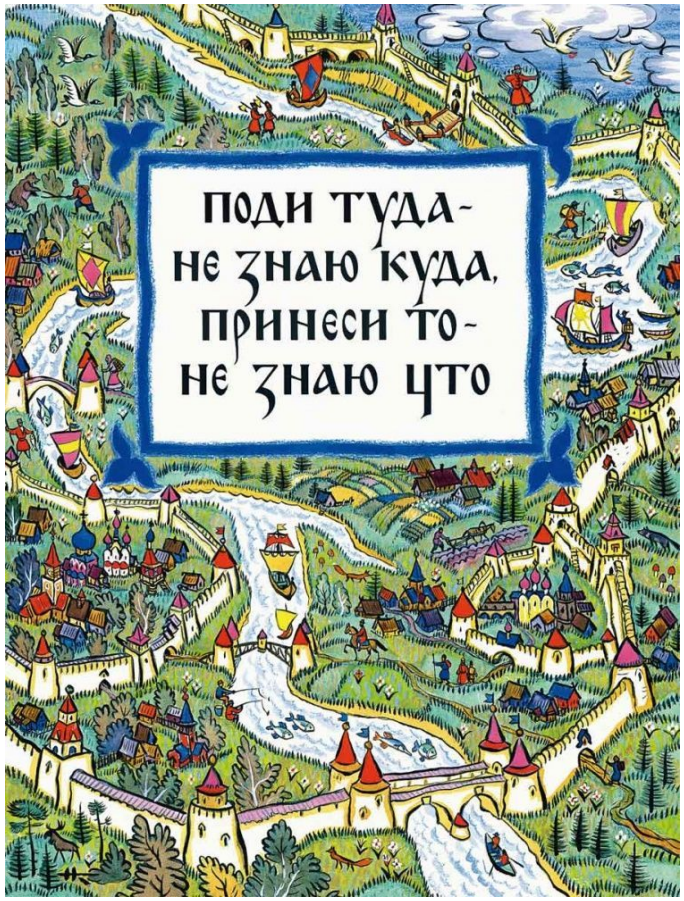




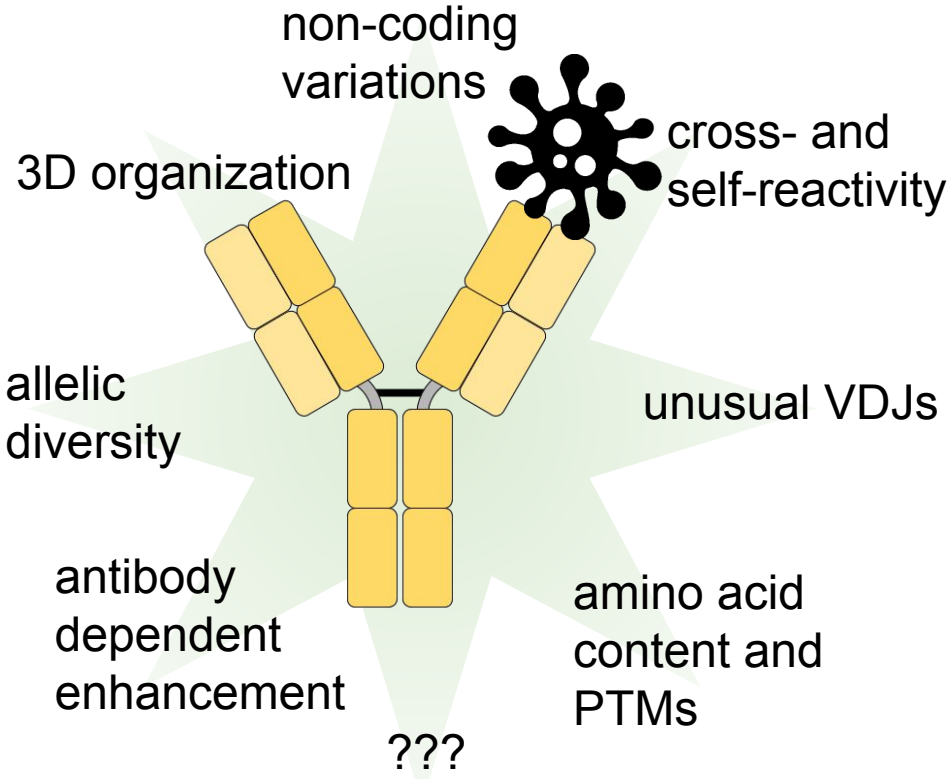
Challenges

- A single IG locus includes many V, D, and J genes. **If a single gene loses functionality, in most cases others can replace it**
- Recent studies report a lack of associations between genomic variations outside IG loci and adaptive immune responses
- Many other factors (age, diet, environment) influence adaptive immune responses
- **Features of antibody repertoires go far beyond genomic variations**

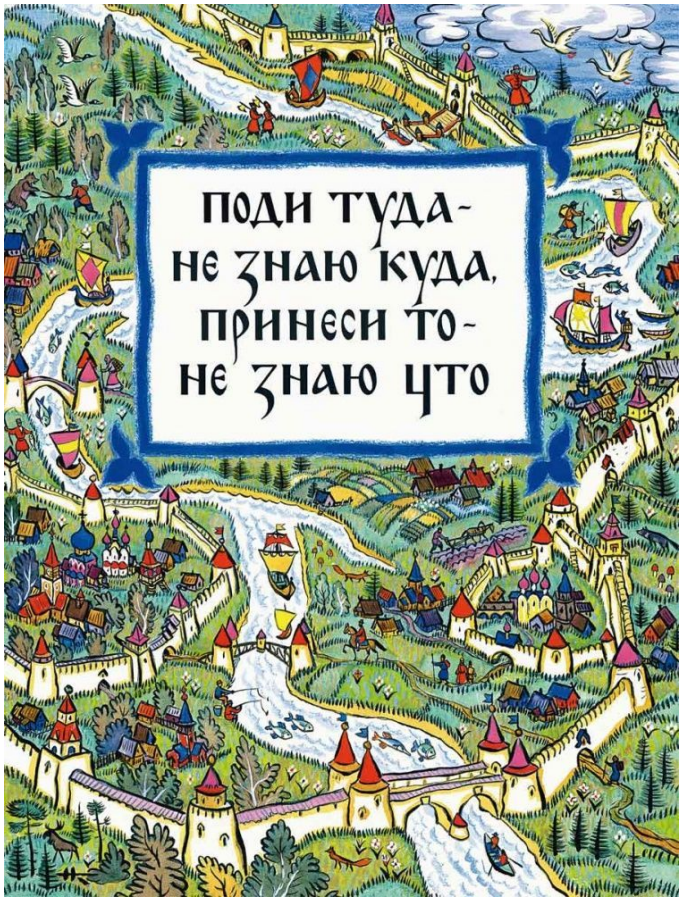
Bioinformatics + immunology = immunoinformatics



Go I Know Not Whither and
Fetch I Know Not What



Bioinformatics + immunology = immunoinformatics



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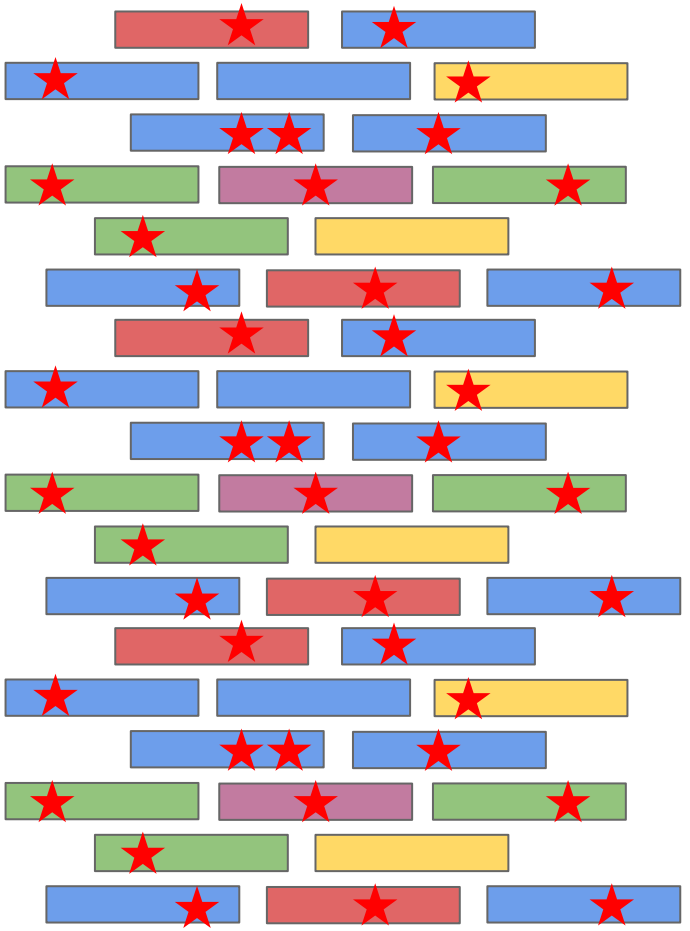
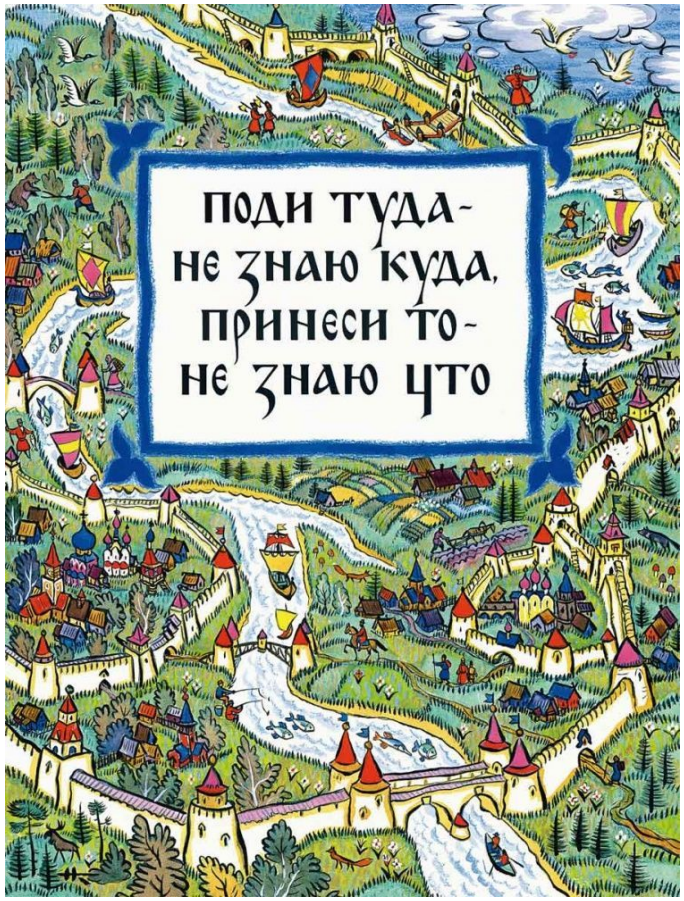


Error-prone immunosequencing reads

Bioinformatics + immunology = immunoinformatics



Go I Know Not Whither and
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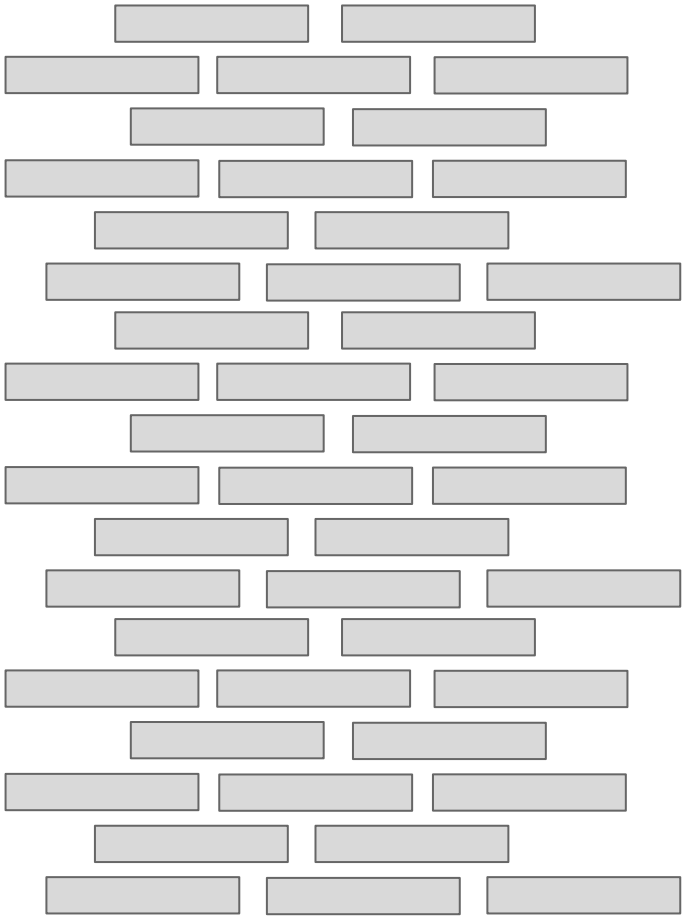

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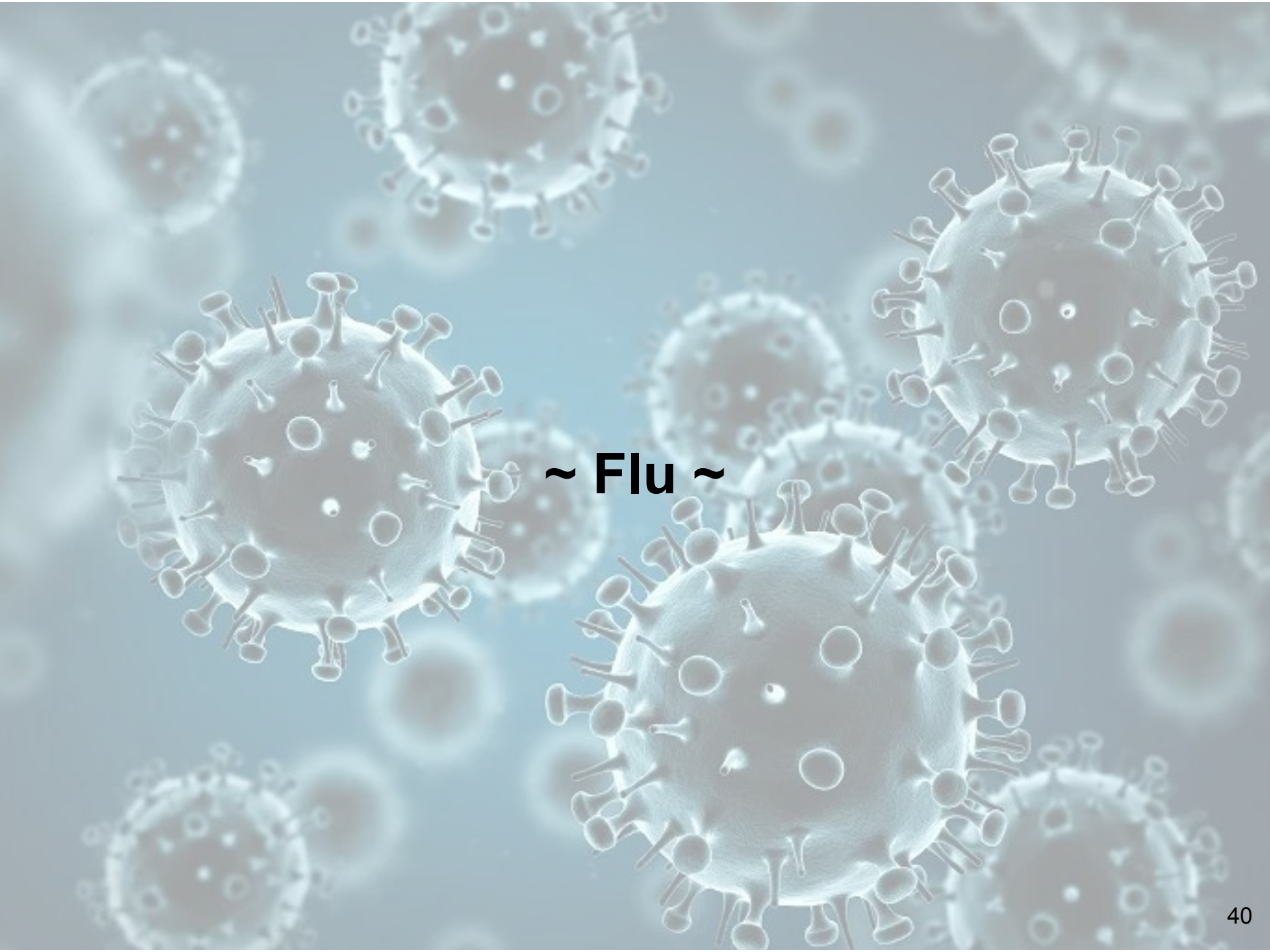
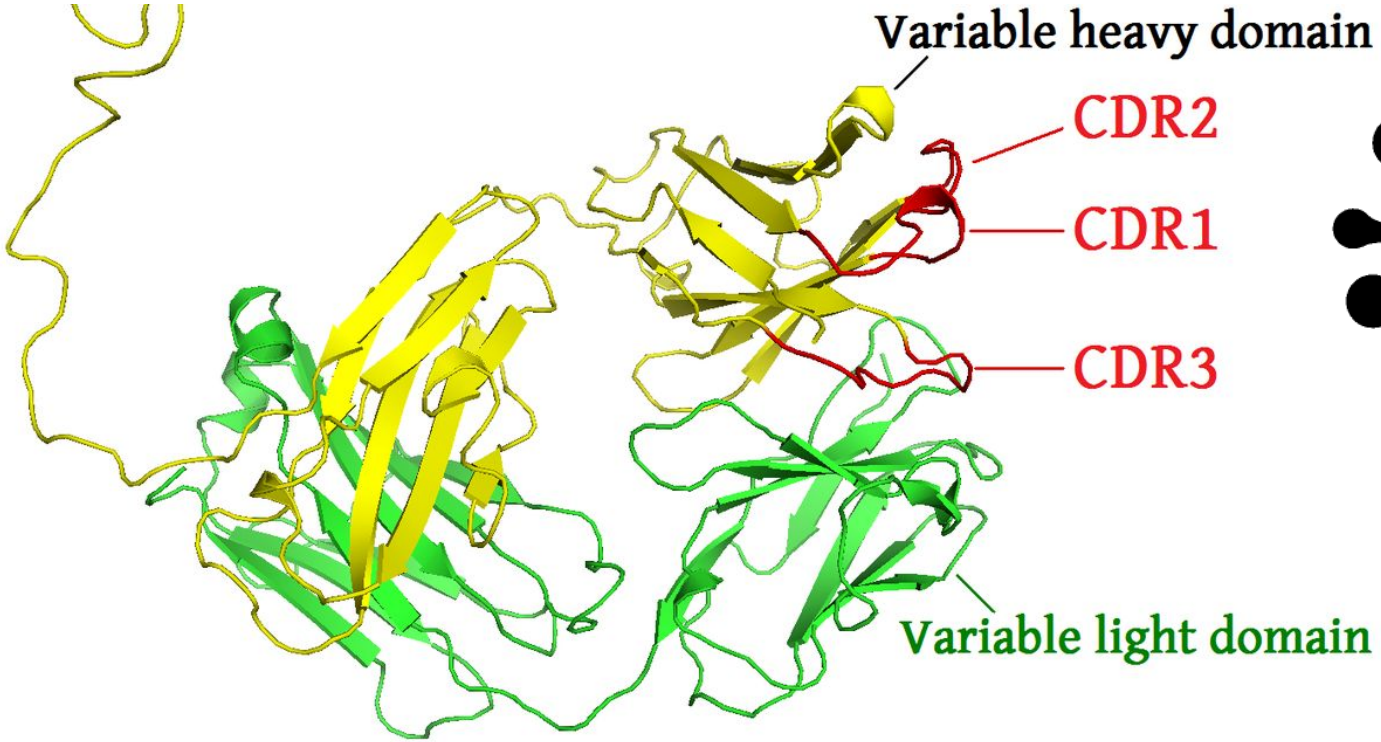
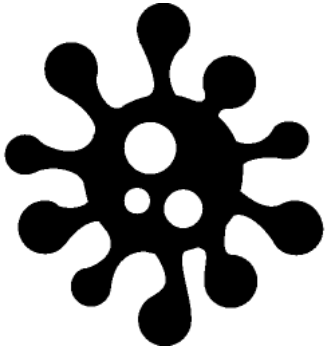
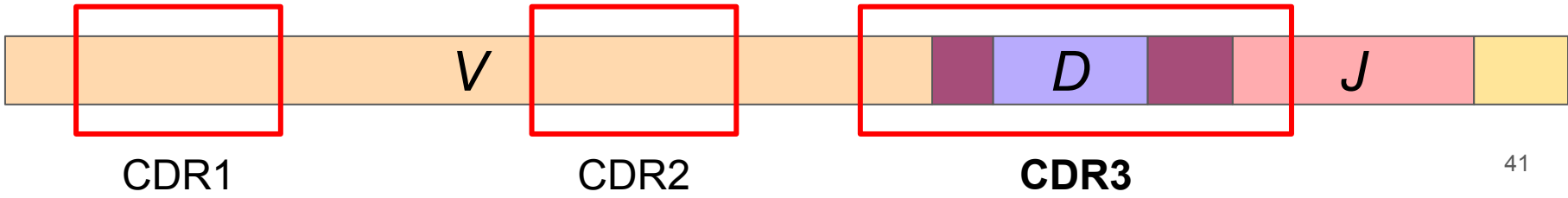

~ Flu ~

CDRs represent antigen-binding sites

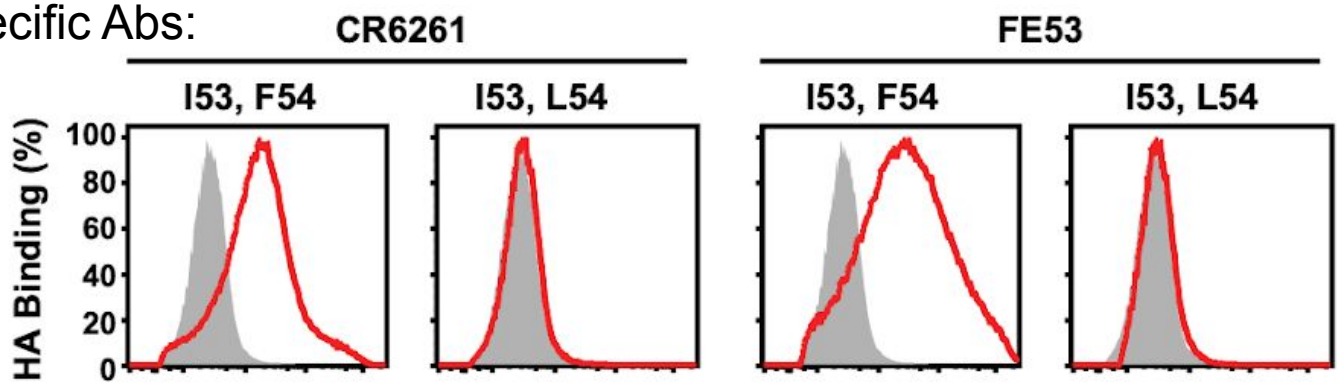






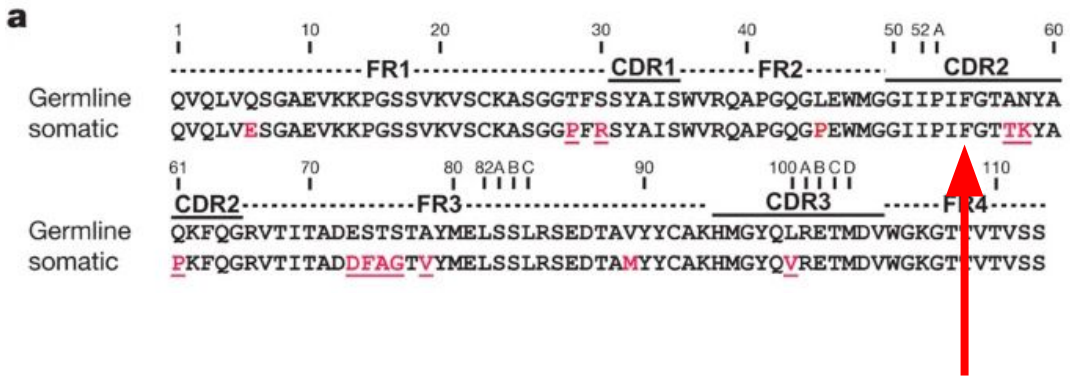
Anatomy of IGHV1-69-guided response to flu

Two flu-specific Abs:



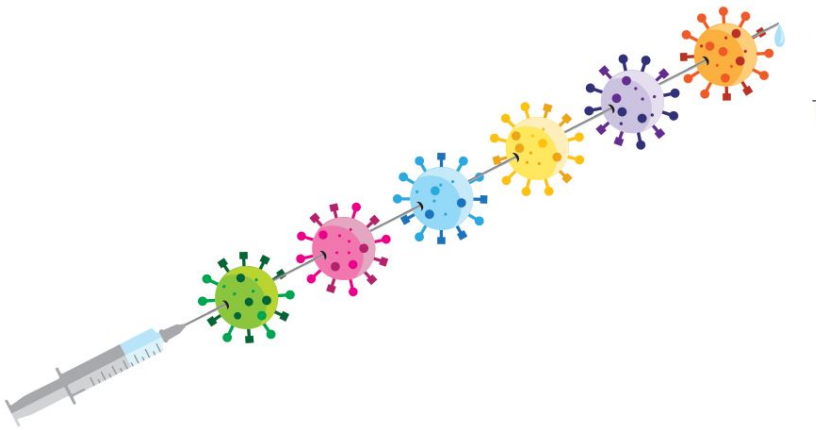
Lindwood et al., Nature, 2012

IGHV1-69*01	54: F
IGHV1-69*02	54: L
IGHV1-69*03	54: F
IGHV1-69*04	54: L
...	
IGHV1-69*14	54: F



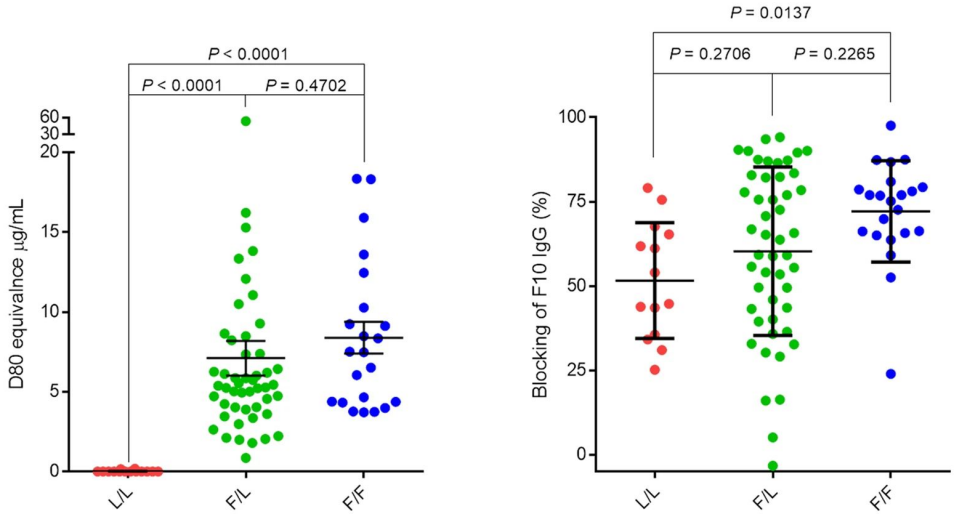
Antibody titers vs IGHV1-69 genotype

The titer data suggests that genotype of IGHV1-69 shapes the response to flu and other V genes do not fully replace the “bad” variant of IGHV1-69



~20% of people have L/L variants of IGHV1-69

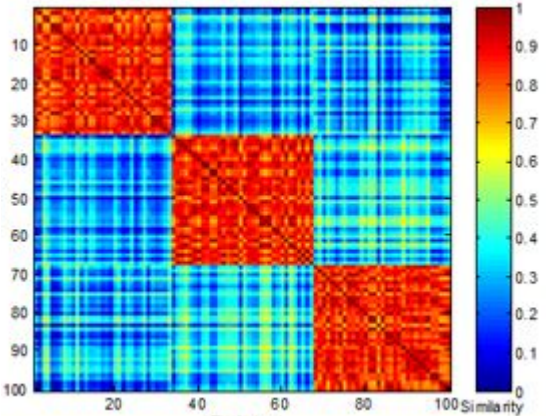
Titers (= antibody counts) before and after immunization

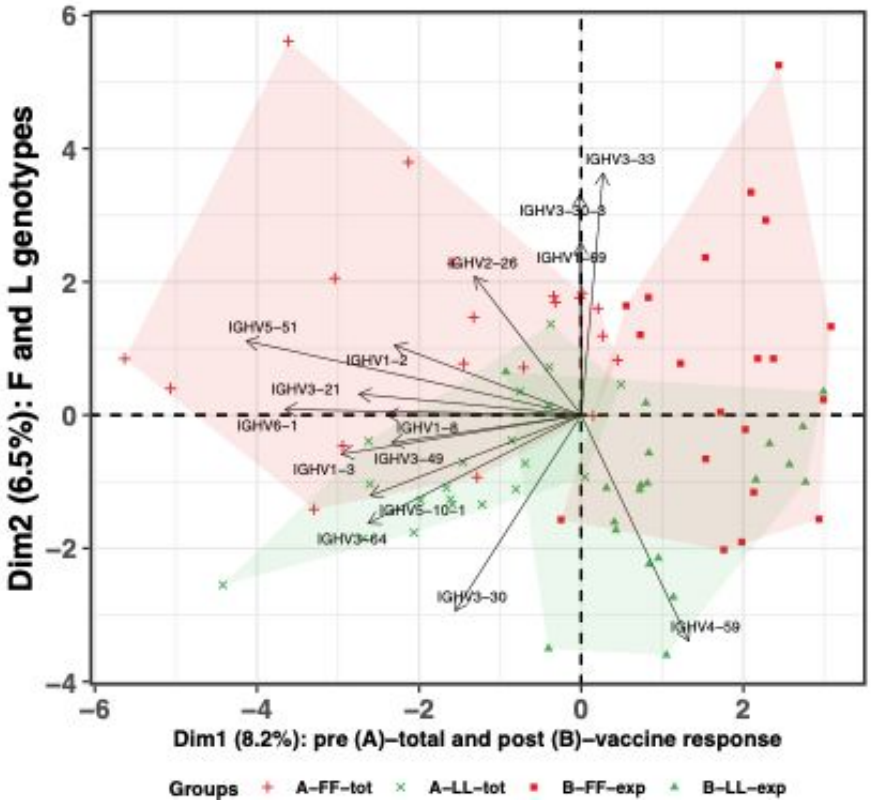


Avnir et al., *Sci Rep*, 2016

Bioinformatics analysis of flu response

- Usage of gene *G* = the fraction of VDJ sequences derived from *G*
- Individual antibody repertoire can be described as a *usage vector* for all existing genes
- We can compare usage vectors for F/F, F/L, and L/L individuals:





Ke, Nouri, Safonova, et al., in preparation

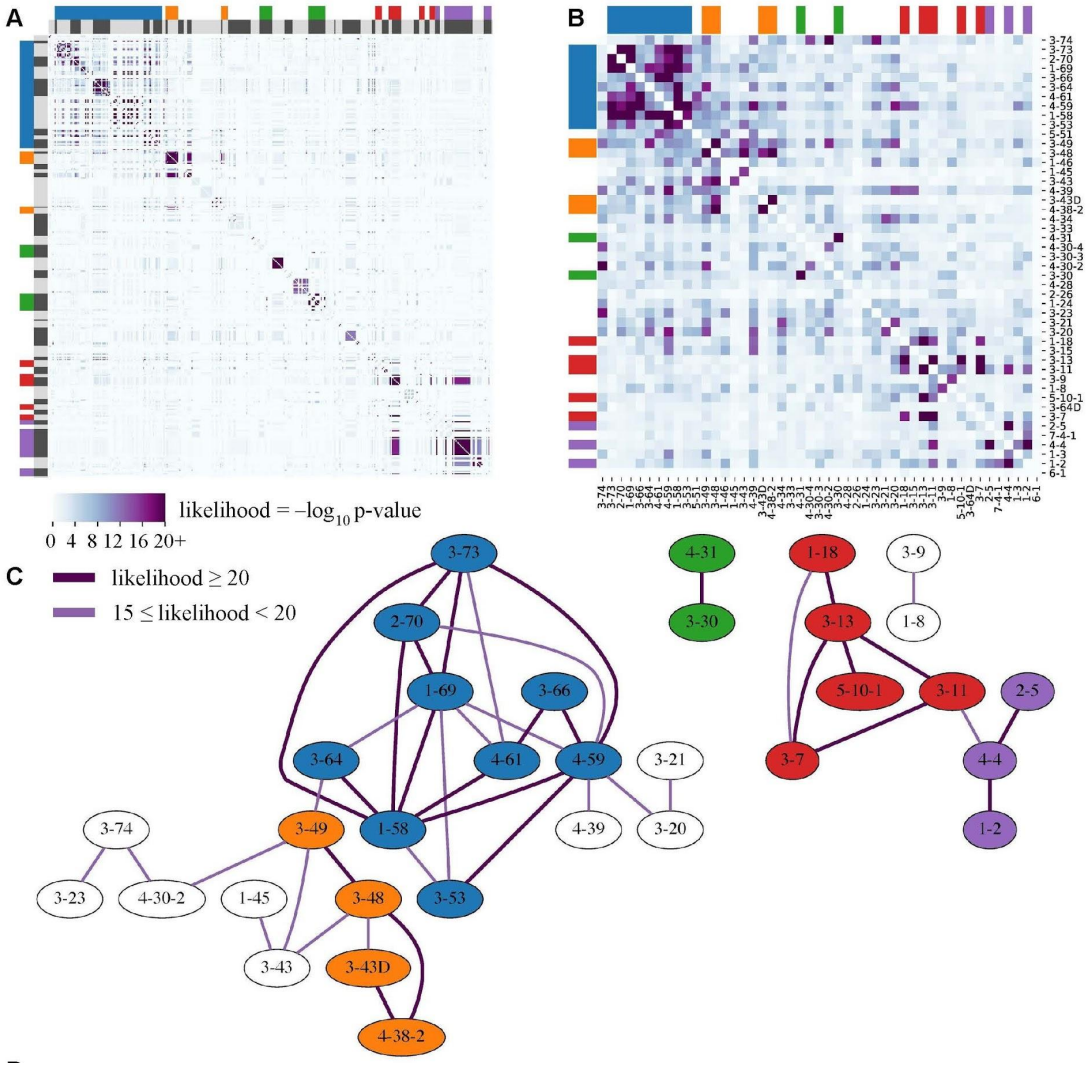
Linked variations of V genes

	A	A/G	G
C	15	10	8
A/C	6	8	3
A	2	5	17

P-value = 0.00967

Blue group is a set of V genes associated with flu response

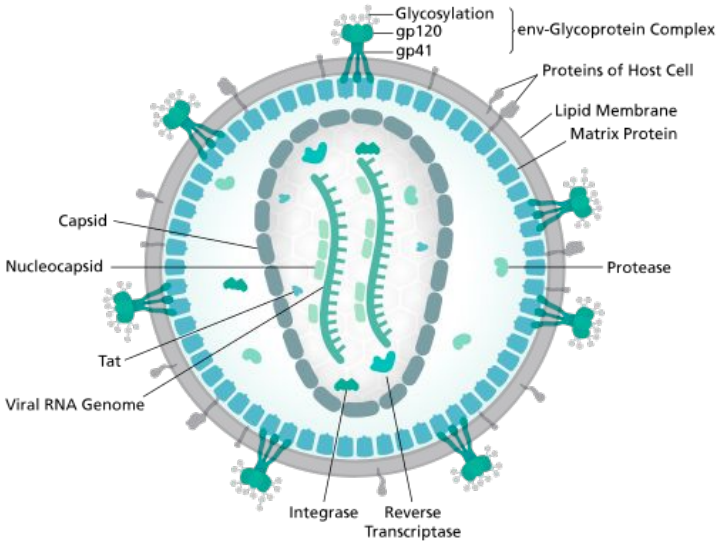
These genes have linked variations and perhaps close specificities

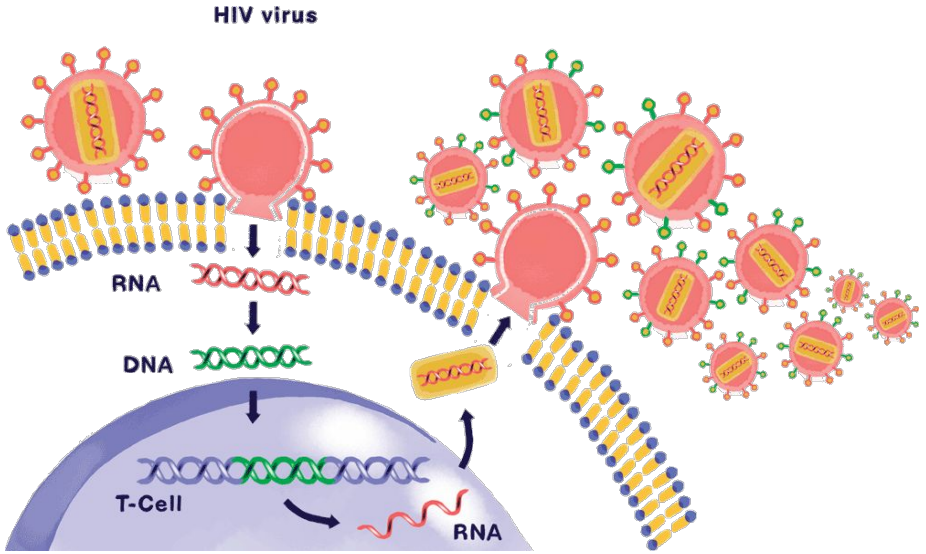


Safonova and Watson, in preparation


~ HIV ~

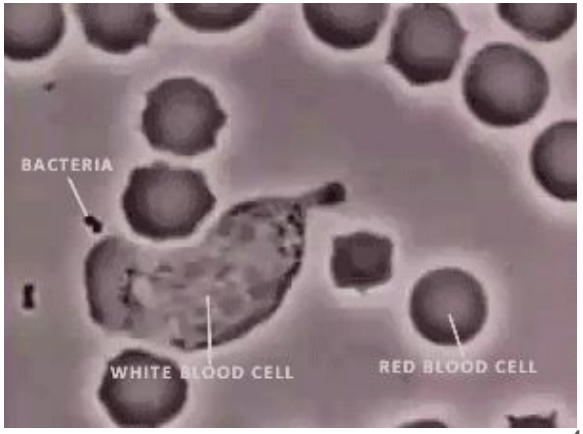
HIV and antibody response



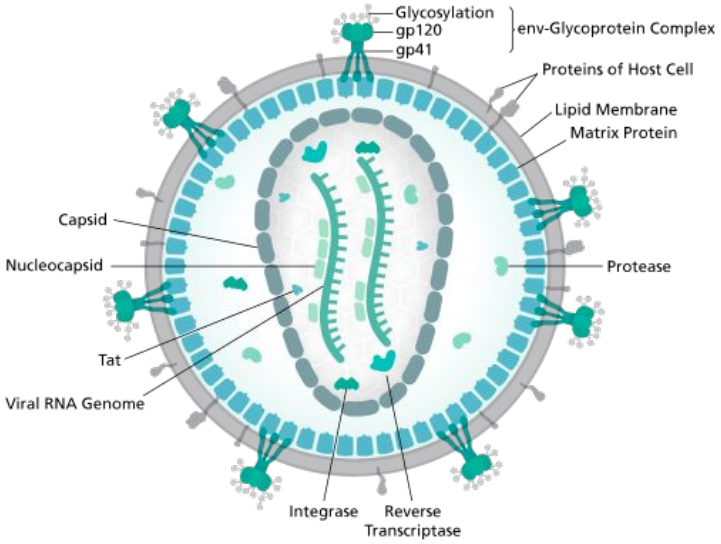


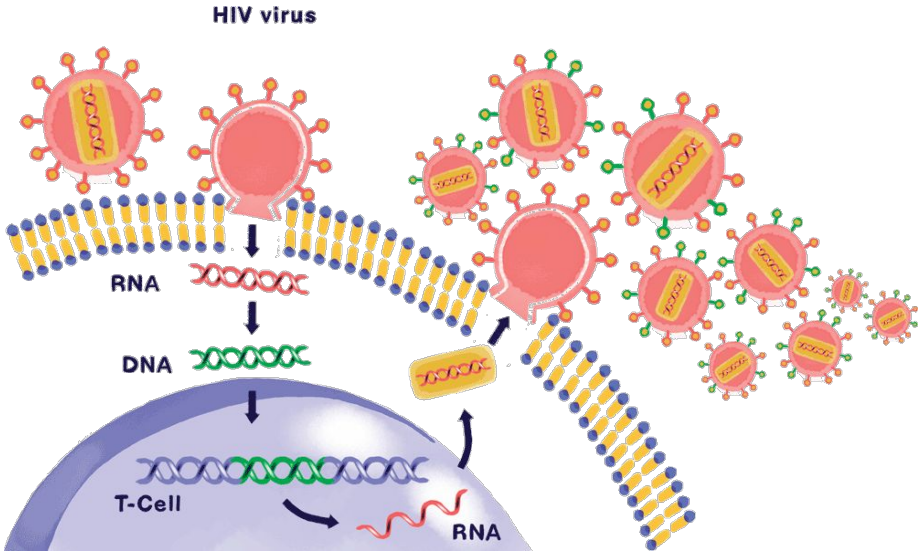
HIV infects immune cells:

- T cells
- Macrophages
- Dendritic cells



HIV and antibody response



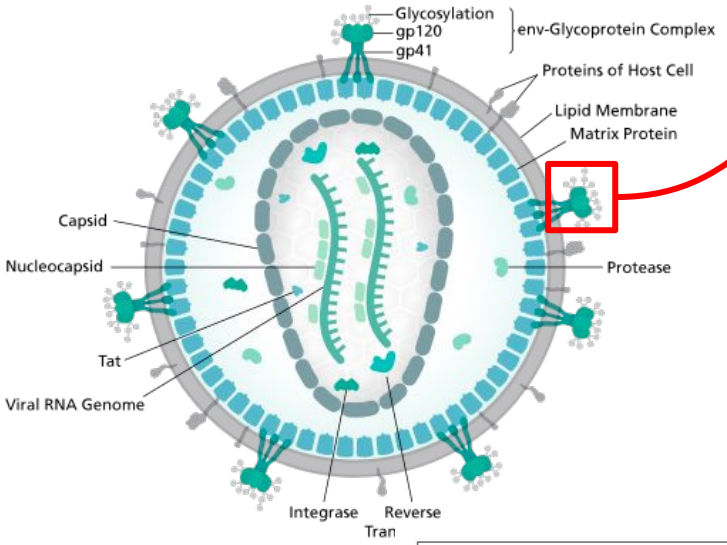


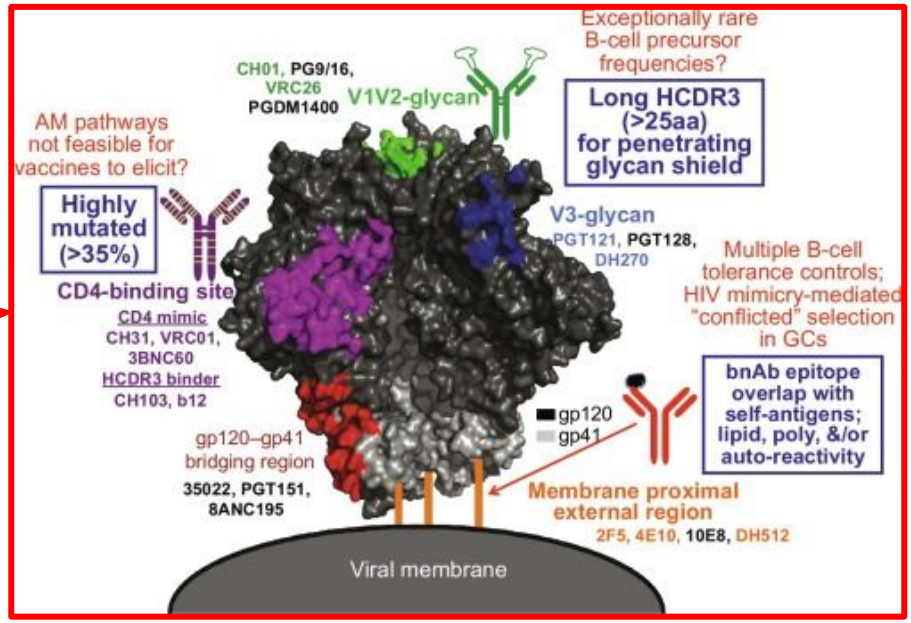
HIV infects immune cells:

- T cells
- Macrophages
- Dendritic cells

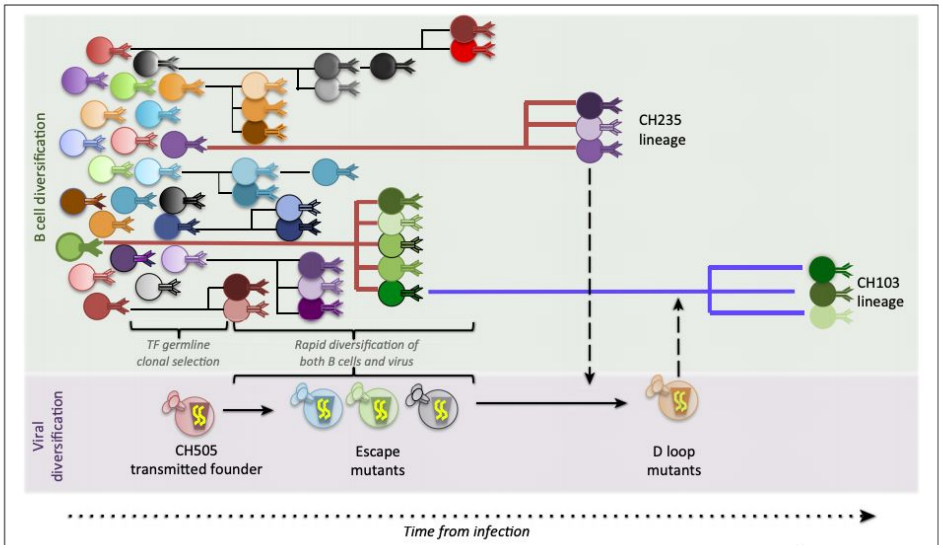
B cells producing antibodies are not affected by HIV but cannot fight it because of a high mutation rate of the HIV genome

Recognition of HIV



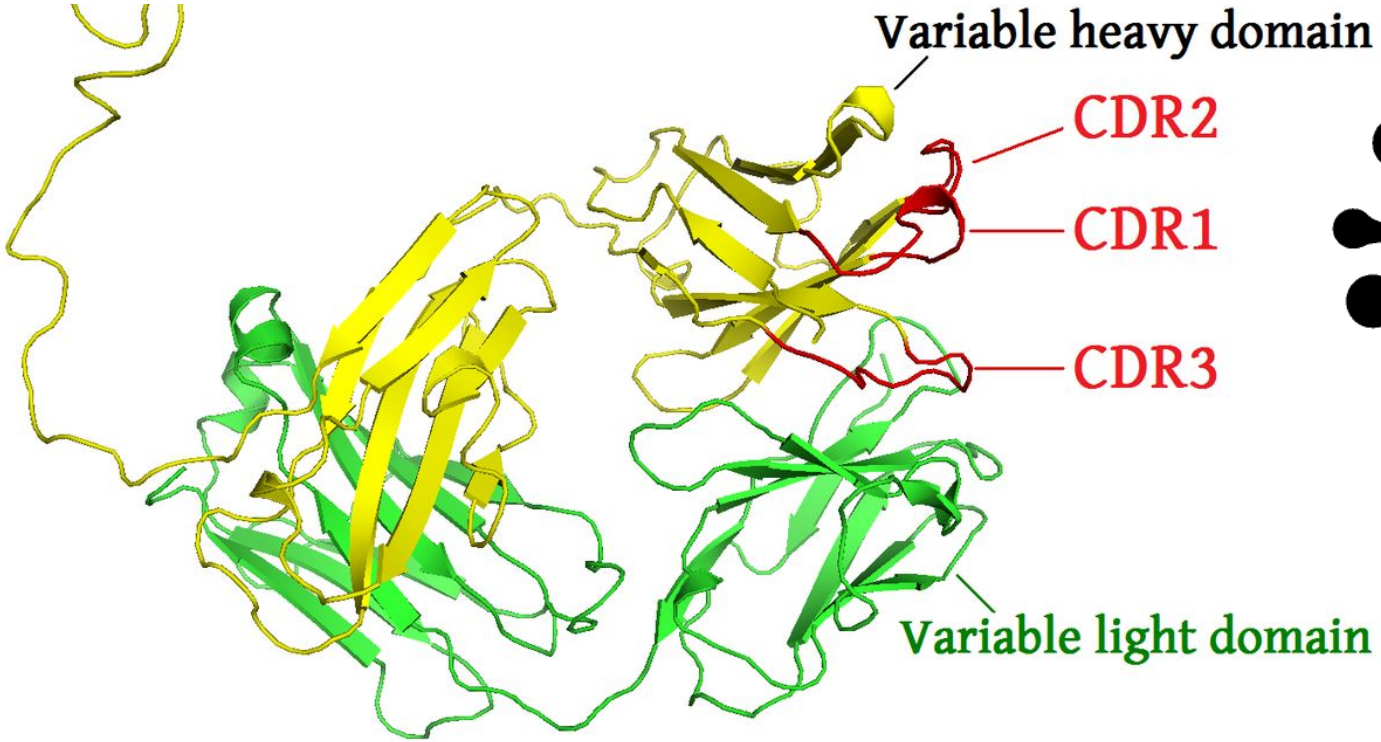


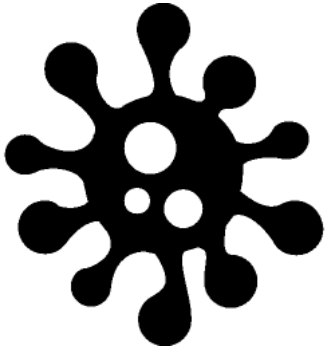
Verkoczy, Adv Immunol, 2017

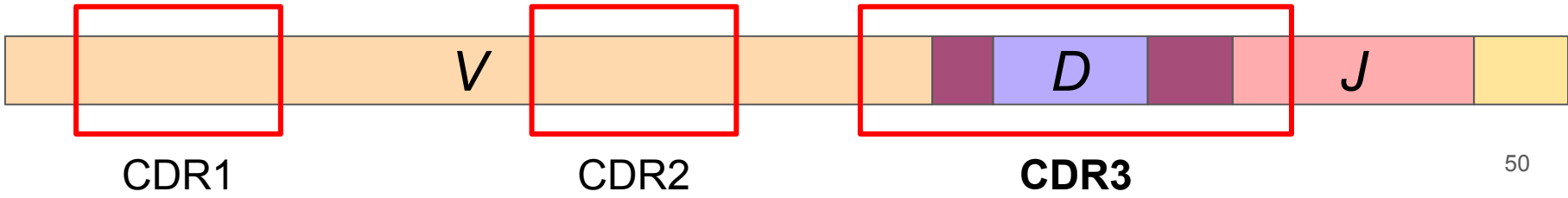


Alter & Ackerman, *Cell*, 2014

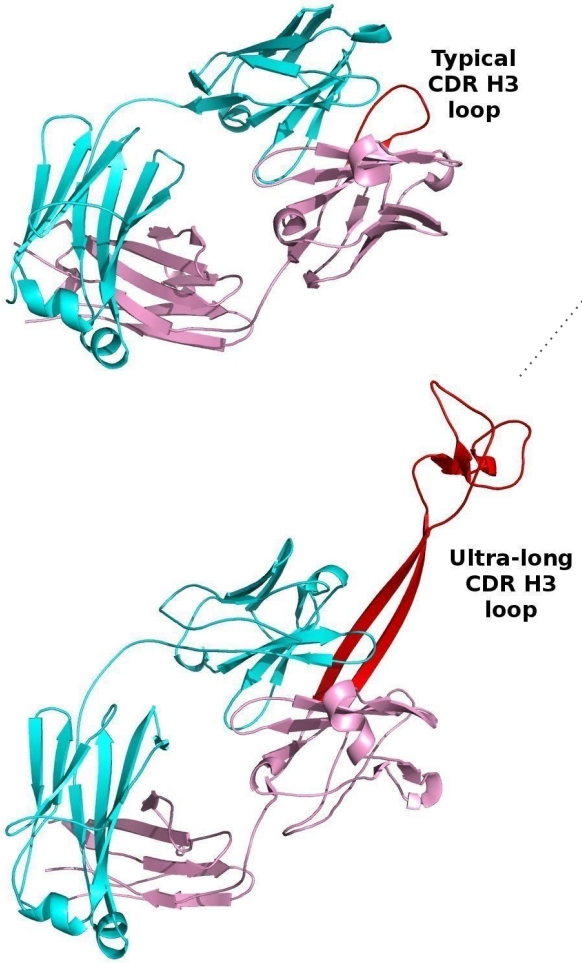
CDRs represent antigen-binding sites







Long CDR3s in response to HIV



Broadly neutralizing antibodies against HIV are characterized by extremely long CDR3s

Ultralong CDR3s in human antibodies present a result of **VDDJ** recombination:

```
D3:                                          GTATTACGATTTTTGGAGTGGTTATtatacc
D5:                                                                  GTGGATACAGCTATGGttac
CDR3:   ACCACAGAACCGCTTCAGTTTAGTCCGTATTACGATTTTTGGAGTGGTTATCAGCCAGTGGATACAGCTATGGACCCGTTGACT
span:                                        GTATTACGATTTTTGGAGTGGTTATCAGCCAGTGGATACAGCTATGG
inter-D insertion:                                                   CAGCCA
```

Sok et al., *Nature*, 2017

Safonova and Pevzner, *Front Immunol*, 2019

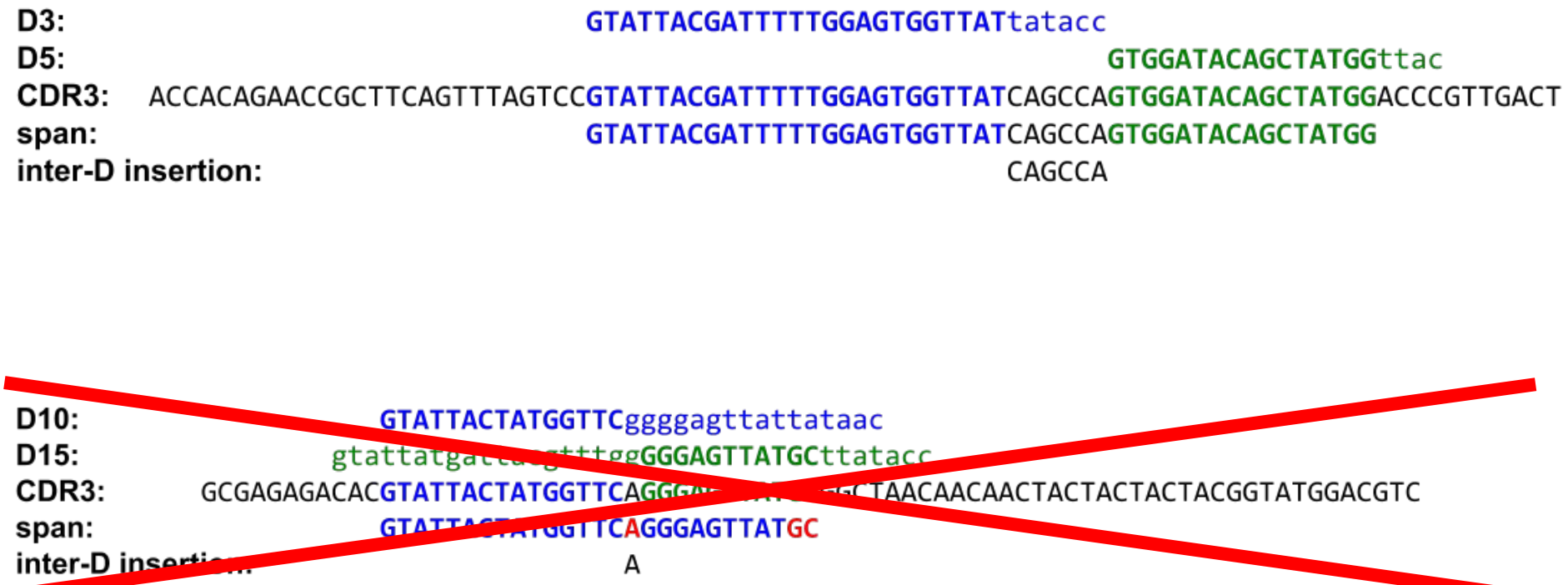
Finding VDDJ recombinations

D3: GTATTACGATTTTTGGAGTGGTTATtatacc
D5: GTGGATACAGCTATGGttac
CDR3: ACCACAGAACCGCTTCAGTTTAGTCCGTATTACGATTTTTGGAGTGGTTATCAGCCAGTGGATACAGCTATGGACCCGTTGACT
span: GTATTACGATTTTTGGAGTGGTTATCAGCCAGTGGATACAGCTATGG
inter-D insertion: CAGCCA

D10: GTATTACTATGGTTCggggagttattataac
D15: gtattatgattacgtttggGGGAGTTATGCttatacc
CDR3: GCGAGAGACACGTATTACTATGGTTCAGGGAGTTATGCGGCTAACAACAACTACTACTACTACGGTATGGACGTC
span: GTATTACTATGGTTCAGGGAGTTATGC
inter-D insertion: A

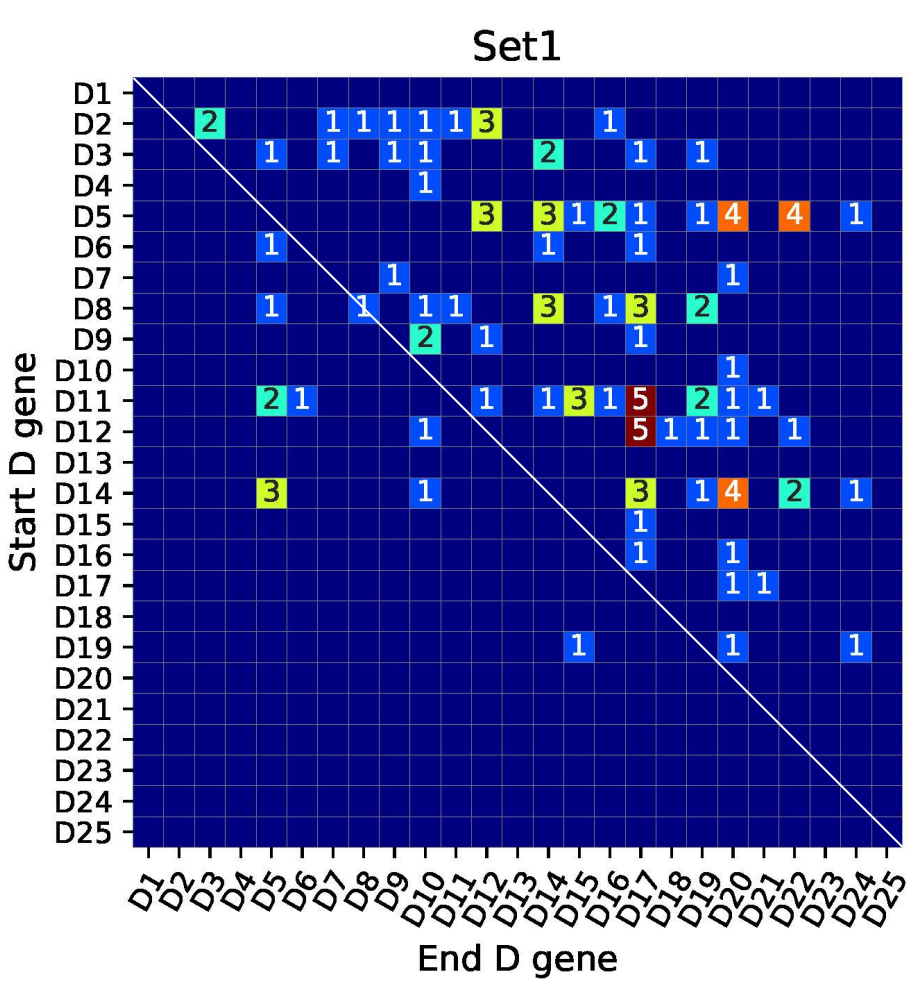
Safonova and Pevzner, *Front Immunol*, 2019

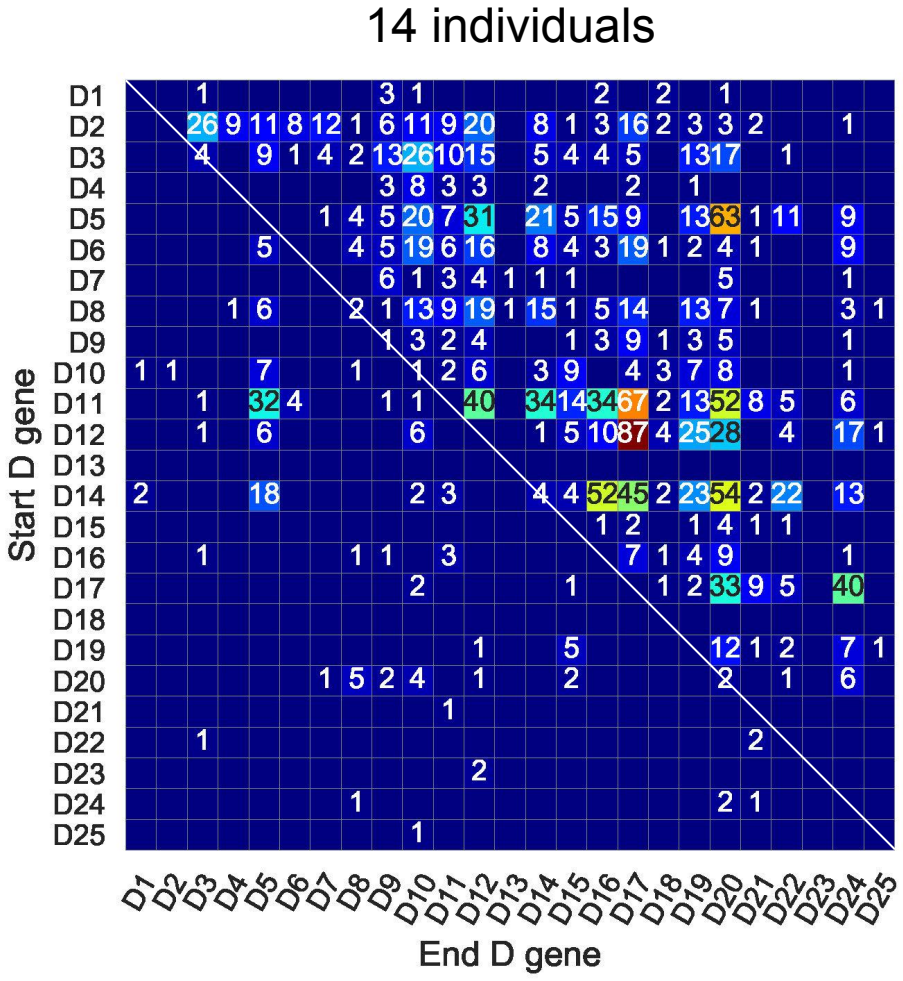
Finding VDDJ recombinations



Safonova and Pevzner, *Front Immunol*, 2019

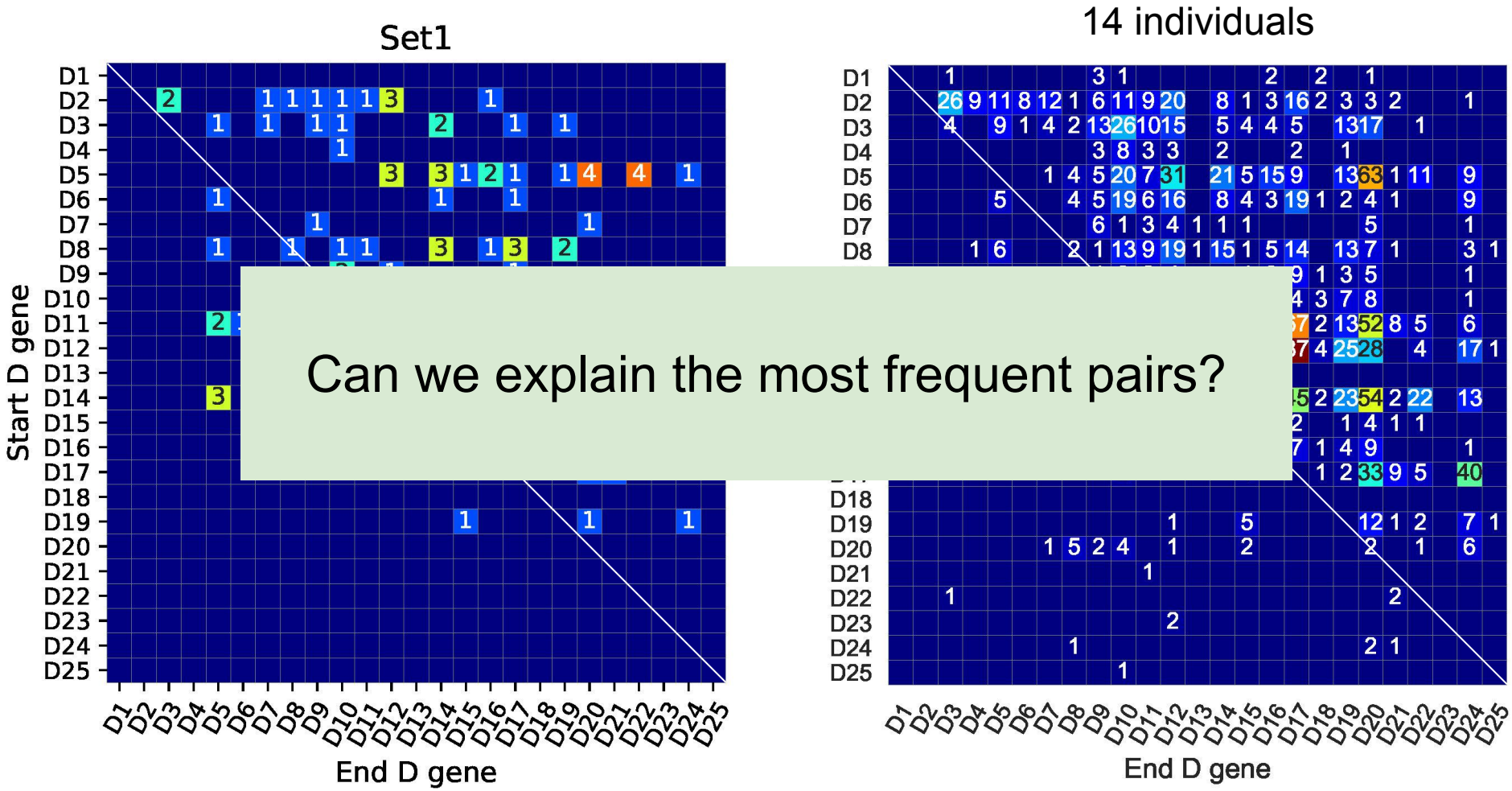
Most D-D pairs follow ordering in IGH locus





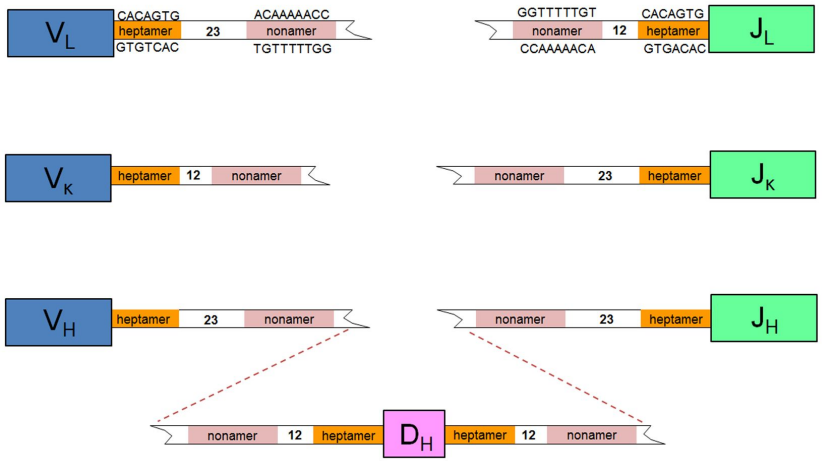
Safonova and Pevzner, *Front Immunol*, 2019

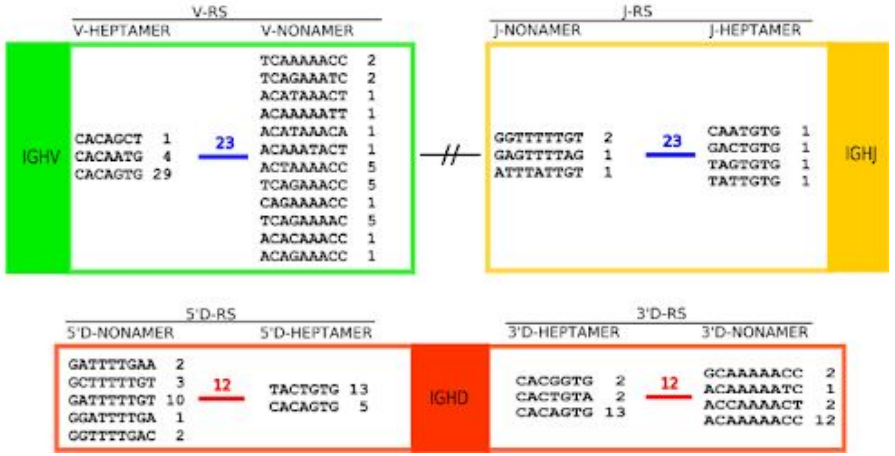
Most D-D pairs follow ordering in IGH locus

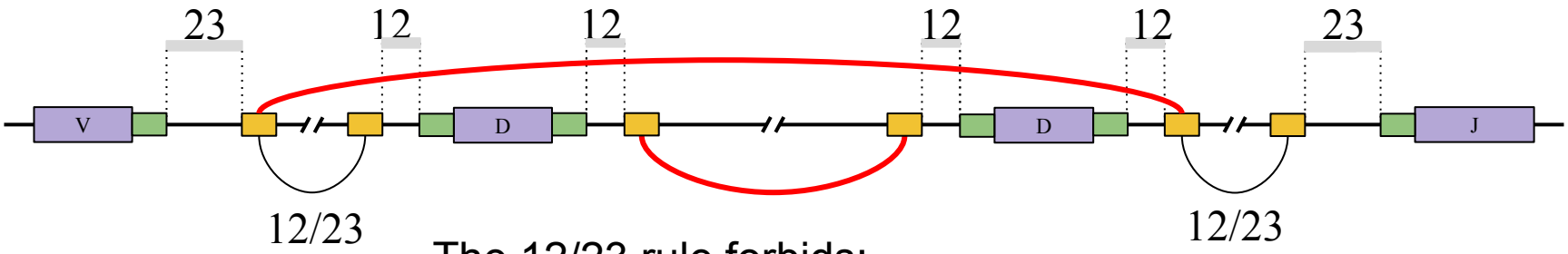


Safonova and Pevzner, *Front Immunol*, 2019

Recombination signal sequences





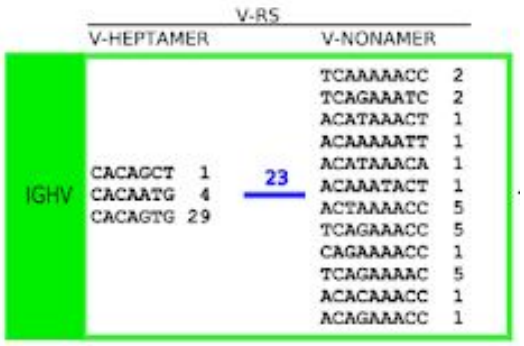


The 12/23 rule forbids:

- V-J pairs
- D-D pairs
- V-V pairs
- J-J pairs

12 nt = 1 turn of DNA
23 nt = 2 turns of DNA

Non-canonical recombination signals

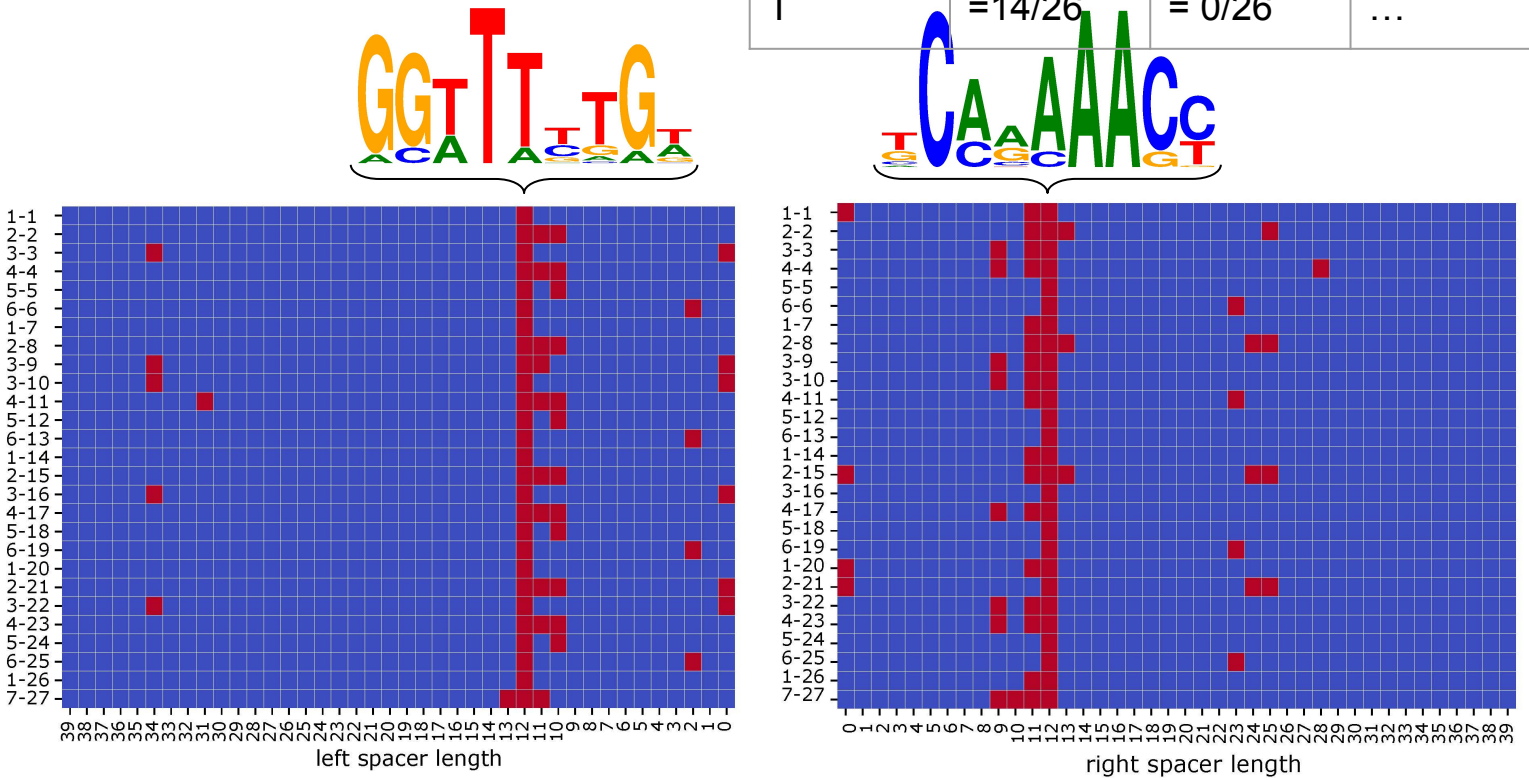


	Pos 1	Pos 2	…
A	= 11/26	= 1/26	…
C	= 1/26	= 25/26	…
G	= 0/26	= 0/26	…
T	=14/26	= 0/26	…

Non-canonical recombination signals

Prob(ACGTACGTA) =
= 11/26 * 25/26 * ...

	Pos 1	Pos 2	…
A	= 11/26	= 1/26	…
C	= 1/26	= 25/26	…
G	= 0/26	= 0/26	…
T	=14/26	= 0/26	…



Safonova and Pevzner, *Genome Res*, 2020

Non-canonical recombination signals

Prob(ACGTACGTA) =
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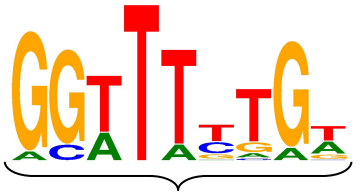
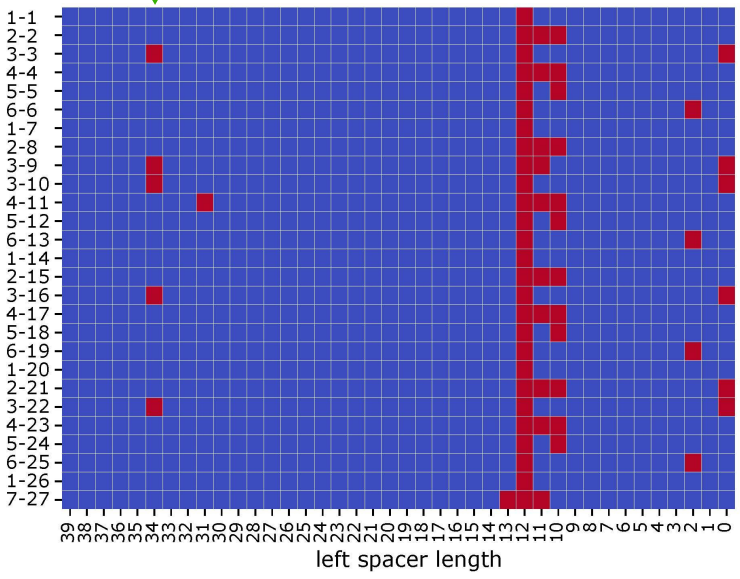
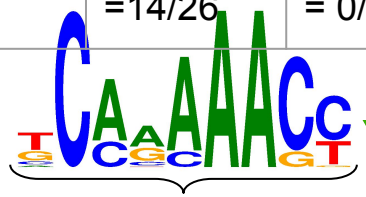
A	= 11/26	= 1/26	…
C	= 1/26	= 25/26	…
G	= 0/26	= 0/26	…
T	=14/26	= 0/26	…

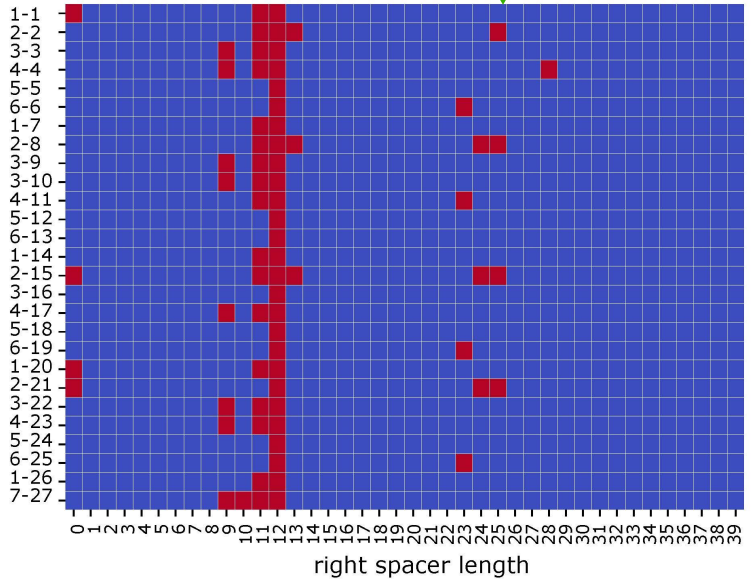
3 DNA turns





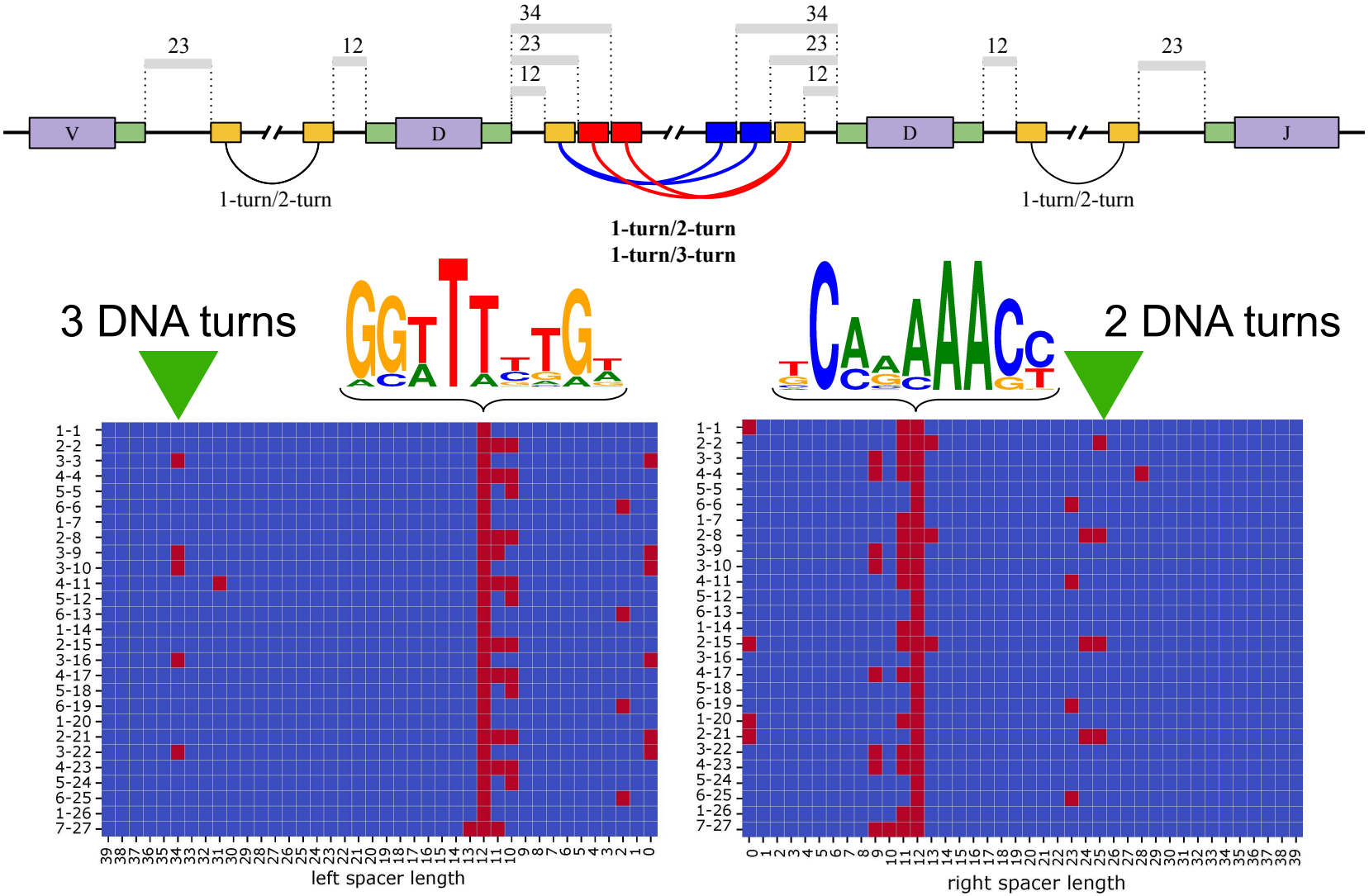
2 DNA turns





Safonova and Pevzner, *Genome Res*, 2020

Non-canonical recombination signals



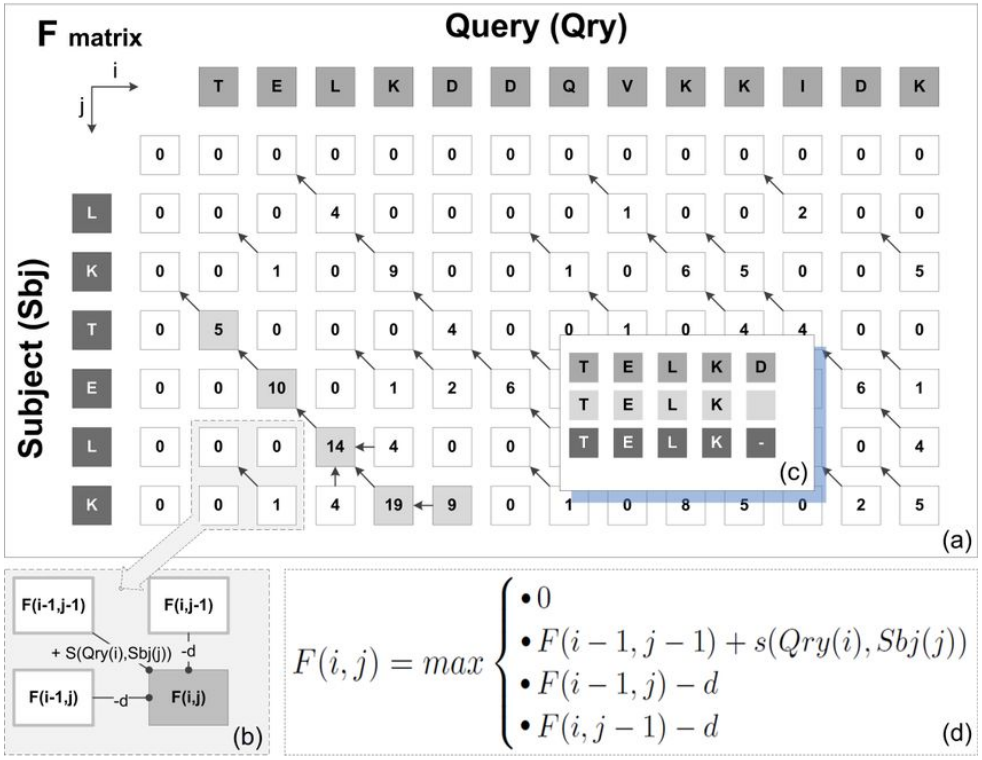
Safonova and Pevzner, *Genome Res*, 2020

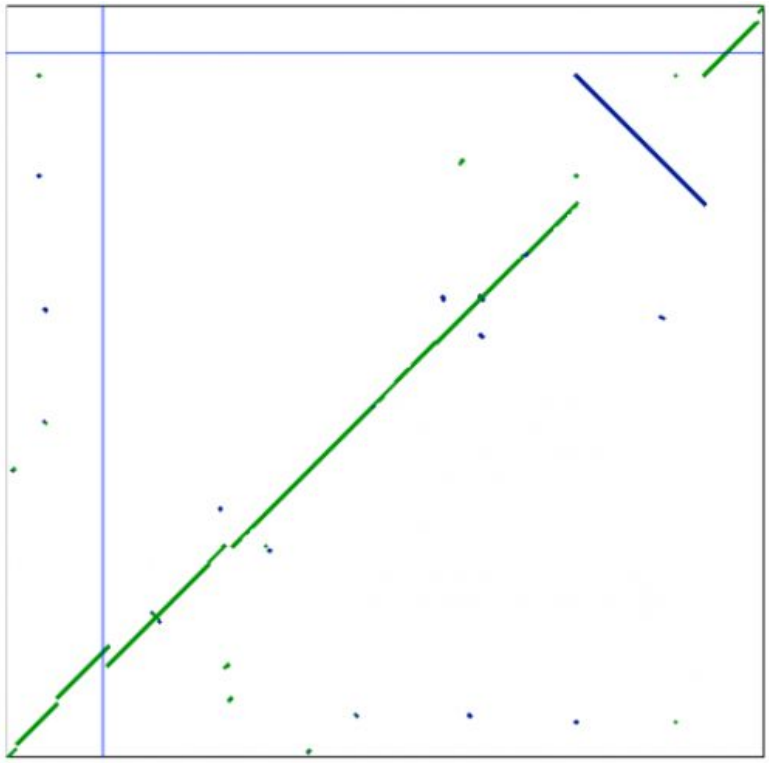
Sequence alignment

Target Sequence

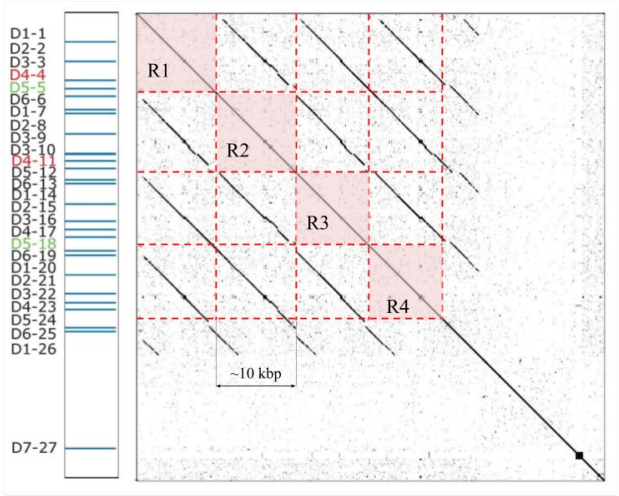
```
5' ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA 3'
   ||||||||||||    |||||||  ||||||||||||||| |||||||
5' ACTACTAGATT----ACGGATC--GTACTTTAGAGGCTAGCAACCA 3'
```

Query Sequence



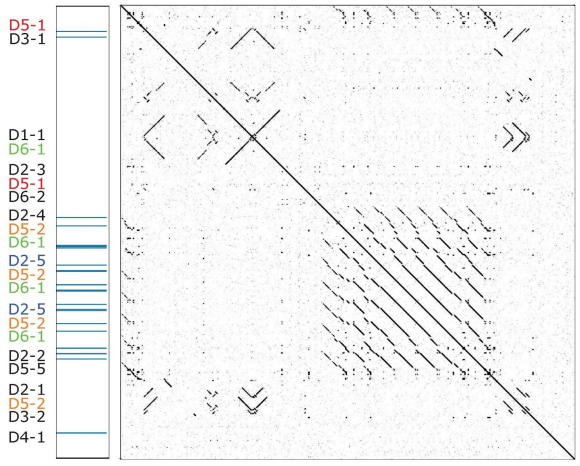


Tandem repeats correlate with D-D fusions

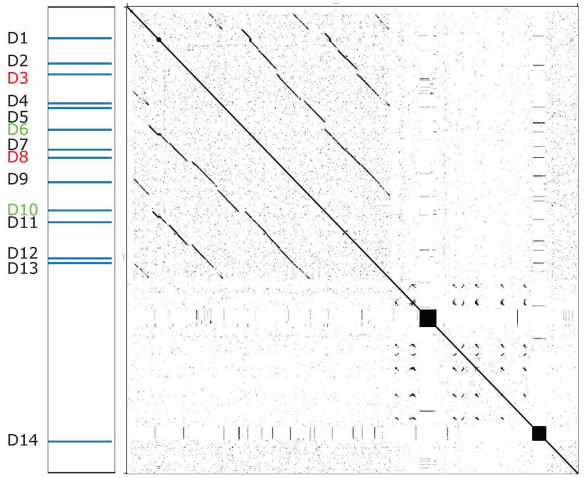


R1	R2	R3	R4
–	D6-6	D6-13	D6-19
D1-1	D1-7	D1-14	D1-20
D2-2	D2-8	D2-15	D2-21
D3-3	D3-9 D3-10	D3-16	D3-22
D4-4	D4-11	D4-17	D4-23
D5-5	D5-12	D5-18	D5-24

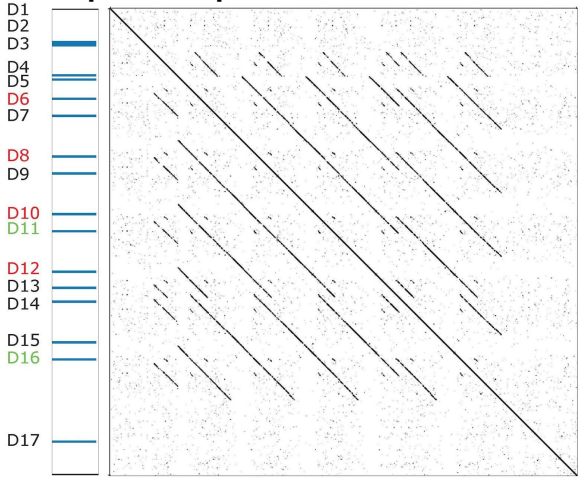
mouse



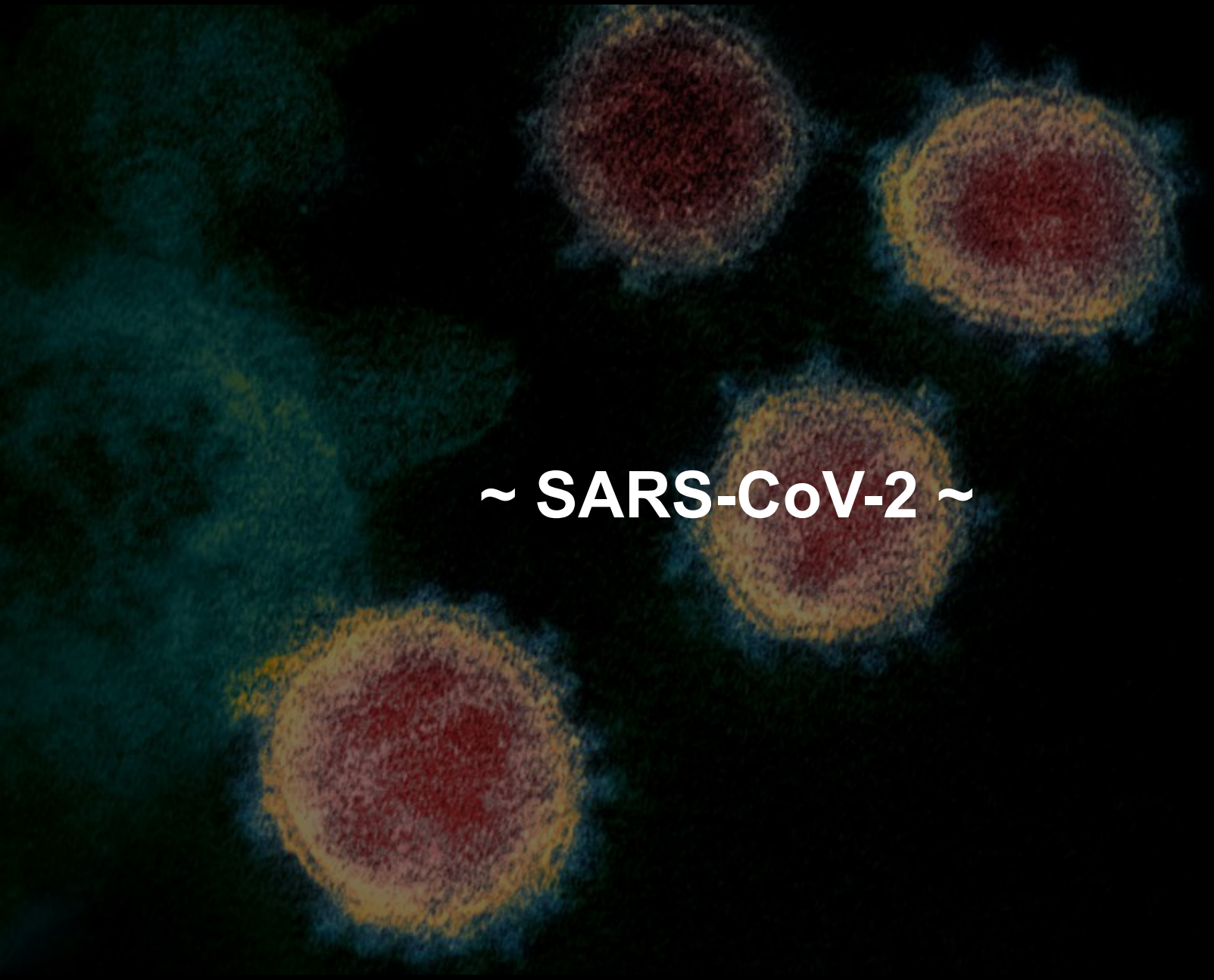
Common marmoset



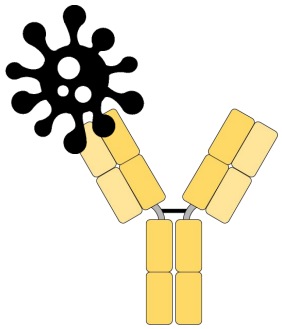
pale spear-nosed bat



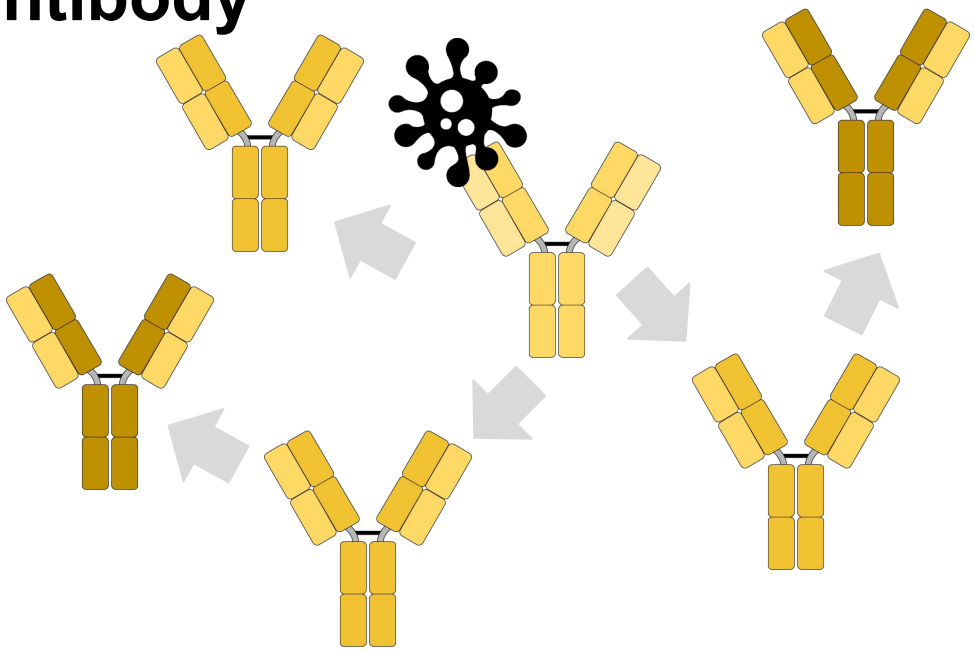
Safonova and Pevzner, *Genome Res*, 2020


~ SARS-CoV-2 ~

Antibodies are subjects of fast evolution

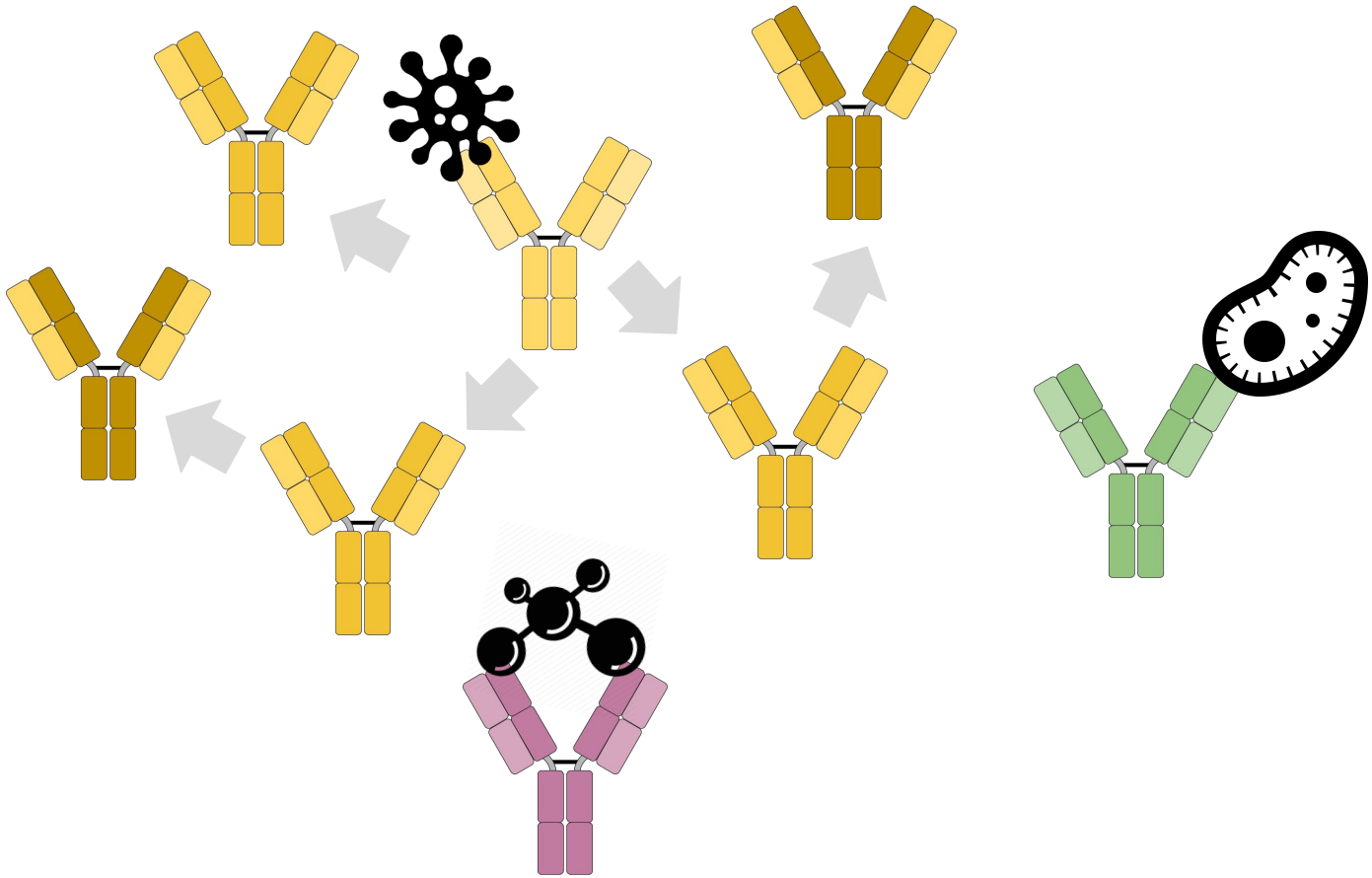


Immune system mutates and amplifies a binding antibody

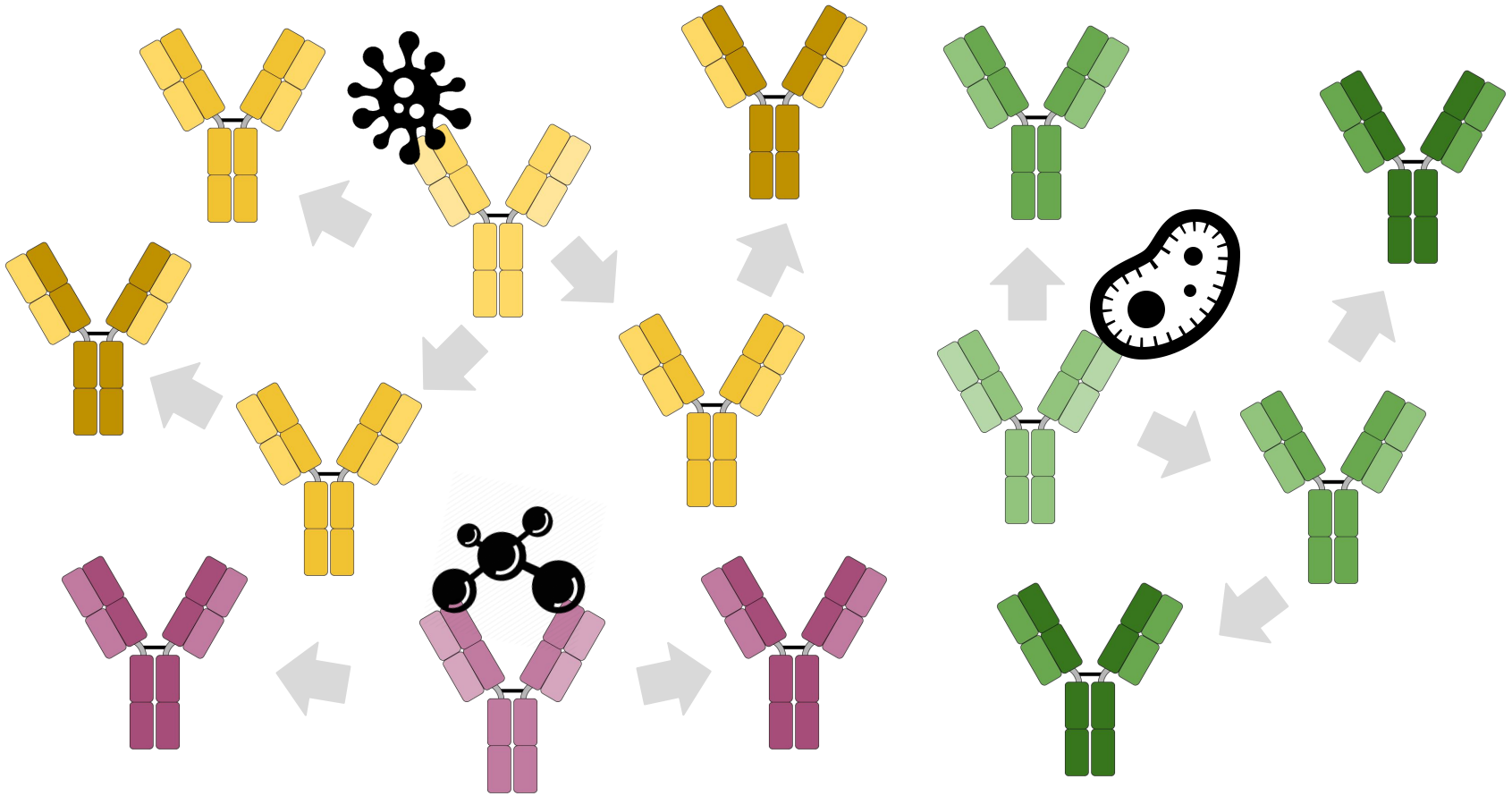


Mutation rate in antibody genes is 3-4 order of magnitude higher than in other genome

One antibody = one antigen

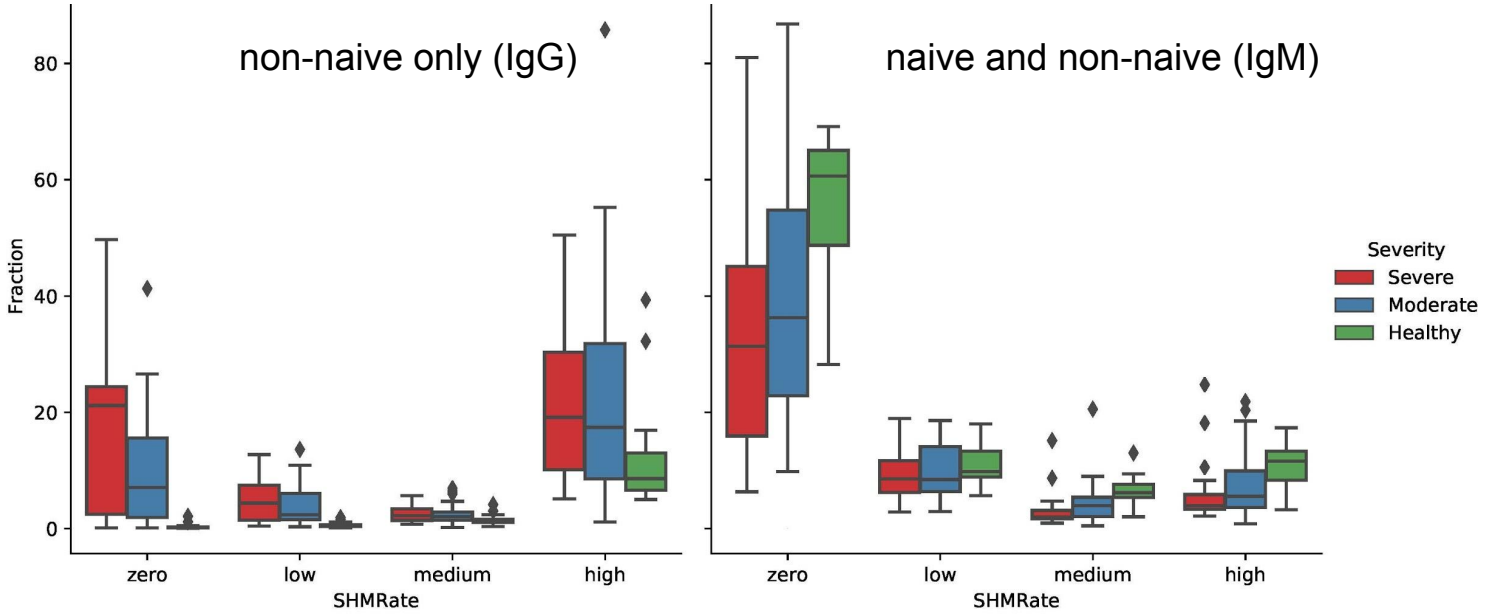


Antibody repertoire is a set of clonal lineages



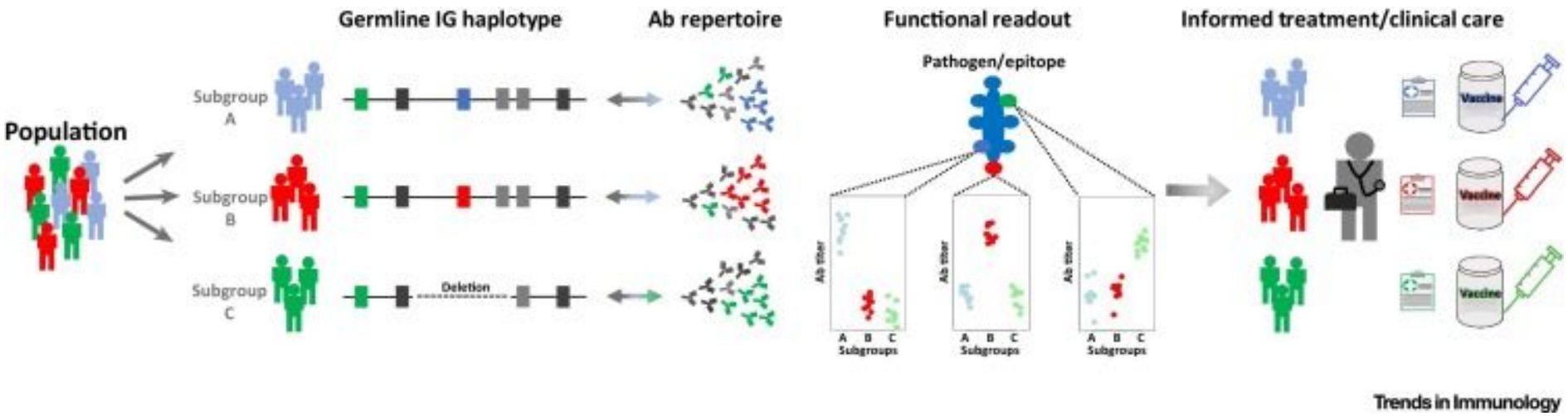
Severity of COVID-19 and Ab response

```
                                                <-------------------------------FR1-IMGT-------------------------------><-------CDR1-IMG
                                                   V  Q  L  V  Q  S  G  G  G  L  V  Q  P  G  G  S  L  R  L  S  C  V  A  S  G  F  T  F  S
                                 Query_1      2    AGGTGCAGCTGGTGCAGTCTGGAGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGCTTCACCTTCAGTA  91
V  90.2% (266/295)               IGHV3-30*02  2    .............G.......G......G.....................................C.........A.............  91
                                                   V  Q  L  V  E  S  G  G  G  V  V  Q  P  G  G  S  L  R  L  S  C  A  A  S  G  F  T  F  S
V  90.2% (266/295)               IGHV3-30-5*02 2    .............G.......G......G.....................................C.........A.............  91
V  89.5% (264/295)               IGHV3-30*18  2    .............G.......G......G..............A......................C...C.....A.............  91

                                                T------><--------------------FR2-IMGT--------------------><-------CDR2-IMGT------><------
                                                N  F  G  M  H  W  V  R  Q  A  P  G  K  G  L  E  W  V  A  M  T  Q  P  D  E  G  T  E  Y  Y
                                 Query_1      92   ATTTTGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGACTGGAGTGGGTGGCAATGACACAACCTGATGAAGGTACTGAATACTATG  181
V  90.2% (266/295)               IGHV3-30*02  92   GC.A.....................................G..............T.T.T..GGTA.....G.A...A.A.........  181
                                                S  Y  G  M  H  W  V  R  Q  A  P  G  K  G  L  E  W  V  A  F  I  R  Y  D  G  S  N  K  Y  Y
V  90.2% (266/295)               IGHV3-30-5*02 92   GC.A.....................................G..............T.T.T..GGTA.....G.A...A.A.........  181
V  89.5% (264/295)               IGHV3-30*18  92   GC.A.....................................G..............G.T.T.TC.TA.....G.A...A.A.........  181

                                                ---------------------------------------------FR3-IMGT-------------------------------------
                                                G  D  S  V  K  G  R  F  T  I  S  R  D  N  S  R  N  T  L  Y  L  Q  M  N  S  L  R  T  E  D
                                 Query_1      182  GAGACTCCGTGAAGGGCCGATTTACCATCTCCAGAGACAATTCCAGGAACACACTGTATCTGCAAATGAACAGCCTGAGAACTGAGGACA  271
V  90.2% (266/295)               IGHV3-30*02  182  C.....................C.....................A......G.....................G.........  271
                                                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
V  90.2% (266/295)               IGHV3-30-5*02 182  C.....................C.....................A......G.....................G.........  271
V  89.5% (264/295)               IGHV3-30*18  182  C.....................C.....................A......G.....................G.........  271
```



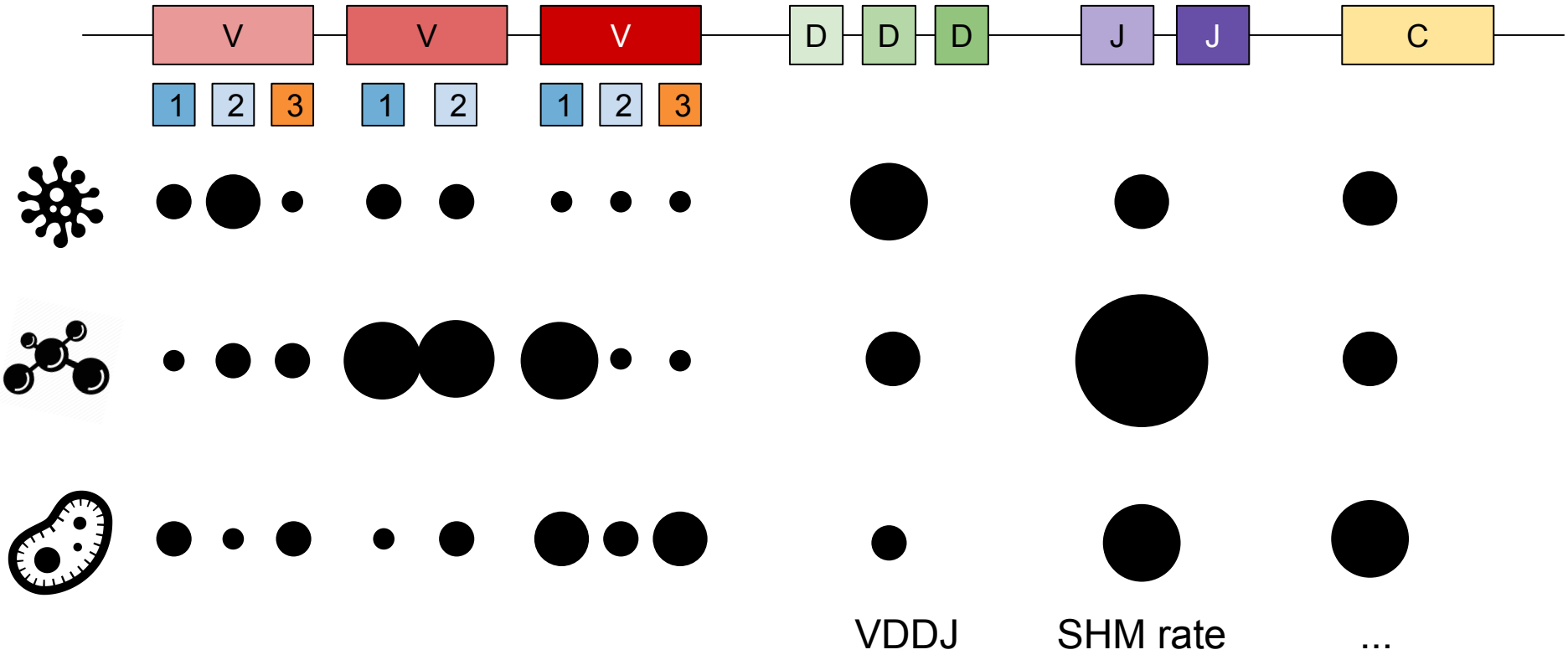
Safonova, Tieri, et al., in preparation

Immunogenomics approach to vaccine design



Watson, Glanville, Marasco, *Trends in Immunol*, 2017

Data science approach to predicting the efficiency of antibody response



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Justin Kos
Oscar Rodriguez
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Katherine Jackson

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Ramesh Rao
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