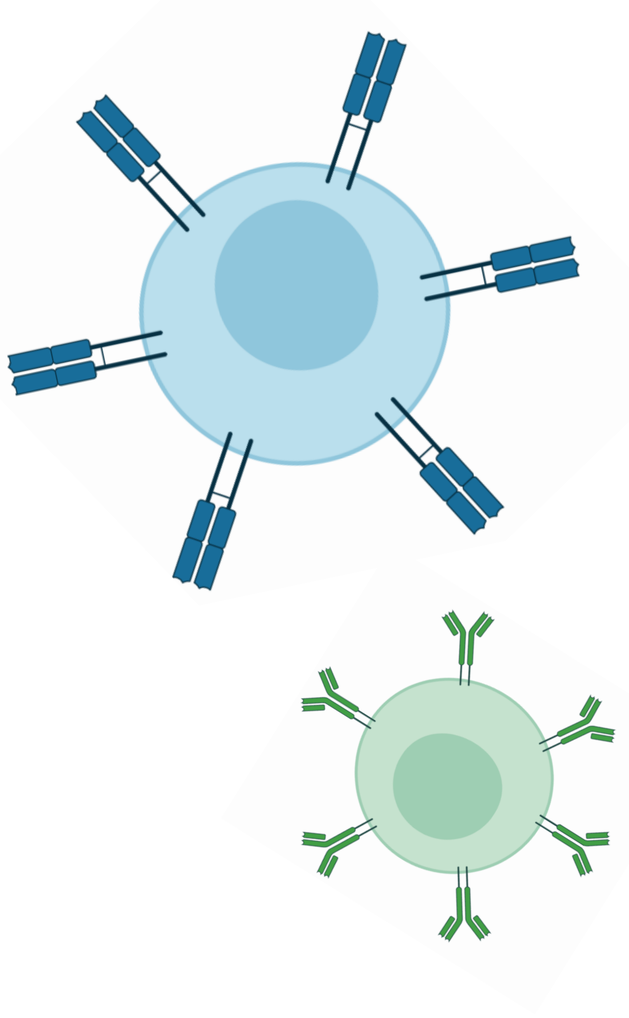
Intro to immune repertoire sequencing and analysis

Maggie Russell

TFCB 2023

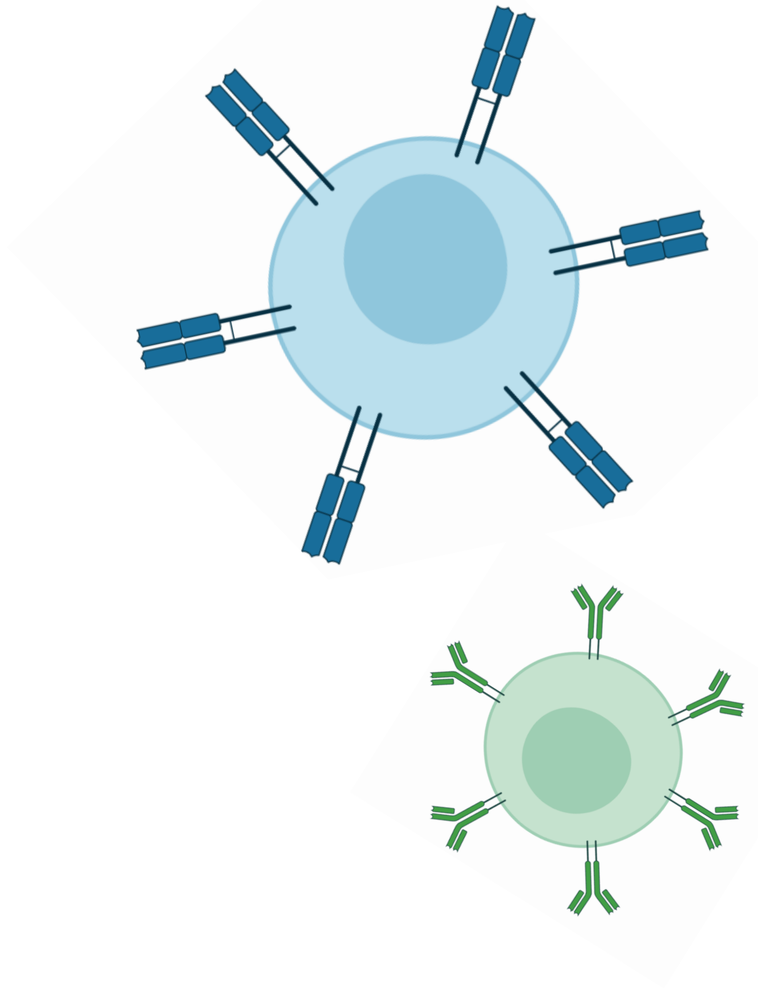
Lecture goals:

1. learn about immune repertoire sequencing
2. familiarize with immune repertoire data
3. work through an example analysis



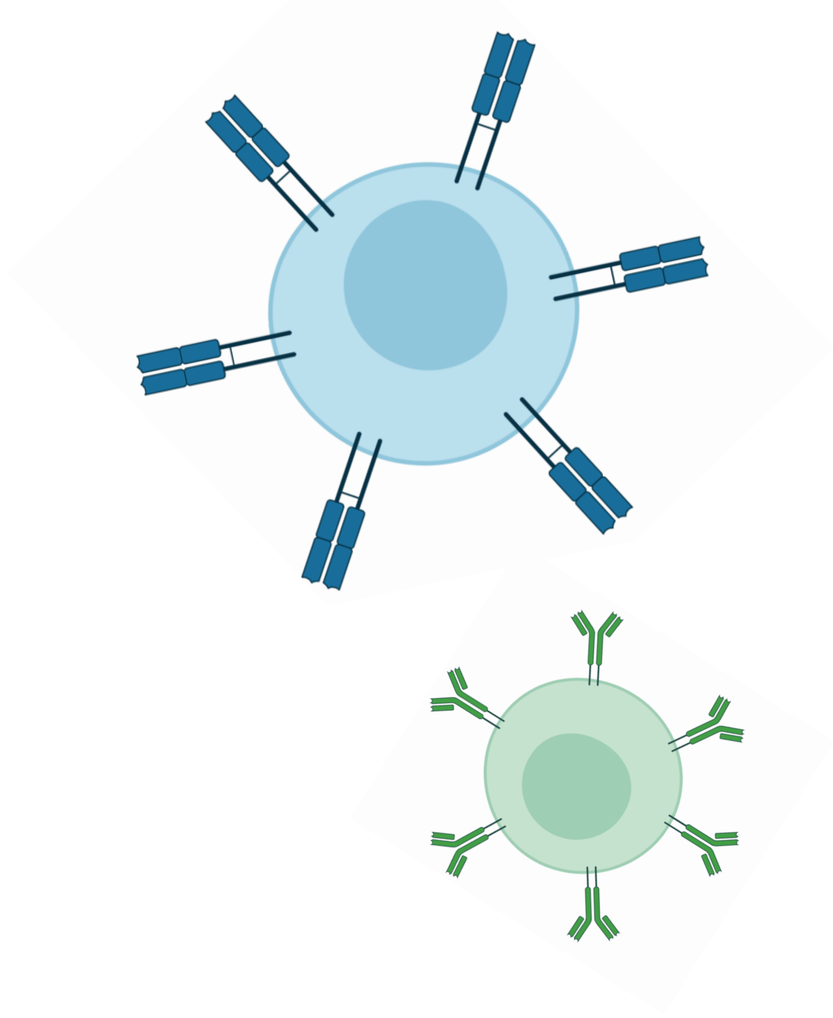
Lecture goals:

1. learn about immune repertoire sequencing

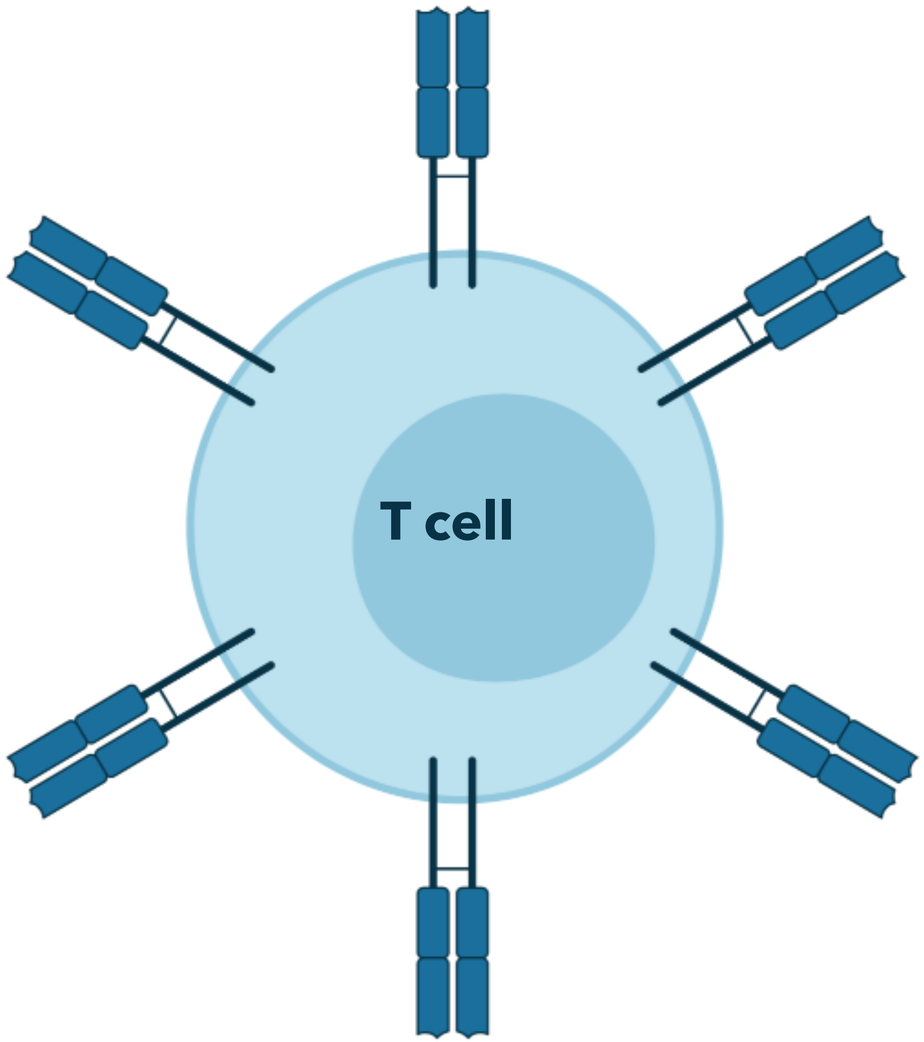


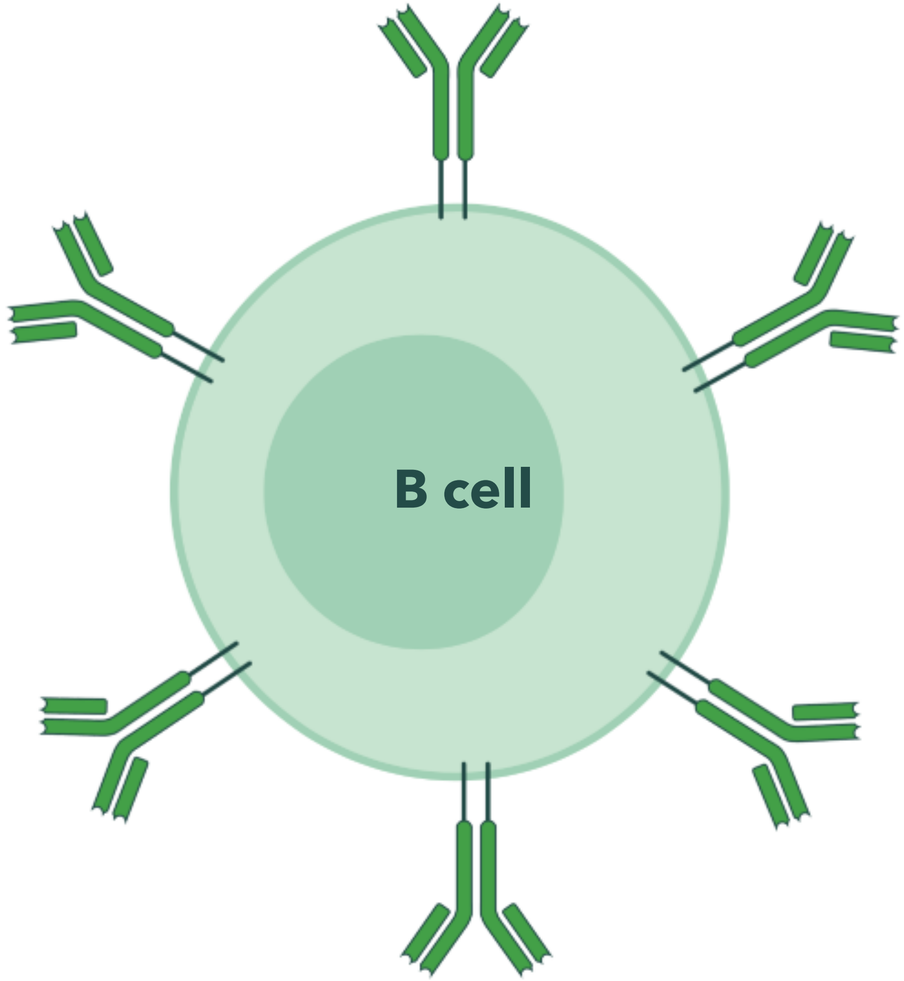
Lecture goals:

1. learn about immune repertoire sequencing
    - **what are immune repertoires?**

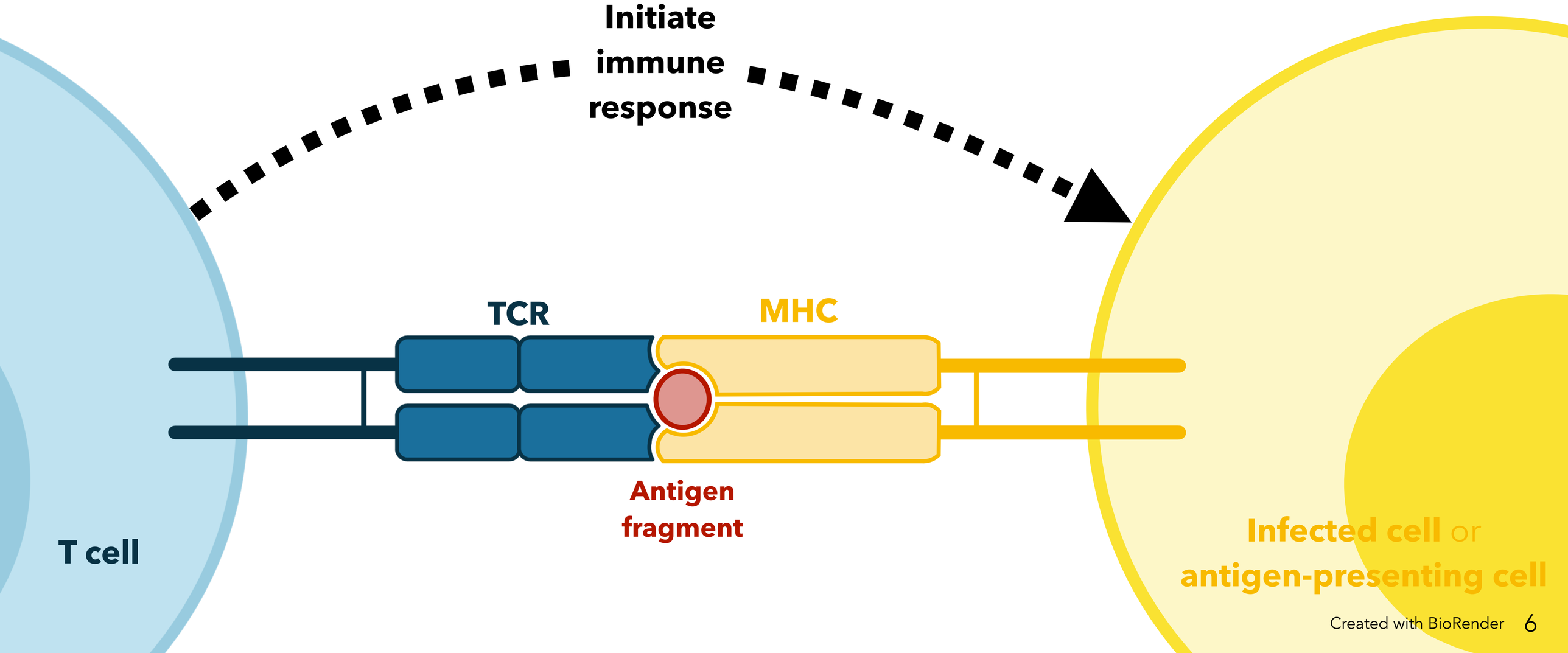


Adaptive immunity is essential for our survival

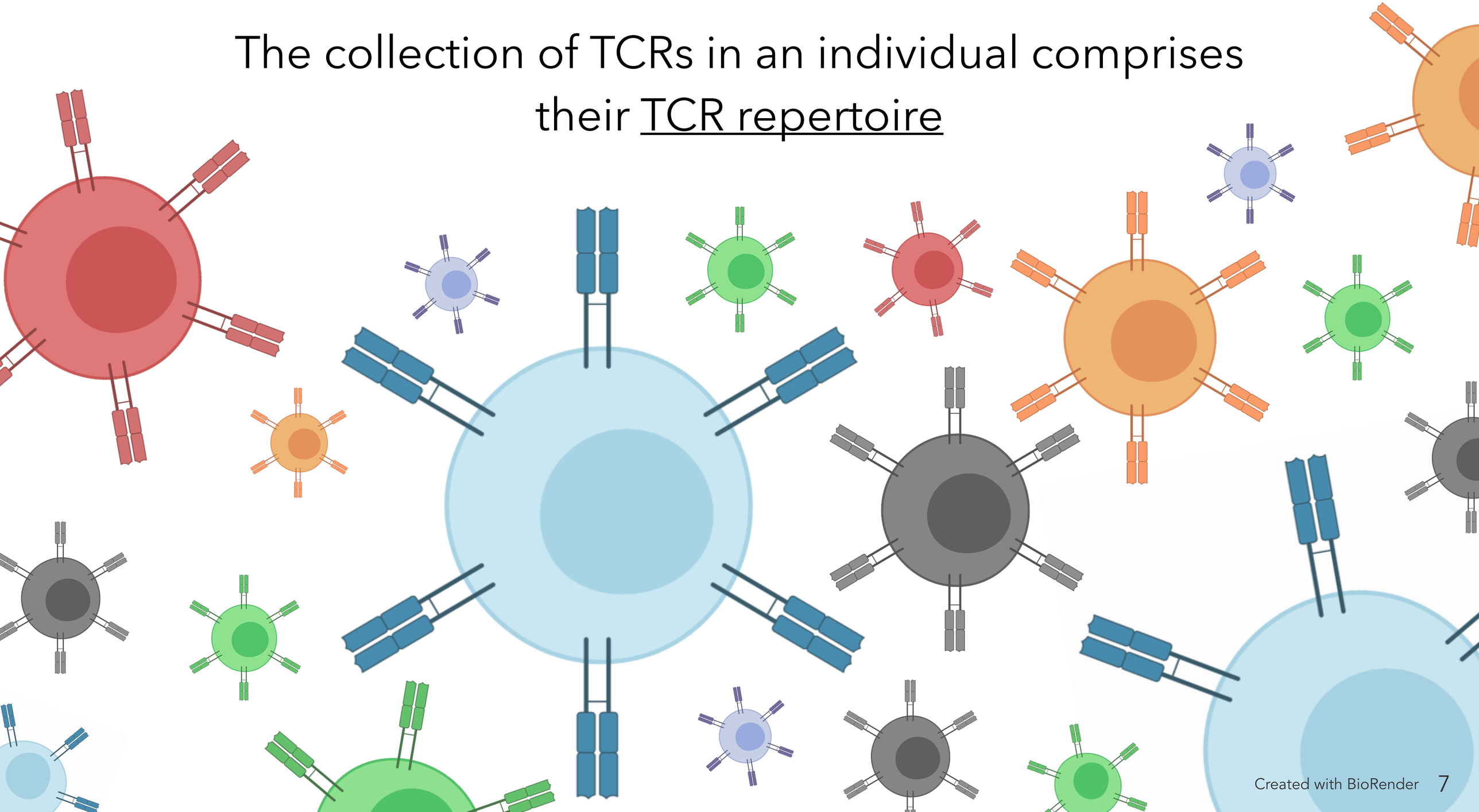




T cell receptors recognize antigen fragments bound to MHC

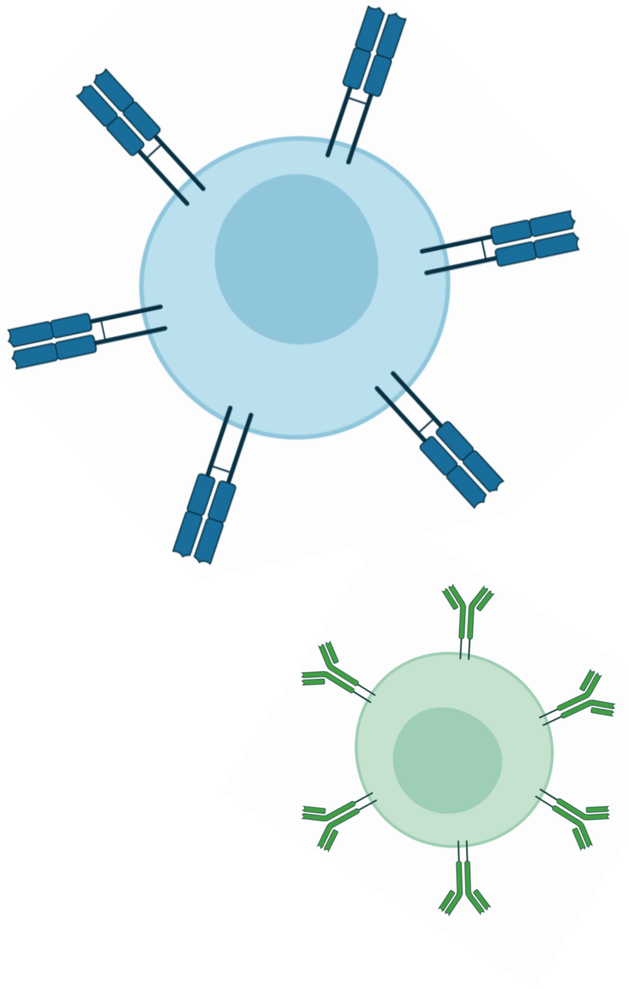


The collection of TCRs in an individual comprises their TCR repertoire



Lecture goals:

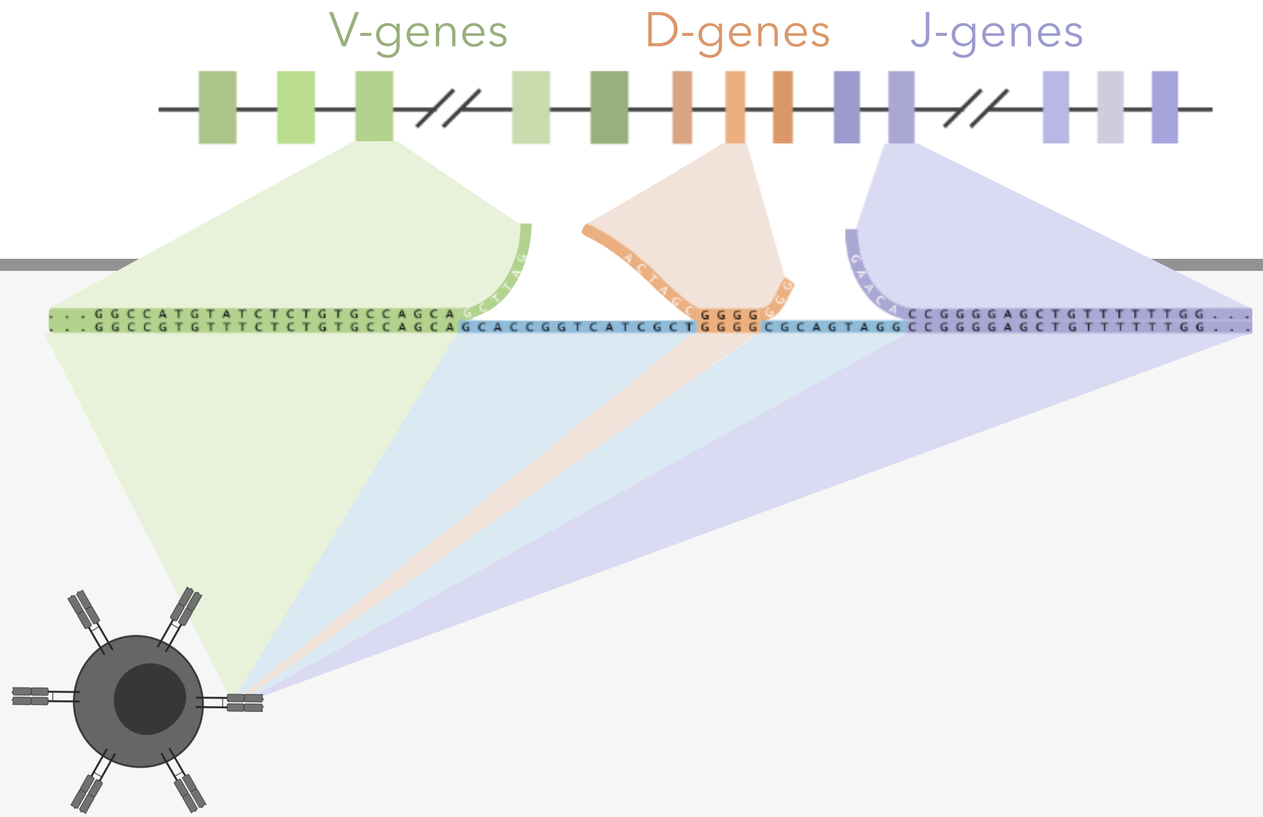
1. learn about immune repertoire sequencing
    - what are immune repertoires?
    - **how are they formed?**



Repertoire composition is influenced by generation, selection, and exposures

Let's use a water pipe as an analogy for TCR repertoire formation…

Repertoire composition is influenced by generation, selection, and exposures



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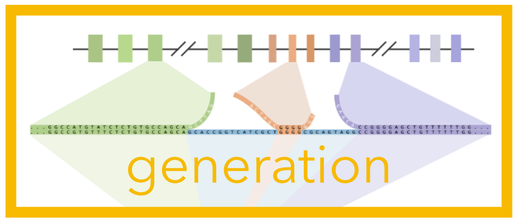

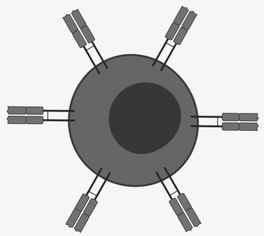

modified from Murugan et. al *PNAS* 2012
Created with BioRender

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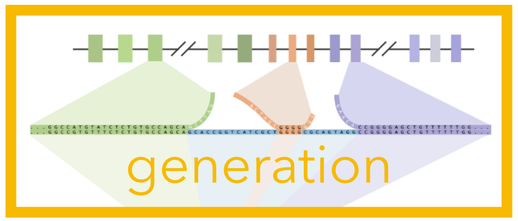

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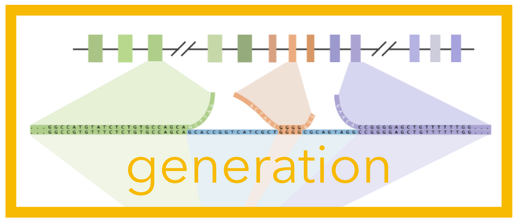

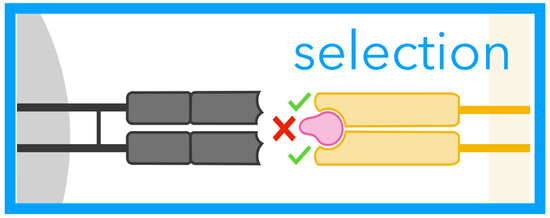

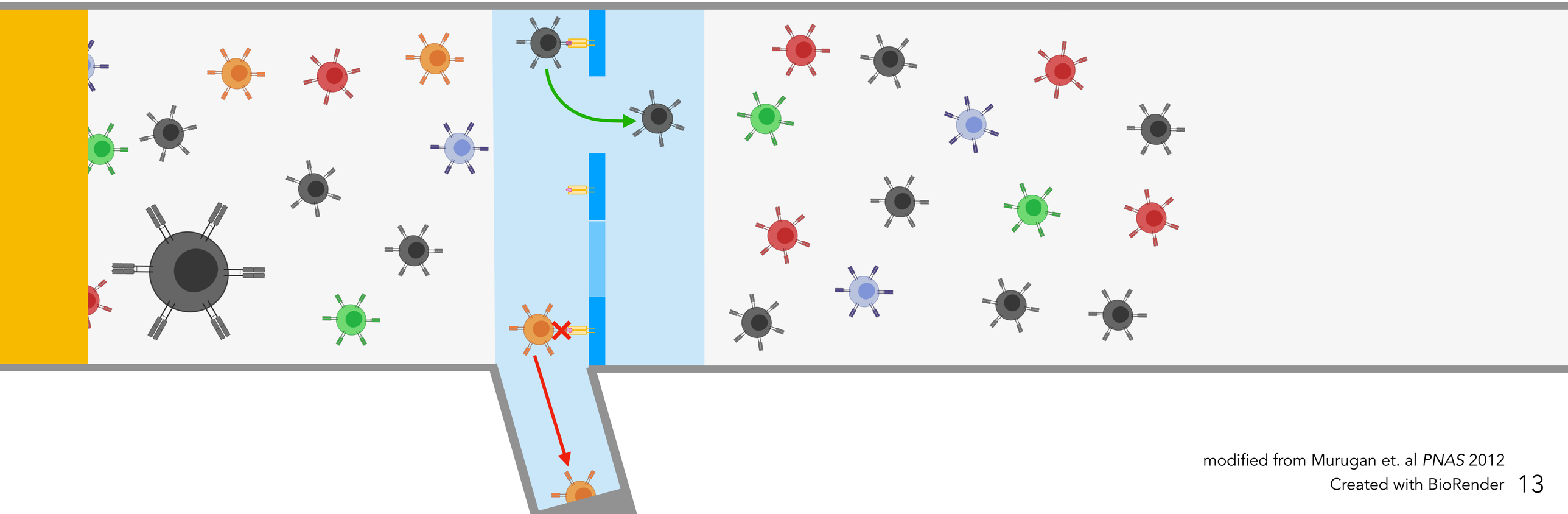

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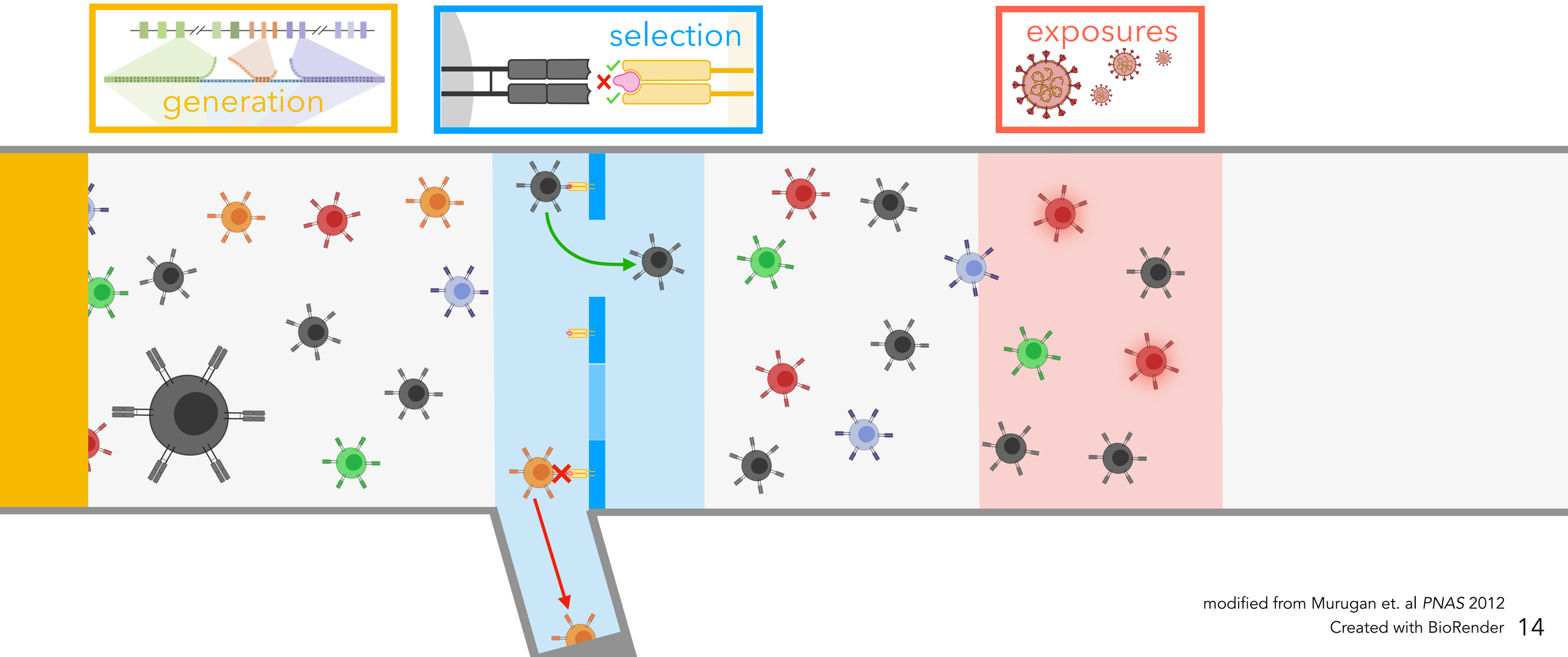
Repertoire composition is influenced by generation, selection, and **exposures**



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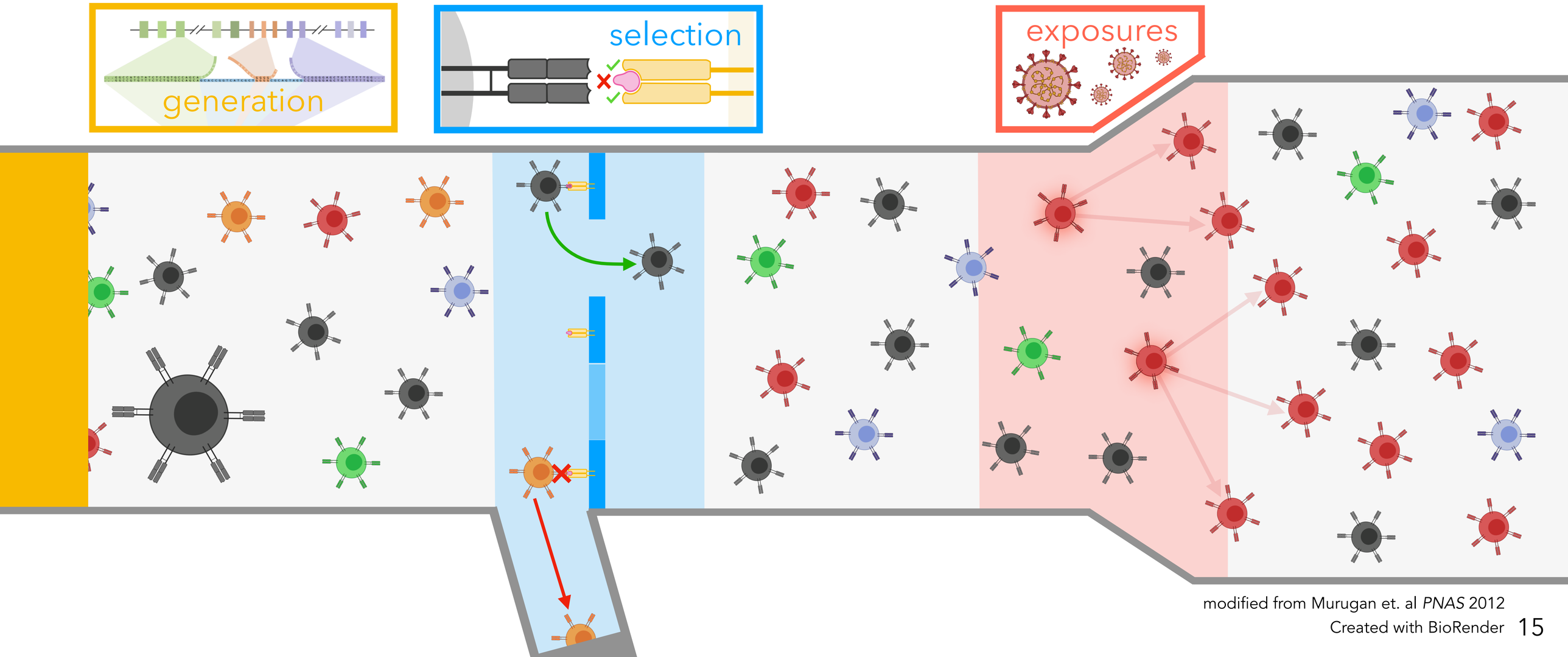

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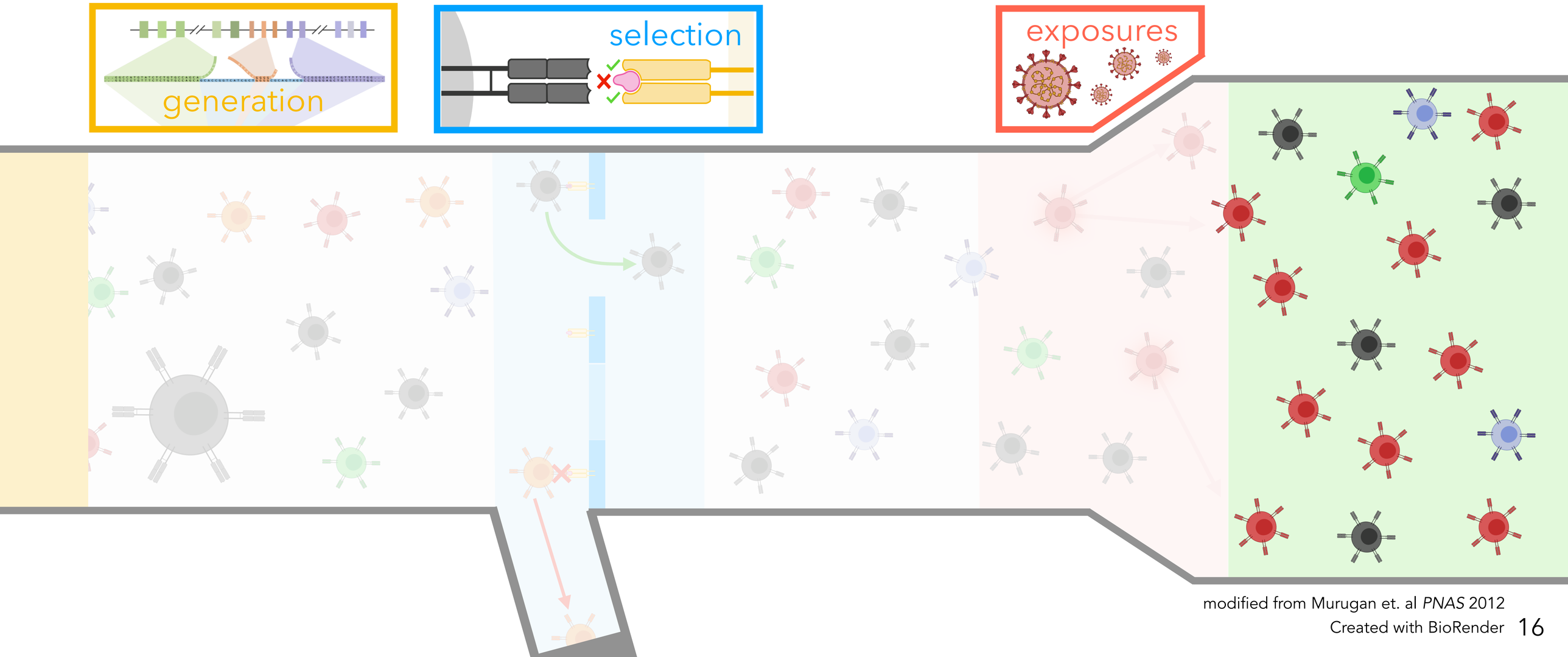
We can sample a **repertoire** using sequencing



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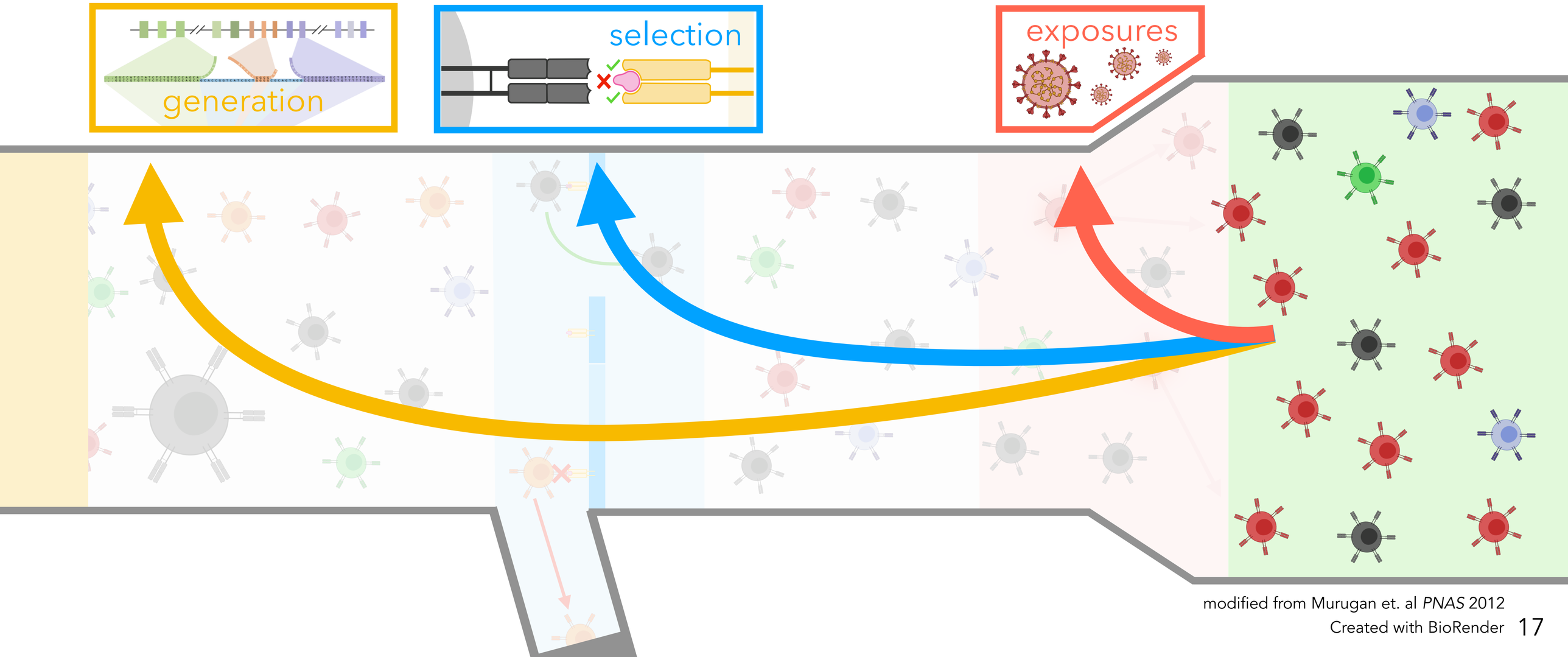

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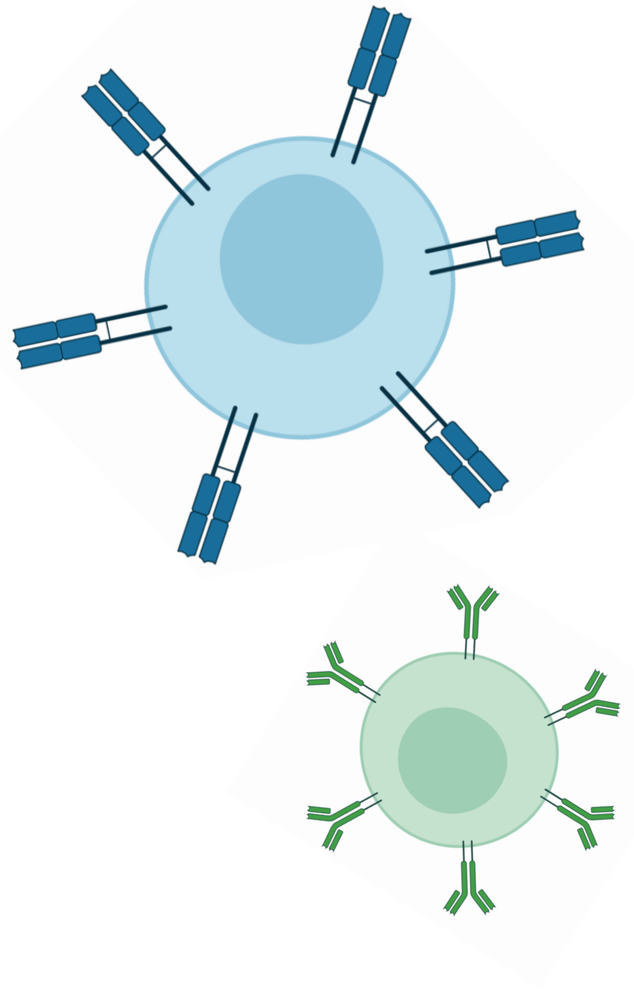

Both **bulk sequencing** and **single-cell sequencing** are possible

	Bulk	**Single-cell**
Repertoire coverage *(e.g. total # of unique sequences that can be identified)*	**High**	**Low**

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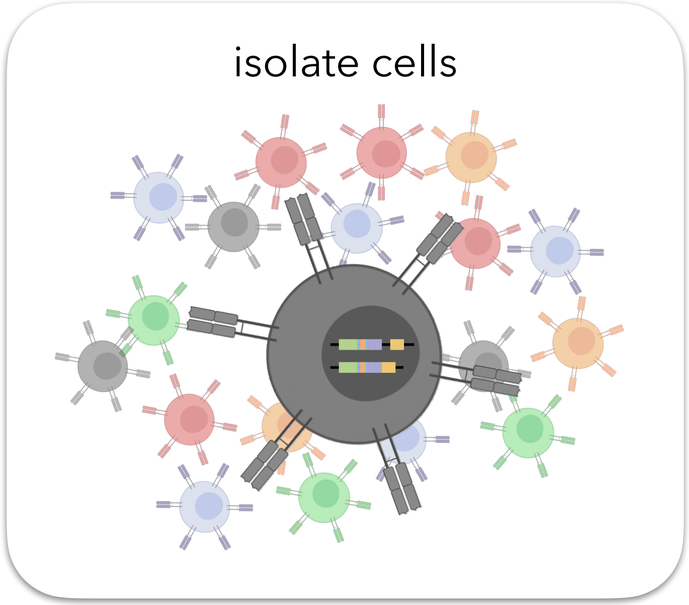
Bulk repertoire sequencing overview



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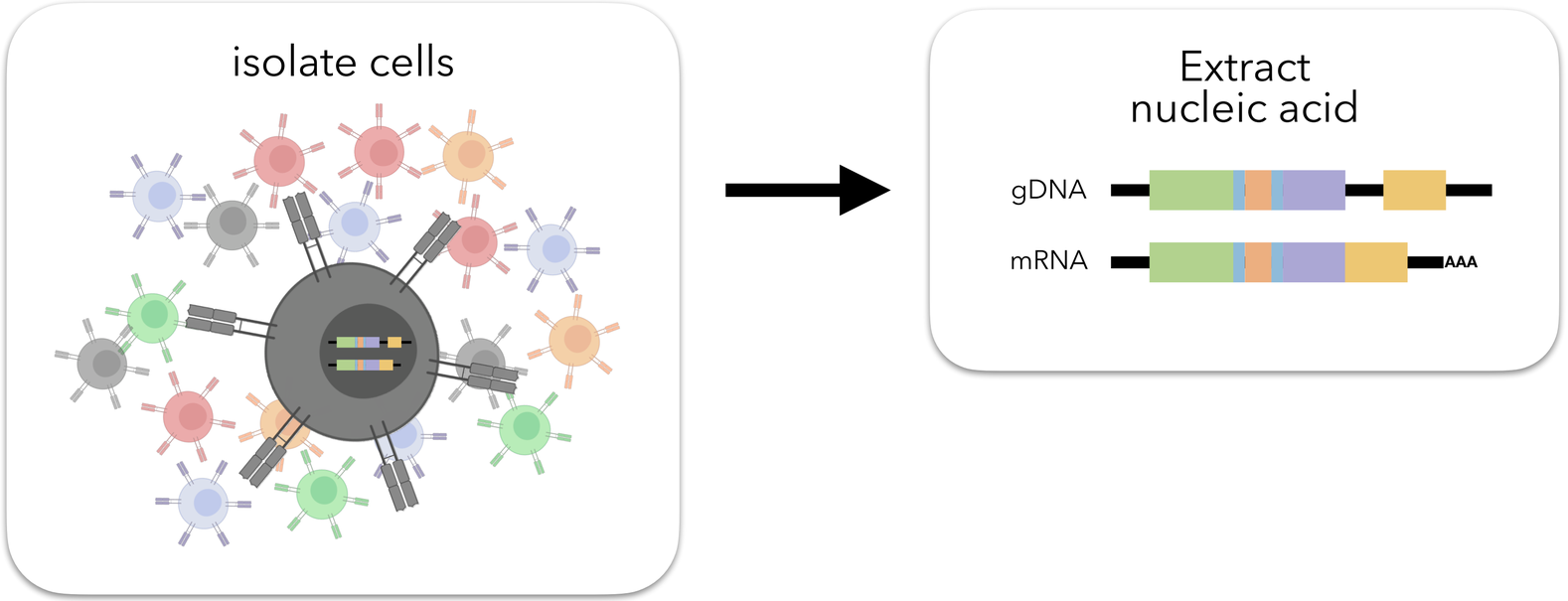

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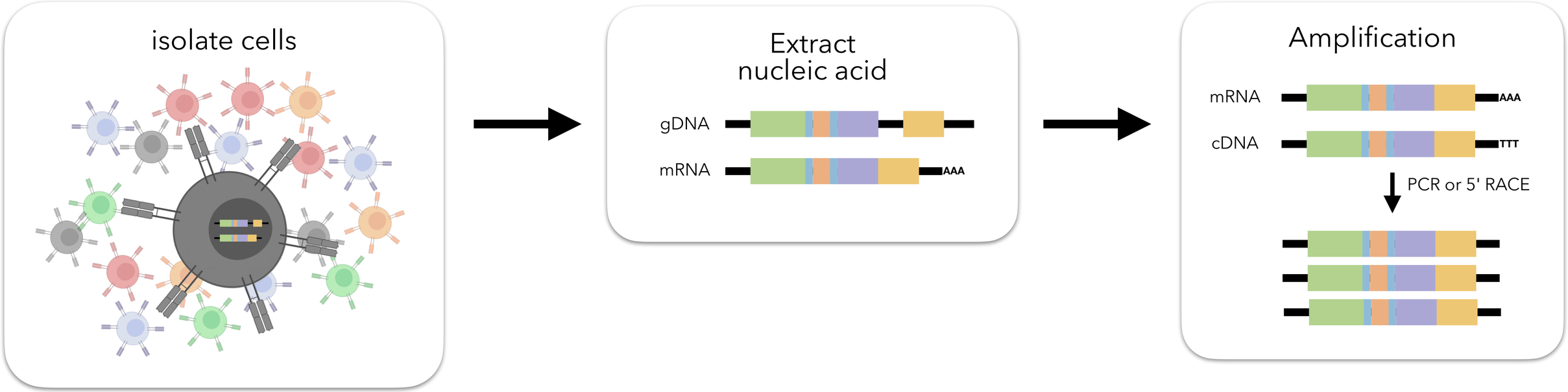

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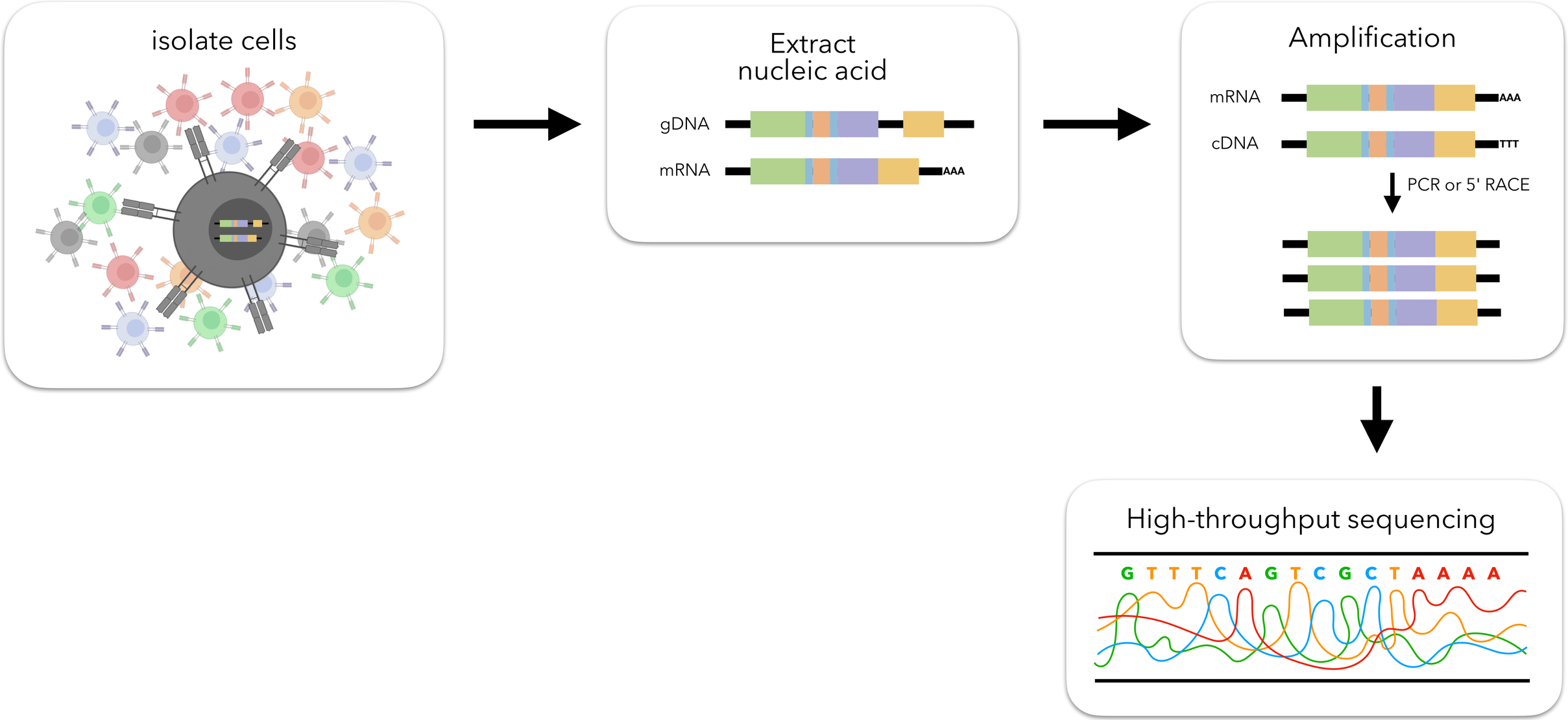
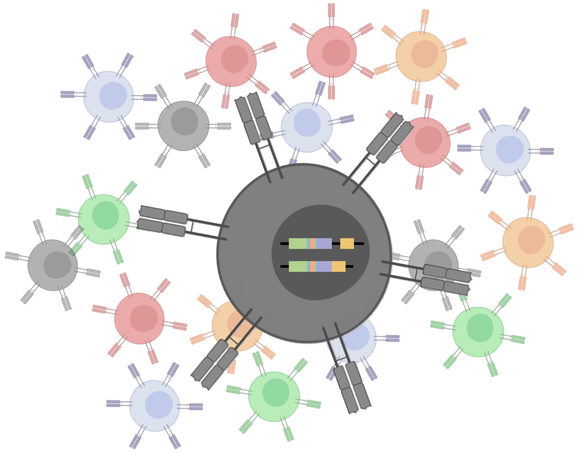
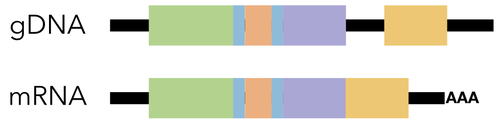


Bulk repertoire sequencing overview

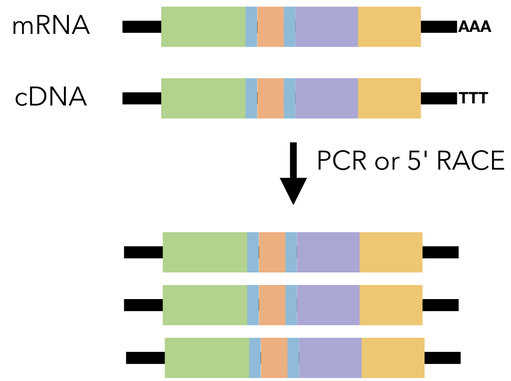
isolate cells



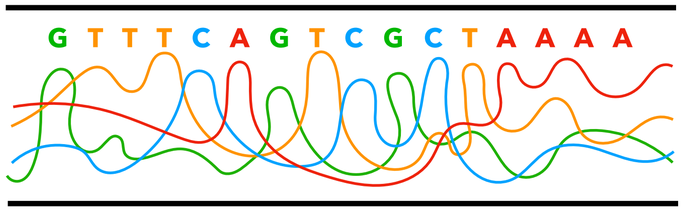
Extract
nucleic acid



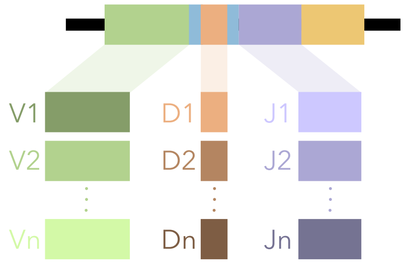
Amplification



High-throughput sequencing



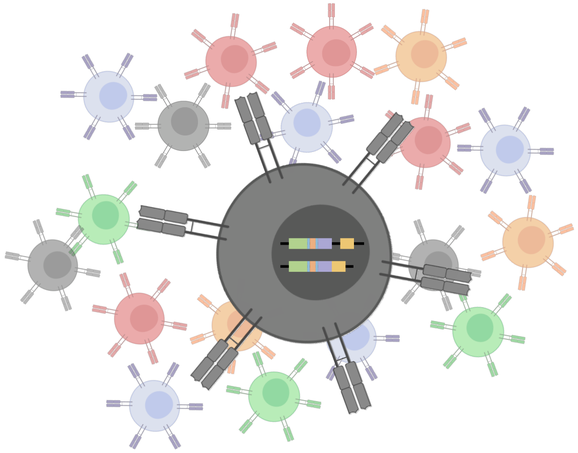
Data preprocessing



(filtering, alignment, annotation,
clustering, etc.)

Bulk repertoire sequencing overview

isolate cells



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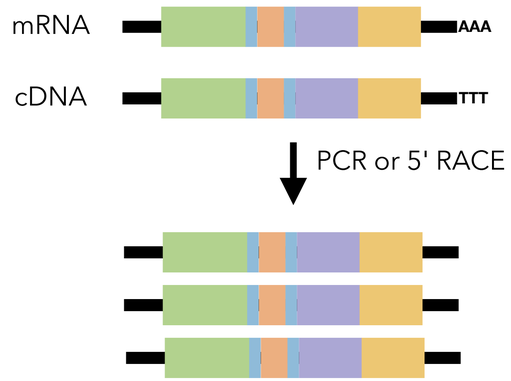

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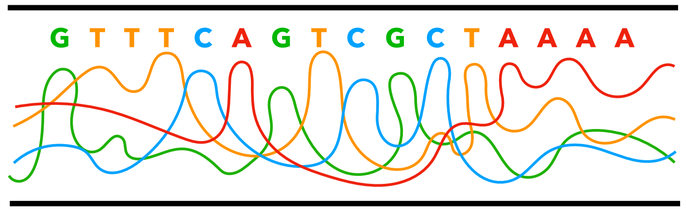

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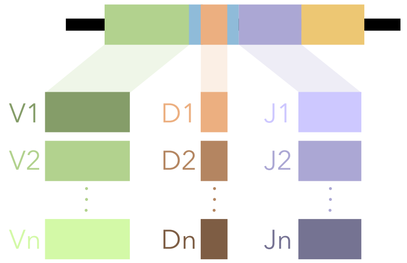
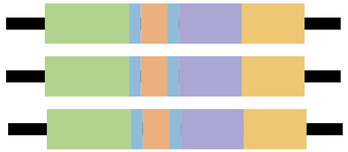


(filtering, alignment, annotation, clustering, etc.)

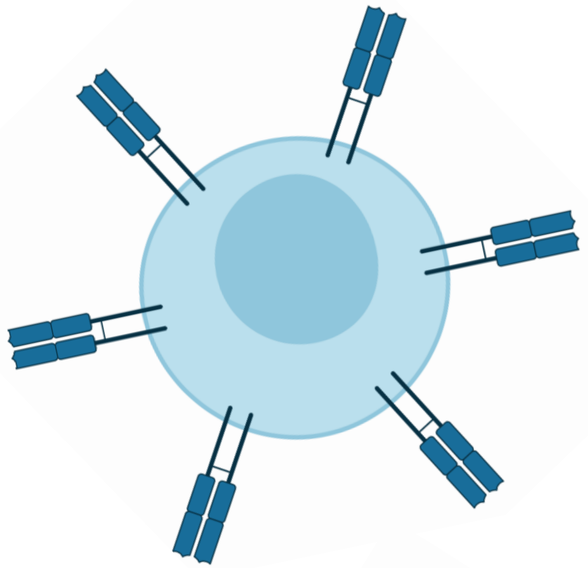
Data analysis

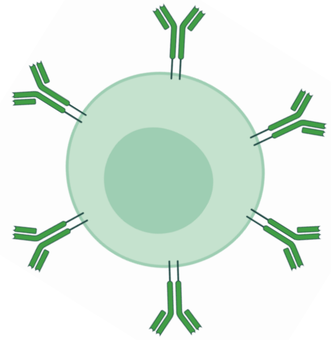
Processed **bulk repertoire sequencing** example output



cdr3_nucseq	cdr3	v_gene	d_gene	j_gene	v_trim	d0_trim	d1_trim	j_trim	vd_insert	dj_insert	vd_insert_nucs	dj_insert_nucs
<chr>	<chr>	<chr>	<chr>	<chr>	<int>	<int>	<int>	<int>	<int>	<int>	<chr>	<chr>
TGTGCCAGCAGCTTGAATCACGAGCAGTACTTC	CASSLNHEQYF	TRBV5-6*01	TRBD2*02	TRBJ2-7*01	1	3	13	5	4	0	AATC	
TGCGCCAGCAGCTTGGCAGAGACCCAGTACTTC	CASSLAETQYF	TRBV5-1*01	TRBD1*01	TRBJ2-5*01	2	9	0	4	0	0		
TGCGCCAGTCGAGCGGCGAGCTCCTACAATGAGCAGTTCTTC	CASRAASSYNEQFF	TRBV5-1*01	TRBD2*01	TRBJ2-1*01	9	6	5	0	4	2	GTCG	GC
TGTGCCAGCAGCTTAAATCTGGTGAGGTACGAGCAGTACTTC	CASSLNLVRYEQYF	TRBV7-2*01	TRBD2*02	TRBJ2-7*01	2	11	1	4	8	0	AATCTGGT	
TGTGCCTGGTCAGGGGGCCCAAACACTGAAGCTTTCTTT	CAWSGGPNTEAFF	TRBV30*01	TRBD1*01	TRBJ1-1*01	5	4	0	2	1	3	T	ACC
TGTGCCACCGAACGAGGGCCCCAAGAGACCCAGTACTTC	CATERGPQETQYF	TRBV2*03	TRBD1*01	TRBJ2-5*01	10	5	3	1	7	2	CCGAACG	CC
TGTGCCAGCATAGCGGGAGGTGAGCAGTTCTTC	CASIAGGEQFF	TRBV28*01	TRBD2*02	TRBJ2-1*01	7	6	3	9	1	2	T	GG
TGTGCCTGGAGCTCCCTCCCTGGCGGGGAGAACAATGAGCAGTTCTTC	CAWSSLPGGENNEQFF	TRBV30*01	TRBD2*01	TRBJ2-1*01	3	7	3	5	11	3	CTCCCTCCCTG	AGA
TGTGCCAGCAGTTATCAGGTCACTGAAGCTTTCTTT	CASSYQVTEAFF	TRBV6-6*02	TRBD1*01	TRBJ1-1*01	4	4	5	4	2	2	AT	TG
TGTGCCAGCGGCCCAGGGCTCGGATACAATCAGCCCCAGCATTTT	CASGPGLGYNQPQHF	TRBV5-5*01	TRBD2*02	TRBJ1-5*01	7	12	0	3	5	8	GGCCC	ATAGGCTC
TGTGCCAGTGCGGGATTCTATGGCTACACCTTC	CASAGFYGYTF	TRBV6-1*01	TRBD1*01	TRBJ1-2*01	9	7	2	4	3	3	TGC	TTA
TGTGCCAGTGCAGGGTACACCGGGGAGCTGTTTTTT	CASAGYTGELFF	TRBV2*03	TRBD1*01	TRBJ2-2*01	9	4	4	3	2	2	TG	TG
TGTGCCATCAGTGAATACAATGAGCAGTTCTTC	CAISEYNEQFF	TRBV10-3*01	TRBD1*01	TRBJ2-1*01	3	3	6	8	2	0	AT	
TGTGCCATCAGTAACACCGGGGAGCTGTTTTTT	CAISNTGELFF	TRBV10-3*02	TRBD2*02	TRBJ2-2*01	6	5	10	2	0	0		
TGTGCCAGTAGCCCTACCCGGTCTGGAAACACCATATATTTT	CASSPTRSGNTIYF	TRBV19*01	TRBD2*02	TRBJ1-3*01	7	5	9	1	4	5	GCCC	GGCCC
TGTGCCACCAGCAGAGGATCGGGGCTAGCGGGTGTTGAGCAGTTCTTC	CATSRGSGLAGVEQFF	TRBV15*02	TRBD2*01	TRBJ2-1*01	1	4	4	9	8	3	GATCGGGG	TGT
TGTGCCAGCAGCTTAACGGTGGGGTCAGGAGAGACCCAGTACTTC	CASSLTVGSGETQYF	TRBV7-3*01	TRBD2*01	TRBJ2-5*01	1	9	3	4	4	5	CGGT	GGACT

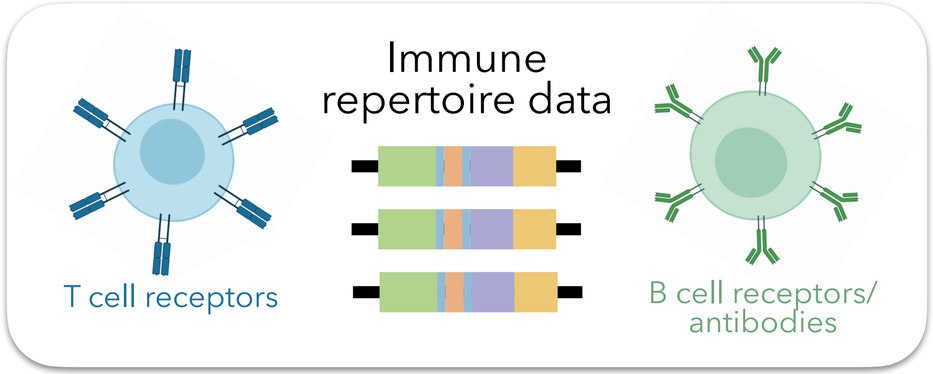
Lecture goals:





1. learn about immune repertoire sequencing
    - what are immune repertoires?
    - how are they formed?
    - how are they sequenced?
    - **what are some common areas of repertoire analysis?**

Immune repertoire analyses often focus on diversity, architecture, evolution, or convergence



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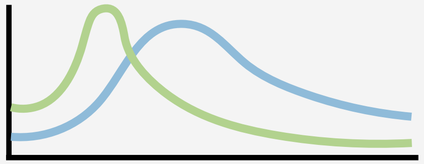
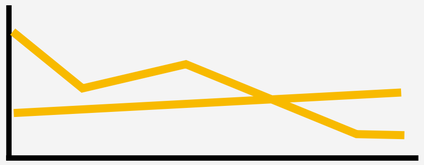
Underlying mechanisms of diversity generation

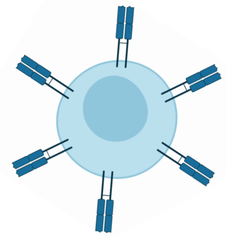
probabilistic sequence annotation



recombination statistics to learn about generation and selection

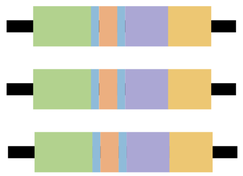


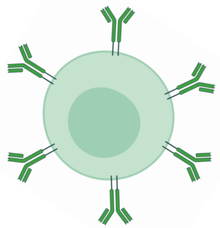
clonotype diversity dynamics



T cell receptors

Immune repertoire data

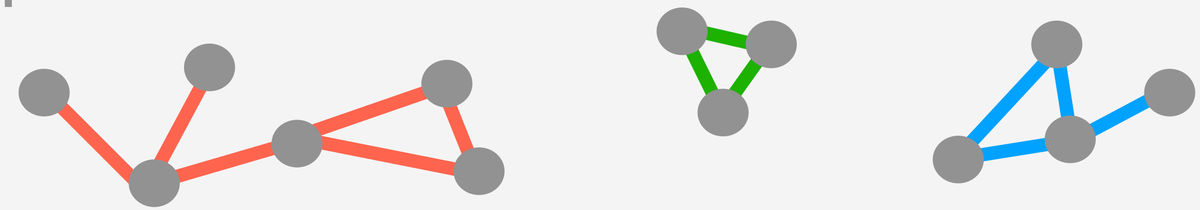




B cell receptors/ antibodies

Immune repertoire analyses often focus on diversity, **architecture**, evolution, or convergence

Repertoire architecture



defining antigen recognition breadth using network analysis

Underlying mechanisms of diversity generation

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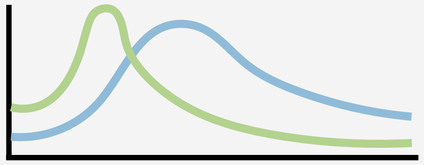

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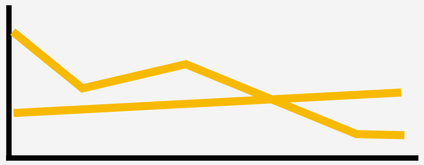
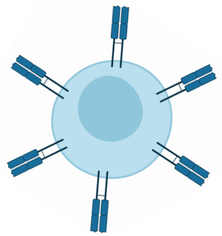
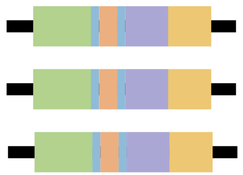


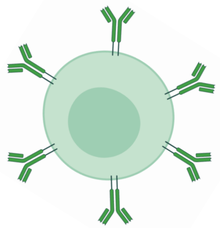
clonotype diversity dynamics



T cell receptors

Immune repertoire data





B cell receptors/ antibodies

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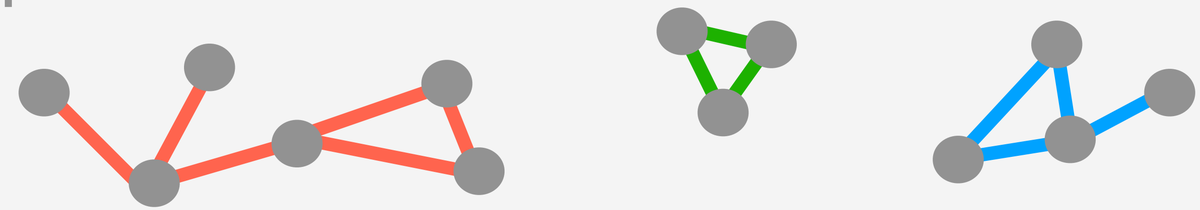

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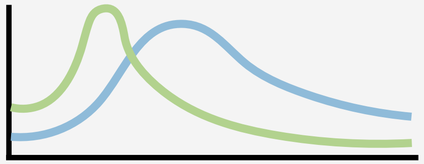

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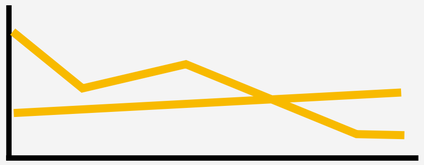

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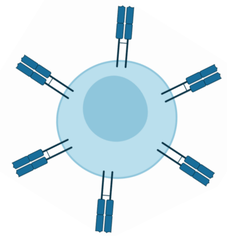

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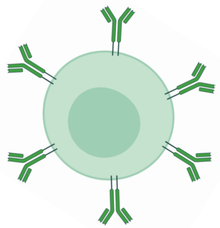

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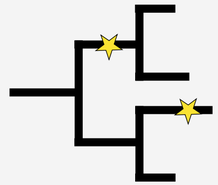
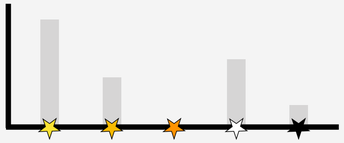
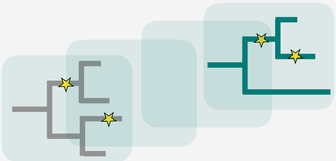
Antibody evolution



reconstruction of phylogenetic trees



inferring mutation statistics



simulating of antibody repertoire evolution

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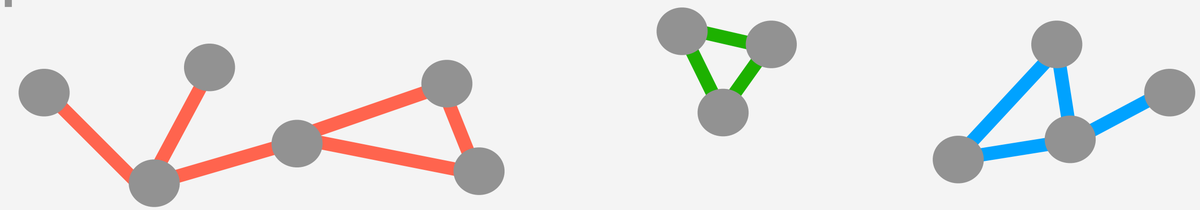

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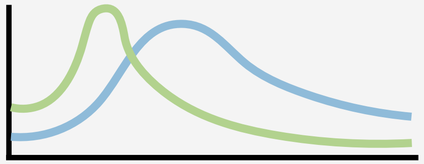

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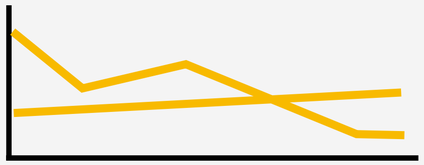

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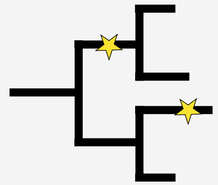

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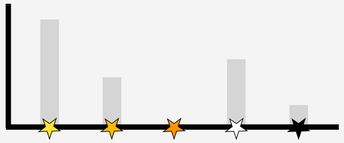

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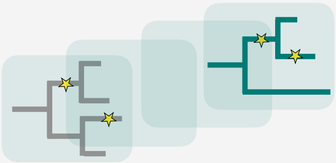

simulating of antibody repertoire evolution

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exploring cross-individual sequence similarity and convergence

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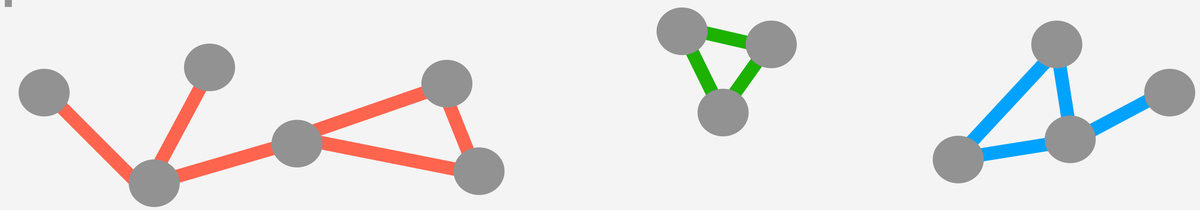

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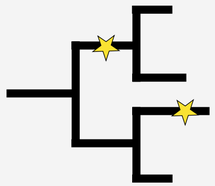

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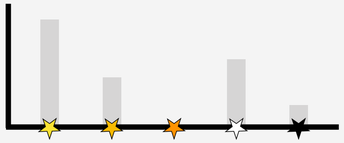

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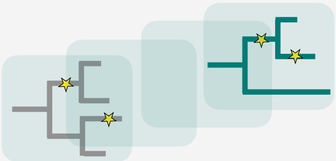

simulating of antibody repertoire evolution

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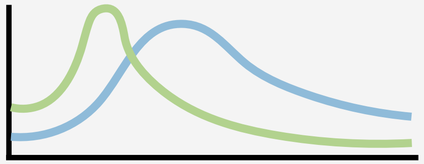

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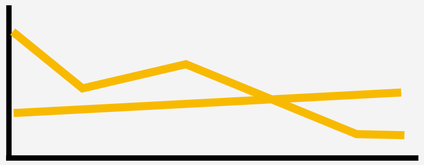

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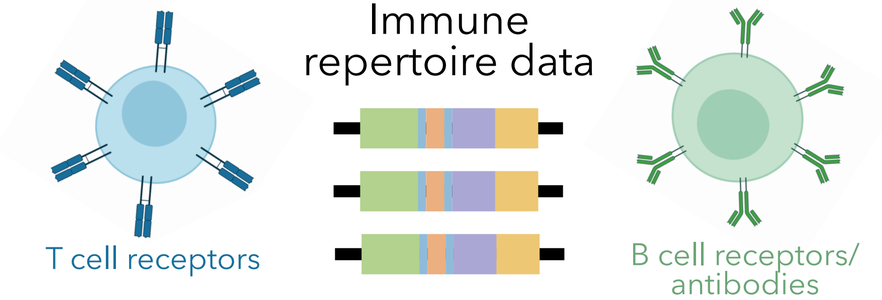
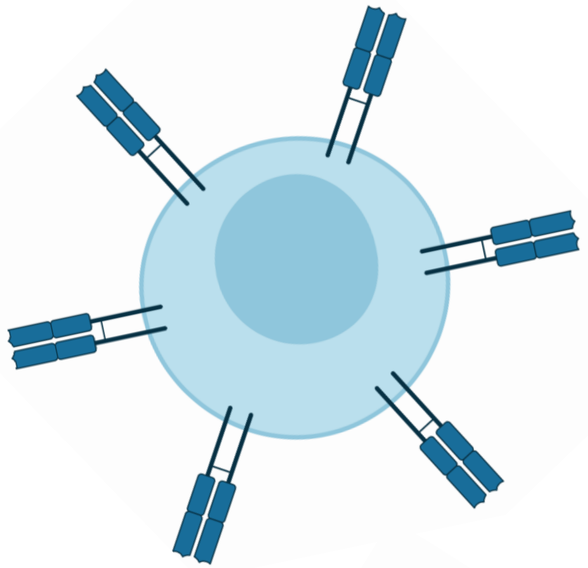
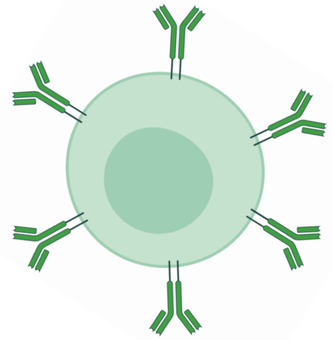


Molecular convergence

exploring cross-individual sequence similarity and convergence

Lecture goals:





1. learn about immune repertoire sequencing
    - what are immune repertoires?
    - how are they formed?
    - how are they sequenced?
    - what are some common areas of repertoire analysis?
2. familiarize with immune repertoire data
3. work through an example analysis