

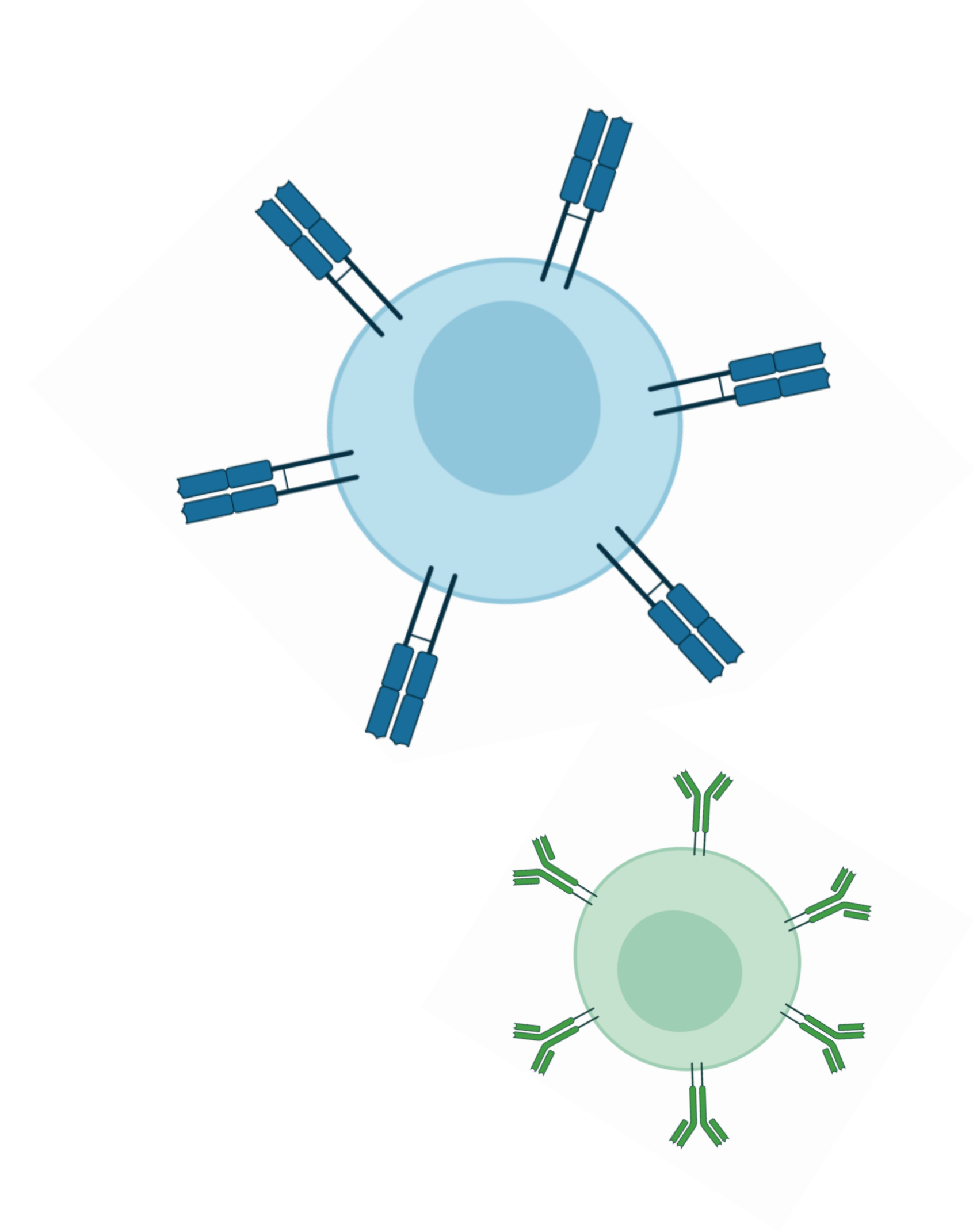
Intro to immune repertoire sequencing and analysis

Maggie Russell

TFCB 2023

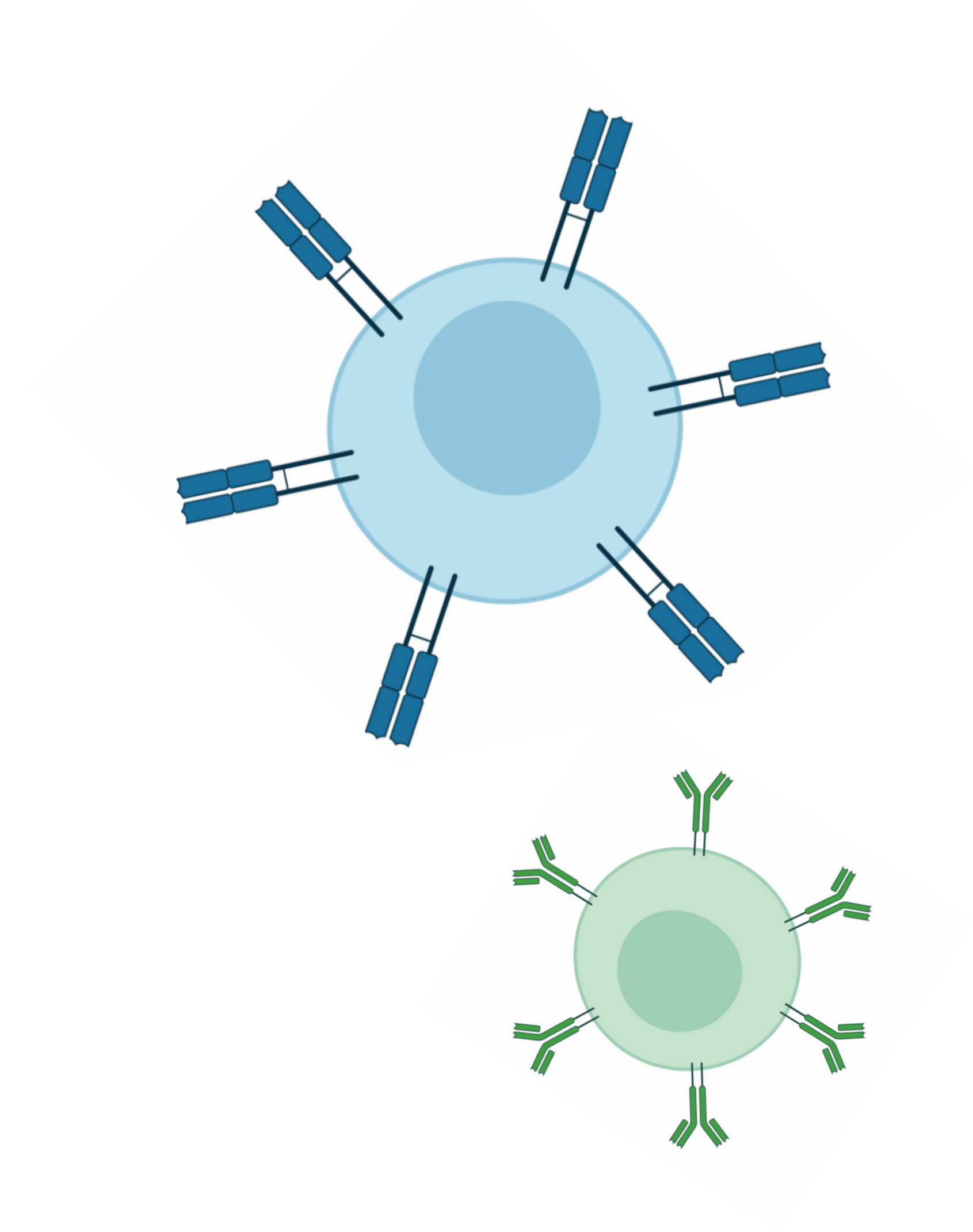
Lecture goals:

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



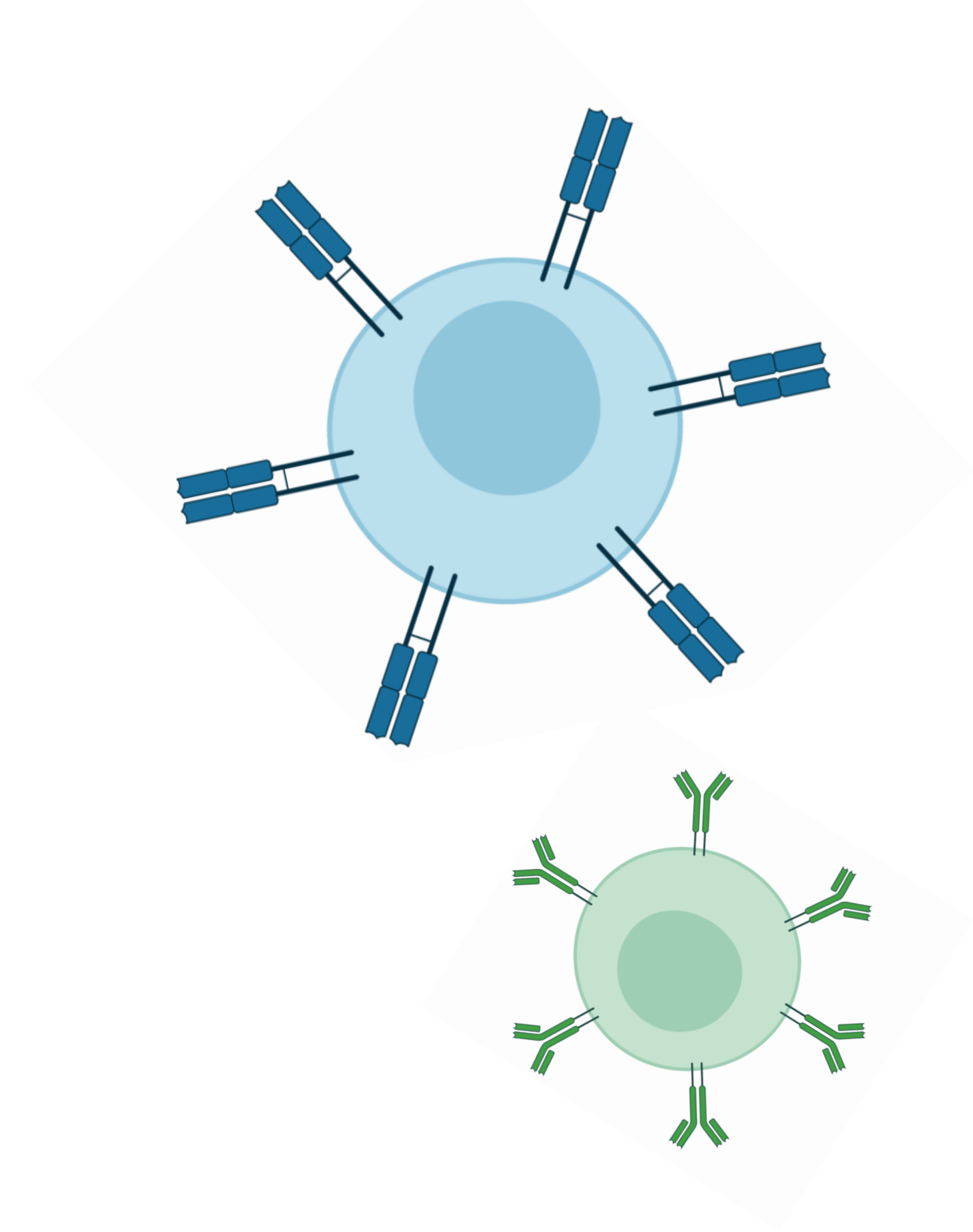
Lecture goals:

1. learn about immune repertoire sequencing

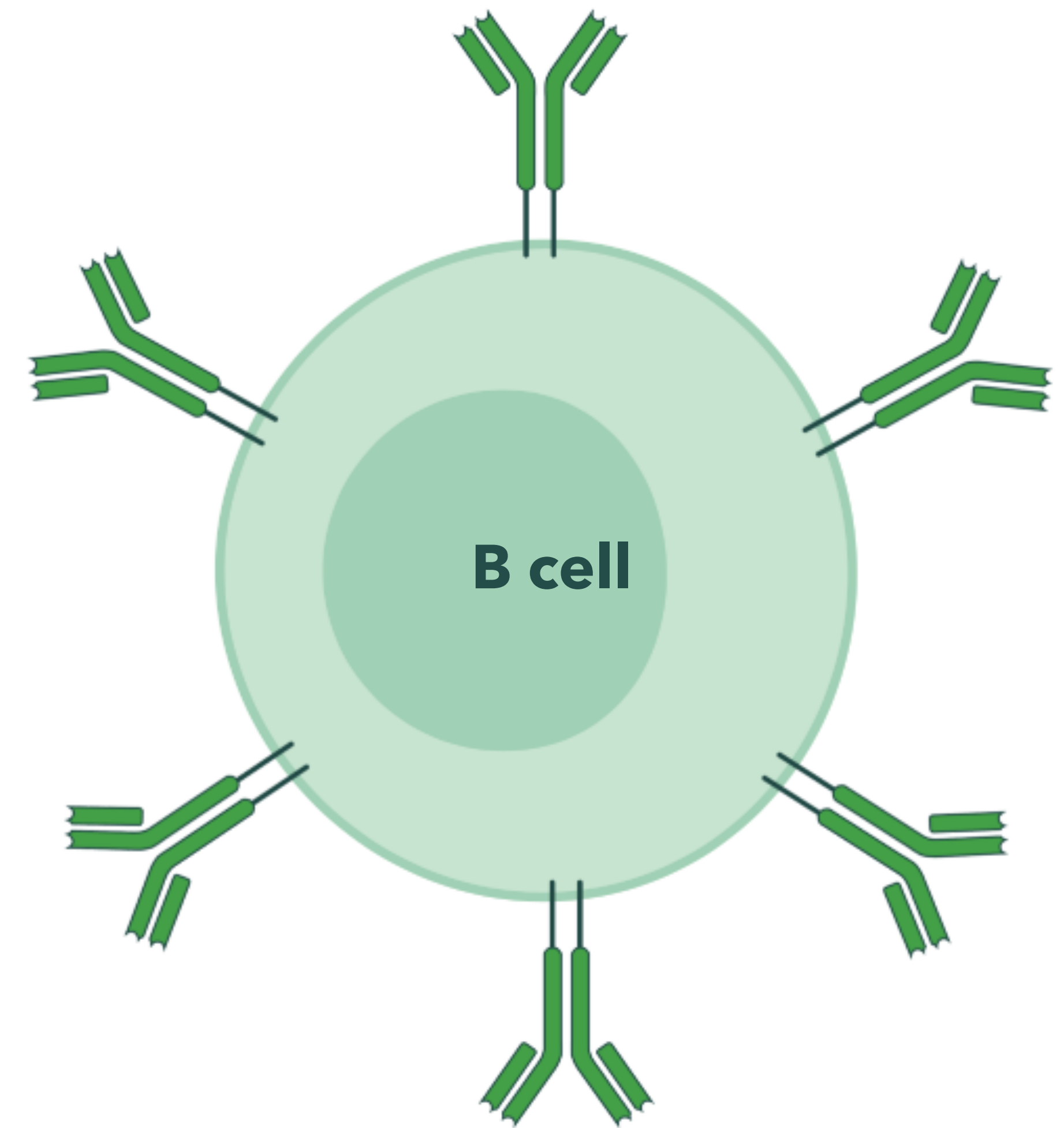
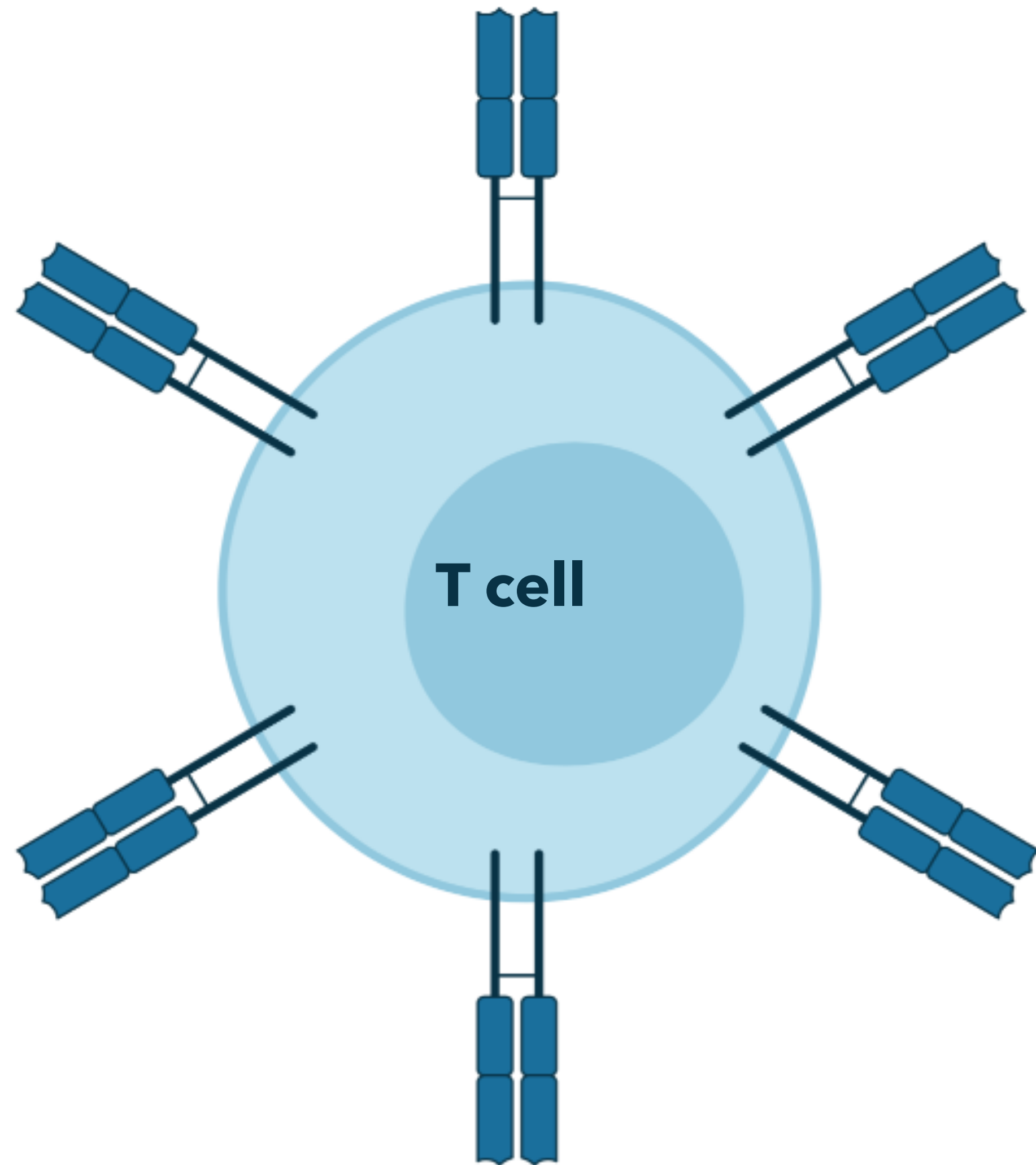


Lecture goals:

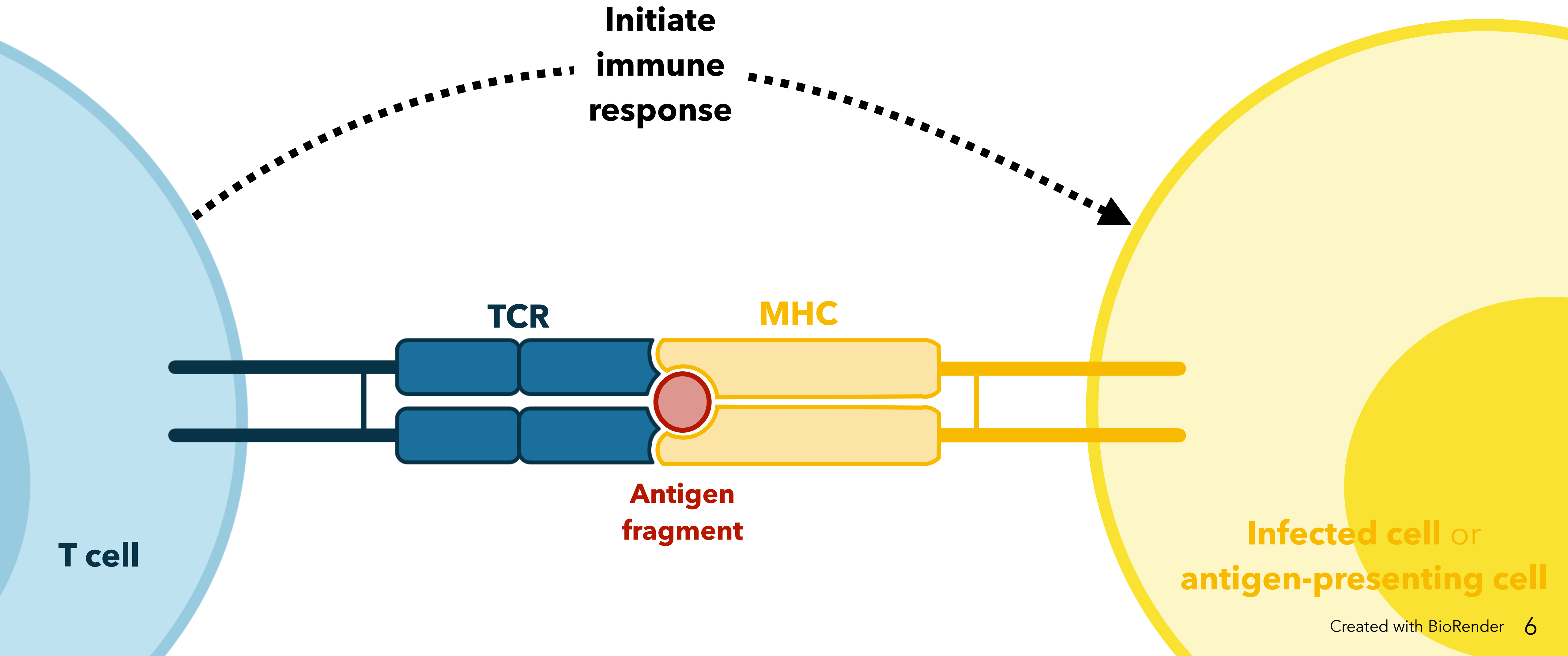
1. learn about immune repertoire sequencing
 - what are immune repertoires?



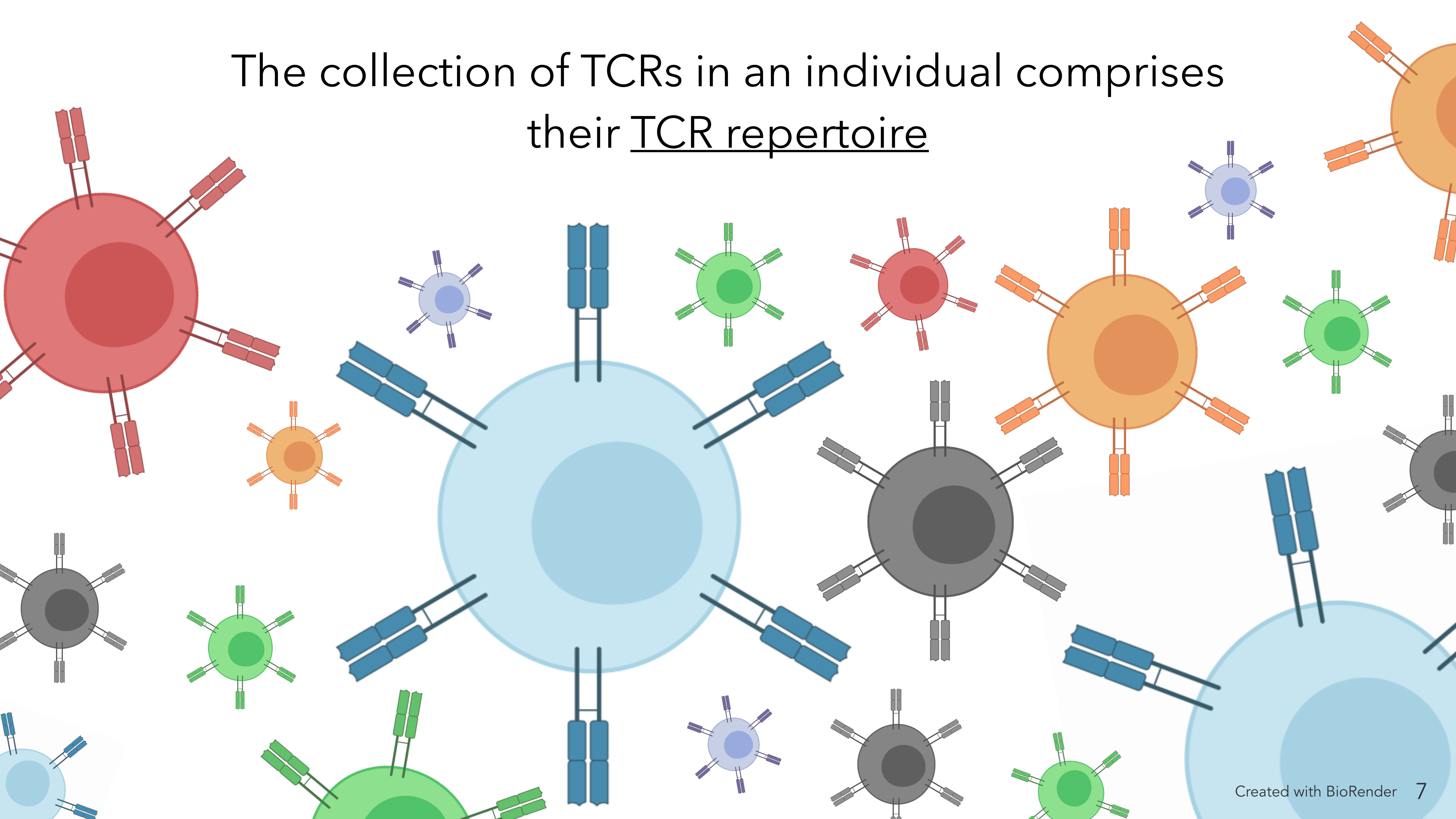
Adaptive immunity is essential for defending against pathogens



T cell receptors recognize antigen fragments bound to MHC

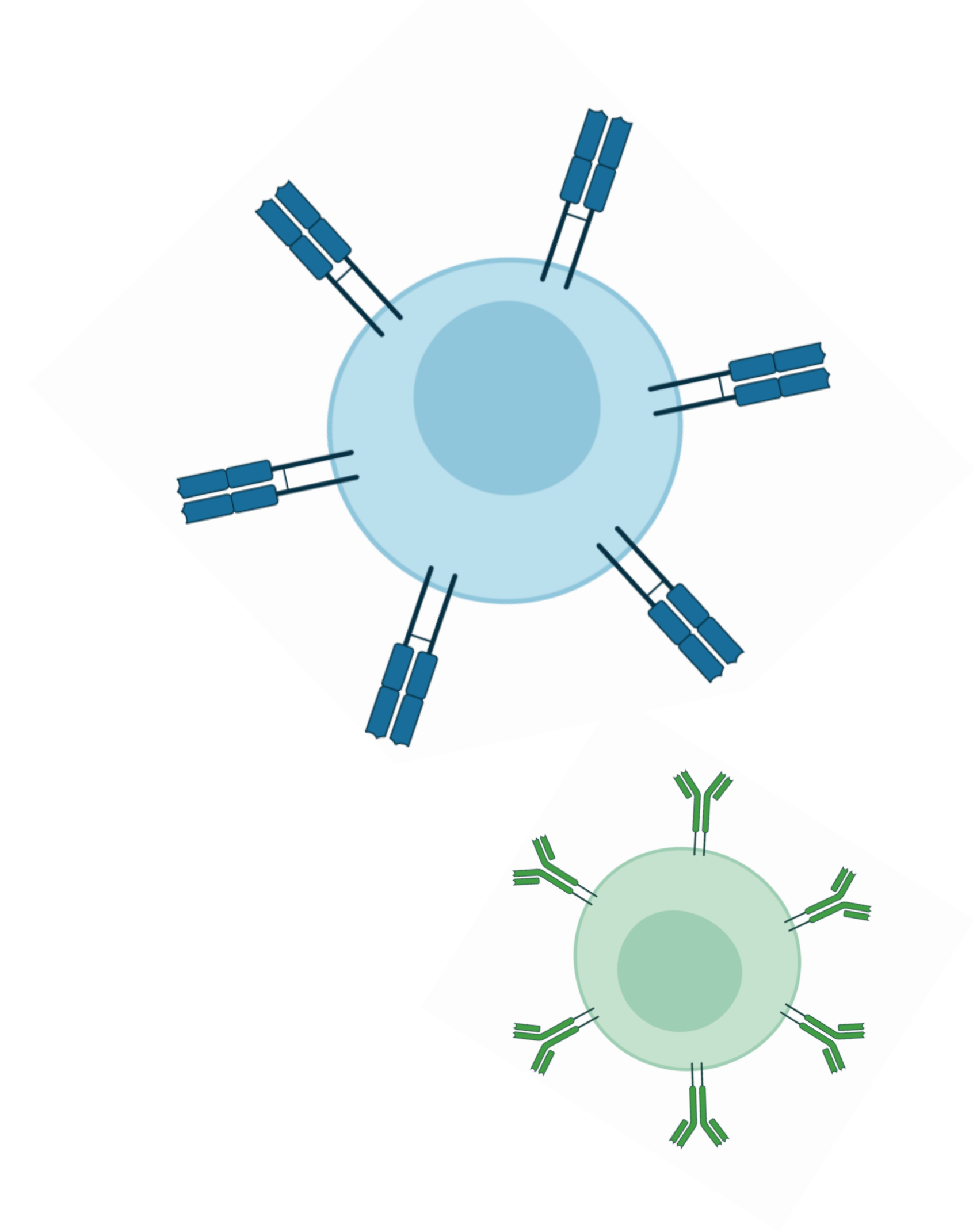


The collection of TCRs in an individual comprises
their TCR repertoire



Lecture goals:

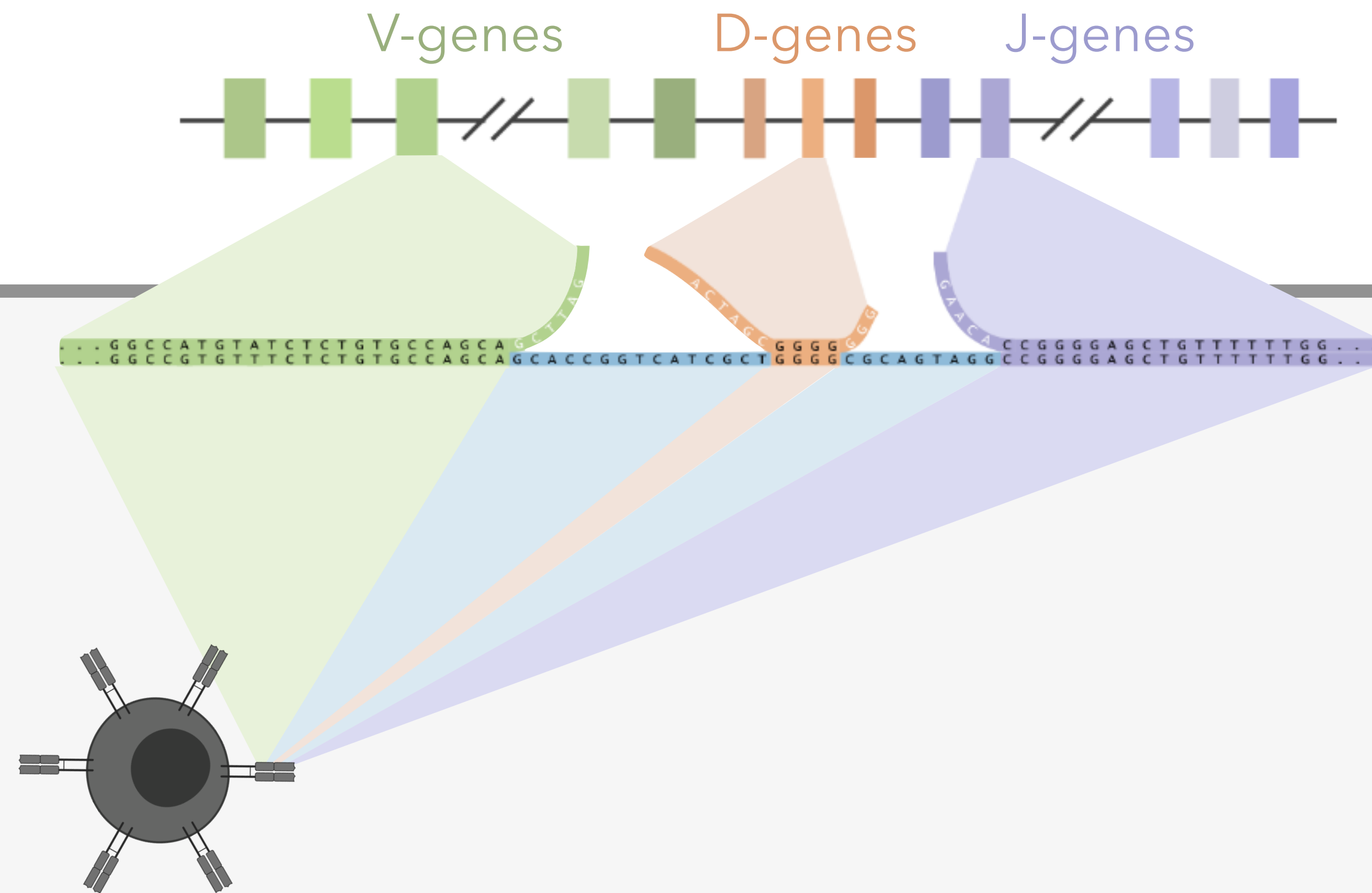
1. learn about immune repertoire sequencing
 - what are immune repertoires?
 - **how are they formed?**



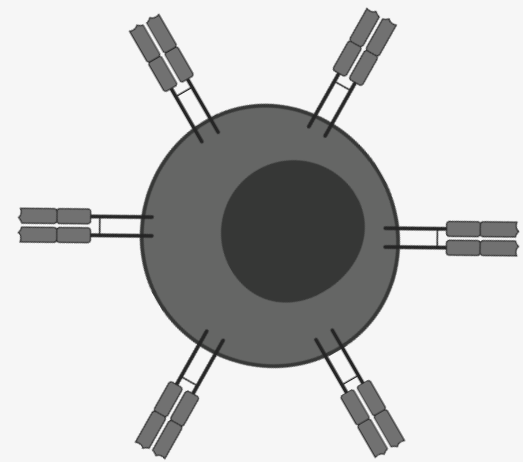
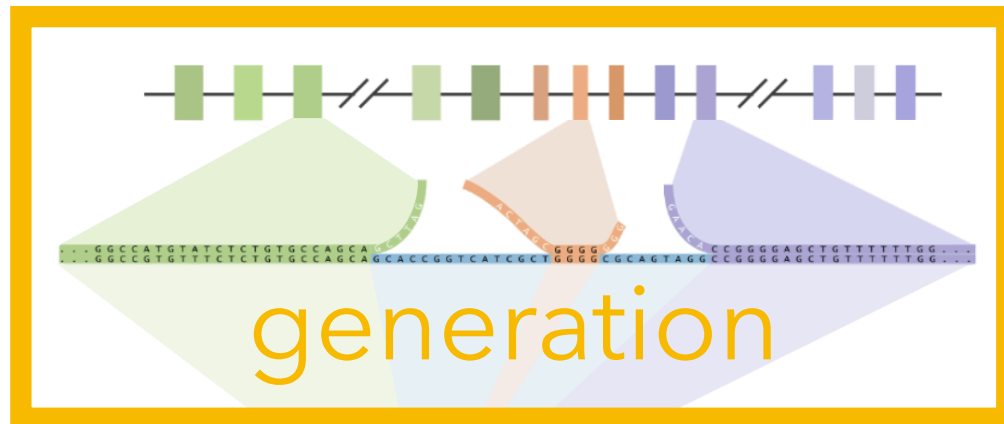
Repertoire composition is influenced by
generation, selection, and exposures

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

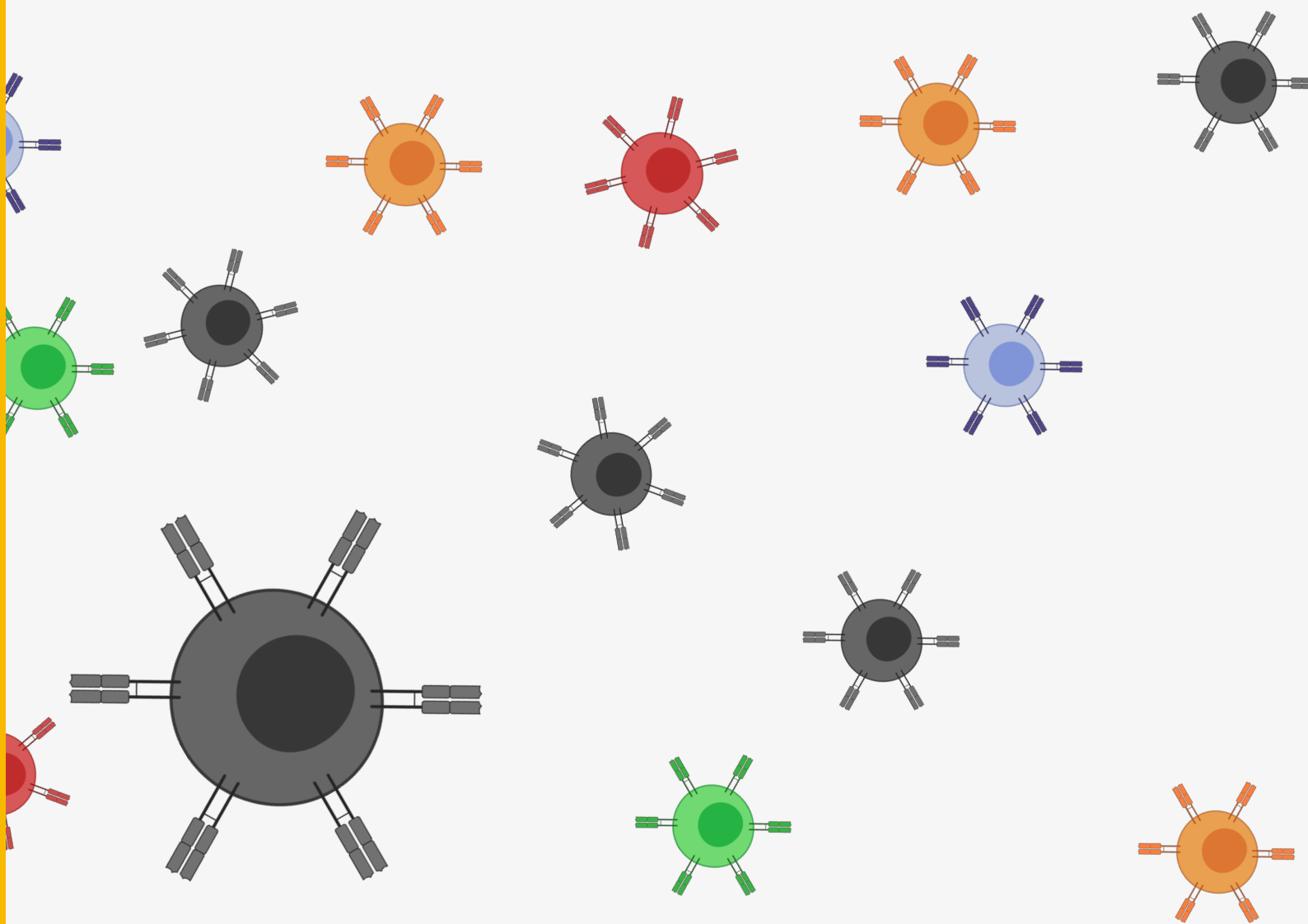
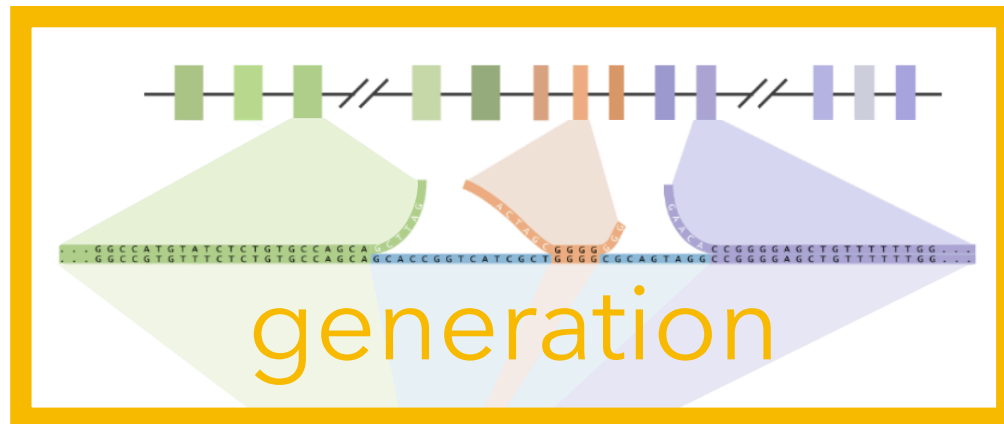
Repertoire composition is influenced by
generation, selection, and exposures



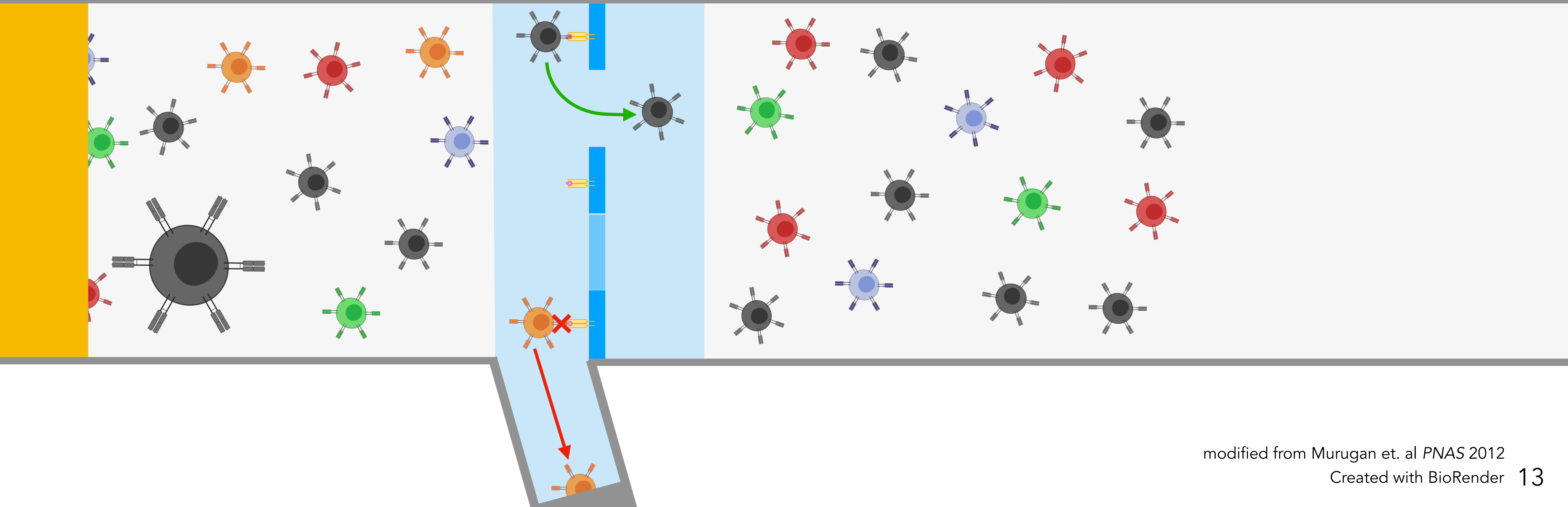
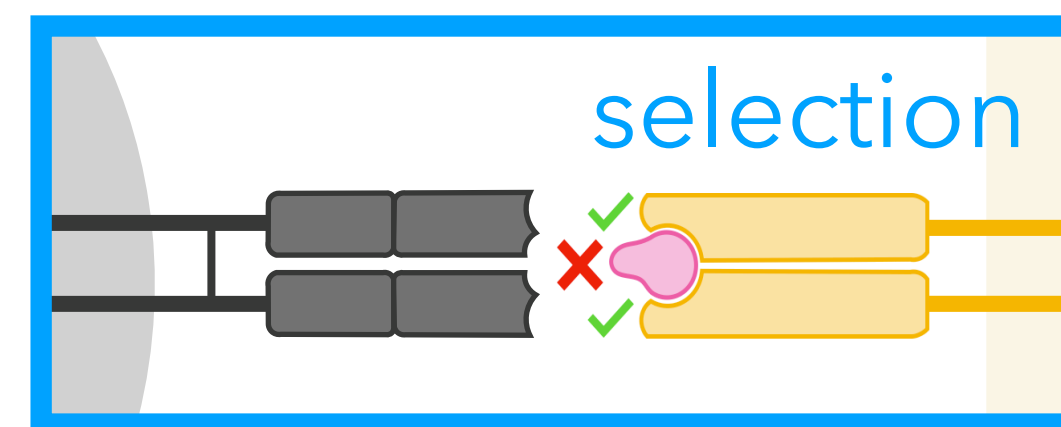
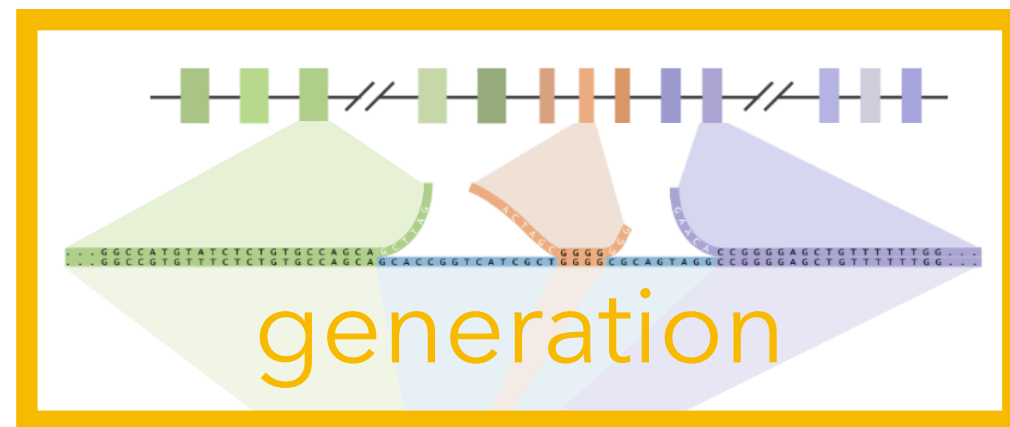
Repertoire composition is influenced by **generation**, selection, and exposures



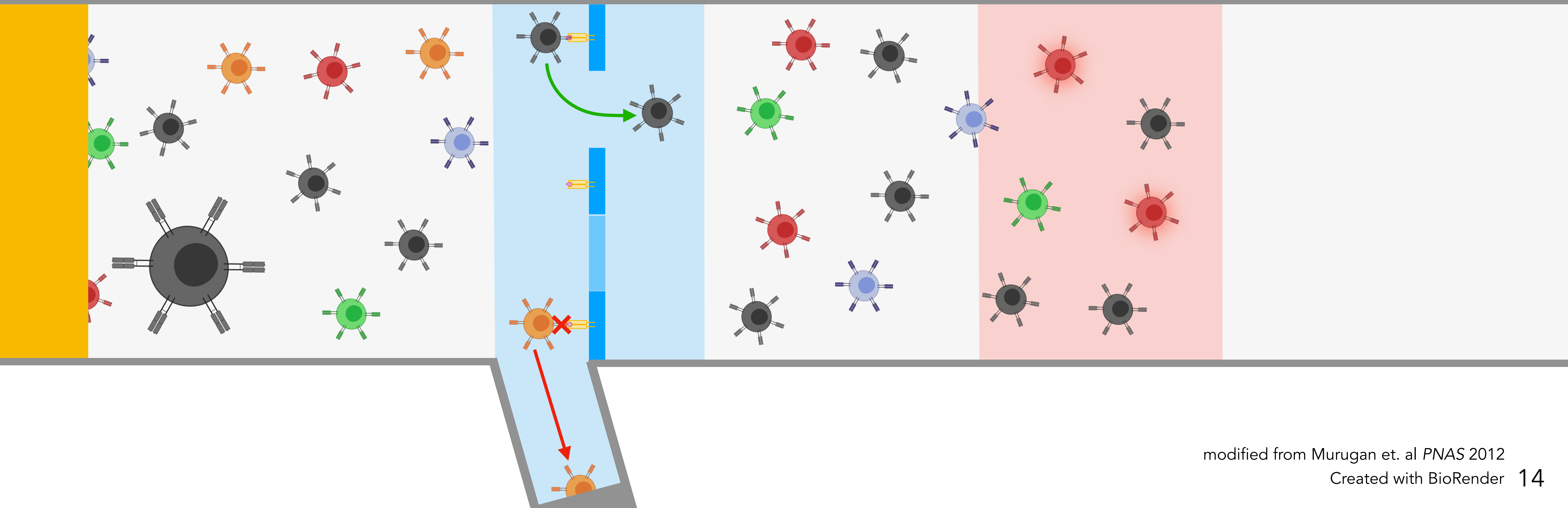
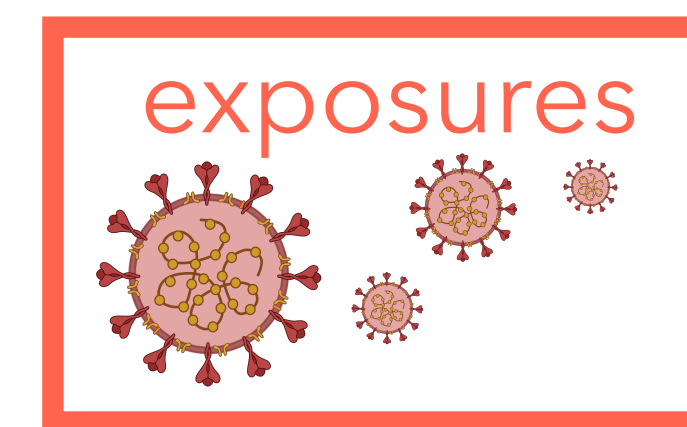
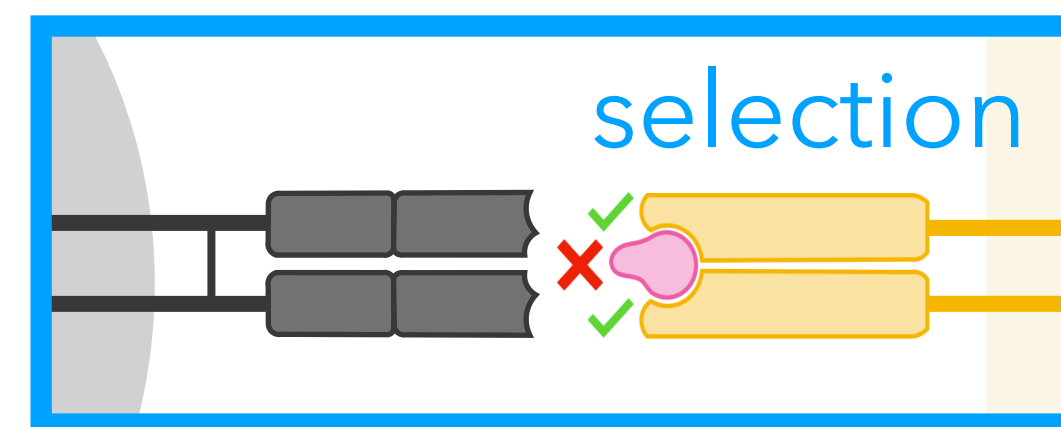
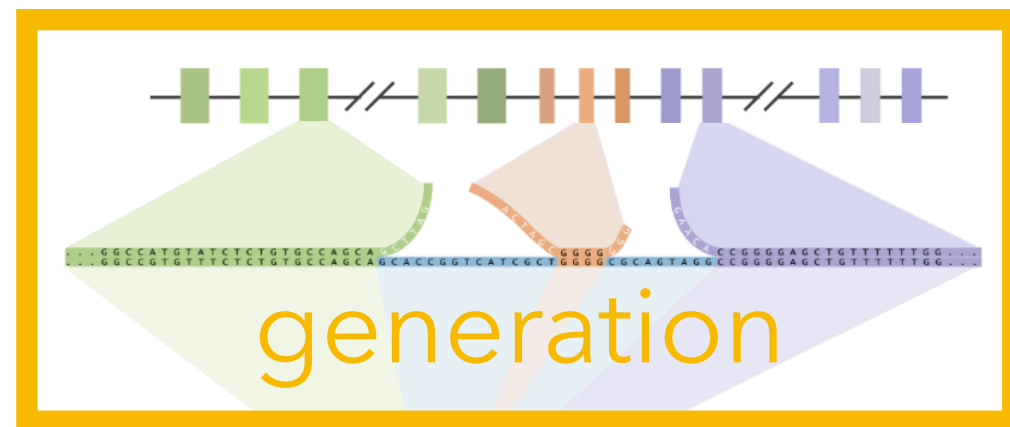
Repertoire composition is influenced by **generation**, selection, and exposures



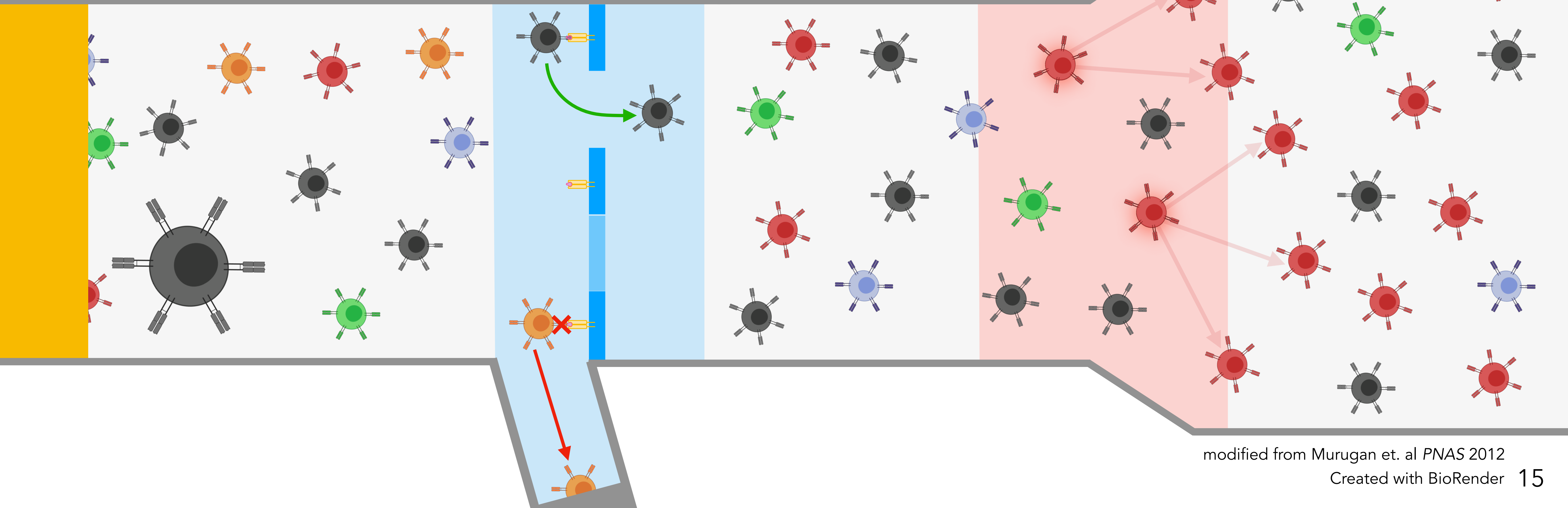
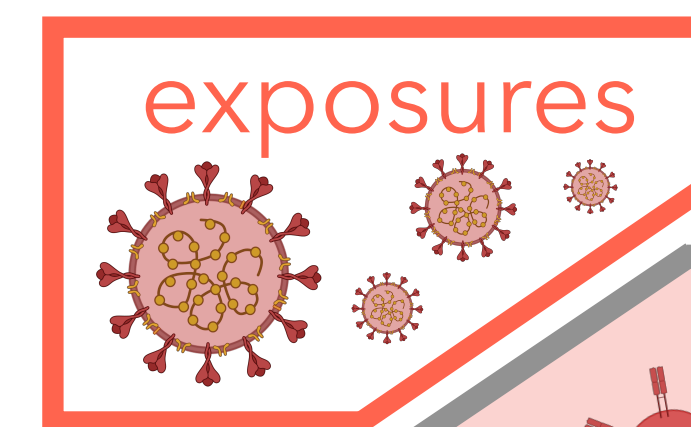
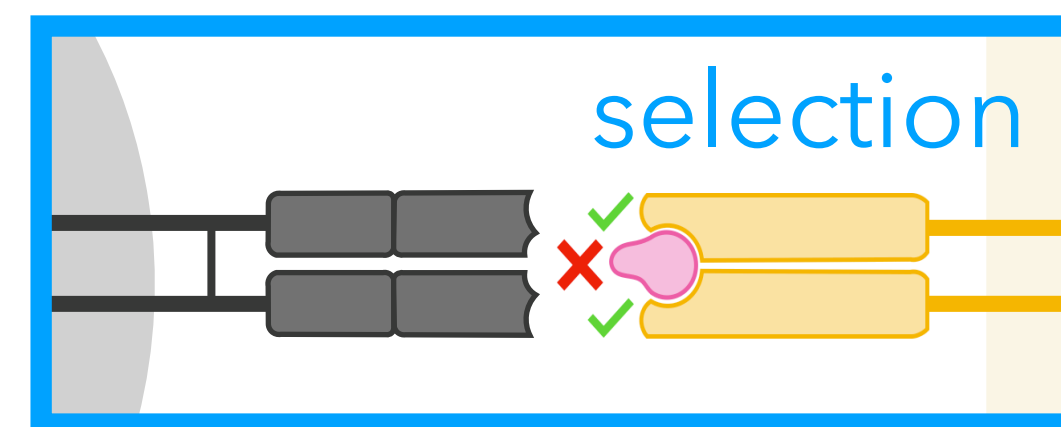
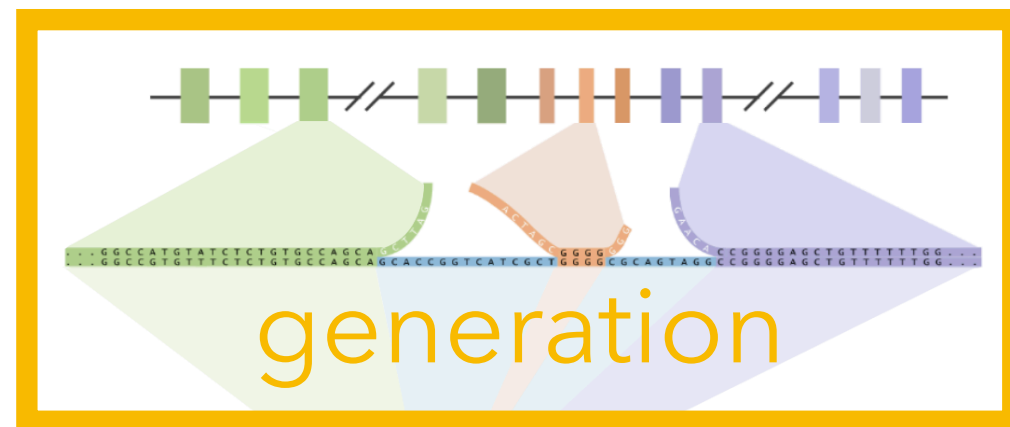
Repertoire composition is influenced by generation, **selection**, and exposures



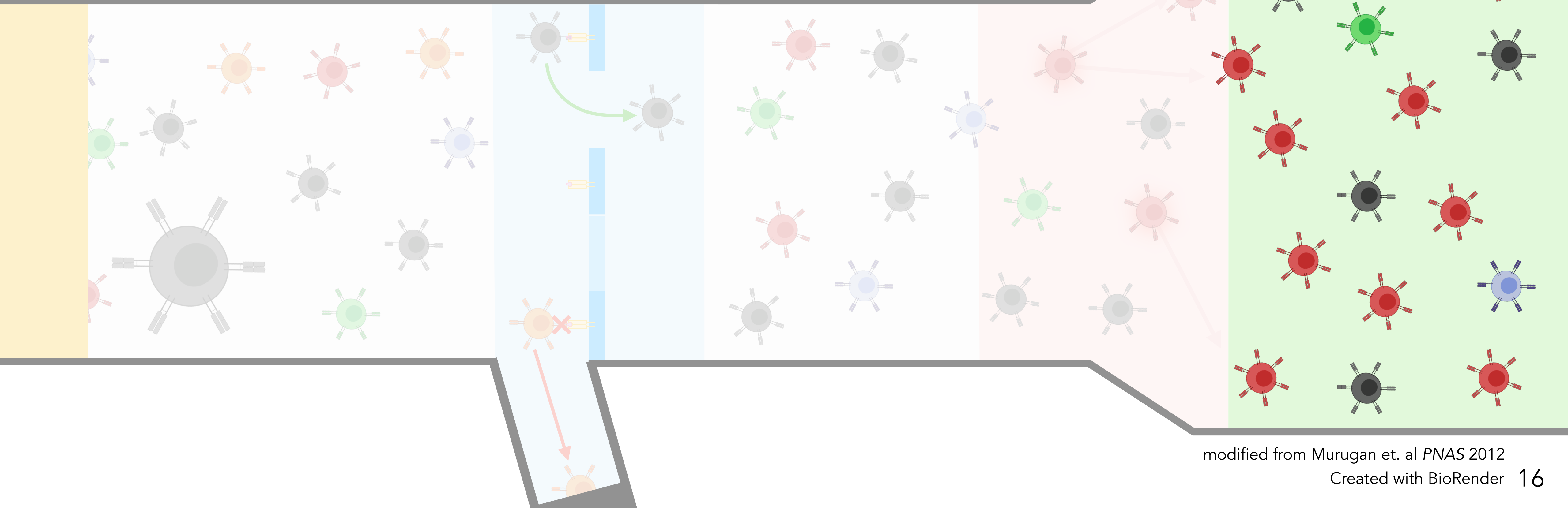
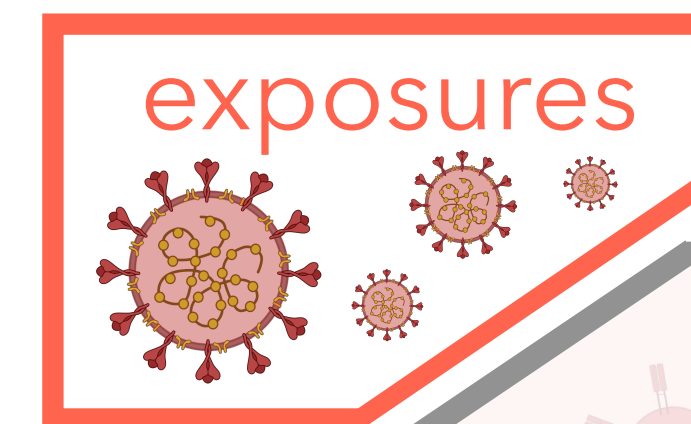
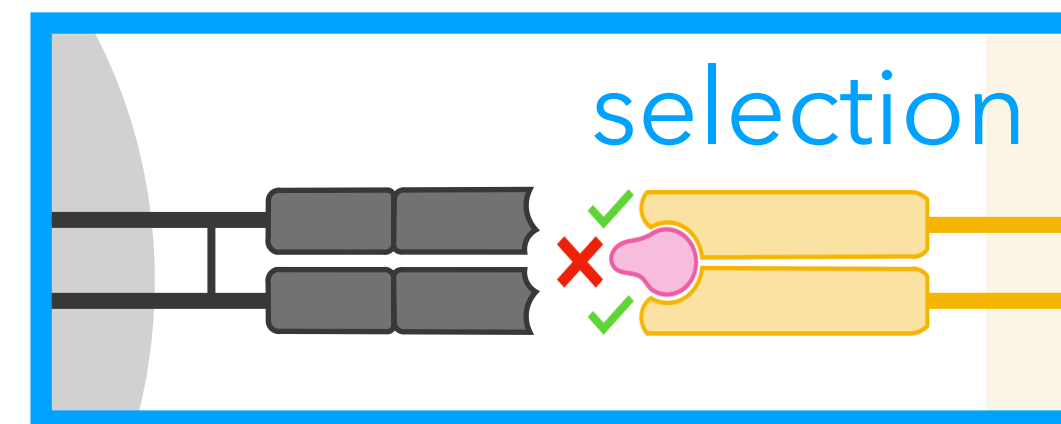
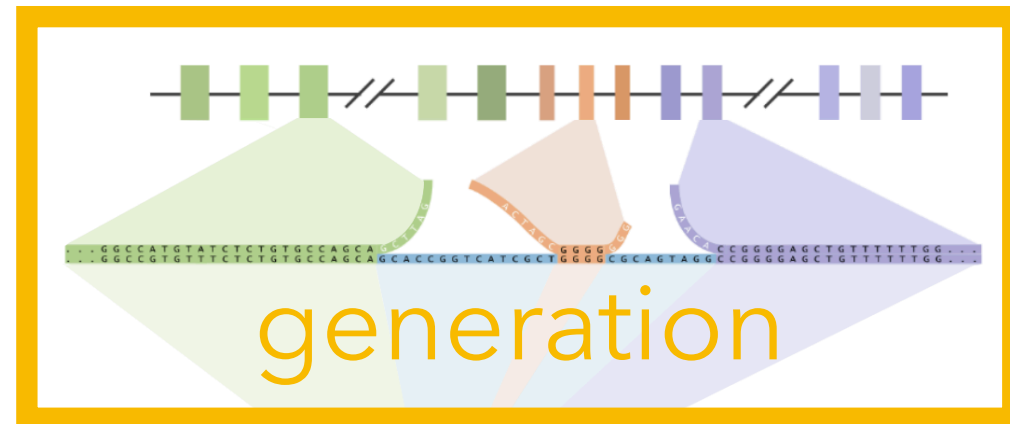
Repertoire composition is influenced by generation, selection, and **exposures**



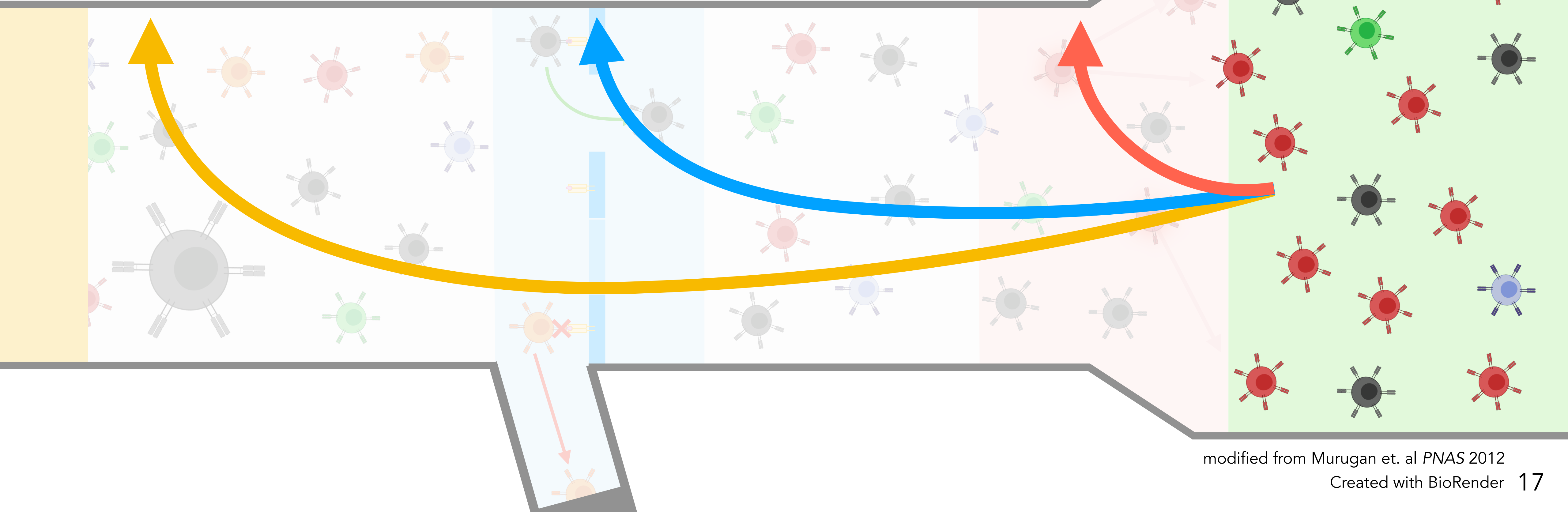
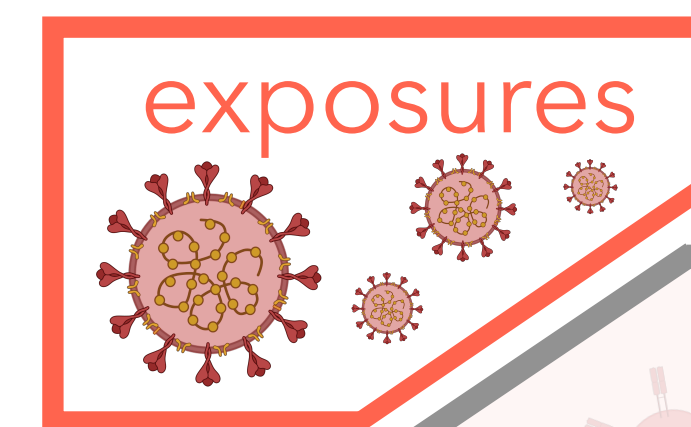
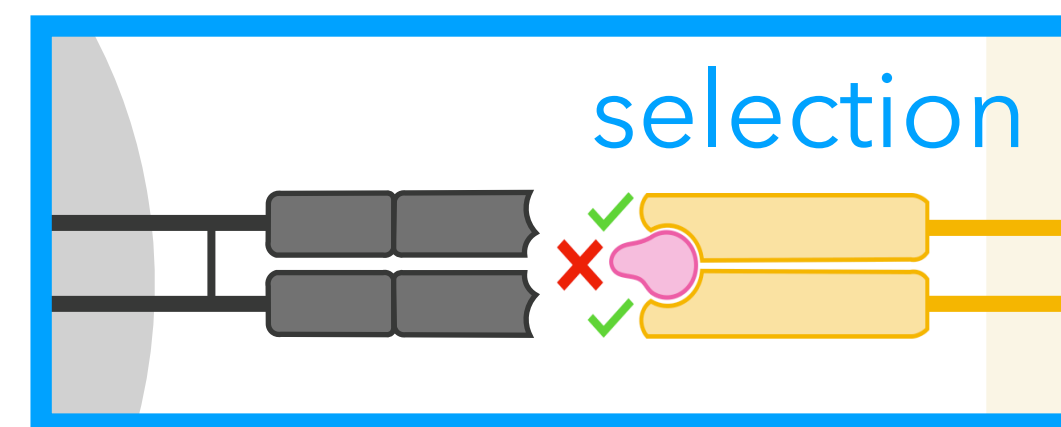
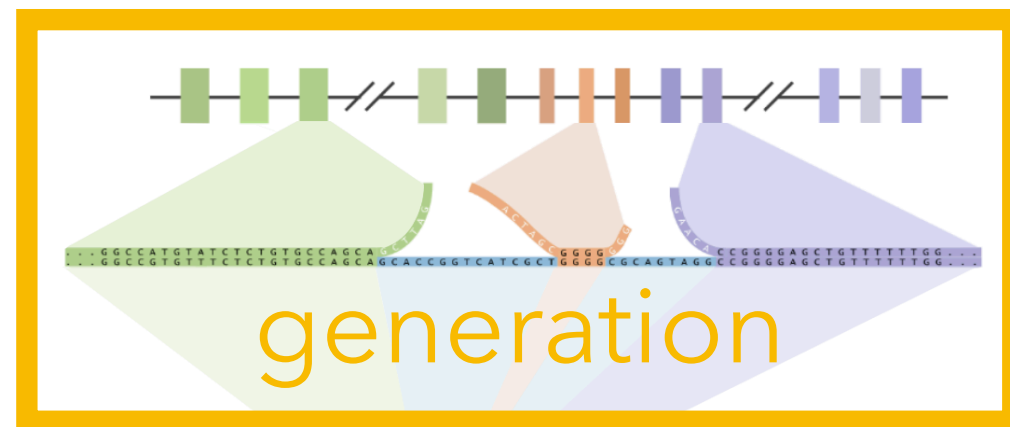
Repertoire composition is influenced by generation, selection, and **exposures**



We can sample a **repertoire** using sequencing

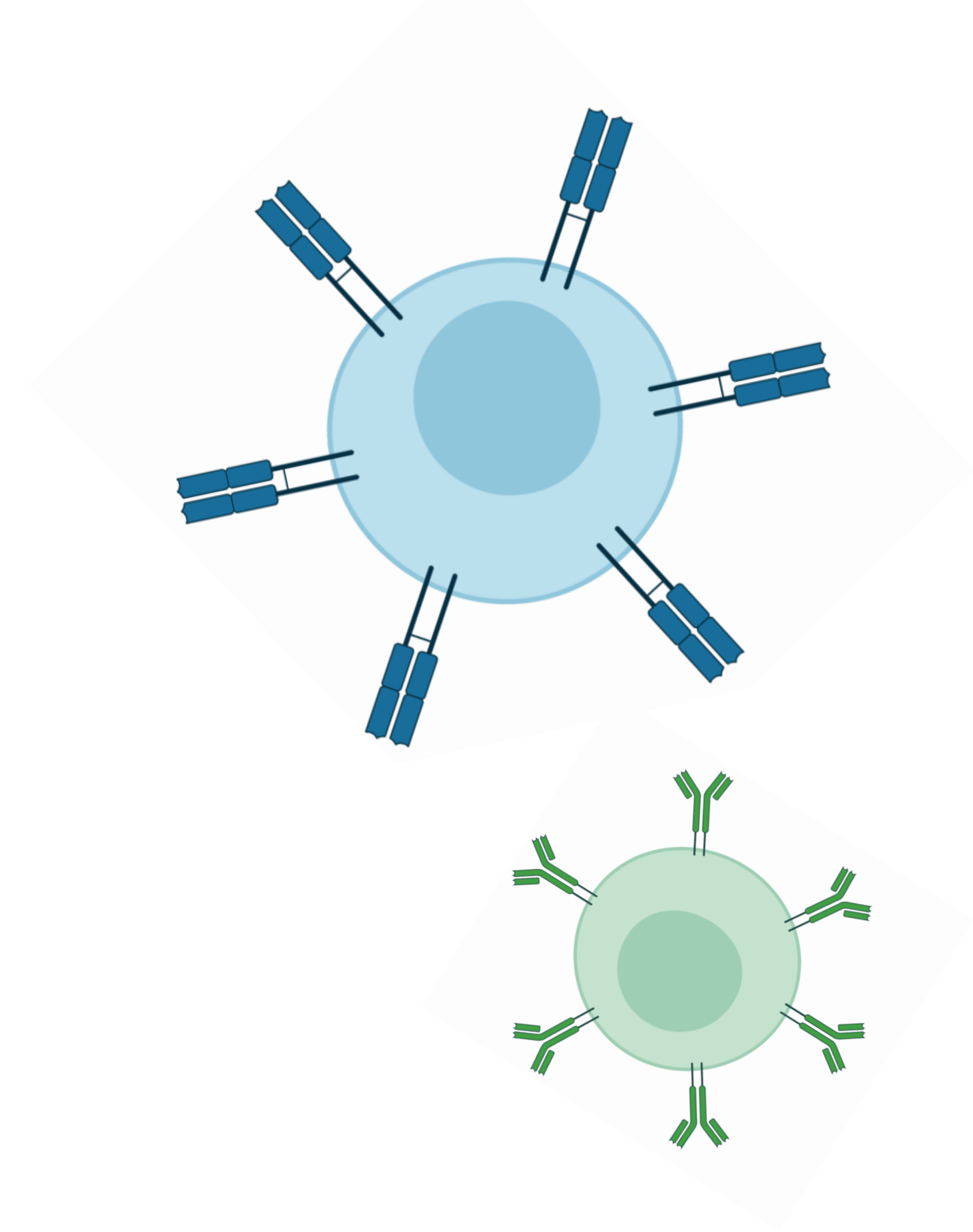


We can use **repertoire statistics** to infer previous dynamics



Lecture goals:

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



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

	Bulk	Single-cell
Sample size <i>(e.g. total # of sampled cells)</i>		
Repertoire coverage <i>(e.g. proportion of total repertoire sequenced)</i>		
Chain pairing <i>(e.g. each receptor consists of two protein chains)</i>		
Cost		

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

	Bulk	Single-cell
Sample size <i>(e.g. total # of sampled cells)</i>	Large	Small
Repertoire coverage <i>(e.g. proportion of total repertoire sequenced)</i>		
Chain pairing <i>(e.g. each receptor consists of two protein chains)</i>		
Cost		

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

	Bulk	Single-cell
Sample size <i>(e.g. total # of sampled cells)</i>	Large	Small
Repertoire coverage <i>(e.g. proportion of total repertoire sequenced)</i>	High	Low
Chain pairing <i>(e.g. each receptor consists of two protein chains)</i>		
Cost		

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

	Bulk	Single-cell
Sample size <i>(e.g. total # of sampled cells)</i>	Large	Small
Repertoire coverage <i>(e.g. proportion of total repertoire sequenced)</i>	High	Low
Chain pairing <i>(e.g. each receptor consists of two protein chains)</i>	No	Yes
Cost		

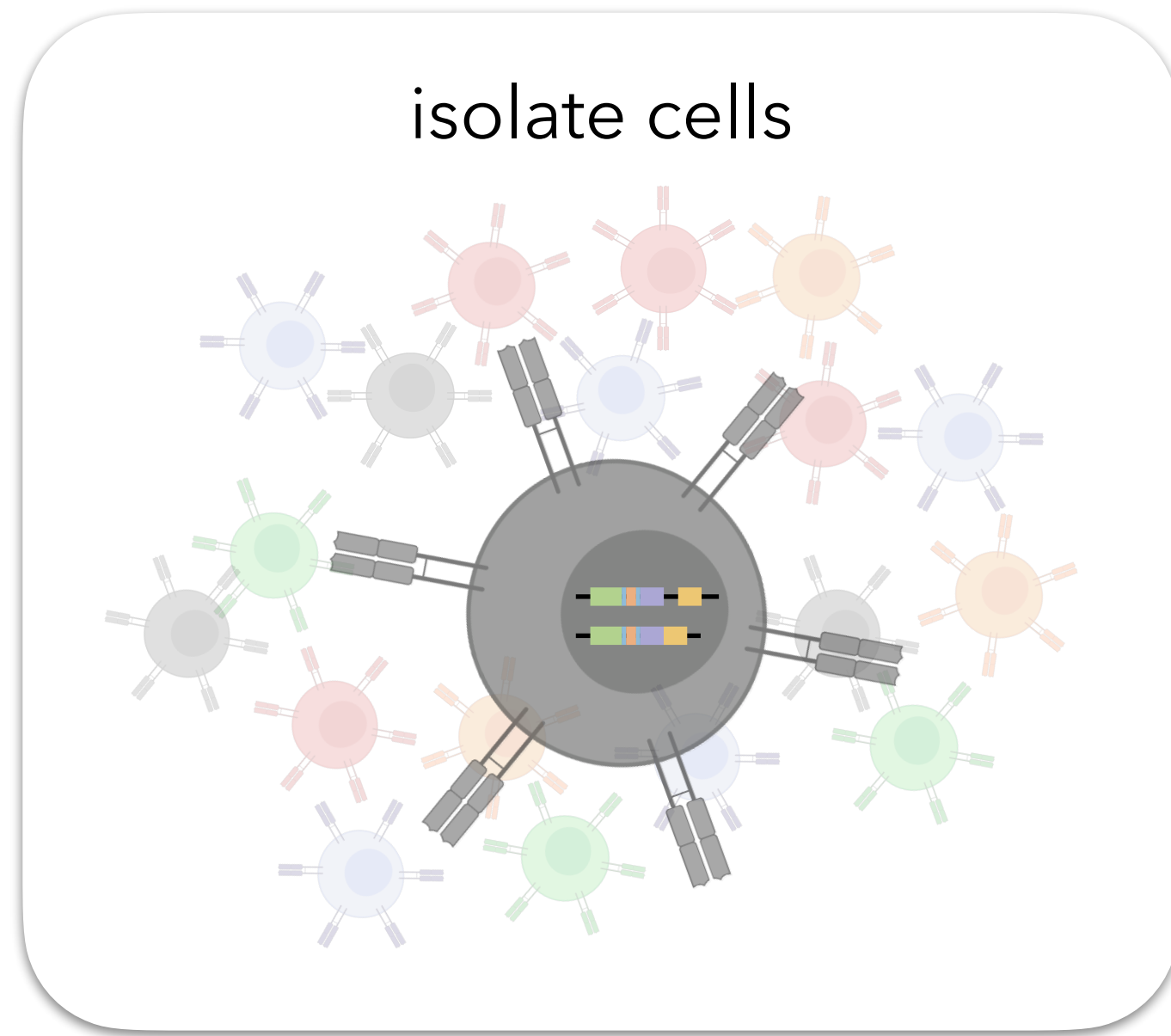
Both **bulk sequencing** and **single-cell sequencing** are possible

	Bulk	Single-cell
Sample size <i>(e.g. total # of sampled cells)</i>	Large	Small
Repertoire coverage <i>(e.g. proportion of total repertoire sequenced)</i>	High	Low
Chain pairing <i>(e.g. each receptor consists of two protein chains)</i>	No	Yes
Cost	Low	High

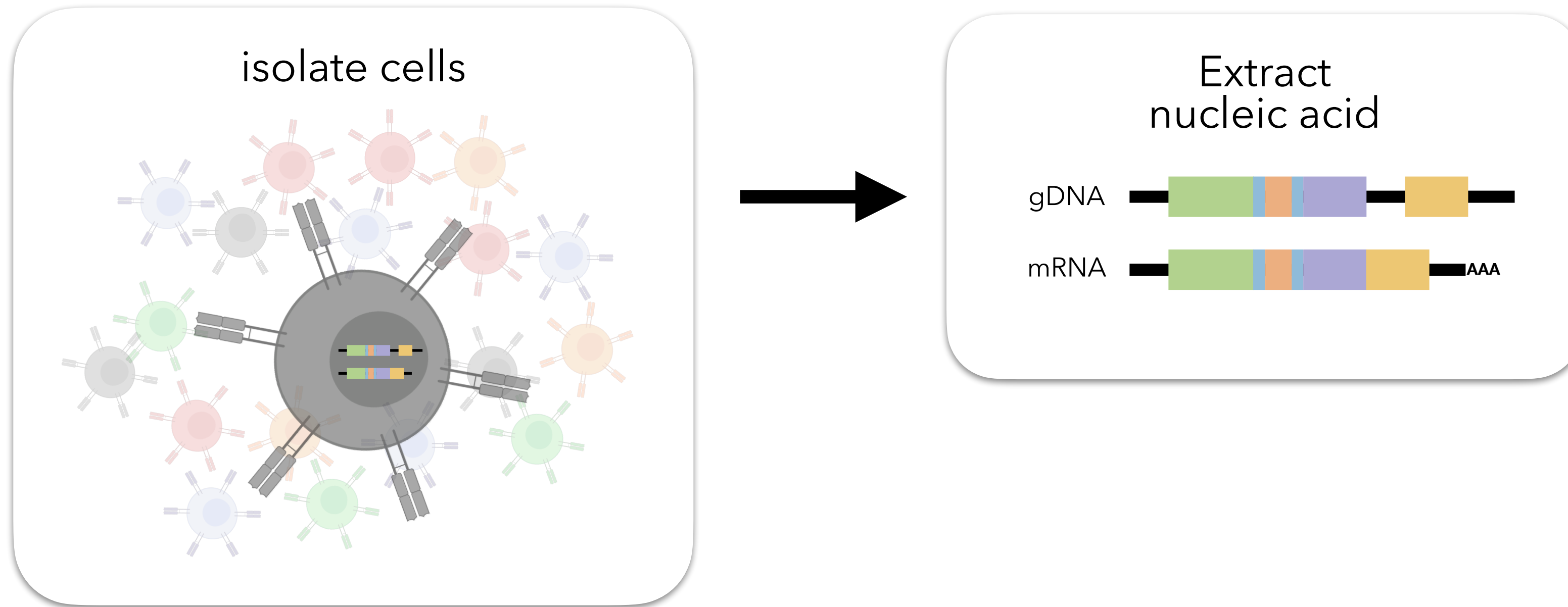
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	Bulk	Single-cell
Sample size <i>(e.g. total # of sampled cells)</i>	Large	Small
Repertoire coverage <i>(e.g. proportion of total repertoire sequenced)</i>	High	Low
Chain pairing <i>(e.g. each receptor consists of two protein chains)</i>	No	Yes
Cost	Low	High

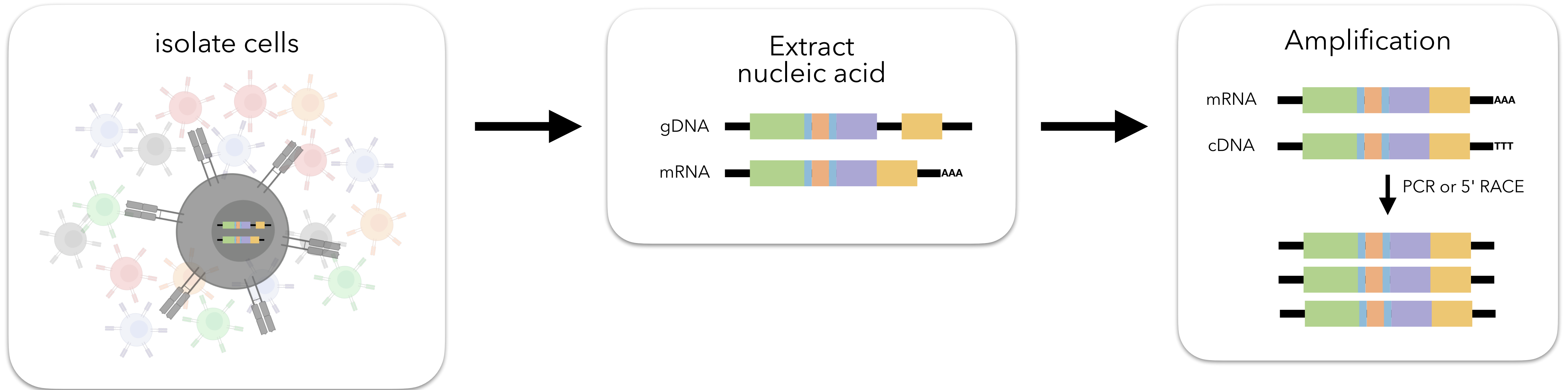
Bulk repertoire sequencing overview



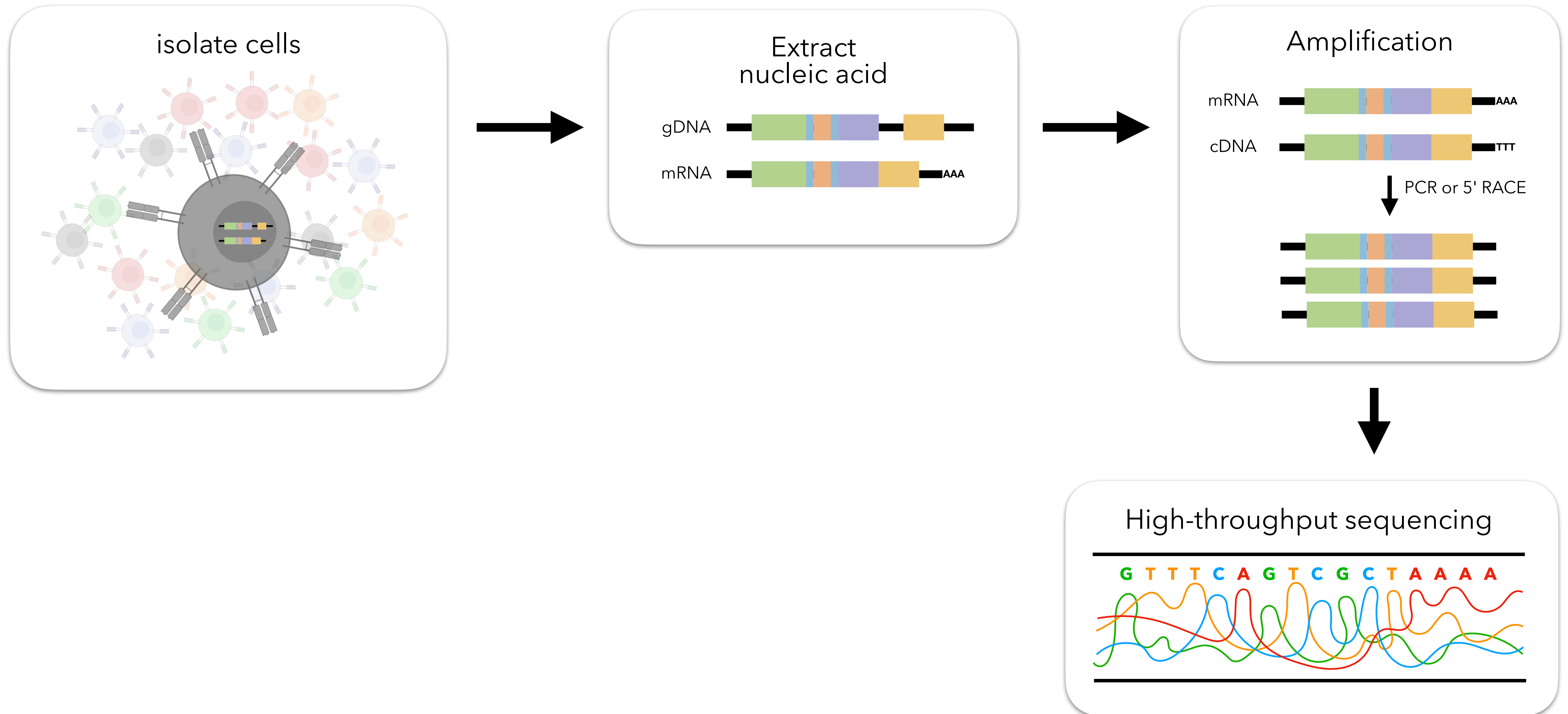
Bulk repertoire sequencing overview



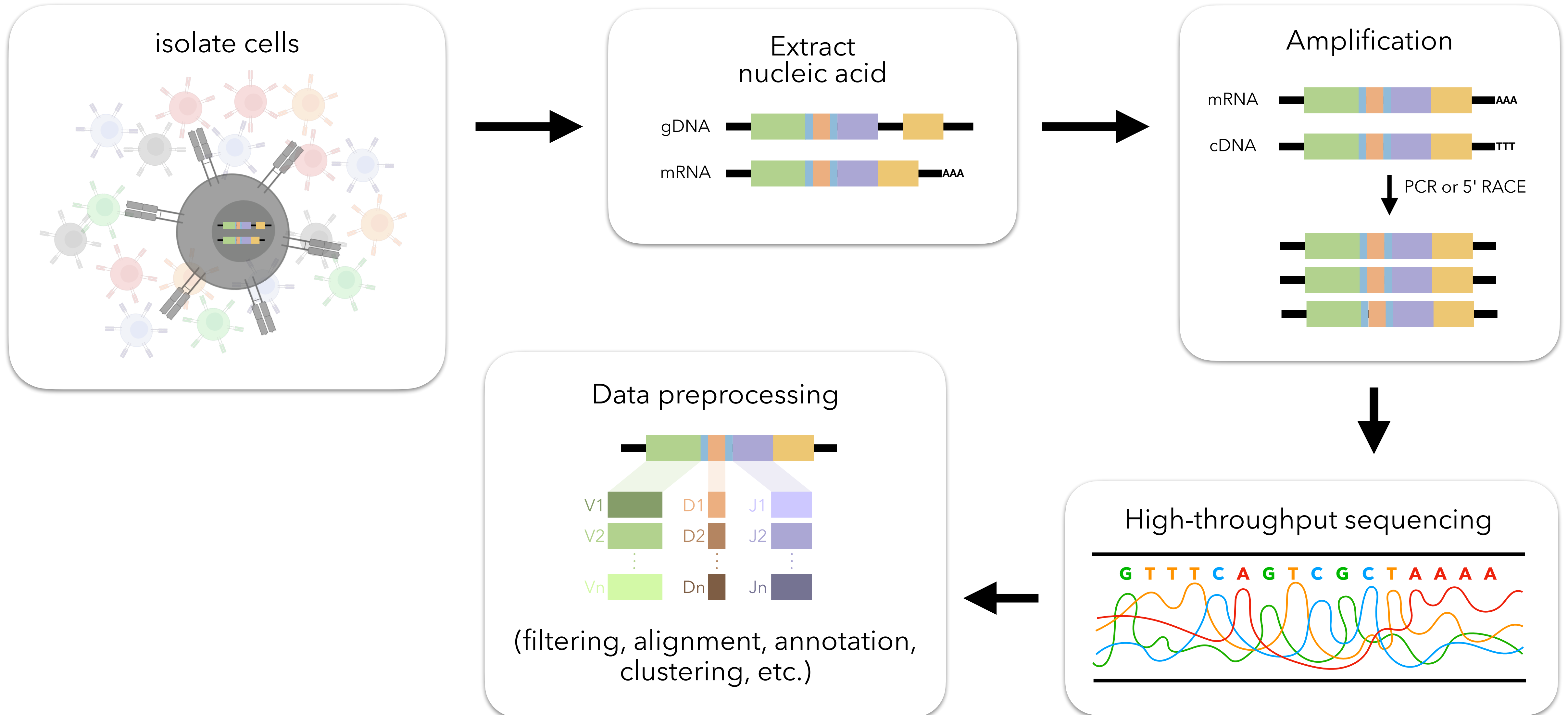
Bulk repertoire sequencing overview



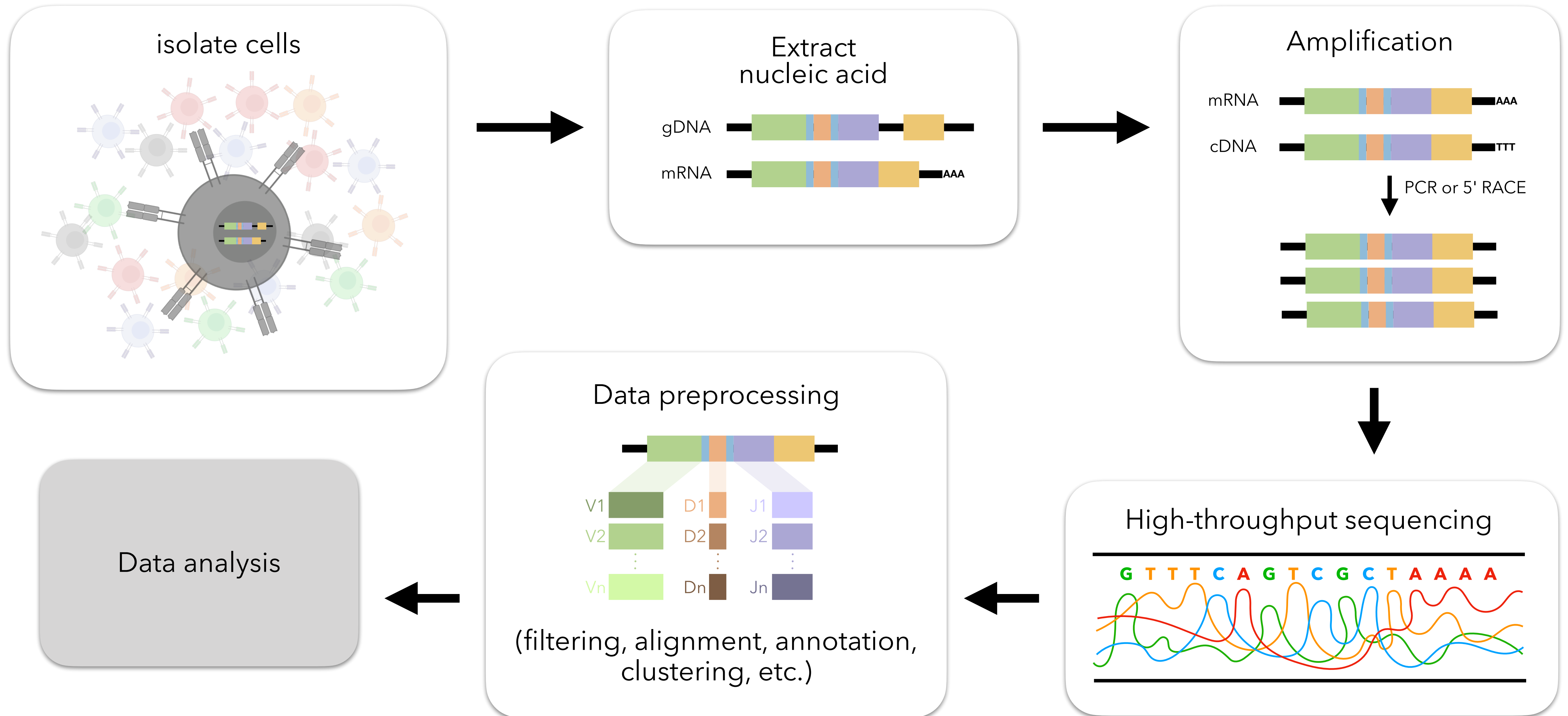
Bulk repertoire sequencing overview



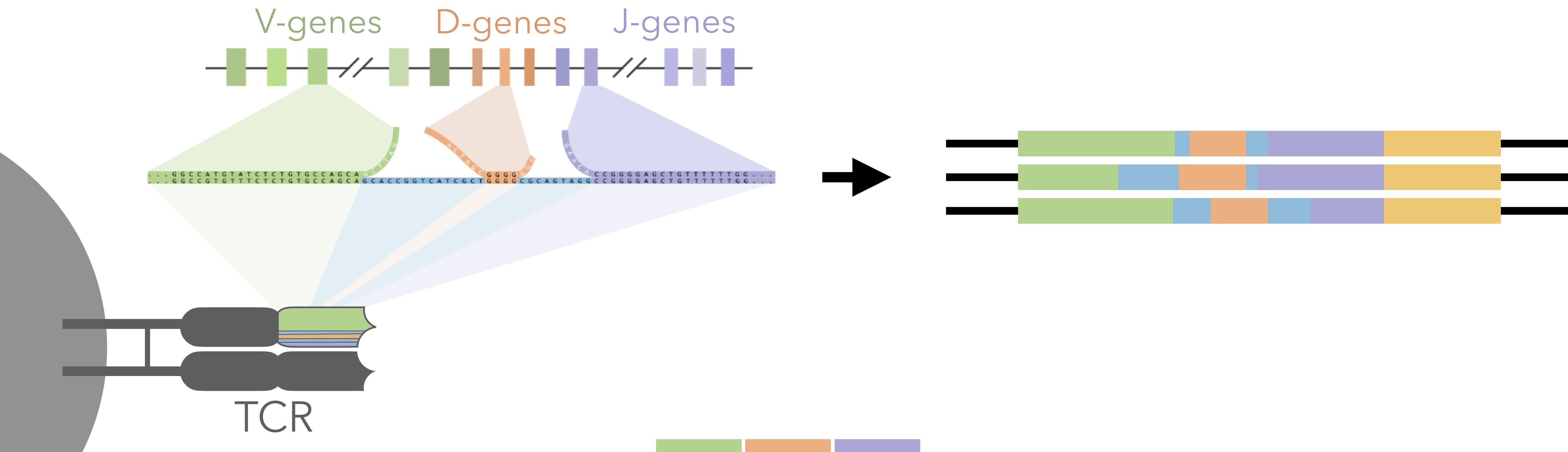
Bulk repertoire sequencing overview



Bulk repertoire sequencing overview

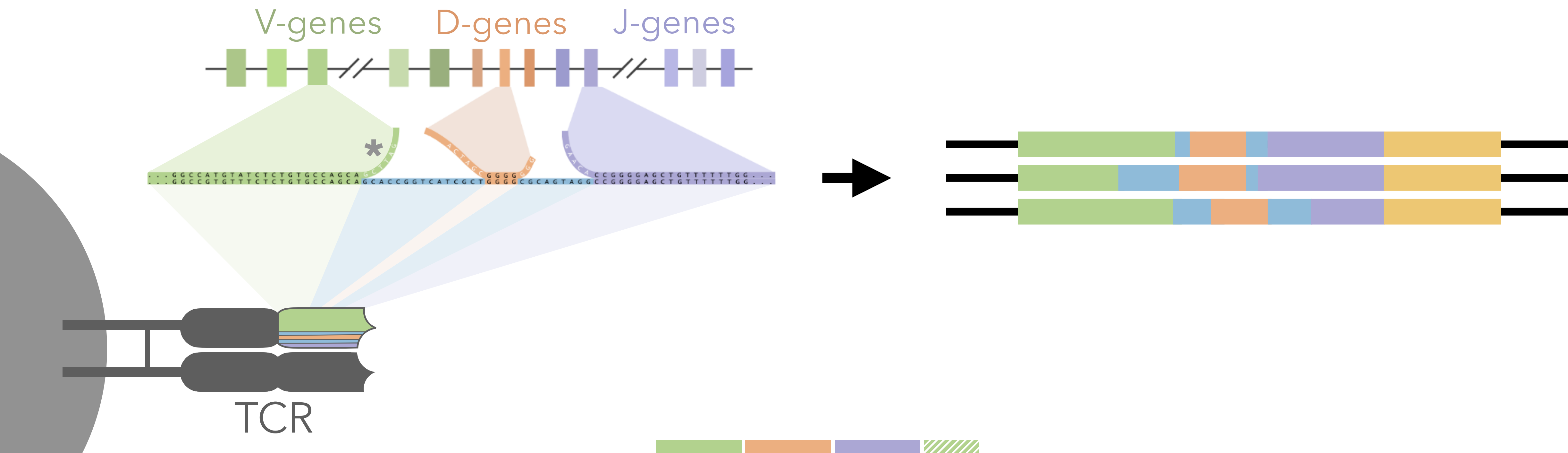


Processed bulk repertoire sequencing example output



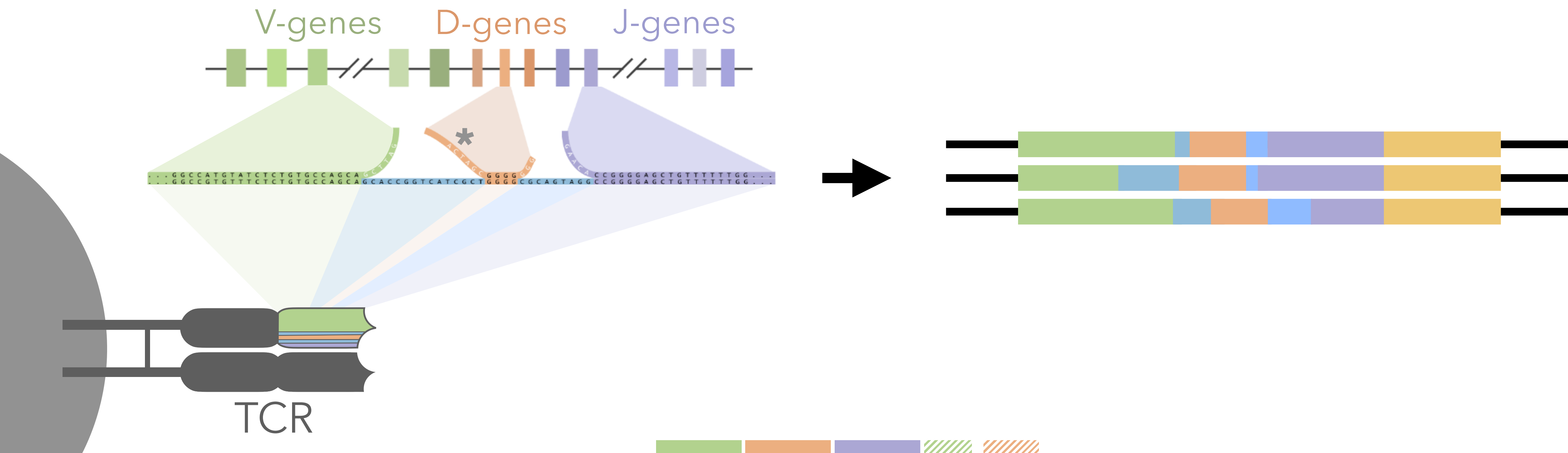
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TGTGCCAGCATAGCGGGAGGTGAGCAGTTCTTC	CASIAGGEQFF	TRBV28*01	TRBD2*02	TRBJ2-1*01
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Processed bulk repertoire sequencing example output



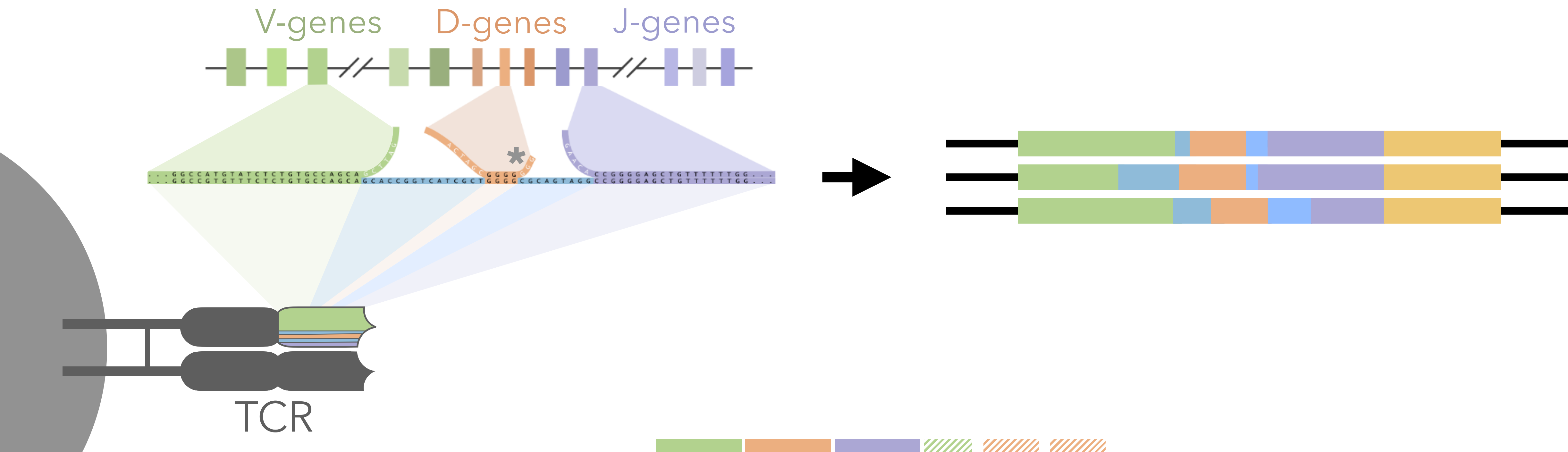
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TGTGCCAGCAGCTTAAATCTGGTGAGGTACGAGCAGTACTTC	CASSLNLVRYEQYF	TRBV7-2*01	TRBD2*02	TRBJ2-7*01	2
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TGTGCCAGCGGCCAGGGCTCGGATAACAATCAGCCCCAGCATTTT	CASGPGLGYNQPQHf	TRBV5-5*01	TRBD2*02	TRBJ1-5*01	7
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Processed bulk repertoire sequencing example output



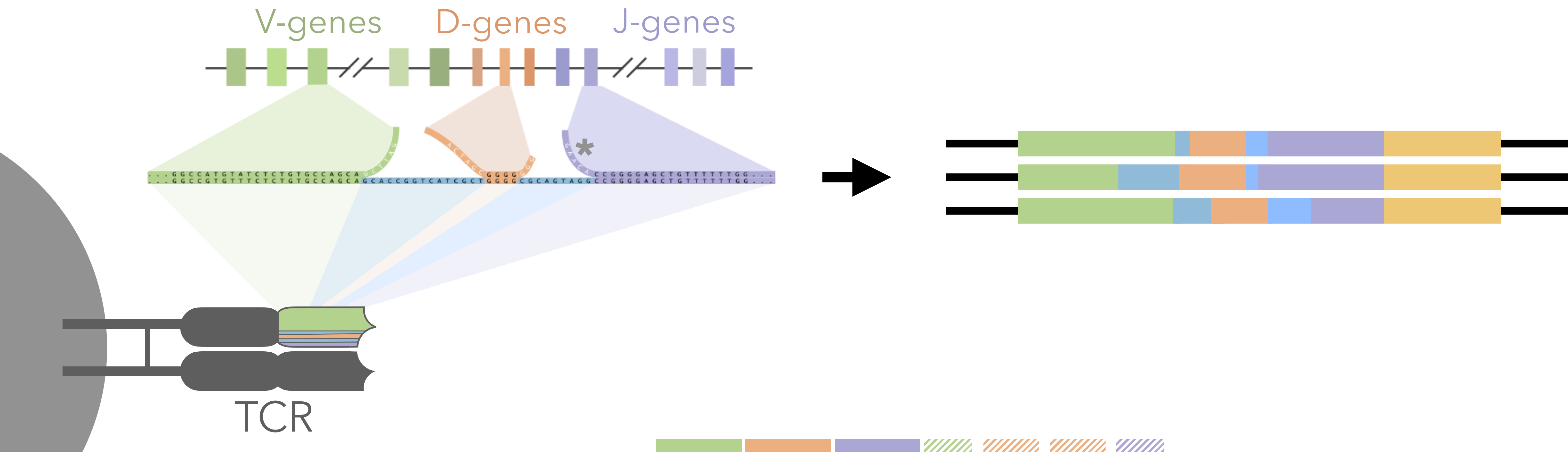
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TGTGCCACCGAACGAGGGGCCCAAGAGACCCAGTACTTC	CATERGPQETQYF	TRBV2*03	TRBD1*01	TRBJ2-5*01	10	5
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Processed bulk repertoire sequencing example output



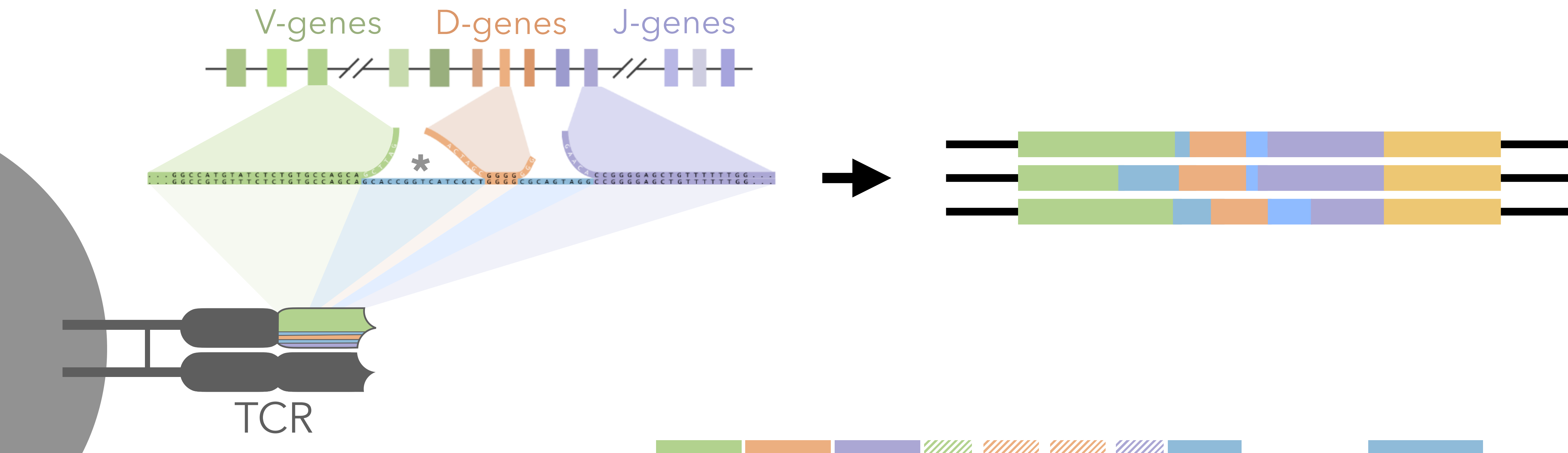
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TGTGCCAGCAGTTATCAGGTCACTGAAGCTTTCTTT	CASSYQVTEAFF	TRBV6-6*02	TRBD1*01	TRBJ1-1*01	4	4	5
TGTGCCAGCGGCCAGGGCTCGGATAACAATCAGCCCCAGCATTTT	CASGPGLGYNQPQHF	TRBV5-5*01	TRBD2*02	TRBJ1-5*01	7	12	0
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Processed bulk repertoire sequencing example output



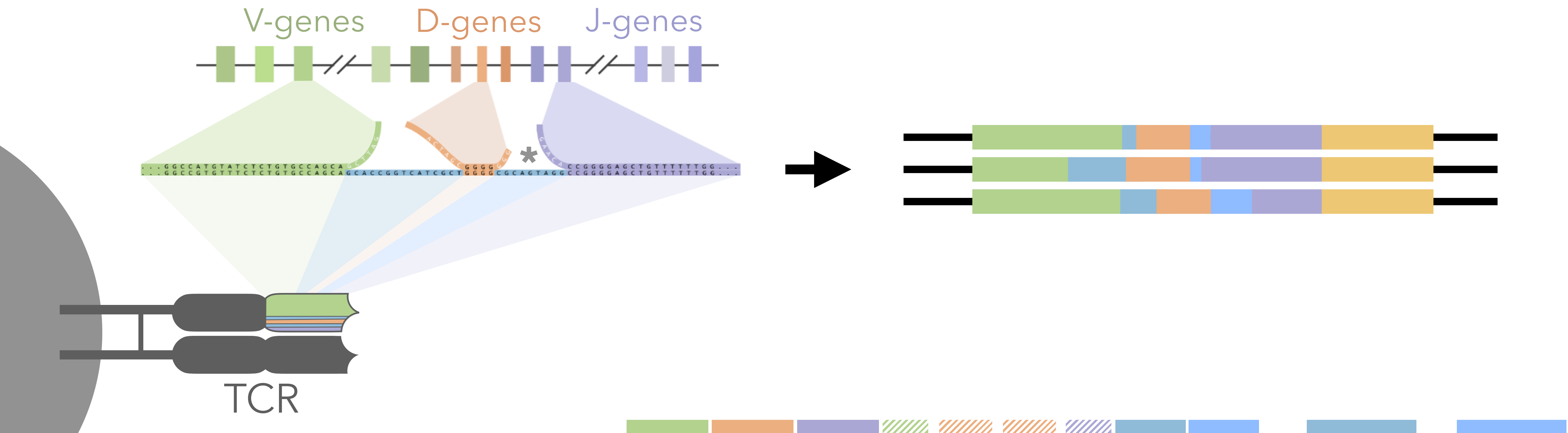
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TGCGCCAGTCGAGCGGCGAGCTCCTACAATGAGCAGTTCTTC	CASRAASSYNEQFF	TRBV5-1*01	TRBD2*01	TRBJ2-1*01	9	6	5	0
TGTGCCAGCAGCTTAAATCTGGTGAGGTACGAGCAGTACTTC	CASSLNLVRYEQYF	TRBV7-2*01	TRBD2*02	TRBJ2-7*01	2	11	1	4
TGTGCCTGGTCAGGGGGCCCAACTGAAGCTTTCTTT	CAWSGGPNTEAFF	TRBV30*01	TRBD1*01	TRBJ1-1*01	5	4	0	2
TGTGCCACCGAACGAGGGGCCCAAGAGACCCAGTACTTC	CATERGPQETQYF	TRBV2*03	TRBD1*01	TRBJ2-5*01	10	5	3	1
TGTGCCAGCATAGCGGGAGGTGAGCAGTTCTTC	CASIAGGEQFF	TRBV28*01	TRBD2*02	TRBJ2-1*01	7	6	3	9
TGTGCCTGGAGCTCCCTCCCTGGCGGGGAGAACAATGAGCAGTTCTTC	CAWSSLPGGENNEQFF	TRBV30*01	TRBD2*01	TRBJ2-1*01	3	7	3	5
TGTGCCAGCAGTTATCAGGTCACTGAAGCTTTCTTT	CASSYQVTEAFF	TRBV6-6*02	TRBD1*01	TRBJ1-1*01	4	4	5	4
TGTGCCAGCGGCCAGGGCTCGGATAACAATCAGCCCCAGCATTTT	CASGPGLGYNQPQHF	TRBV5-5*01	TRBD2*02	TRBJ1-5*01	7	12	0	3
TGTGCCAGTGCGGGATTCTATGGCTACACCTTC	CASAGFYGYTF	TRBV6-1*01	TRBD1*01	TRBJ1-2*01	9	7	2	4

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	vd_insert_nucs
<chr>	<chr>	<chr>	<chr>	<chr>	<int>	<int>	<int>	<int>	<int>	<chr>
TGTGCCAGCAGCTTGAATCACGAGCAGTACTTC	CASSLNHEQYF	TRBV5-6*01	TRBD2*02	TRBJ2-7*01	1	3	13	5	4	AATC
TGCGCCAGCAGCTTGGCAGAGACCCAGTACTTC	CASSLAETQYF	TRBV5-1*01	TRBD1*01	TRBJ2-5*01	2	9	0	4	0	
TGCGCCAGTCGAGCGGCGAGCTCCTACAATGAGCAGTTCTTC	CASRAASSYNEQFF	TRBV5-1*01	TRBD2*01	TRBJ2-1*01	9	6	5	0	4	GTCG
TGTGCCAGCAGCTTAAATCTGGTGAGGTACGAGCAGTACTTC	CASSLNLVRYEQYF	TRBV7-2*01	TRBD2*02	TRBJ2-7*01	2	11	1	4	8	AATCTGGT
TGTGCCTGGTCAGGGGGCCCAAACTGAAGCTTTCTTT	CAWSGGPNTEAFF	TRBV30*01	TRBD1*01	TRBJ1-1*01	5	4	0	2	1	T
TGTGCCACCGAACGAGGGGCCCAAGAGACCCAGTACTTC	CATERGPQETQYF	TRBV2*03	TRBD1*01	TRBJ2-5*01	10	5	3	1	7	CCGAACG
TGTGCCAGCATAGCGGGAGGTGAGCAGTTCTTC	CASIAGGEQFF	TRBV28*01	TRBD2*02	TRBJ2-1*01	7	6	3	9	1	T
TGTGCCTGGAGCTCCCTCCCTGGCGGGGAGAACAATGAGCAGTTCTTC	CAWSSLPGENNEQFF	TRBV30*01	TRBD2*01	TRBJ2-1*01	3	7	3	5	11	CTCCCTCCCTG
TGTGCCAGCAGTTATCAGGTCACTGAAGCTTTCTTT	CASSYQVTEAFF	TRBV6-6*02	TRBD1*01	TRBJ1-1*01	4	4	5	4	2	AT
TGTGCCAGCGGCCAGGGCTCGGATAACAATCAGCCCCAGCATTTT	CASGPGLGYNQPQHF	TRBV5-5*01	TRBD2*02	TRBJ1-5*01	7	12	0	3	5	GGCCC
TGTGCCAGTGCGGGATTCTATGGCTACACCTTC	CASAGFYGYTF	TRBV6-1*01	TRBD1*01	TRBJ1-2*01	9	7	2	4	3	TGC

Processed bulk repertoire sequencing example output

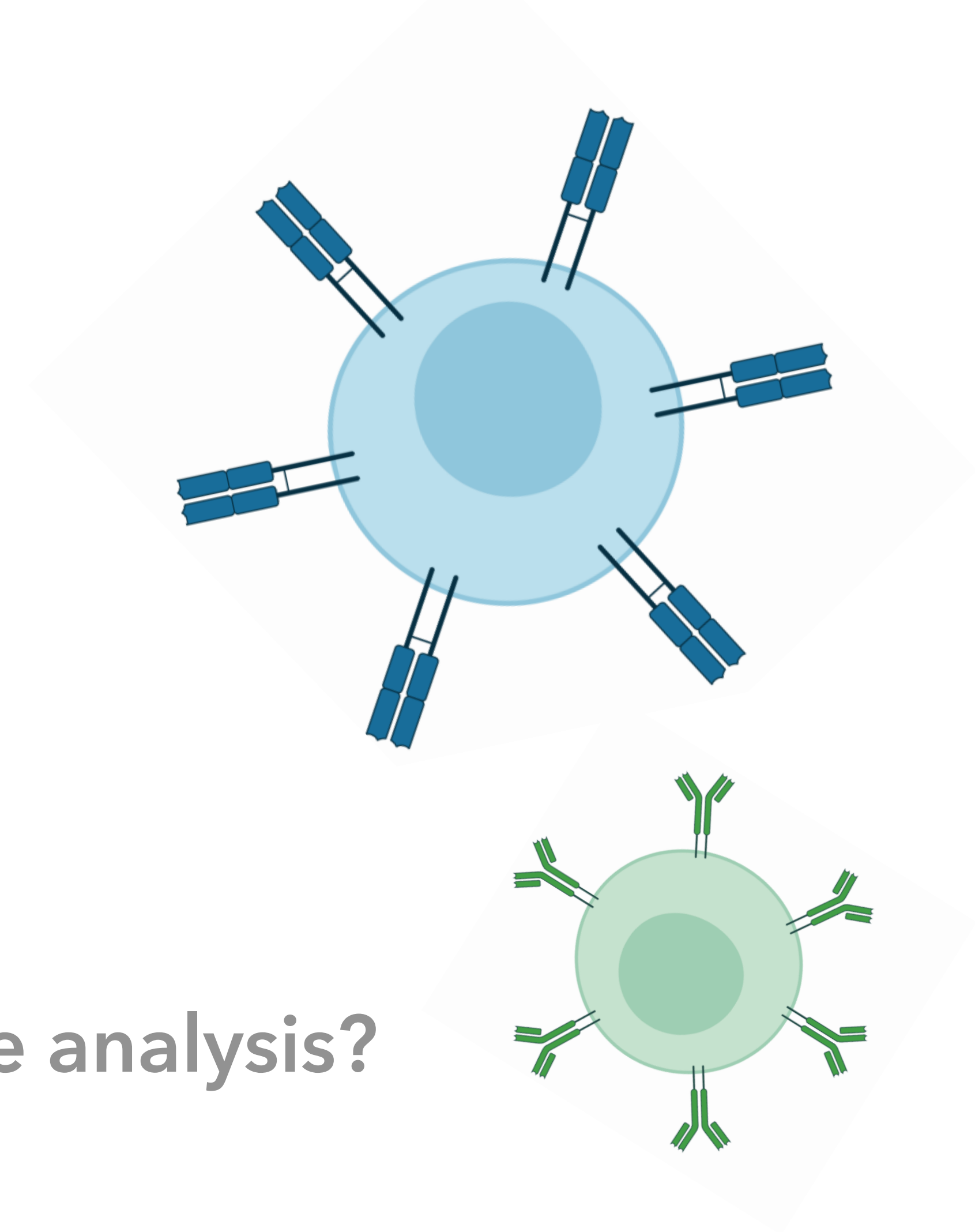


cdr3_nucseq	cdr3	v_gene	d_gene	j_gene	v_trim	d0_trim	d1_trim	j_trim	vd_insert	dj_insert	vd_insert_nucs	dj_insert_nucs
<chr>	<chr>	<chr>	<chr>	<chr>	<int>	<int>	<int>	<int>	<int>	<int>	<chr>	<chr>
TGTGCCAGCAGCTTGAATCACGAGCAGTACTTC	CASSLNHEQYF	TRBV5-6*01	TRBD2*02	TRBJ2-7*01	1	3	13	5	4	0	AATC	
TGCGCCAGCAGCTTGGCAGAGACCCAGTACTTC	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	
TGTGCCTGGTCAGGGGGCCCAAACTGAAGCTTTCTTT	CAWSGGPNTEAFF	TRBV30*01	TRBD1*01	TRBJ1-1*01	5	4	0	2	1	3	T	ACC
TGTGCCACCGAACGAGGGGCCCAAGAGACCCAGTACTTC	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
TGTGCCAGCGGCCAGGGCTCGGATAACAATCAGCCCCAGCATTTT	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

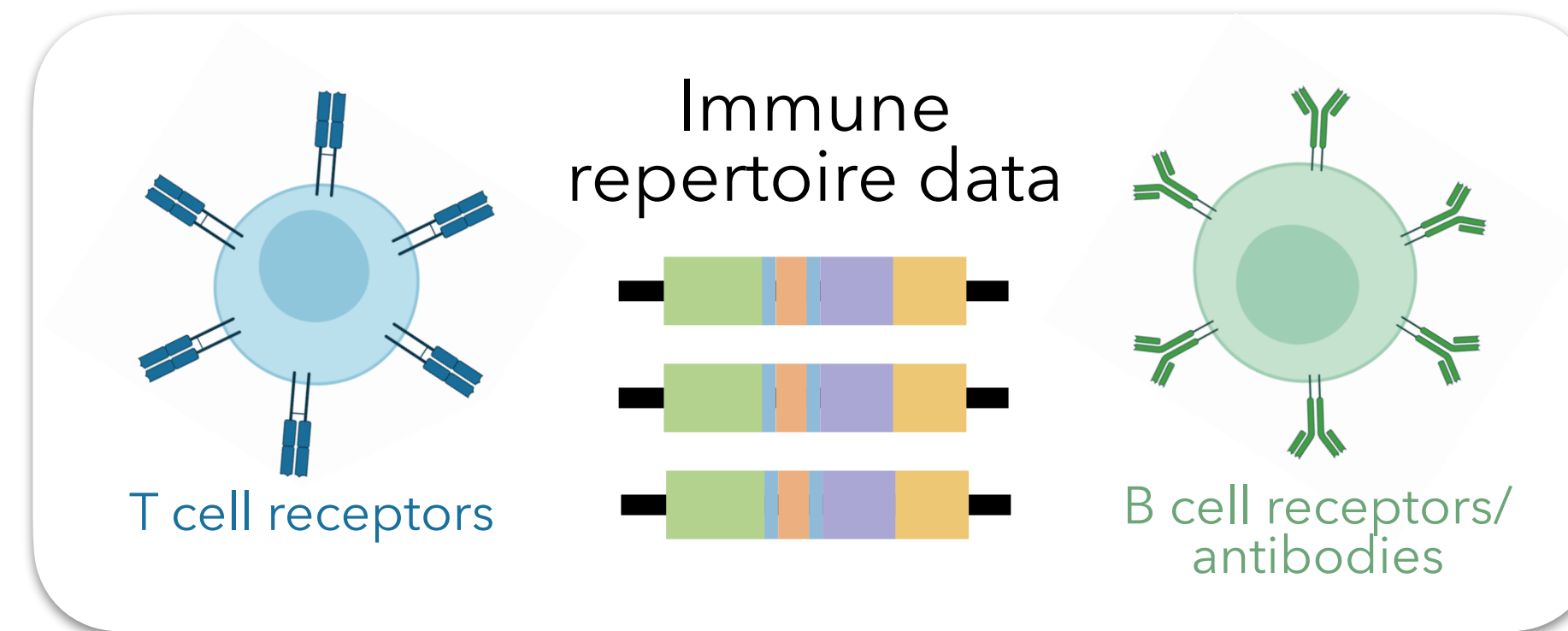
Lecture goals:

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?**

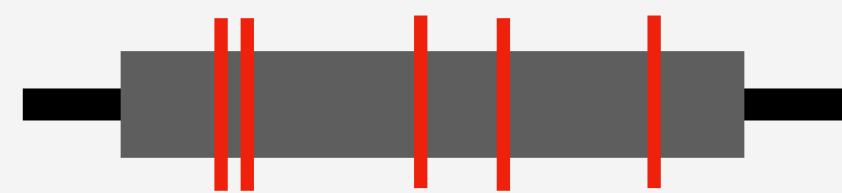


Immune repertoire analyses often focus on diversity, architecture, evolution, or convergence

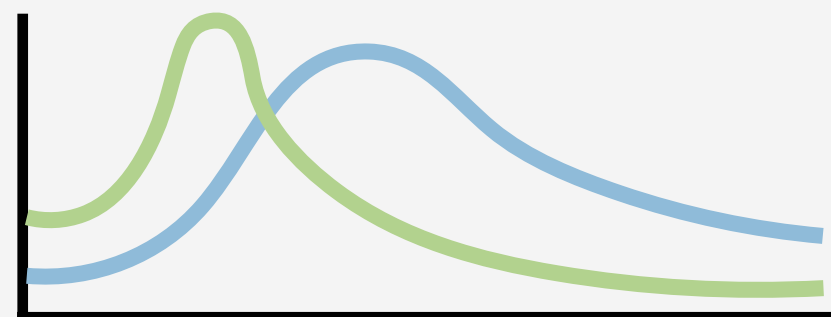


Immune repertoire analyses often focus on **diversity**, architecture, evolution, or convergence

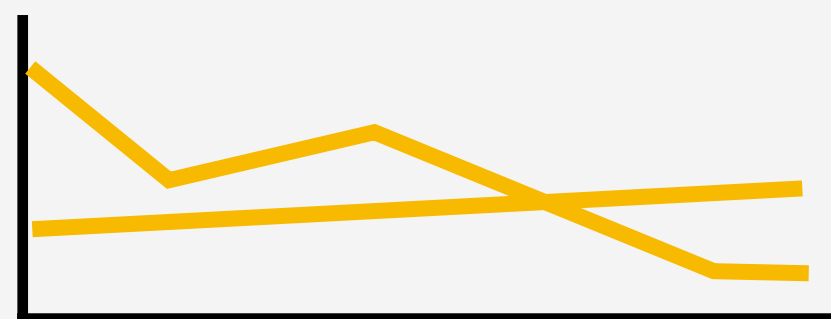
Underlying mechanisms of diversity generation



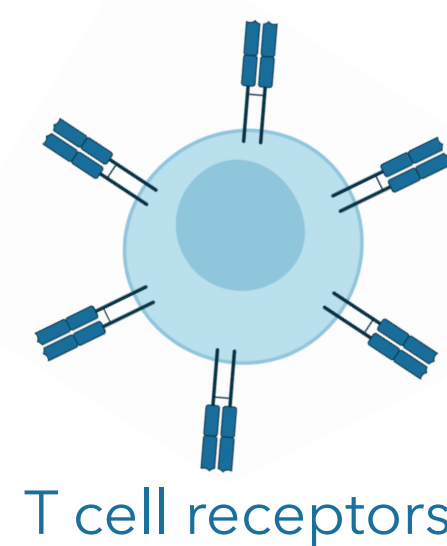
probabilistic
sequence
annotation



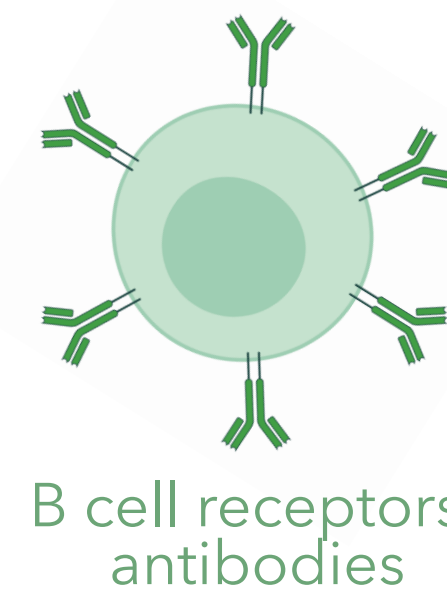
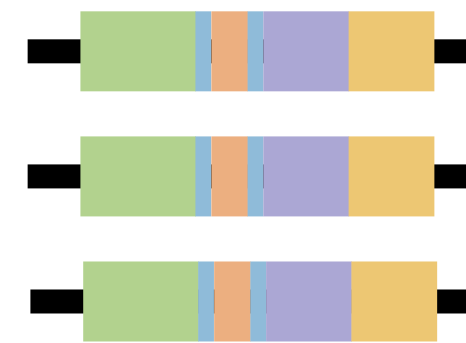
recombination
statistics to learn
about generation
and selection



clonotype
diversity
dynamics

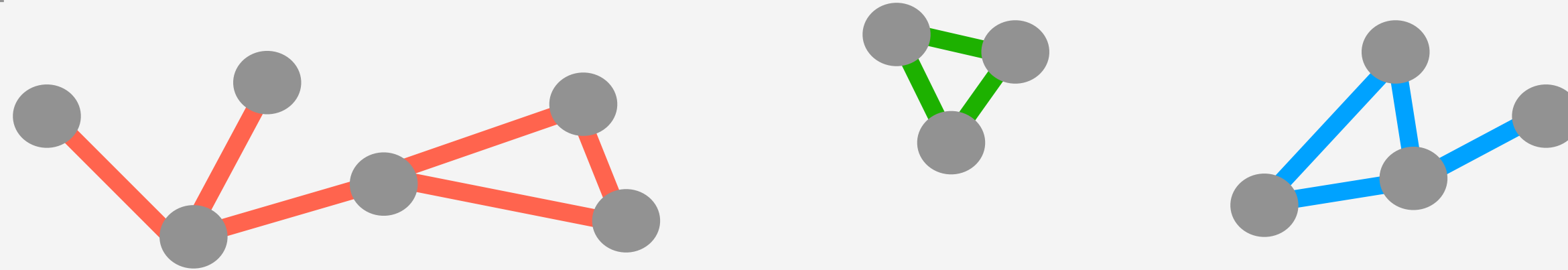


Immune
repertoire data



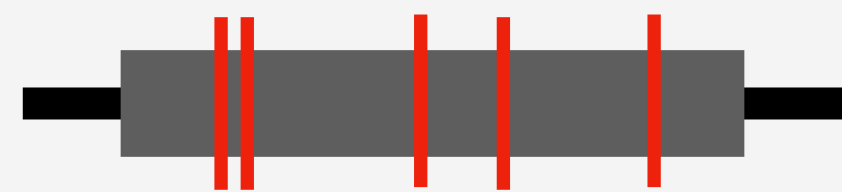
Immune repertoire analyses often focus on diversity, **architecture**, evolution, or convergence

Repertoire architecture

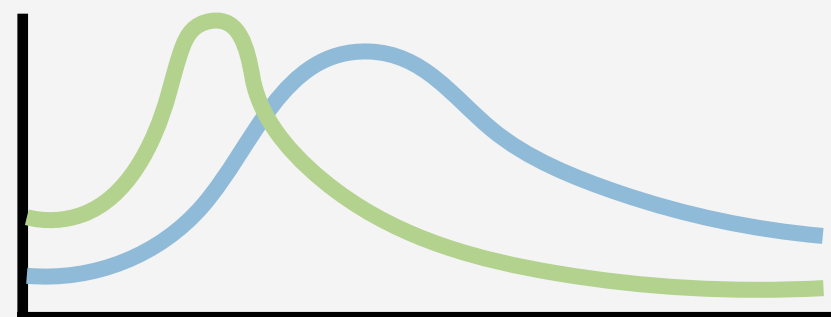


defining antigen
recognition breadth
using network
analysis

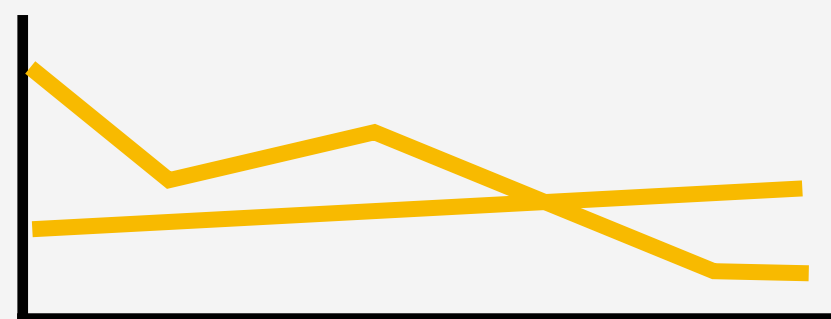
Underlying mechanisms of diversity generation



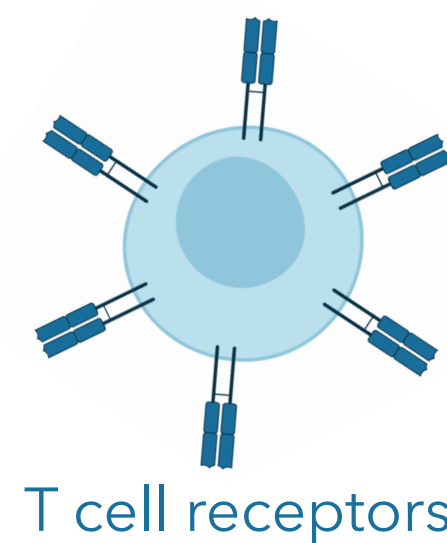
probabilistic
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annotation



recombination
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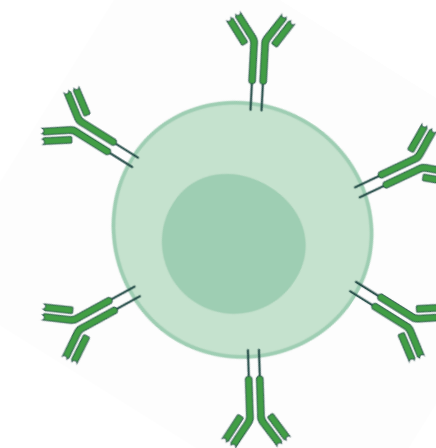
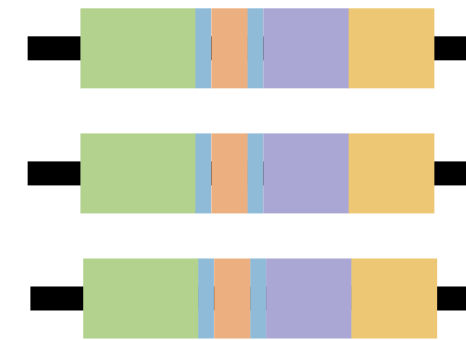


clonotype
diversity
dynamics



T cell receptors

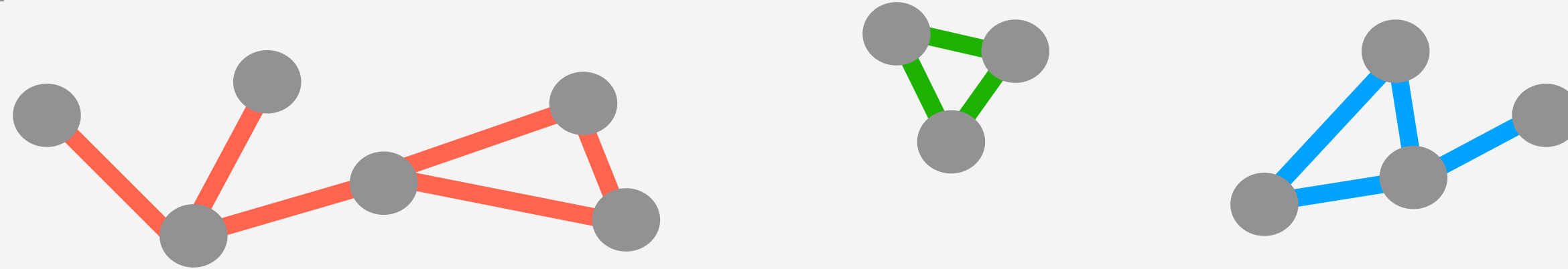
Immune repertoire data



B cell receptors/
antibodies

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

Repertoire architecture

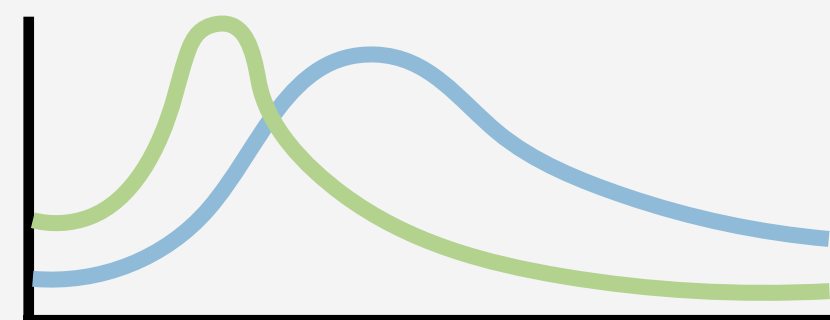


defining antigen
recognition breadth
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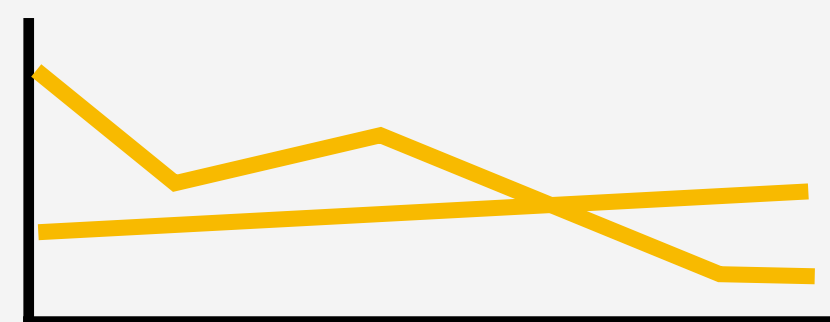
Underlying mechanisms of diversity generation



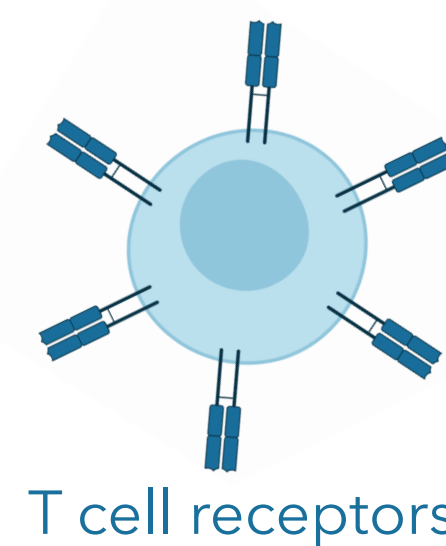
probabilistic
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recombination
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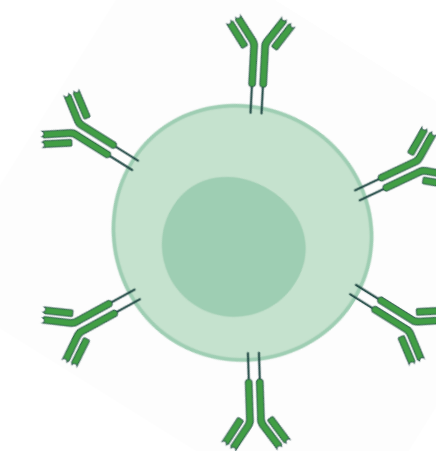
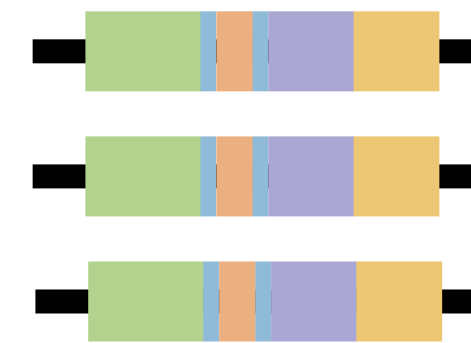


clonotype
diversity
dynamics



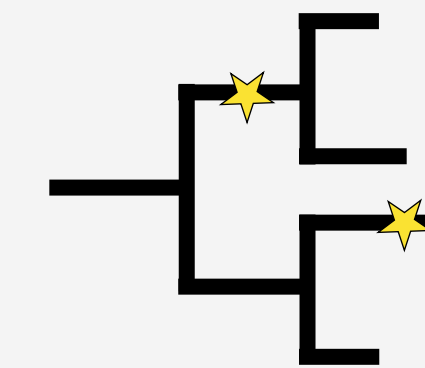
T cell receptors

Immune
repertoire data

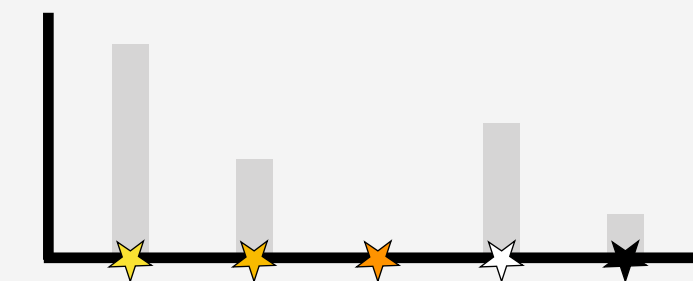


B cell receptors/
antibodies

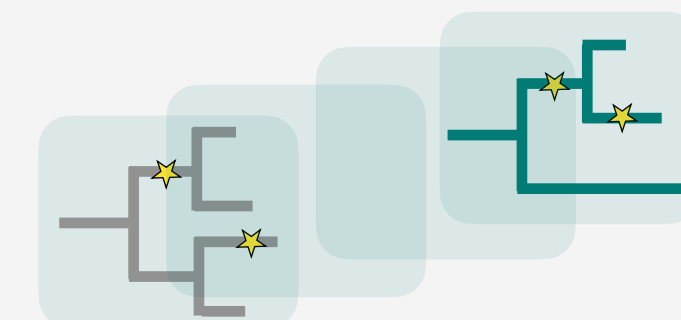
Antibody evolution



reconstruction of
phylogenetic trees



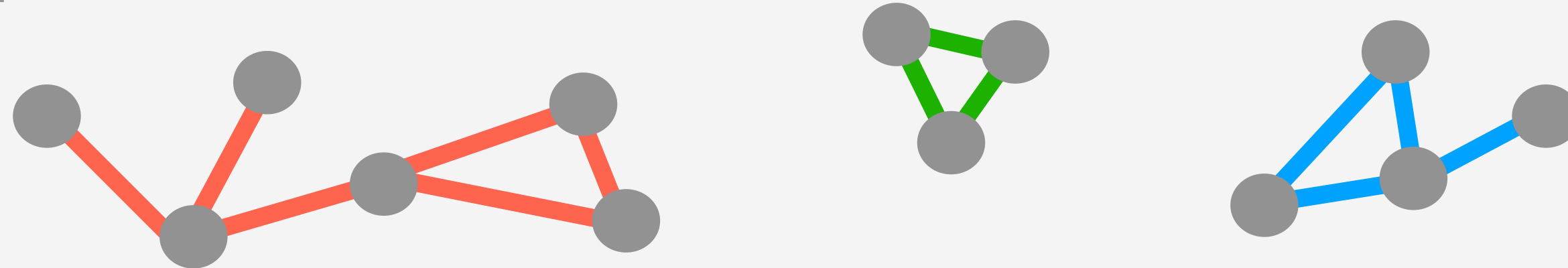
inferring mutation
statistics



simulating antibody
repertoire evolution

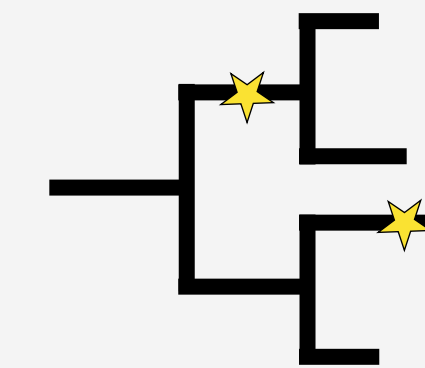
Immune repertoire analyses often focus on diversity, architecture, evolution, or **convergence**

Repertoire architecture

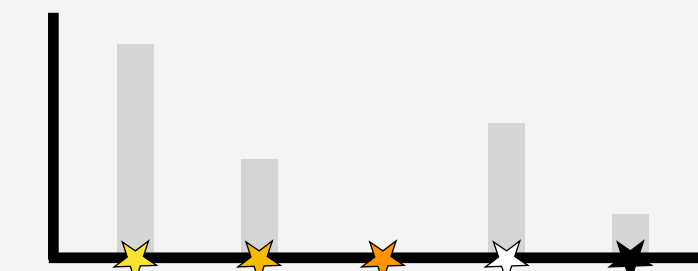


defining antigen
recognition breadth
using network
analysis

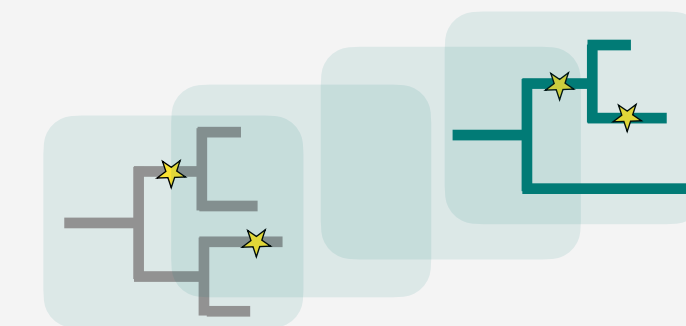
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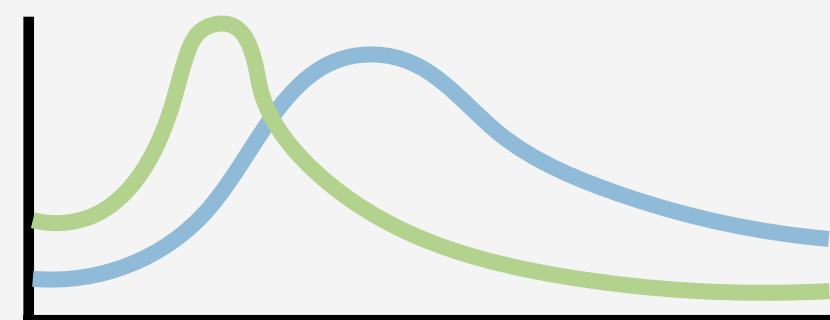


simulating antibody
repertoire evolution

Underlying mechanisms of diversity generation



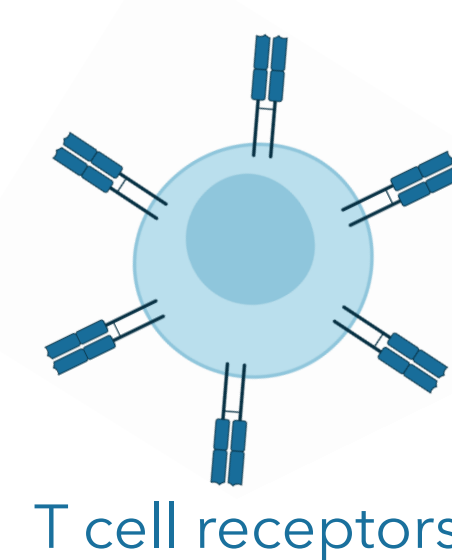
probabilistic
sequence
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recombination
statistics to learn
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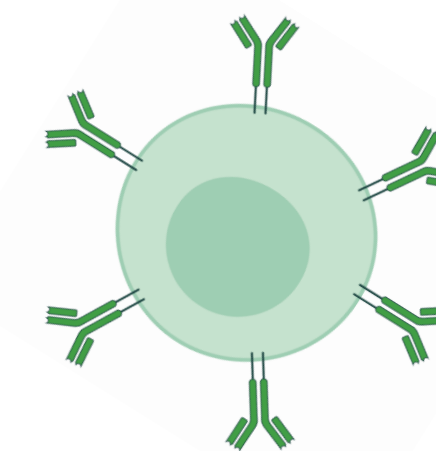
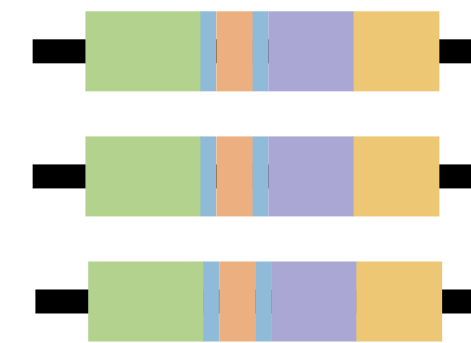


clonotype
diversity
dynamics



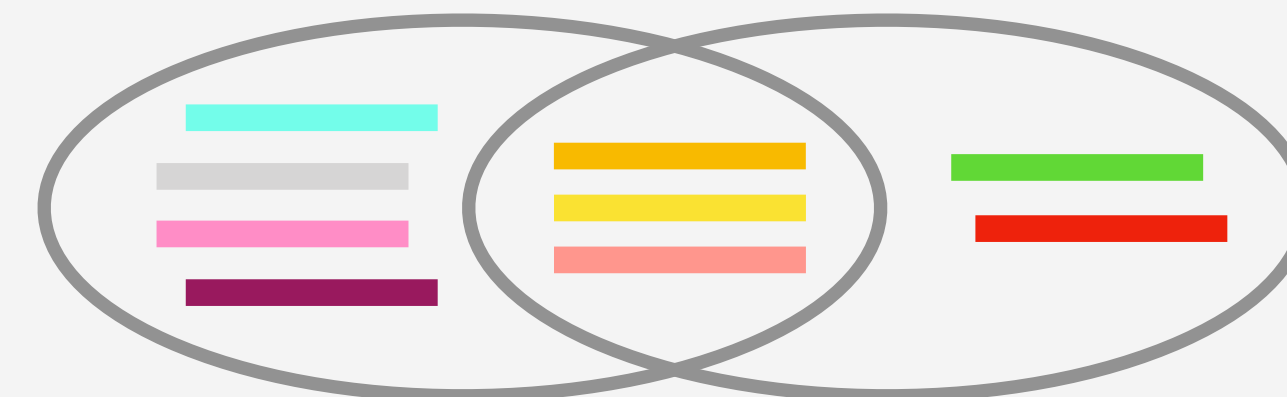
T cell receptors

Immune
repertoire data



B cell receptors/
antibodies

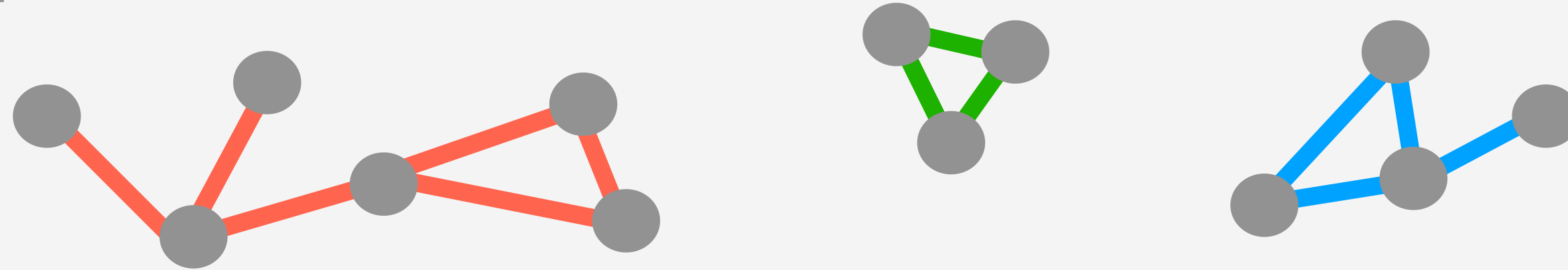
Molecular convergence



exploring cross-individual
sequence similarity and
convergence

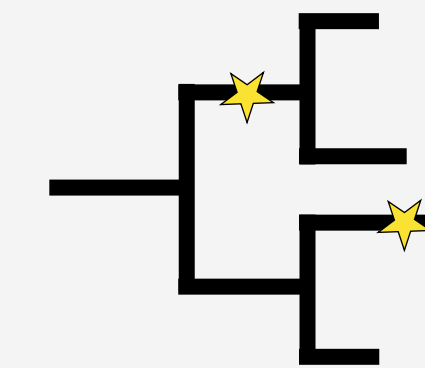
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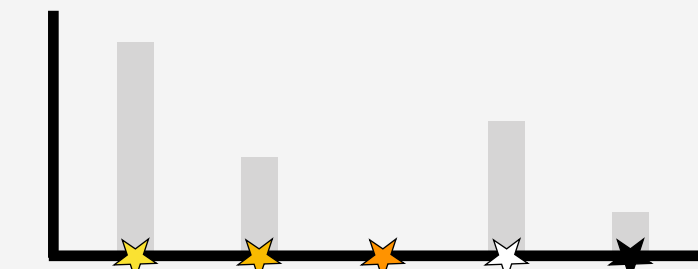


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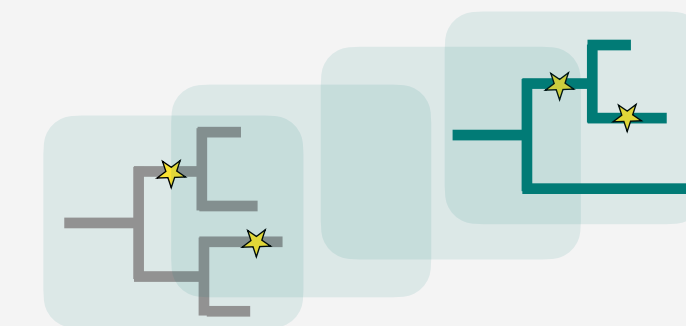
Antibody evolution



reconstruction of
phylogenetic trees

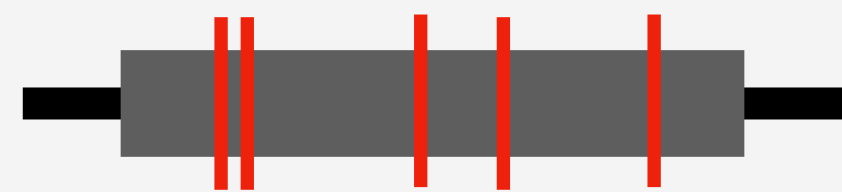


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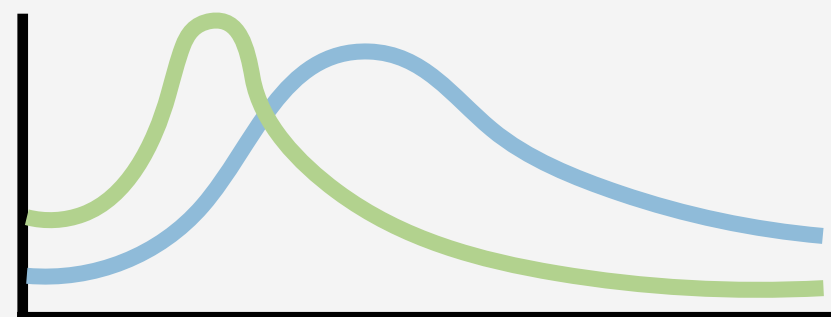


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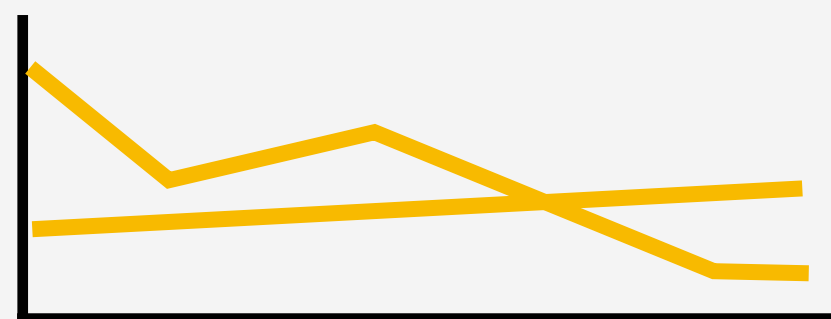
Underlying mechanisms of diversity generation



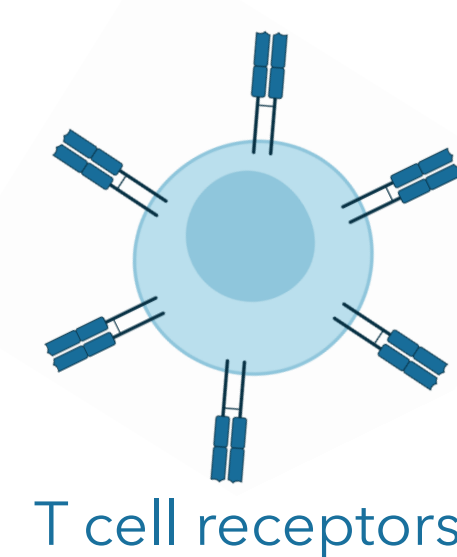
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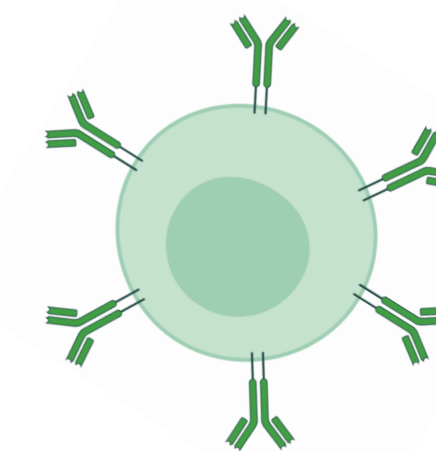
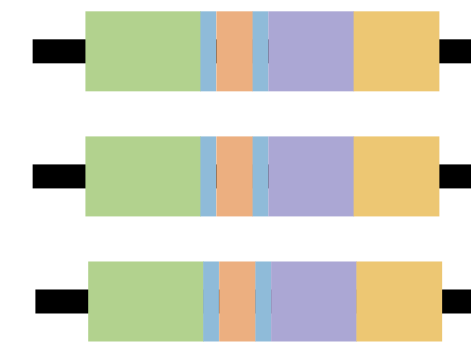


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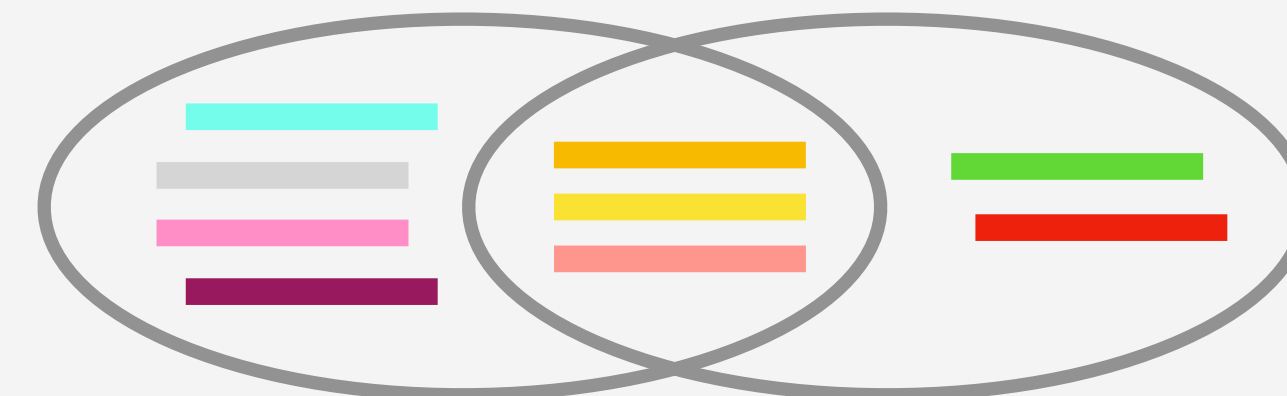
T cell receptors

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B cell receptors/
antibodies

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

