Identifying the antibody specificity repertoire

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You have a unique antibody repertoire that changes as immune responses occur

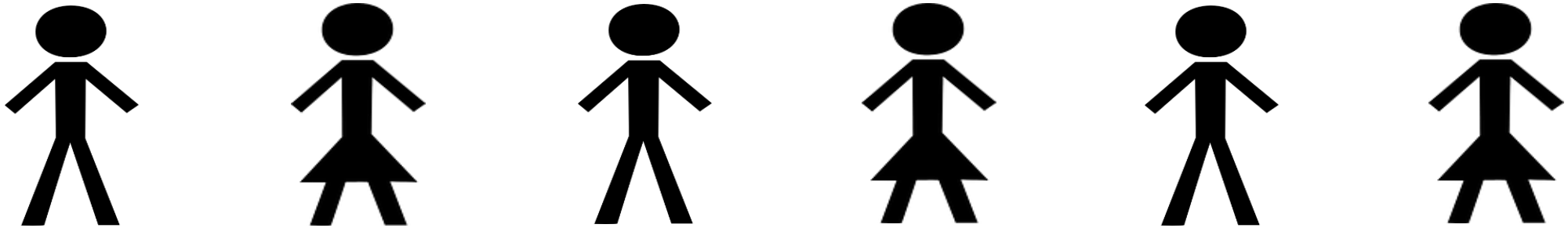
Immune factors

Pathogens

Allergens

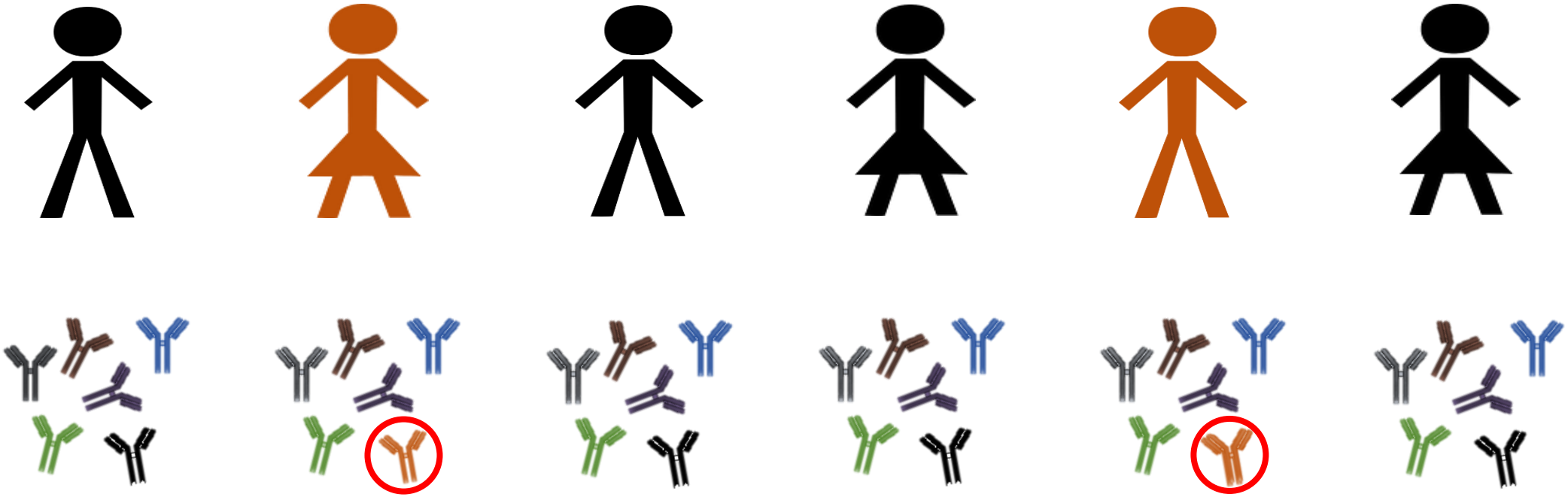
Environmental

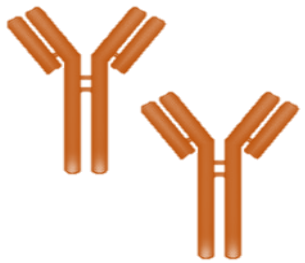
You



Antibody repertoire

By comparing patient's serums, antibodies can be isolated as disease specific

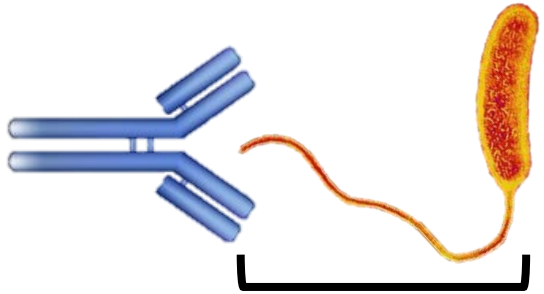




Potential Biomarkers:

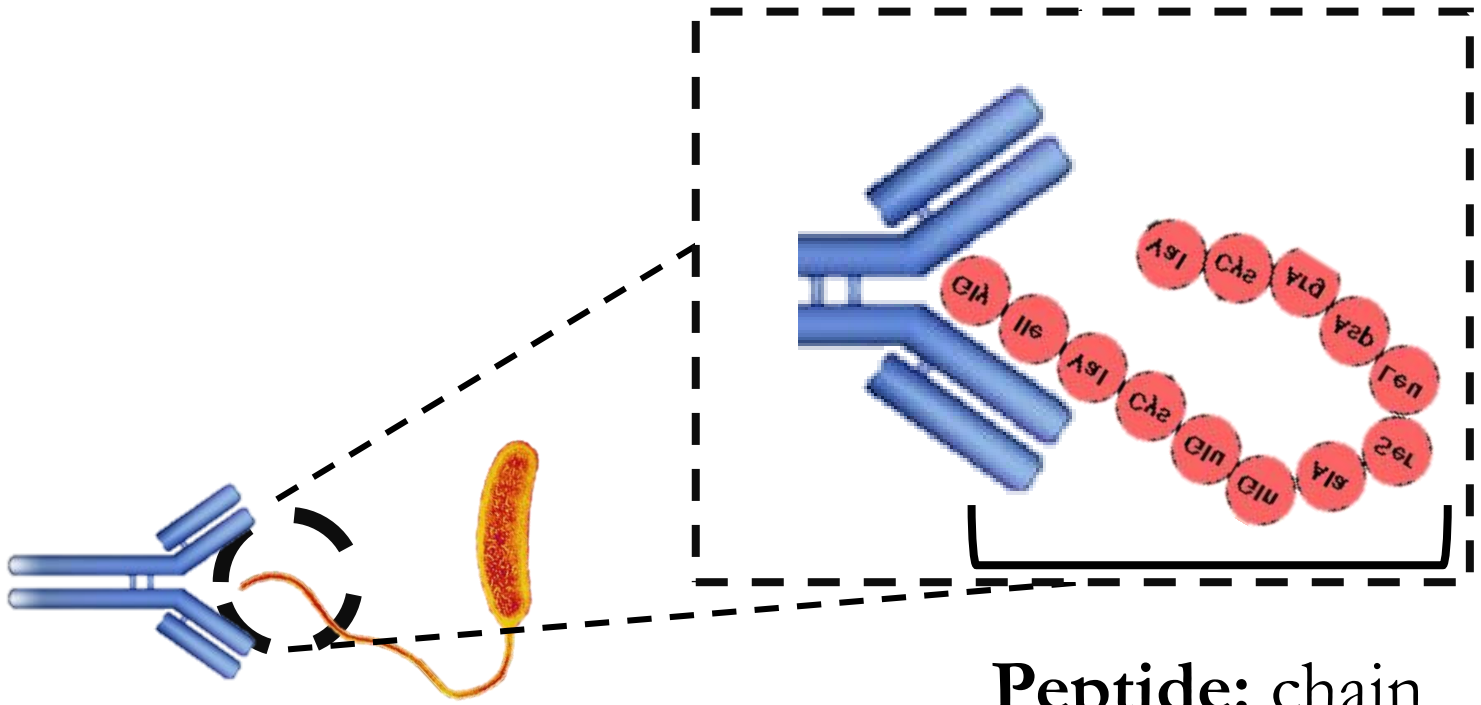
- develop therapeutics
- diagnostic tools
- mechanisms of pathogenesis

All antibodies bind to a unique antigen dependent on their affinity



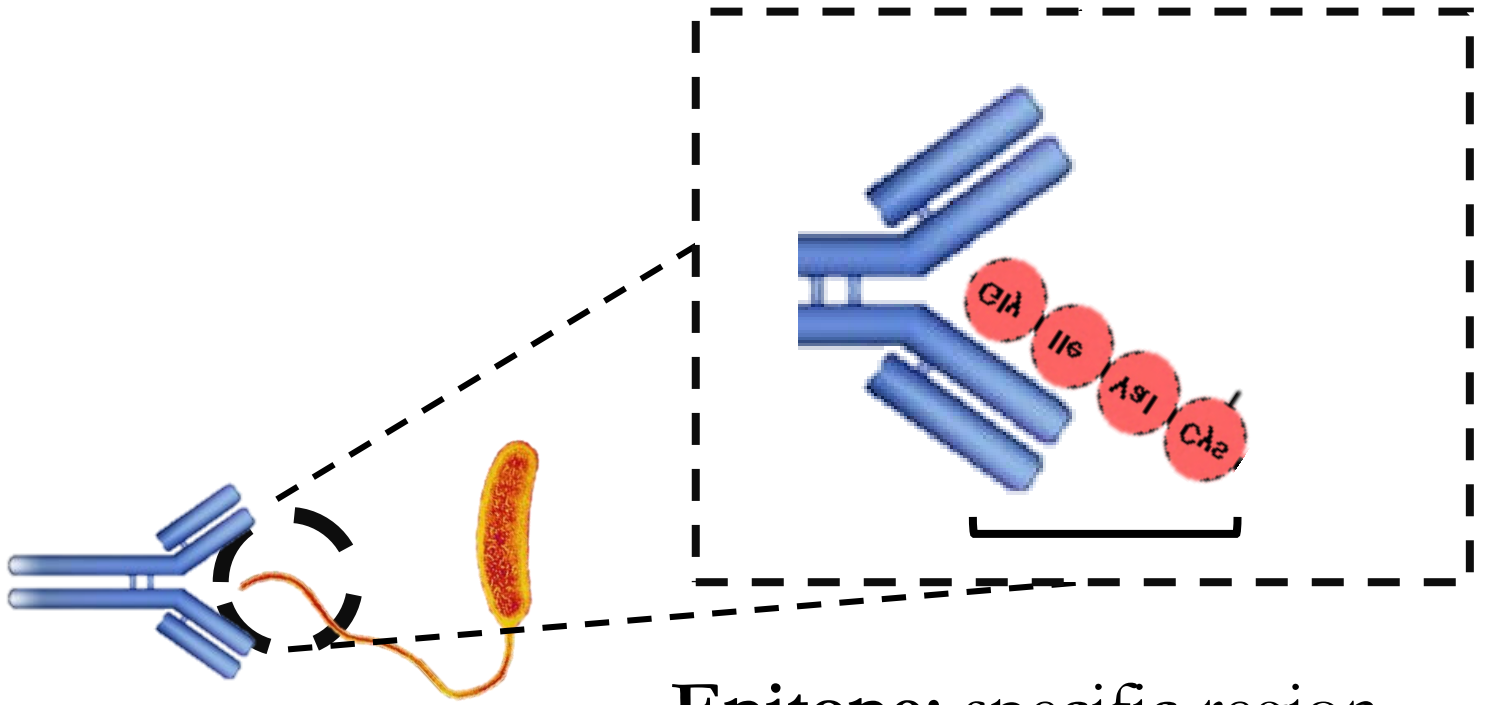
Antigen: a foreign substance
that induces an immune response

All antibodies bind to a unique antigen dependent on their affinity



Peptide: chain of amino acids

All antibodies bind to a unique antigen dependent on their affinity



Epitope: specific region responsible for antibody binding

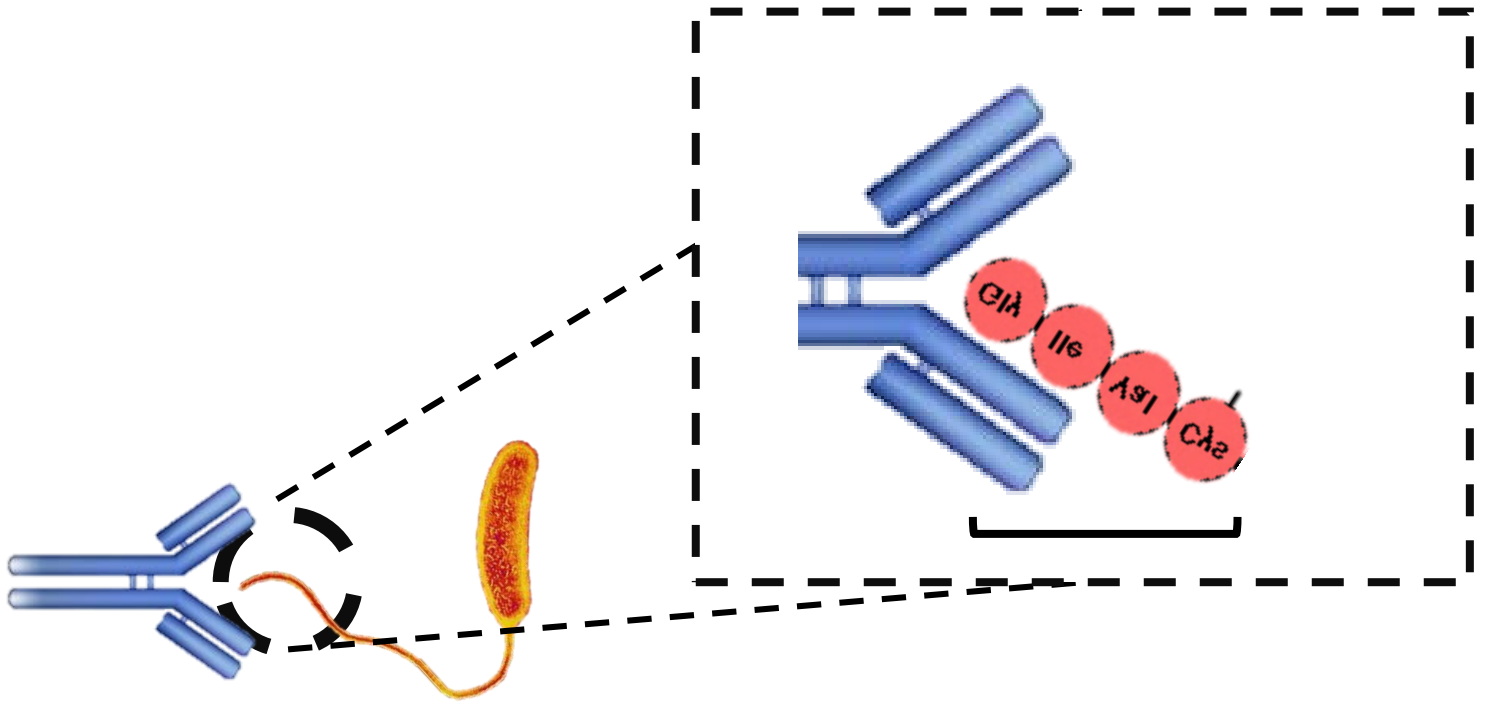
All antibodies bind to a unique antigen dependent on their affinity

Definitions to remember:

-**Antigen:** cause of immune response

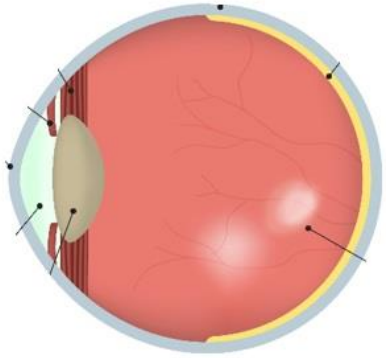
-**Peptide:** chain of amino acids

-**Epitope:** amino acids responsible for binding



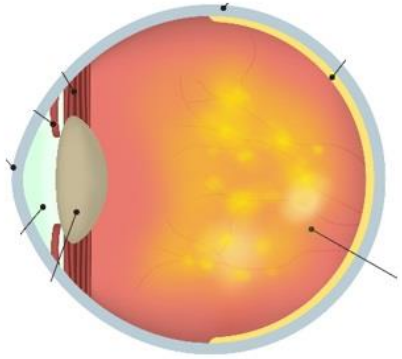
Summer Project: Determine candidates for motifs which are specific to the disease Aged Macular Degeneration (AMD)

Healthy eye



Macula

Eye with AMD



Damaged Macula

Wet AMD:

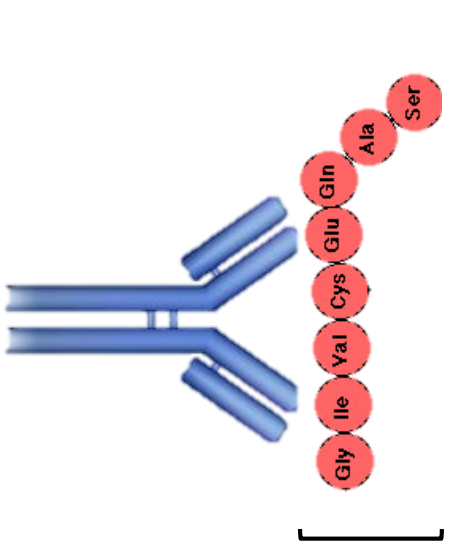
- Neo Vascular
- caused by swelling of blood vessels

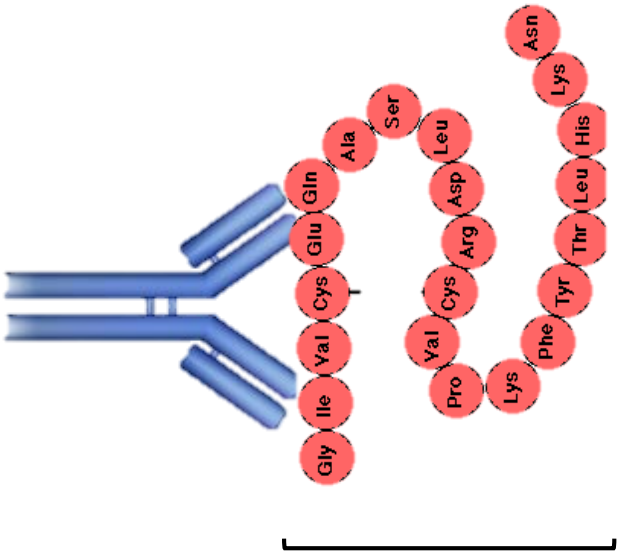
Dry AMD:

- Geographic Atrophy
- caused by aggregation of Drusen

Life with AMD on the right

Research Goal: Develop a systematic method of obtaining disease specific and medically relavant biomarkers



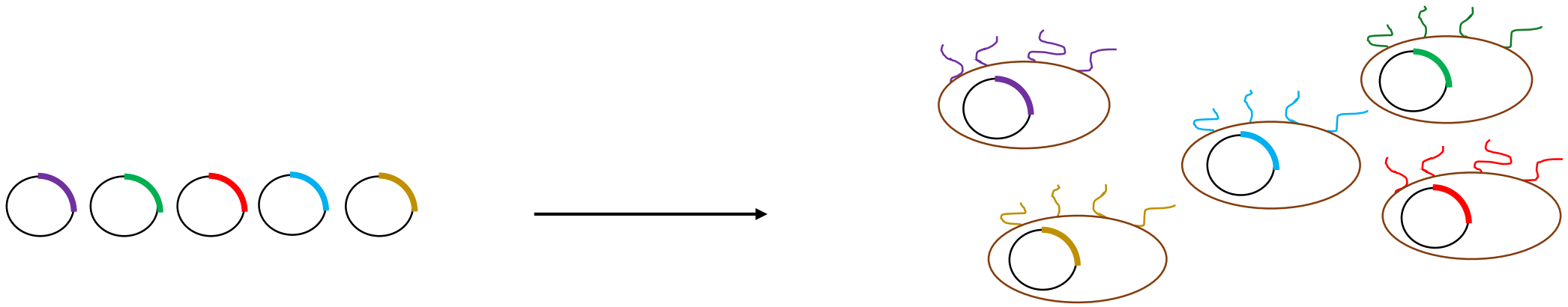


Identify disease specific peptide

Determine complimentary antigen

Accomplish with bacterial display

Engineering bacteria to display peptides for antibody identification



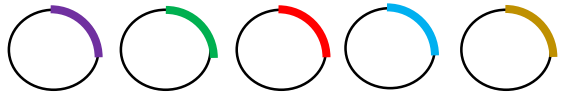
Library of peptides

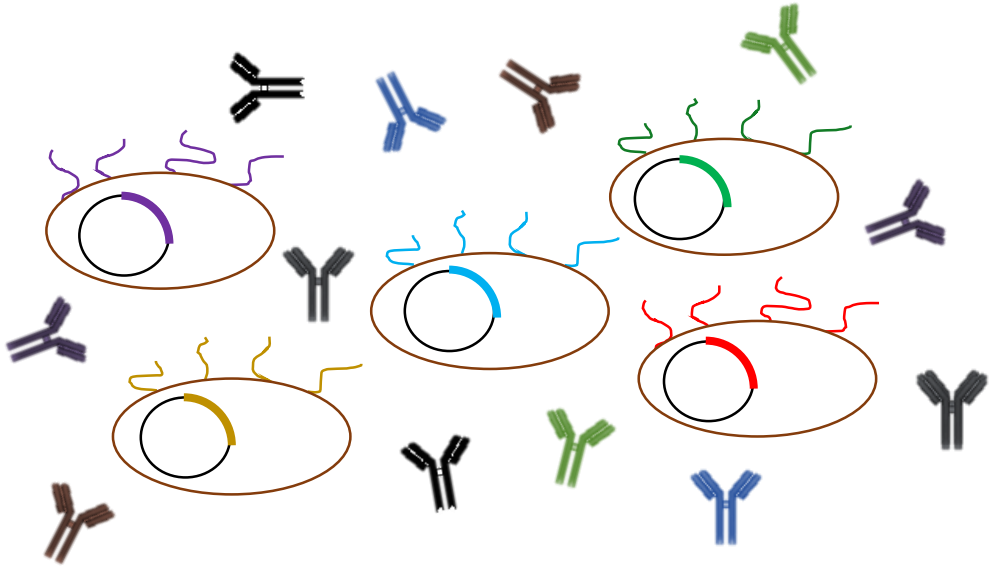
- 7.6 billion unique peptides
- 12 amino acids in length

Engineer bacteria to display peptide library

- Cells display a unique peptide
- Screen for antibody binding

Engineering bacteria to display peptides for antibody identification





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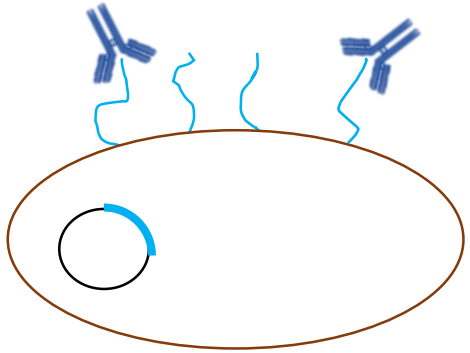
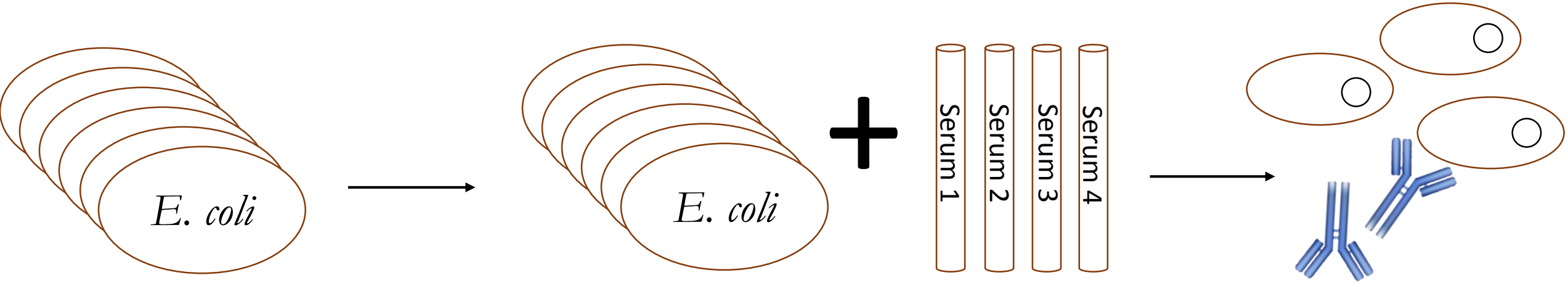


Isolate and sequence
these antibody-peptide interactions

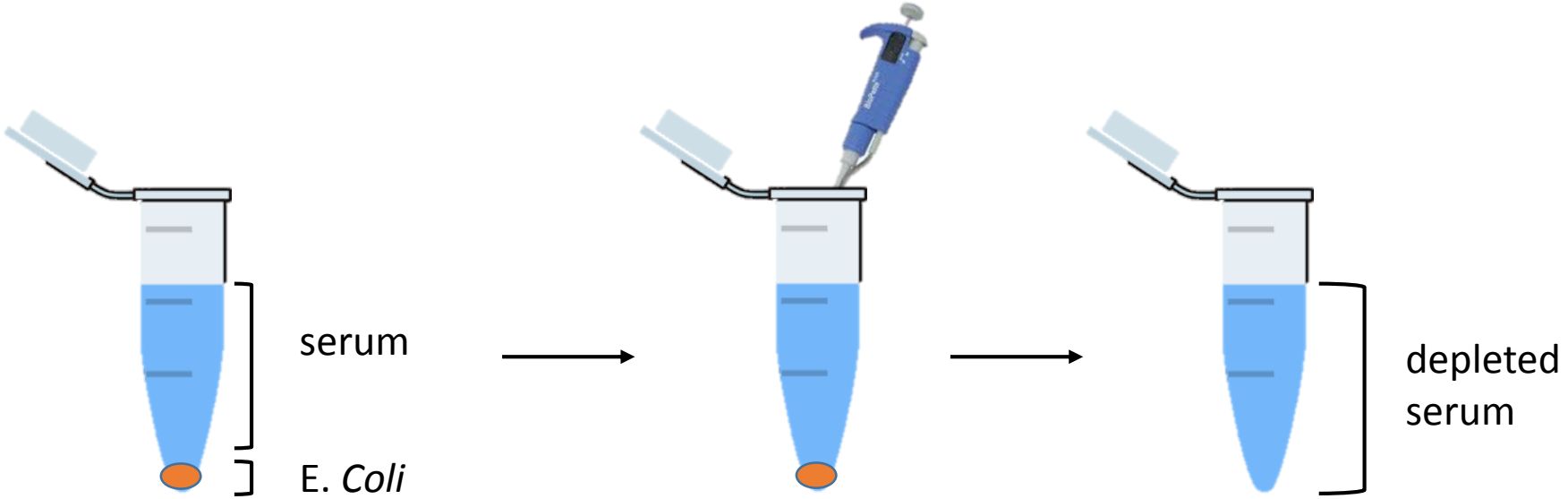
Deplete patient's serum of antibodies that would naturally bind to *E. coli* cells



Grow culture

Combine serum and bacteria in vials

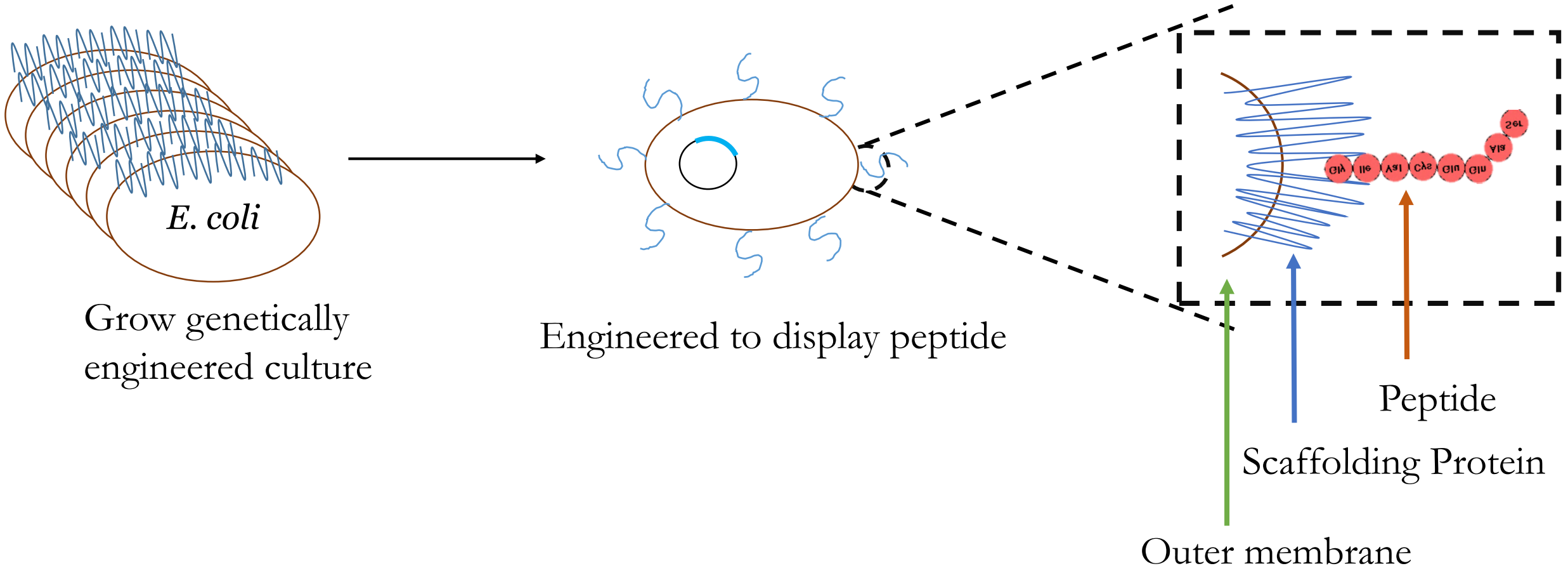
Some antibodies will bind



Spin down cells

Extract the serum

Combine patient's serum with a vast library of peptides and magnetically separate cells that bound to antibodies



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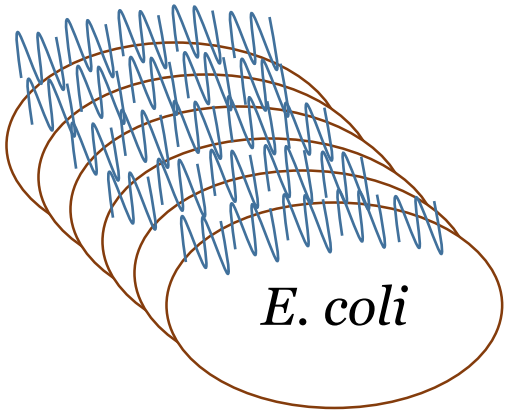
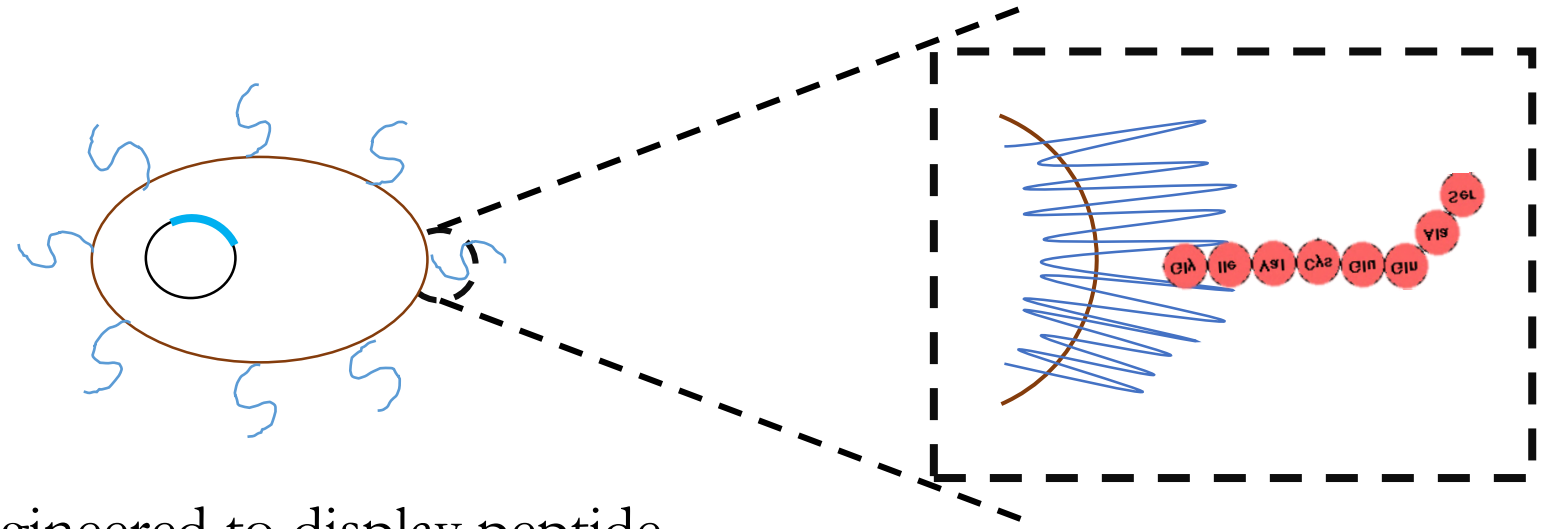
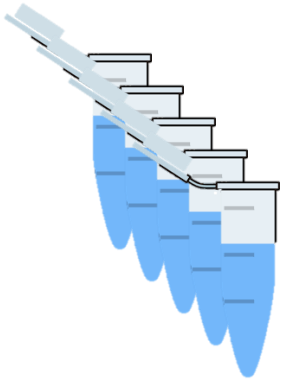


Grow genetically engineered culture



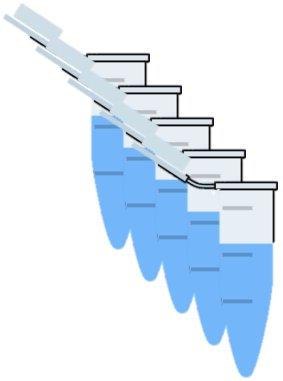
Engineered to display peptide



Primary Binding Event:

- Peptide Library
- Patient Serum

- Incubate 45 minutes

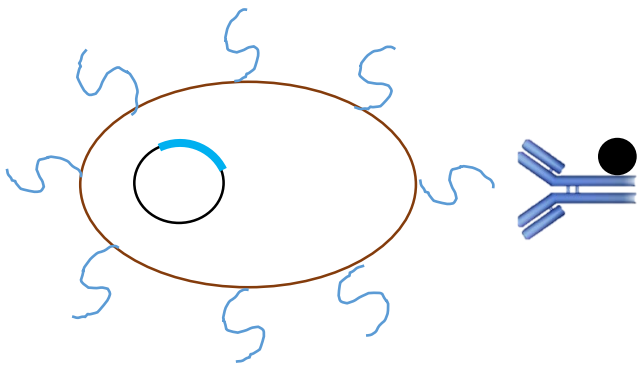


Secondary Binding Event:

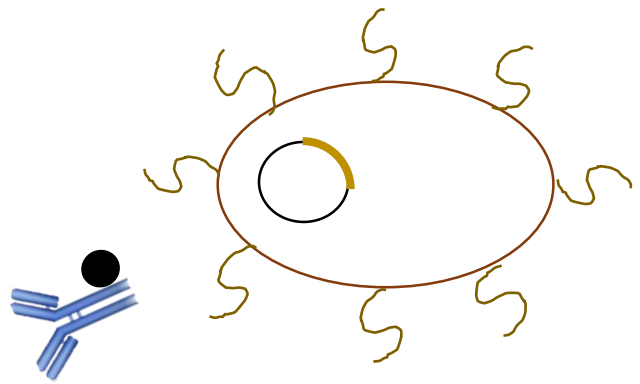
- Peptide Library
- Patient Serum
- Magnetic Beads

- Incubate 45 minutes

Separate cells that bound with antibodies from cells that didn't bind to antibodies via magnetic separation



two possible interactions



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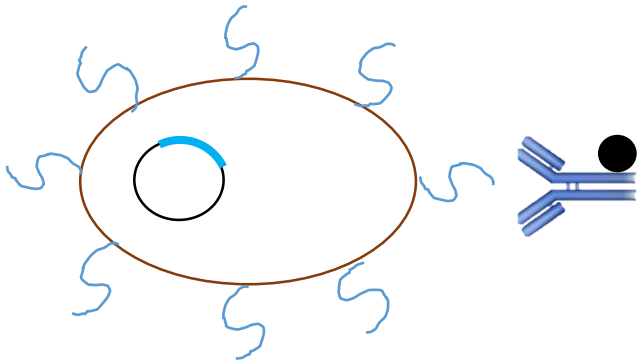

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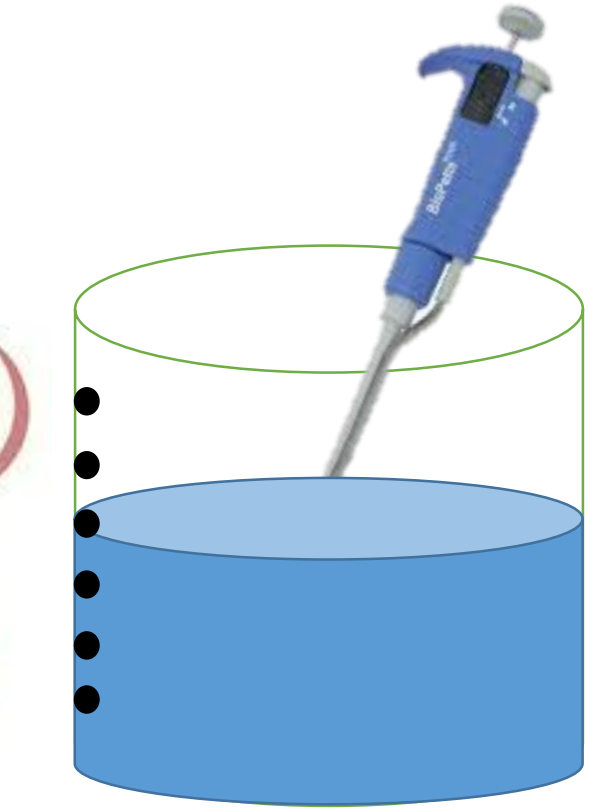
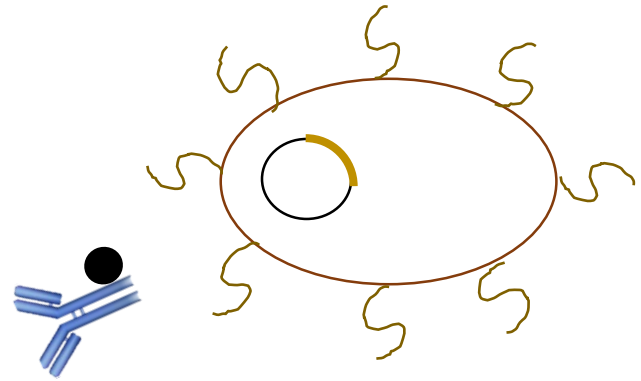
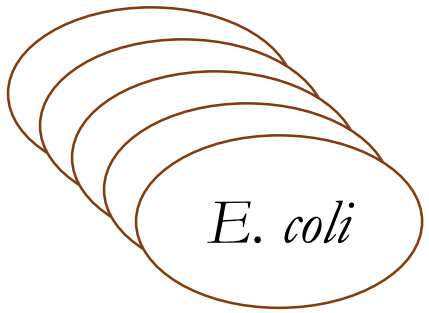
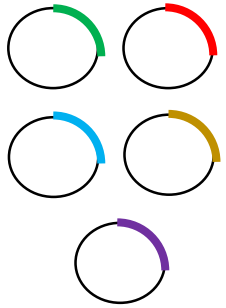




Any cells that did not bind to an antibody will not have a magnet and can be extracted in the supernatant

Isolate library DNA so that the peptide can be sequenced and the genetic information accessed





Plasmid DNA

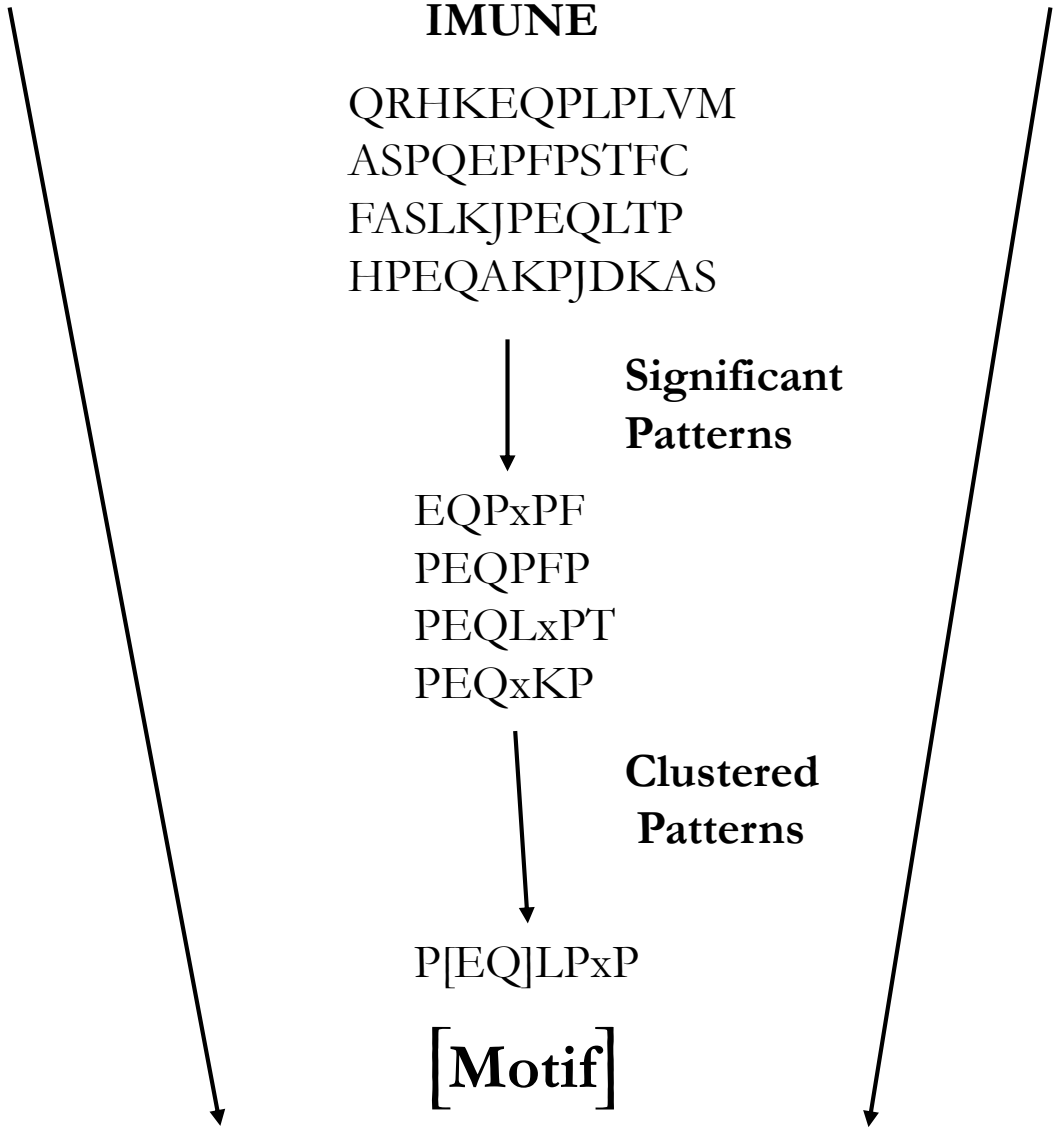
Isolate the region of the plasmid that encodes for the peptide

Then use PCR to amplify the strands

KPFCDCRGLCPF
IVTLYAGCTKCD
KLGCLCTVYPAF
VPPKLPCKGTVL
.
.
KPCDCLTVYAG

Use Ilumina NGS to sequence the peptides

Process the data by converting peptide sequences into amino acids using bioinformatics



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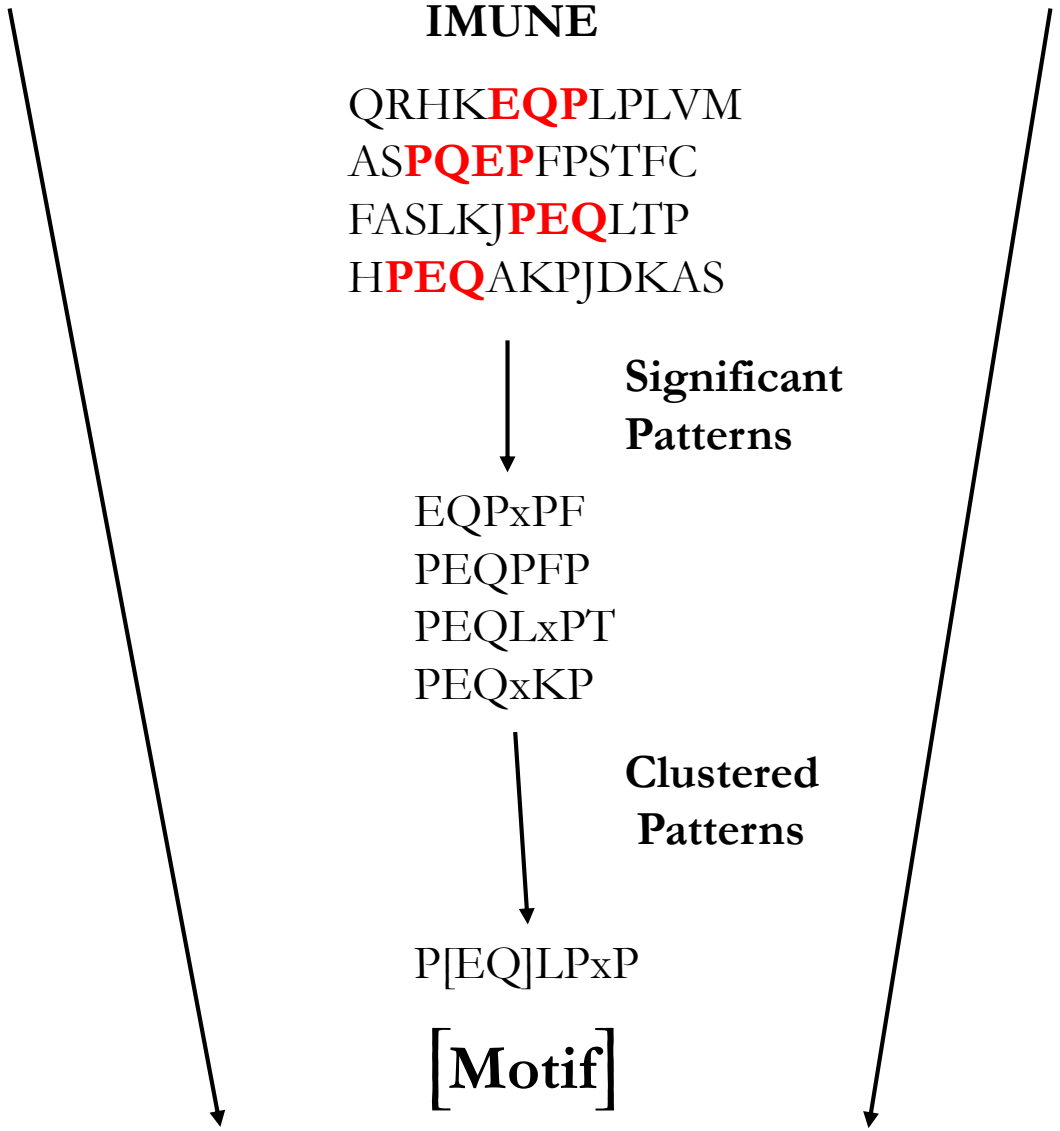

Assess the data by organizing patients into groups and comparing groups for unique sequences

Group 1:

Diseased Patients

AVCDCFWPRPGW
YEPWRDGFVDCG
HWFLSGHEQGWF
YEPTPWWFKLMF
WPRPGWRDFVDC
HRVGREPCDCWH

List of epitopes

Group 2:

Healthy Patients

HRVGREPCDCWH
KCDCVLPFWHRT
YEPTPWWFKLMF
AVCDCFWPRPGW
TVYALPCDCMFH
CPLFMAHDCDWL

List of epitopes

Assess the data by organizing patients into groups and comparing groups for unique sequences

Group 1:

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AVCDCVALWPGW
YEPWRDGFVDCG
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HRVGREPCDCWH

HRVGREPCDCWH
KCDCVLPFWHRT
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AVCDCVALWPGW
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CPLFMAHDCDWL

Comparison Algorithm

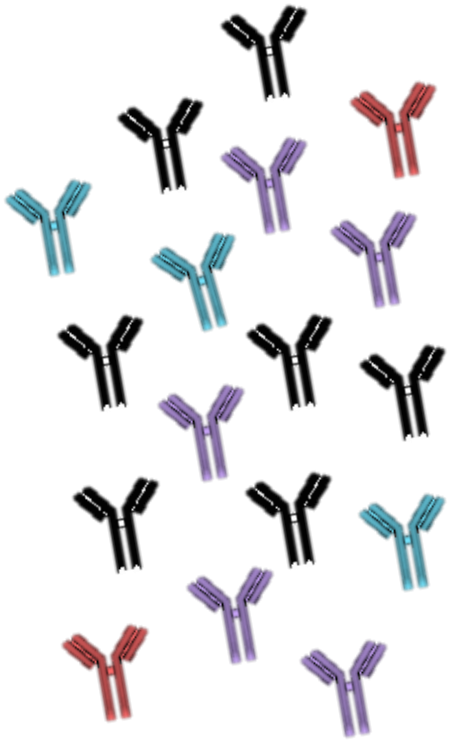
Group 2:

Healthy Patients

Assess the data by organizing patients into groups and comparing groups for unique sequences

Group 1:

Diseased Patients



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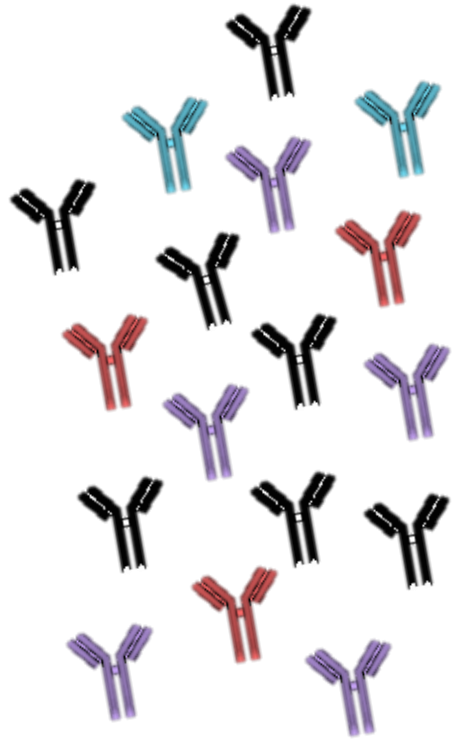
AVCDCVALWPGW
TVYALPCDCMFH
CPLFMAHDCDWL

Comparison Algorithm

Group 2:

Healthy Patients



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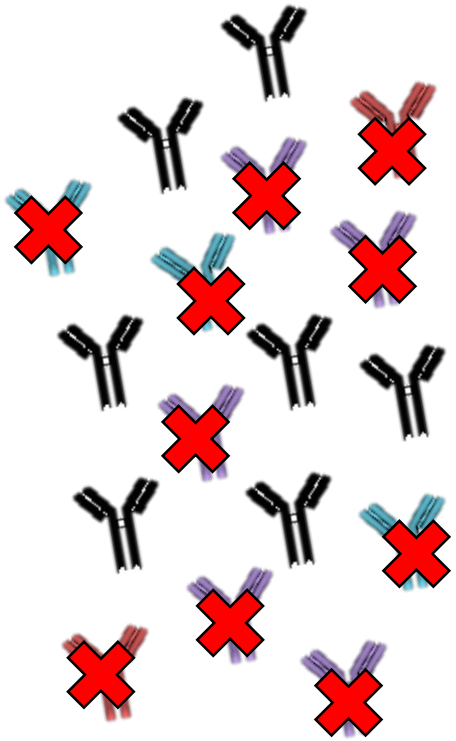
Diseased Patients



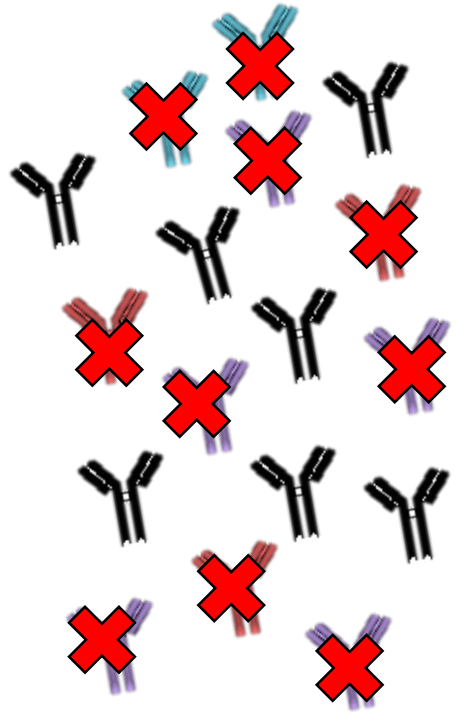
~~AVCDCVALWPGW~~
YEPWRDGFVDCG
HWFLSGHEQGWF
~~YEPTPWWFKLMF~~
WPRPGWRDFVDC
~~HRVGREPCDCWH~~

~~HRVGREPCDCWH~~
KCDCVLPFWHRT
~~YEPTPWWFKLMF~~
~~AVCDCVALWPGW~~
TVYALPCDCMFH
CPLFMAHDCDWL

Comparison Algorithm

Group 2:

Healthy Patients



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Group 1:

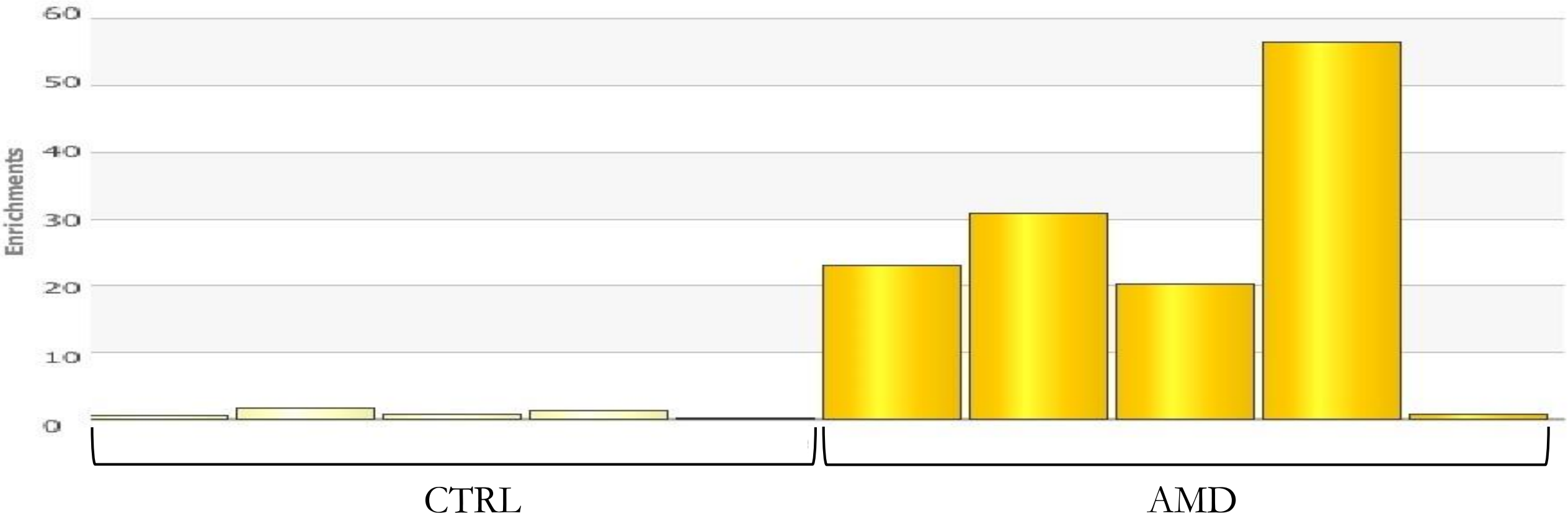
Diseased Patients

YEPWRDGFVDCG
HWFLSGHEQGWF
WPRPGWRDFVDC

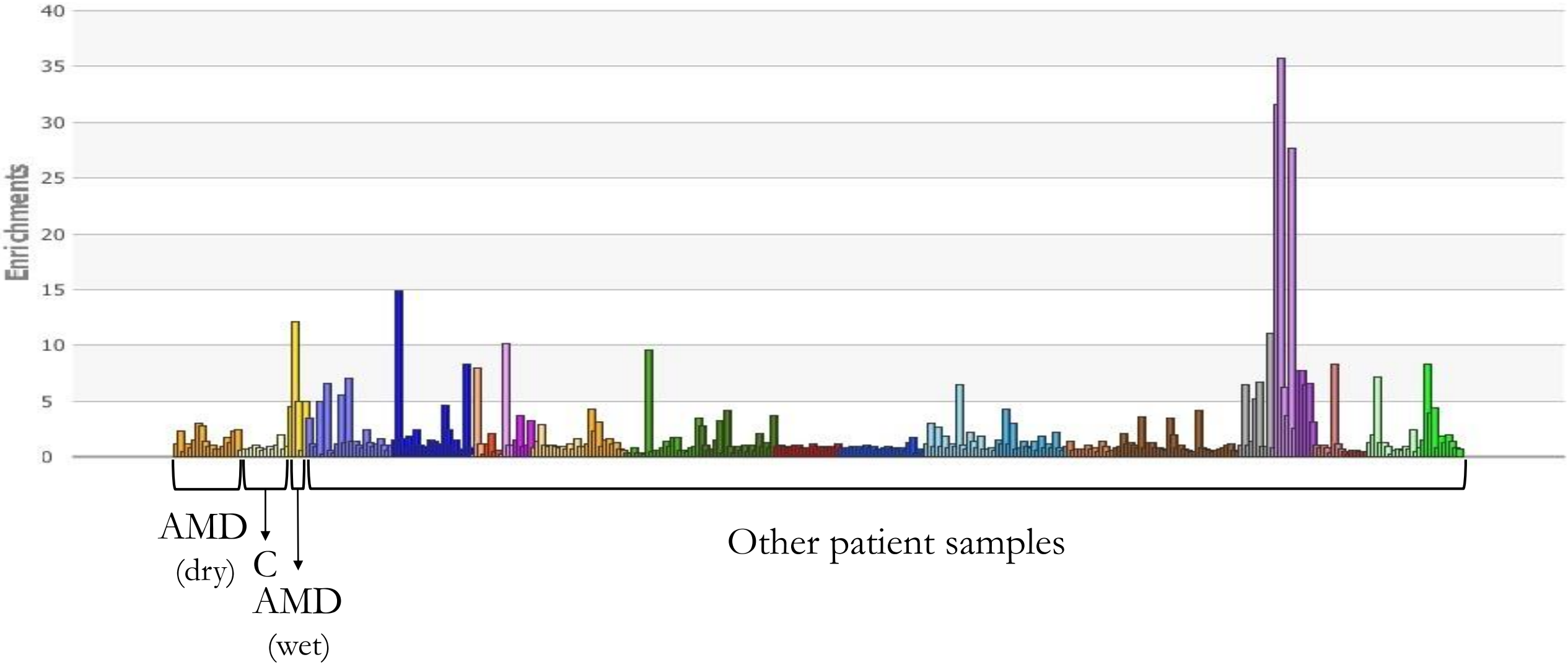
<u>Potential disease specific epitopes/antibodies</u>

Compare motif enrichment across patients to find AMD-specific reactivity

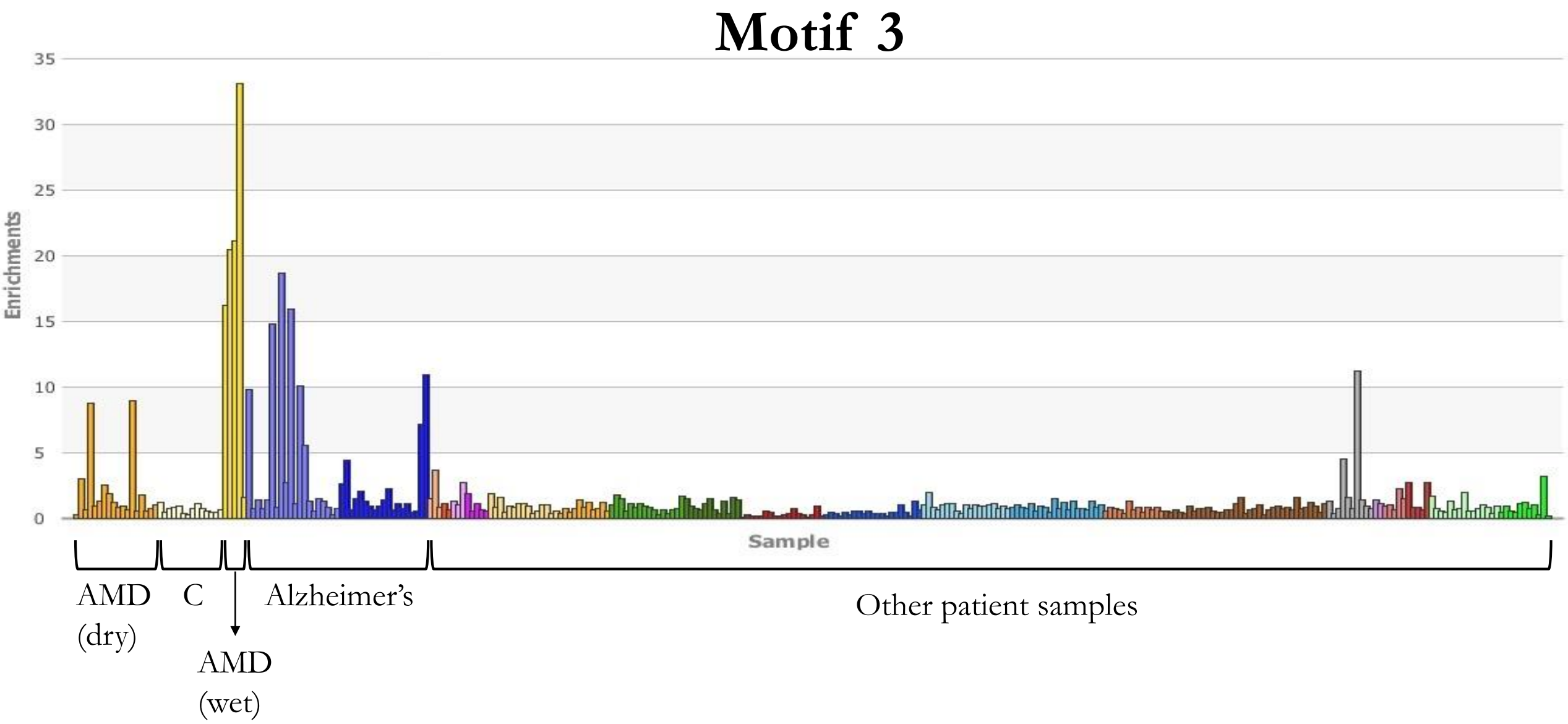
Enrichment = Observed/Expected



Utilizing the database allows us to eliminate motifs that appear AMD specific but are enriched in other samples



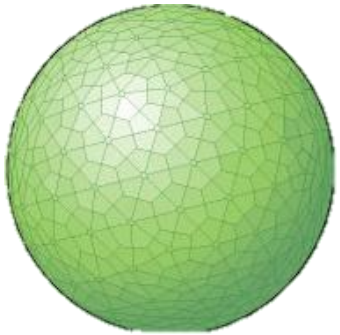
Interesting motif that shows potential AMD specificity



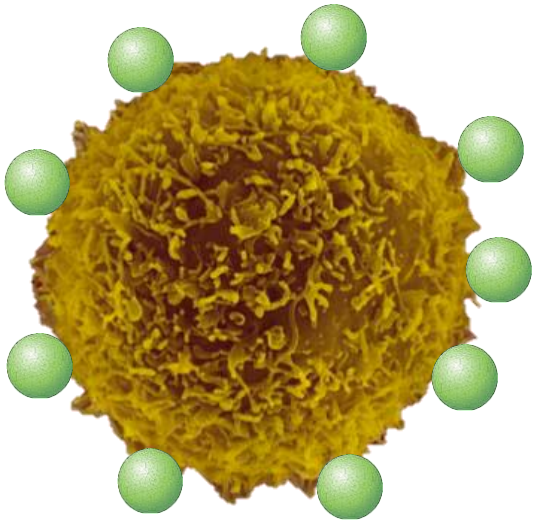
Use protein databases, BLAST, to search for a possible antigen for the peptide

Antigen-to-Protein Identification

Epitope for Human Rhinovirus: ExLVVPNI



Capsid
(antigen)

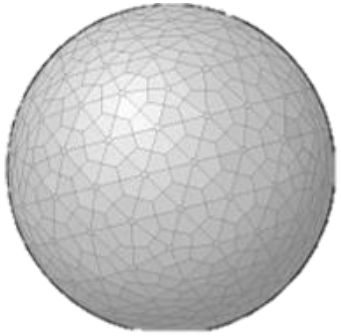


Rhinovirus
(common cold)

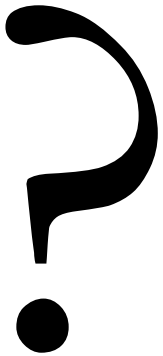
In the future we hope to discover a primary candidate disease specific epitope

AMD motif candidate

-Currently not close to realistically searching this database for a protein that is specific to AMD



Unknown Antigen



Acknowledgements

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- Arica Lubin
- Kelly Ibsen
- So many more

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