Identifying the antibody specificity repertoire

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You have a unique antibody repertoire that changes as immune responses occur. Immune factors include pathogens, allergens, and environmental factors. Different antibody repertoires are shown for each category.
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  Eye with AMD

Macula  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 relevant biomarkers

Identify disease specific peptide

Determine complimentary antigen

Accomplish with bacterial display
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

1. **Grow culture**
   - *E. coli*

2. **Combine serum and bacteria in vials**
   - Serum 1, Serum 2, Serum 3, Serum 4
   - *E. coli*

3. **Some antibodies will bind**

4. **Spin down cells**
   - *E. coli* in serum

5. **Extract the serum**
   - Depleted serum
Combine patient’s serum with a vast library of peptides and magnetically separate cells that bound to antibodies.

Grow genetically engineered culture

Engineered to display peptide

Outer membrane

Peptide

Scaffolding Protein
Combine patient’s serum with a vast library of peptides and magnetically separate cells that bound to antibodies.

**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.
Separate cells that bound with antibodies from cells that didn’t bind to antibodies via magnetic separation.

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.

Isolate the region of the plasmid that encodes for the peptide. Then use PCR to amplify the strands. Use Ilumina NGS to sequence the peptides.

KPFCDCRGLCPF
IVTLYAGCTKCD
KLGCLCTVYPAF
VPPKLPCKGTVL

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

**IMUNE**

QRHKEQPLPLVM
ASQPSEQPFPSTFC
FASLJKPEQQLTP
HPEQAKPJDKAS

**Significant Patterns**

EQPxPF
PEQPF
PEQLxPT
PEQxKP

**Clustered Patterns**

P[EQ]LPxP

[**Motif**]
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**IMUNE**

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ASPQEPFPSTFC
FASLKJ**PEQL**TP
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**Motif**

**Clustered Patterns**

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**Significant Patterns**

EQPxPF
PEQPF
PEQLxPT
PEQxKP
Assess the data by organizing patients into groups and comparing groups for unique sequences.

Group 1: Diseased Patients

- AVCDCFWPRPGW
- YEPWRDGFVDCG
- HWFLSGHEQGWF
- YEPTPWWFKLMF
- WPRPGWRDFVDC
- HRVGREPCDCWH

Group 2: Healthy Patients

- HRVGREPCDCWH
- KCDCVLPFWHRT
- YEPTPWWFKLMF
- AVCDCFWPRPGW
- TVYALPCDCMFH
- CPLFMAHDCDWL

List of epitopes

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Potential disease specific epitopes/antibodies
Compare motif enrichment across patients to find AMD-specific reactivity.

Motif 1

Enrichment = 
Observed/Expected

CTRL

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

Motif 2
Interesting motif that shows potential AMD specificity

Motif 3

AMD (dry) C Alzheimer’s Other patient samples
AMD (wet)
Use protein databases, BLAST, to search for a possible antigen for the peptide

**Epitope for Human Rhinovirus:** ExLVVPNI

**Antigen-to-Protein Identification**

**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](image)
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