Targeted amplicon next-generation sequencing of phage display libraries reactive with IgG against tick tissues

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Background

- Massive parallel sequencing of libraries encoding phage surface proteins is an approach to high throughput screening for peptides that bind to ligands.
- This project is part of a long-term effort to search for potential epitopes or mimotopes in tick tissues targeted by host sera

Problem

PCR gels reveal more than one band

Objective

Identify what band(s) represent target amplicon

Working Hypothesis

The higher molecular weight band is the target amplicon

Methods

Antisera from host exposed to tick tissues

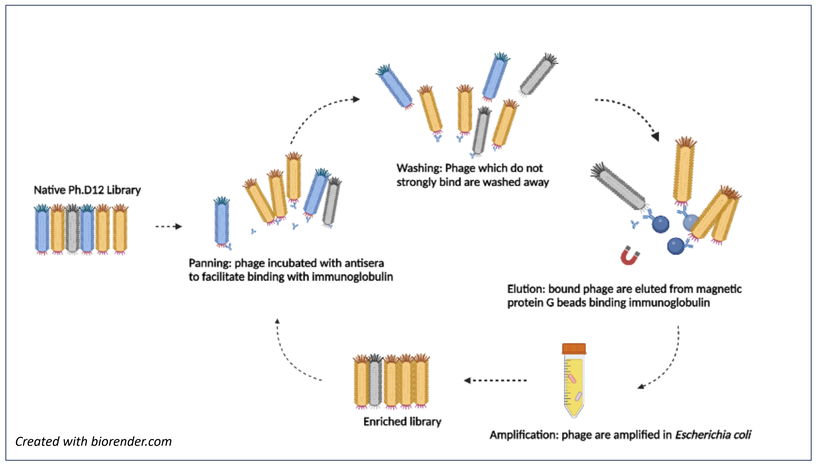

Figure 1: Phage Display: M13KE bound to bovine IgG were eluted from protein G-coated magnetic beads

PCR

M13KE + Primers + Illumina adaptors

PCR

Cloning Amplicons

Sanger Sequencing to confirm target sequence is being amplified

Illumina Next Generation Sequencing for peptide comparison

Results

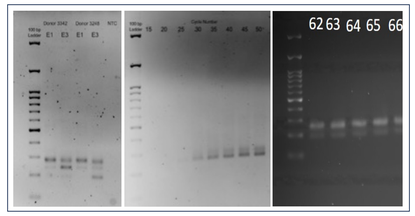

Figure 2: PCR revealed 2-3 bands. Through optimization it was reduced to two consistent bands with one close to target size (240bp), and one slightly lower (~200 bp).

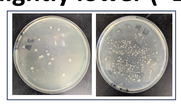

***Figure 3: E. coli* cloning colonies of amplicon product**

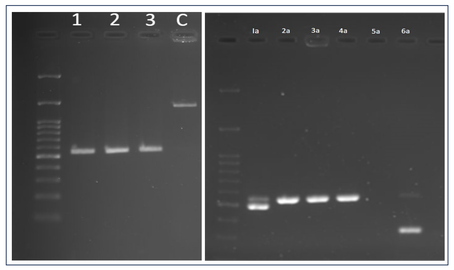

Figure 4: PCR of cloned colonies often results in one defined band, but variable placement of multiple bands is also seen in some colonies.

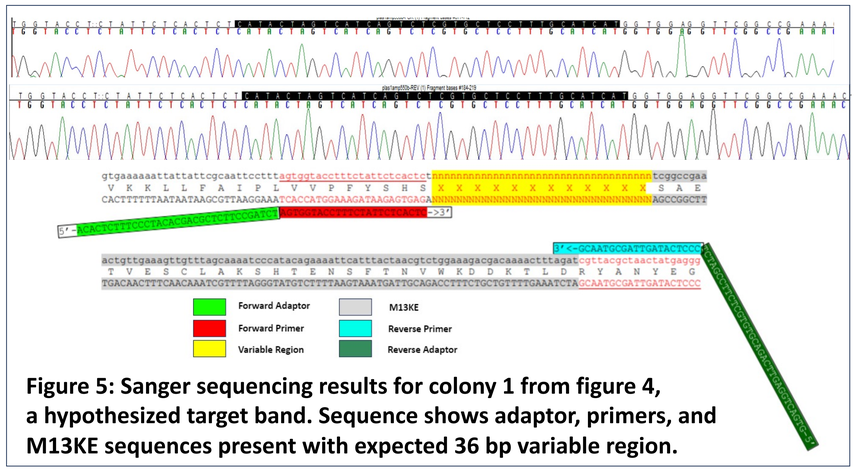

Figure 5: Sanger sequencing results for colony 1 from figure 4, a hypothesized target band. Sequence shows adaptor, primers, and M13KE sequences present with expected 36 bp variable region.

Sanger Sequencing: Potential Outcomes

A	B	C
One band is target	Both bands target	Neither band target
Isolate just target for Illumina sequencing	Illumina sequencing for both bands	Re-evaluate phage display techniques

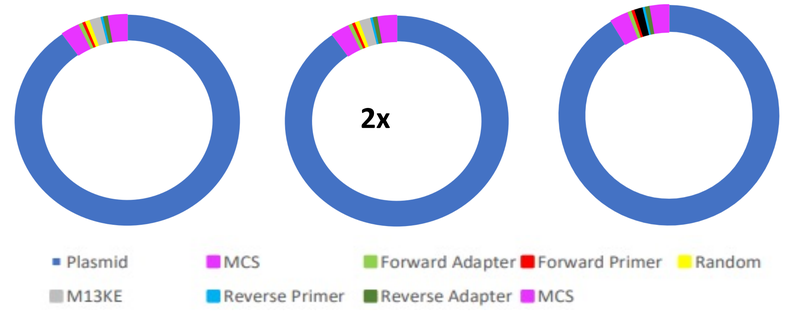

Figure 6: Expected results with

- If the amplicon is the target, this is identified by the presence of the M13KE DNA found next to the insertion region, as well as the forward and reverse primers and Illumina adaptors added
- The 36 base pairs found between the forward and reverse primers are the variable region, which should express the protein on the pIII surface of the phage to which was binding IgG in antisera
- Each amplicon will likely have a different variable region, which could be representing different epitopes or mimitopes
- Non-target sequences will not include this pattern of nucleotides

Next Steps

- More Sanger sequencing of amplicon product to confirm target product, redesign primers
- Secondary PCR with NGS-compatible primers and adapters flanking amplicon library-specific barcode sequences
- Illumina sequencing of pooled libraries
- Bioinformatic identification, sorting and characterization of individual M13KE-derived amplicon libraries.