Next generation sequencing of phage display libraries uniquely reactive to IgG from cattle protected against biologic transmission of *Anaplasma marginale*

Veterinary Research Scholars Program
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Background

- *Anaplasma marginale,* the primary etiologic agent of bovine anaplasmosis, is biologically transmitted to cattle by Rhipicephaline ticks
- IgG is an effector in immune resistance to ticks
- Host immunity to ticks is a strategy to control transmission of pathogens
- M13KE phage display libraries are used to identify peptides mimetic of reactive epitopes
- Next generation phage display is high-throughput approach for sequencing complex libraries

Objective

- Identify peptides uniquely reactive to antisera from cattle protected from biologic transmission of *Anaplasma marginale*

Methods

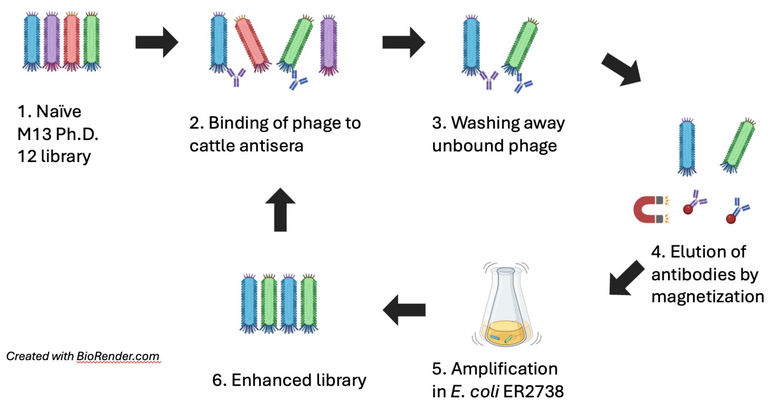

Figure 1. Selection of phage reactive to bovine IgG. Random M13KE phage bind to bovine IgG, with any unbound phage washed away. Bovine IgG bind to Protein G-coated magnetic beads and the reactive phage are eluted from the magnetic beads and amplified in *E. coli*. This product is the enriched library. This process is repeated to select for more selection of phage reactive to the bovine antisera.

Serum from cattle exposed to tick tissues

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Phage display (Figure 1)

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PCR of phage (Figures 2-3)

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Purification of PCR samples (Figure 4)

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Quantification of samples (Figure 5)

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NovaSeq X Next Generation Sequencing (Figures 6-7)

Target Amplicon

Figure 2. Target Amplicon. The M13KE target sequence is ~ 220 bp, in addition to the ~30 bp Illumina adapters. The M13KE forward primer is 1 nucleotide upstream of the variable regions that have random 36 bp sequence in the pIII protein on the phage surface, which is the region selected for reactivity to bovine IgG.

PCR

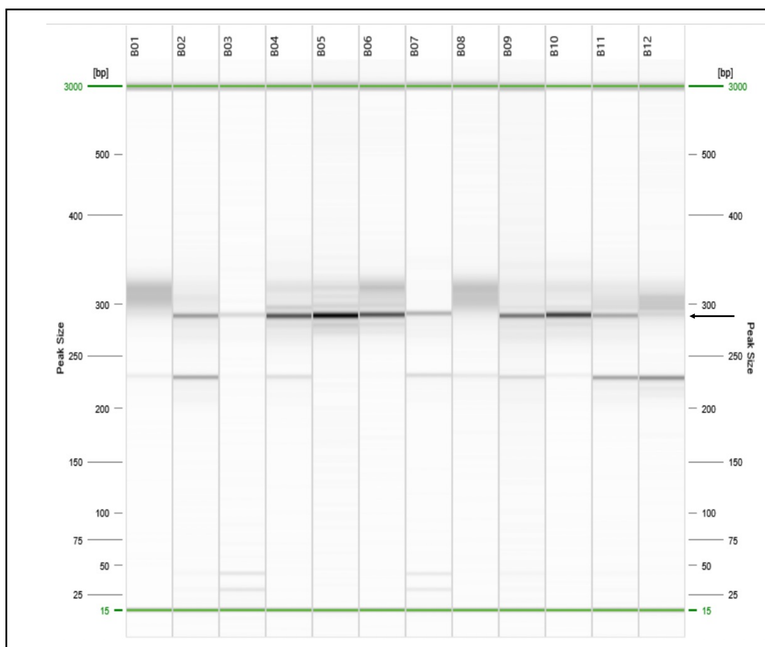

Figure 3. M13KE-derived amplicons. The target amplicon (~280bp) is consistent with the higher band.

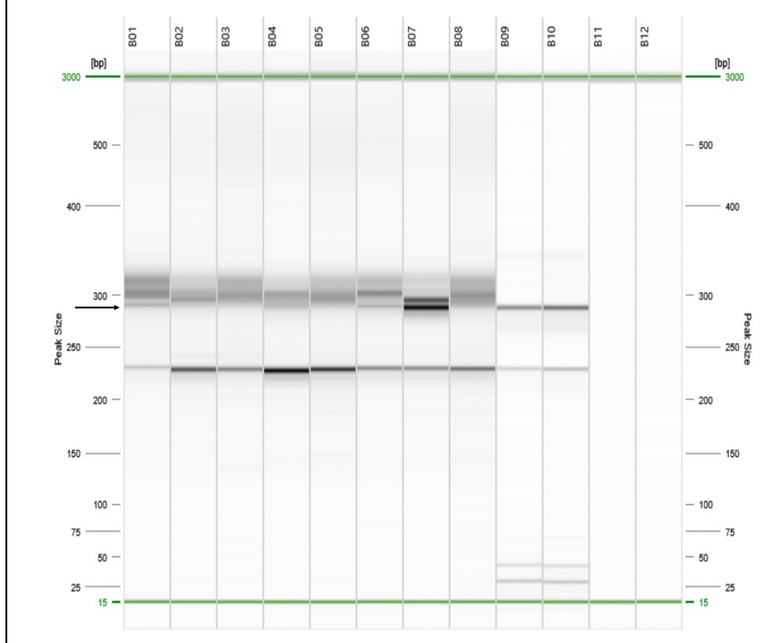

Figure 4. Electrophoresis after DNA purification to confirm presence of target amplicon.

Quantification

Sample Name	Total Samples	Original Sample Conc. (ng/μL)
S1	8	75.6
S2	8	0.228
S3	8	51.8
S4	8	7.16
S5	8	20.6
S6	8	106
S7	8	8
S8	8	92
S9	8	83.2
S10	8	58.6

Figure 5. Quantification of PCR products with a Qubit Fluorometer. All samples were diluted to 1 ng/ul in 60ul for NovaSeq Next Generation Sequencing.

Next Generation Sequencing

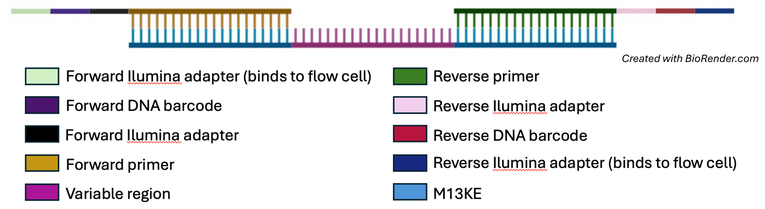

Figure 6. Amplicon-Targeted Next Generation Sequencing. Another round of PCR is performed with primers specific to the first set of Illumina adapters incorporated when targeting MKE pIII gene fragment, this time with a different Illumina adapter that anneals to the flow cell, along with a unique DNA barcode for each amplicon library.

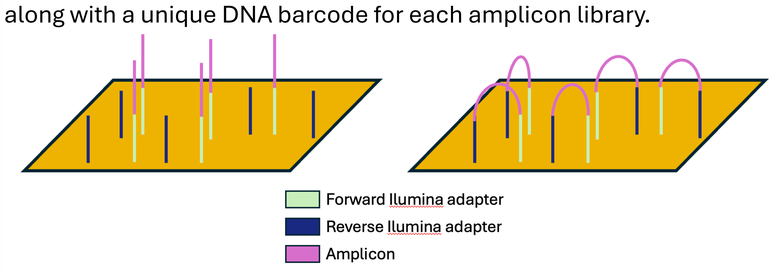

Figure 7. The amplicon replication process. The second set of adapters anneal to the flow cell. Preparatory to massive parallel sequencing, amplicons undergo bridging between the forward and reverse adapters to create clonal clusters of the DNA fragment.

Next Steps

- Bioinformatic analysis of sequences derived from the Illumina
  - Analyze artifact sequences to refine M13KE-specific primers
  - Find differences between cattle that were protected vs. unprotected
  - Find similarities between cattle that were protected
- Continuation of Next Generation Sequencing on biologic replicates
- Similar analyses with other phage display libraries

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