Apr 24th, 3:15 PM - 4:30 PM

Analyzing Wisp DNA Sequences and Building Primers for DNA Separation

Paige Spicer
Ouachita Baptist University

Payton Crum
Ouachita Baptist University

Analyzing Wisp DNA Sequences for DNA Separation

Payton Crum, Paige Spicer, and Dr. Ruth Plymale

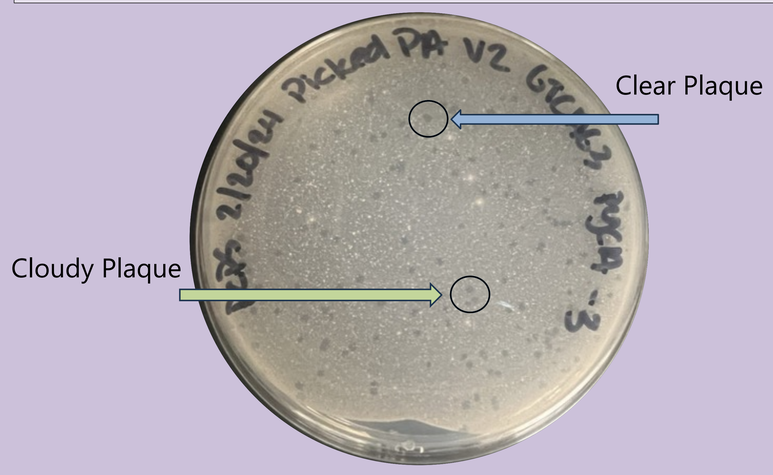
OUACHITA BAPTIST UNIVERSITY

Abstract

A bacteriophage is a virus that infects bacteria. Bacteriophage DNA was isolated from a lysate sample, but **two separate, unrelated genome sequences were returned**. Plaque assays and plaque streaking were used to in an effort to separate the phages. After plaque isolation was unsuccessful, NCBI BLAST and IDT software were used to build unique primers for each of the two unrelated genomes. After receiving the primers, PCR and gel electrophoresis were run on plaques and lysate from each phage **to classify each phage in the correct cluster and sub cluster as identified by the original DNA sequencing.**

Experimental Design

The first method used to separate the two DNA sequences was plaque assays. These plates contain dilutions of original Wisp lysate with plaques decreasing in frequency as dilution increases. Each plaque indicates successful phage infection. **Each phage creates a plaque with unique turbidity and morphology. Because each plaque is started by one phage, each can be used to identify the infecting DNA sequence.** To separate the two phage, a cloudy plaque and a clear plaque were chosen from the first plaque assay. The DNA sequencing report indicated a virulent and temperate phage were present in the lysate sample, which correspond to clear and turbid plaques, respectively. The plaque isolation protocol was repeated six times, in attempt to separate the two phage. The plates continued to produce both clear and cloudy plaques regardless of the turbidity of the picked plaque.



Plaque assay plate with clear and cloudy plaques.

Experimental Design

Primer Design

Primers are unique sequences of nucleotides that are designed to be specific to a phage cluster. **Two groups of primers were designed, one that bound only to cluster CV and one that bound only to cluster DE3.** Using the tape measure sequences of the two unrelated genomes, IDTDNA software generated five sets of primers for each genome. Two primer sets from each cluster were ordered for PCR. The primers were then used in amplification of control phage Chickadee, a DE3 phage, to ensure that the primers only attached to the expected sequence.

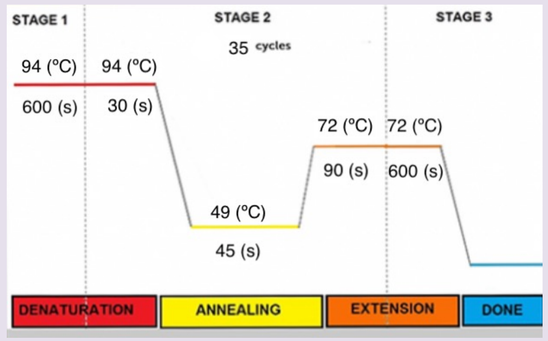
CV and DE3 Primers

Assay Set 1 (Wisp_CV_tmp)	Forward Primer	CAACGCCATCAAATGGGTTATC
Assay Set 1 (Wisp_CV_tmp)	Reverse Primer	GTGACGATGGTGTCGAAGAA
Assay Set 1 (Wisp_CV_tmp)	Product	384 base pairs

Assay Set 2 (Wisp_DE3_tmp)	Forward Primer	TCGGTGCGTTCAAGGTATTC
Assay Set 2 (Wisp_DE3_tmp)	Reverse Primer	CGATCACGTTCGCAACAAATC
Assay Set 2 (Wisp_DE3_tmp)	Product	891 base pairs

Polymerase Chain Reaction

A specific protocol was constructed for each Wisp primer based on its unique melting temperature. The DE3 protocol is shown below. After running separate PCR for the DE3 and CV primers, the products were collected to be run for gel electrophoresis.

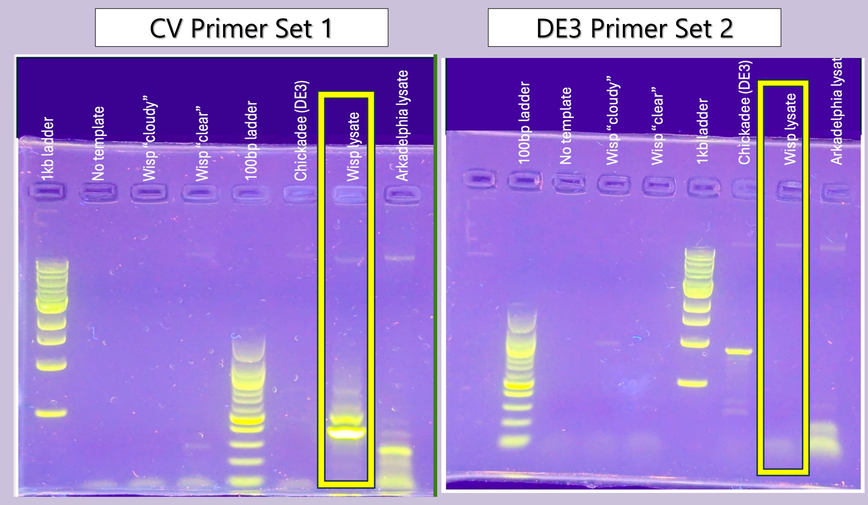


Gel Electrophoresis

The amplified DNA products from PCR were run on agarose gels to separate the sequences by length. **The band length corresponds to the PCR product size, which is specific for CV and DE3 clusters.** By analyzing the size of the DNA bands, the primer that bound, and therefore the cluster of the phage, can be determined.

CV Primer Set 1

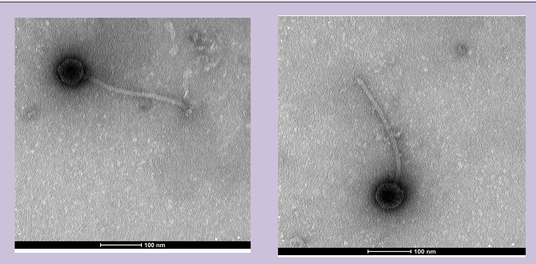
DE3 Primer Set 2



Gel electrophoresis results with Wisp boxed.

Results

As indicated by the gel electrophoresis pictures above, **Wisp lysate produced DNA bands only with the CV primer**. There was no Wisp amplification with the DE3 primer. The DE3 gel did contain bands for the control phage, Chickadee, as expected. This gel also contained bands for phage Arkadelphia, another phage isolated at Ouachita. No bands appeared on either gel for the negative control, indicating that the DNA results are accurate.



Wisp electron microscopy photographs.

Conclusions

Based on these results, it was determined that phage Wisp does belong to the CV cluster. The DE3 DNA originally sequenced with Wisp does not appear to belong to Wisp. The DE3 DNA may have originated from phage Arkadelphia which was isolated in close proximity to Wisp. Because the gel electrophoresis results were inconclusive, further testing must be done to verify the source of the DE3 subcluster DNA.