An Investigation of K2 Mycobacteriophage Lysin A Proteins

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Abstract

Within the genetically similar grouping of subcluster K2 mycobacteriophages, the mycobacteriophage Mufasa has demonstrated an anomalous hyper-infection of other K2 subcluster *Mycobacterium smegmatis* mc² 155 lysogens. Additionally, attempts to use bacteriophage recombineering with electroporated DNA (BRED) to create immunity repressor knockouts of Mufasa have been unsuccessful, with mutants detected, but not viable to purification. A hypothesized source of these irregularities is thought to come from Mufasa's Lysin A protein, which belongs a different phamily than most other K2 subcluster phages, excluding TM4 and BoostSeason. Lysin A proteins function as endolysins, an essential component of mycobacteriophage lysis machinery necessary for the degradation of the peptidoglycan cell wall of mycobacteria and release of progeny phage. With its critical role in the bacteriophage life cycle, the Lysin A protein offers a logical starting point to explain varying phenotypes of phage infection among genetically similar K cluster phages. In this project, Mufasa and ZoeJ Lysin A proteins will be cloned into an expression vector to be purified, the structures of each Lysin A will be determined by X-Ray crystallography for comparison, and purified Lysin A protein samples will be assayed for potential antibiotic activity against *M. smegmatis*.

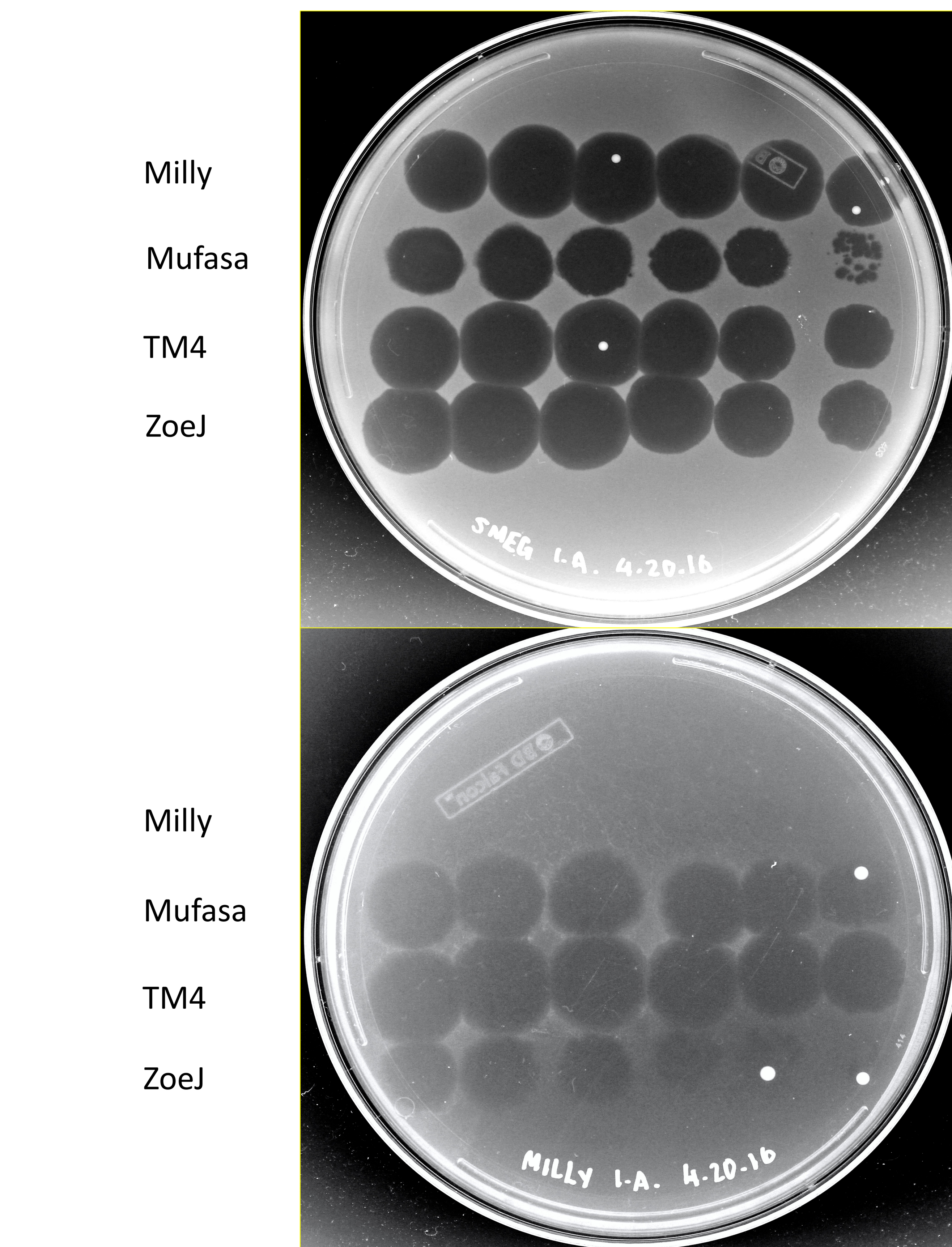

Figure 1. An immunity assay with a Milly (K2) lysogen. Compare infection against *M. smegmatis mc² 155* (upper), with infection against lysogenic *M. smegmatis* (lower).

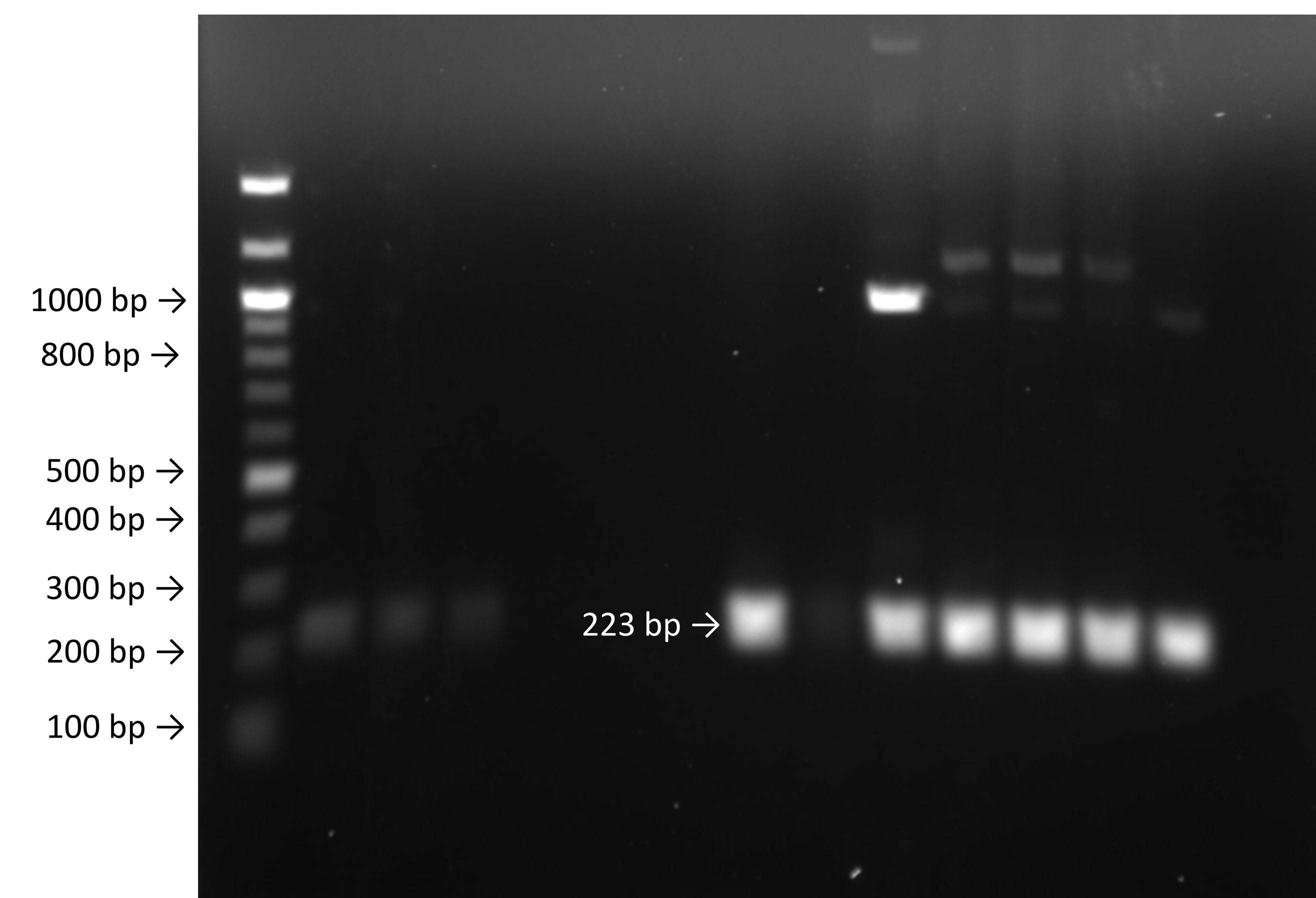

Figure 2. Attempts at using bacteriophage recombineering with electroporated DNA (BRED) to delete the Mufasa immunity repressor gene (gp 46) were unsuccessful, with only the first round of screening yielding the expected amplicon.

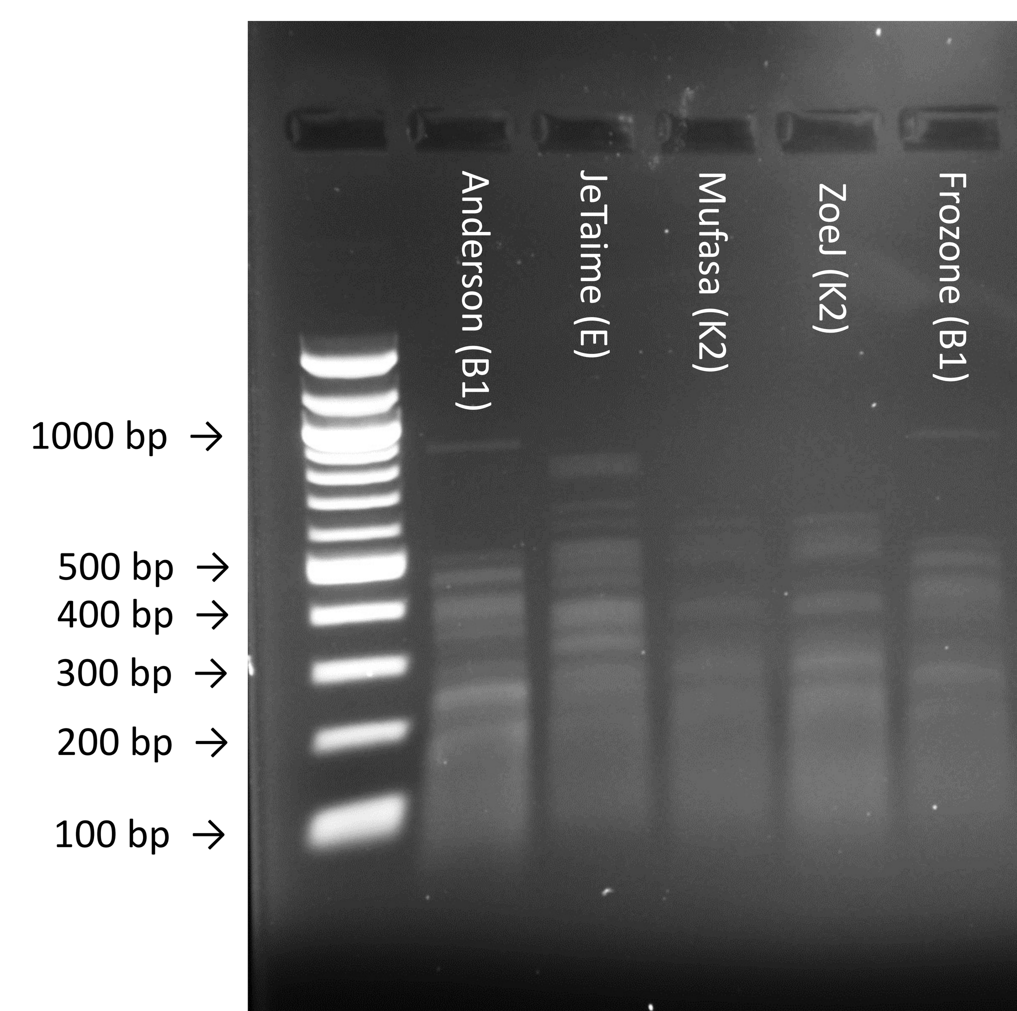

Figure 3. A Hae III restriction digest of Mufasa DNA. The restriction digest pattern of Mufasa DNA can be compared to other non-K cluster phages to assess purity of the sample.

Hypothesis

Mufasa seems to exhibit a hyper-infection phenotype against lawns of other K2 subcluster phage lysogens. Additionally, it has been recalcitrant to immunity repressor deletion using BRED. These observations warrant further investigation of Mufasa's mechanisms of host-cell infection. Isolation of Mufasa and ZoeJ Lysin A proteins will allow for assays of potential antibiotic activity, and future Lysin A structural determinations might provide valuable information to explain these observations.

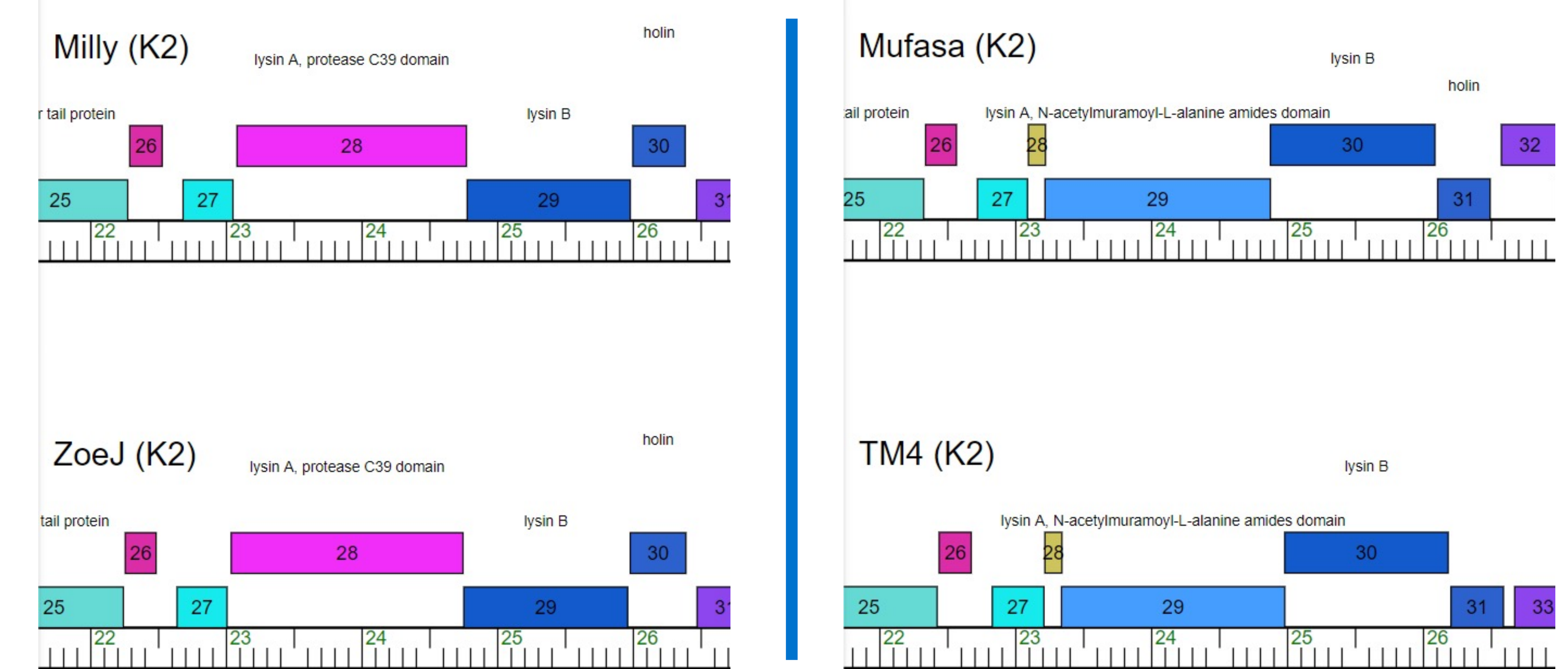

Figure 4. A Phamerator map of K2 subcluster Lysin A proteins. The phages Mufasa, TM4, and BoostSeason have a Lysin A that belongs to a different phamily than other K2 subcluster phages, such as ZoeJ and Milly.

Methods

Primers that include the 5' and 3' regions of the Lysin A gene and an overhang with sequence homology to the pET28a plasmid DNA will be used in PCR with Mufasa DNA as a template to create and amplify the insert. An empty pET28a plasmid vector will also be prepared by doing a plasmid isolation on *E. coli* containing pET28a. The empty pET28a vector will then be linearized by digestion with the restriction enzyme EcoRI and treated with calf intestinal alkaline phosphatase (CIP) to remove a phosphate from the 5' end of the plasmid DNA to prevent the cut plasmid from re-ligating without the insert. Next, Gibson Assembly will be performed using the linearized pET28a vector and the Lysin A insert to construct a pET28a plasmid containing the bacteriophage Lysin A gene. This plasmid will then be transformed into DH5α *E. coli* cells for plasmid replication. Once pET28a containing the Lysin A protein has been replicated, another plasmid isolation will be done to obtain the Lysin A pET28a plasmid from *E. coli*. This plasmid will then be transformed into competent Xjb cells, which will synthesize the Lysin A protein from the pET28a Lysin A plasmid. Finally, the protein will be purified from the Xjb cellular lysate with affinity chromatography using a nickel column. Purified protein samples will be exposed to a lawn of *M. smegmatis* and Lysin A enzyme antibiotic activity will be monitored.

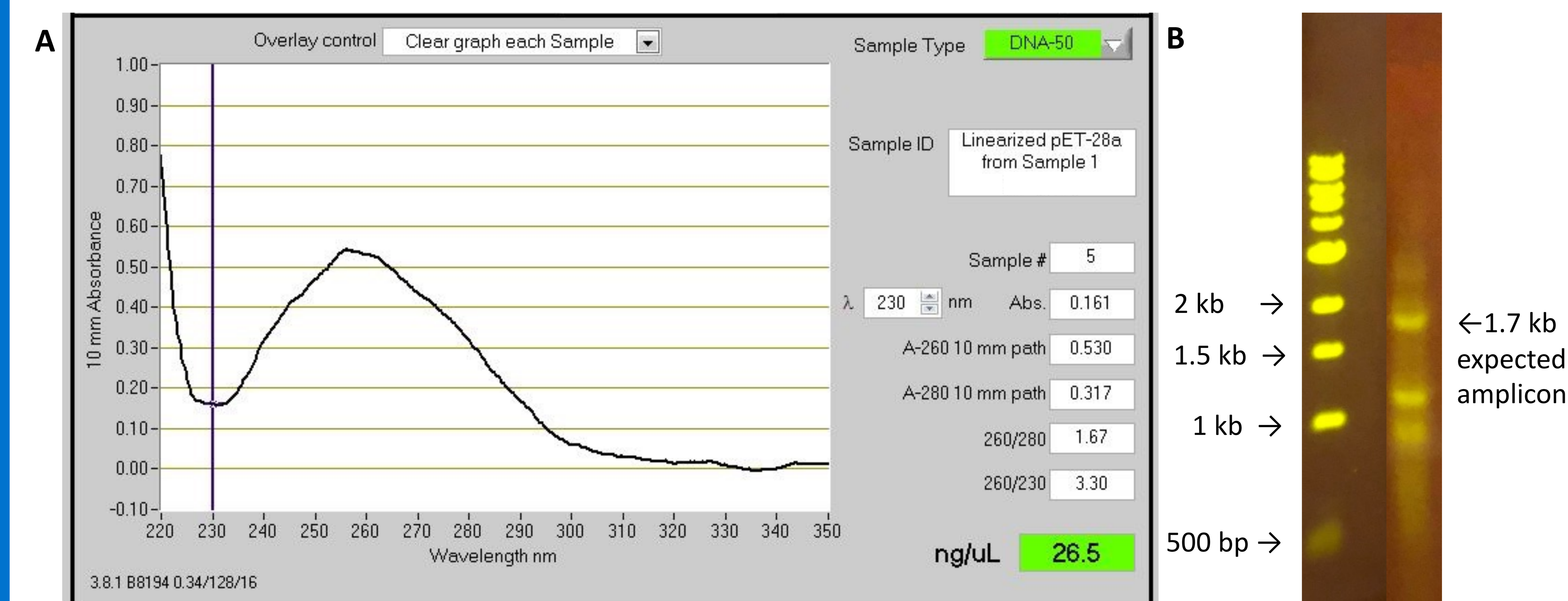

Figure 5. A) Linearized pET28a plasmid which will be used in Gibson Assembly. B) PCR amplification of Mufasa Lysin A Insert for use in Gibson Assembly. The Lysin A protein will be overexpressed once it has been inserted to the pET28a expression vector.

References

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Payne, K. M.; Hatfull, G. F. Mycobacteriophage Endolysins: Diverse and Modular Enzymes with Multiple Catalytic Activities. *Plos One*. **2012,** 7 (3), e34052. DOI: 10.1371/journal.pone.0034052
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