

Cloning a gene involved in antigen presentation to be expressed by an oncolytic virus

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Introduction

Often in cancers, there is a lack of recognition of cancer cells by the immune system because of a dysfunctional antigen presentation pathway, resulting in inefficient anti-tumor responses. Many cancers have undergone genetic or epigenetic alterations resulting in silencing of genes involved in regulating the antigen presentation pathway. Oncolytic viruses (OV) are potent cancer-killing self-replicating therapeutics that are capable of triggering anti-tumor responses. However, anti-tumor responses following OV infection rely upon a functional antigen presentation pathway.

Objectives

The objective of this project is to clone a gene involved in regulating the antigen presentation pathway into a mammalian expression vector.

Long-term aims of this project are to express this gene in an oncolytic virus. Mouse tumor models will be evaluated in terms of increased tumor regression, survival, and increased anti-tumor response following infection by oncolytic vaccinia virus expressing this gene compared to the parental virus.

Methods

The gene of interest contained within a gateway expression vector for bacterial systems was used to transform competent DH5-alpha *E. coli* cells. These cells were grown in spectinomycin spiked LB plates. Plasmid DNA was isolated by a minipreparation. PCR was used to amplify and flank the gene with restriction sites for two restriction enzymes. The amplified gene and mammalian expression vector were each double digested using the same two restriction enzymes, and the double digested gene was subsequently ligated into the double digested mammalian expression vector.

Results

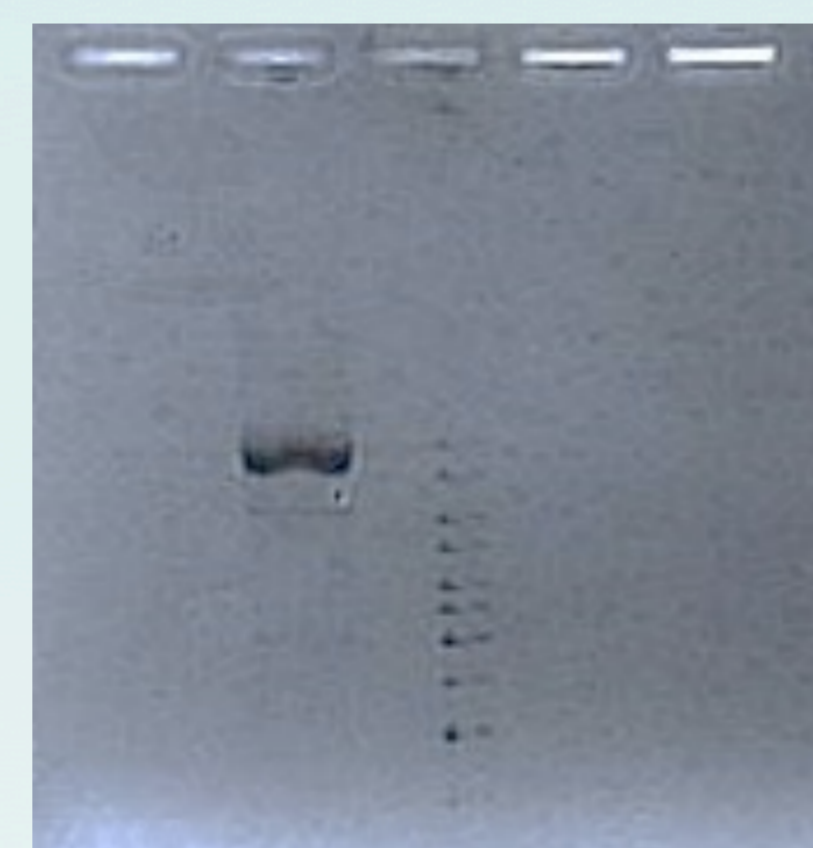


Figure 1: 1% agarose gel electrophoresis of miniprep product. Plasmid DNA from a colony that was successfully grown in spectinomycin spiked medium was isolated by miniprep. By comparison to a 1kB GeneRuler DNA ladder (lane 3), plasmid DNA in elution buffer (lane 2) was confirmed to have a size of approximately 6500 bp.

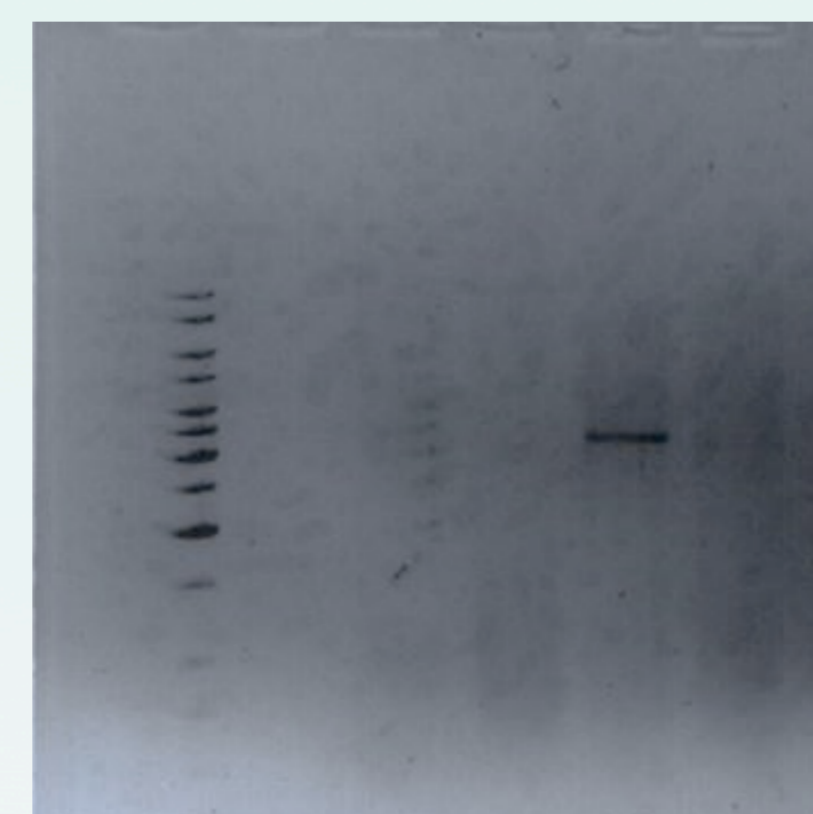


Figure 2: 1% agarose gel electrophoresis of PCR amplicon. Of the four PCR products analyzed in this gel (lanes 3, 4, 5, and 6), only one successfully amplified the gene (lane 5). By comparison to a 1kB GeneRuler DNA ladder (lane 1), the PCR amplicon was confirmed to have a size of approximately 3500 bp, the size of the gene of interest.



Figure 3: 1% agarose gel electrophoresis of digestion products. The double digested mammalian expression vector (lane 3) was confirmed to have a size of approximately 5500 bp by comparison to a 1kB GeneRuler DNA ladder (lane 1). The double digested gene (lane 2) is too dilute to be visualized.



Figure 4: 1% agarose gel electrophoresis of ligation product. The ligation product (lane 3) has the expected size of the mammalian expression vector containing the gene of interest (8000-9000 bp), judged by comparison to a 1kB GeneRuler DNA ladder (lane 1). It is also evident that the ligation product is larger than the empty linearized vector as visualized in Figure 3.

Conclusion

The gene of interest was successfully cloned from the bacterial gateway expression vector into a mammalian expression vector that can be manipulated for OV expression and infection into mouse tumor cells.

- A PCR protocol was designed that successfully amplified the gene of interest
- The gene and mammalian expression vector were each successfully double digested using restriction enzymes
- The double digested gene was successfully ligated into the double digested mammalian expression vector

Further confirmation of successful cloning of the gene of interest can be provided by sequencing and expression by Western blot analysis

References

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2. Kim, DH and Thorne, SH. 2009. Targeted and armed oncolytic poxviruses: a novel multi-mechanistic therapeutic class for cancer. 1:64-71

