Construction of Recombinant Vaccinia Virus for Oncolyitc Therapy

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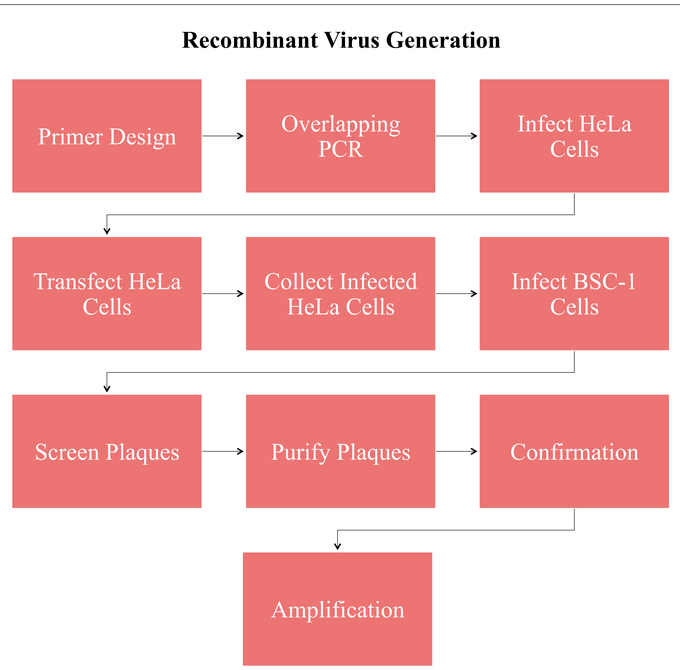
Abstract

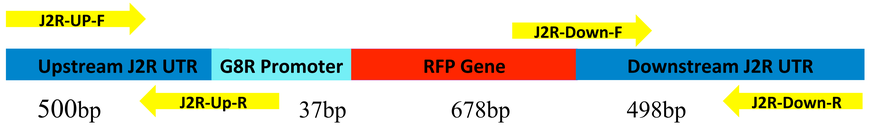
Homologous recombination is a mechanism conducted to exchange nucleotides between two similar sequences, which allows researchers to knock out and add genetic sequences of their interest in vaccinia virus (VACV). In this study, a Western Reserve VACV strain with inserted genes encoding Red Florescence Protein (RFP) and Green Florescence Protein (GFP), will be targeted and replaced with the Thymidine Kinase gene, J2R as well as C11 gene, respectively. This will set the foundation for continual removal of genes as well as additions to make a virus that will more effectively target cancer cells without affecting healthy cells. To conduct this experiment, the J2R gene was constructed via overlapping PCR and shared homology with the Western Reserve strain allowing for the homologous recombination to occur. HeLa cells are then infected and transfected with the VACV and the J2R overlapping PCR fragment, respectively. The transfection of the overlapping J2R PCR fragment into the infected HeLa cells will allow the virus to recombine. The infected cells are then collected and used to infect BS-C-1 cells, which allowed formation of plaques. The green (GFP) plaques without RFP are to be collected and undergo several rounds of purification. Upon purification, the DNA will be extracted using an SQ Blood Kit and enhanced via PCR. To confirm the virus recombined appropriately, the DNA will be sent out for sequencing and the procedure will be repeated for the replacement of GFP with C11 overlapping PCR fragment. Upon successful recombinant virus construction, the virus will undergo additional knockouts as well as additions that will allow the virus to target cancerous cells more effectively.

Methods



Results

A. RFP gene within J2R gene with Primers



B. Expected Recombination with Overlapping Primer

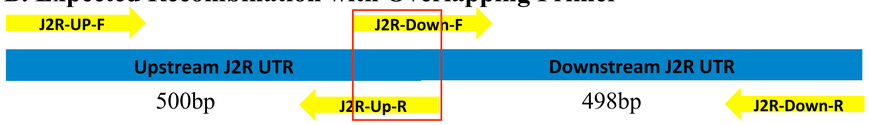


Figure 1: Schematics of Red Florescence Protein integrated in VACV and Expected Recombination with Overlapping Primer.
A. Schematic contains the current schematic of the Western Reserve Vaccinia Virus with the Red Florescent protein integrated along with a G8R promoter. The primers are included, two of which were design for overlapping PCR.
B. The expected recombination of the vaccinia virus with the overlapping PCR segment. This then allows the J2R upstream and downstream green segments to rejoin.

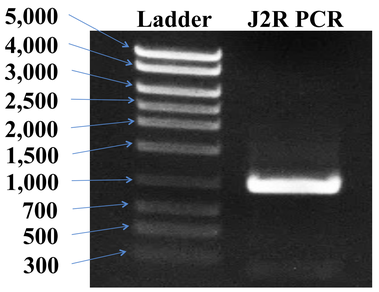
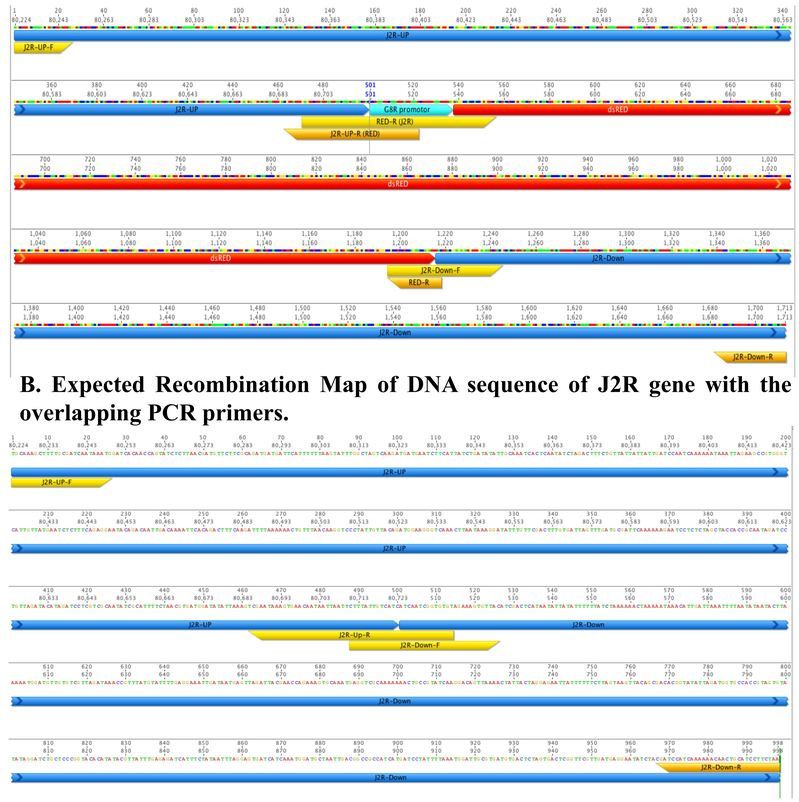


Figure 2: Screening and Conformation of Overlapping PCR Fragment using Gel Electrophoresis
The electrophoresis gel demonstrates the correct fragment from the overlapping PCR reaction. This will be used transfect the HeLa cells.

A. DNA Sequence Construct of Red Florescent Protein within the J2R gene with the designed primers



B. Expected Recombination Map of DNA sequence of J2R gene with the overlapping PCR primers.

Figure 3: DNA sequences of the initial Vaccinia Virus with RFP and the expected recombination map of the J2R gene.

Conclusion and Future Research

- After the removal of the Red Florescent Protein from the Western Reserve Vaccinia Virus, the procedure will be repeated with the removal of Green Florescent Protein using the same method used in this experiment.
- This research is setting the foundation for additional gene knockouts as well as gene implementations that will allow the virus o more specifically target cancer cells.
- The genes that will be targeted include viral immunoregulating genes of Vaccinia Virus including Virus Growth Factor (VGF) and K1L gene.

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