Engineering *E. coli* to Produce Virus-Like Particles for Gene Transfer into Mammalian Cells

Gaurav Basnet

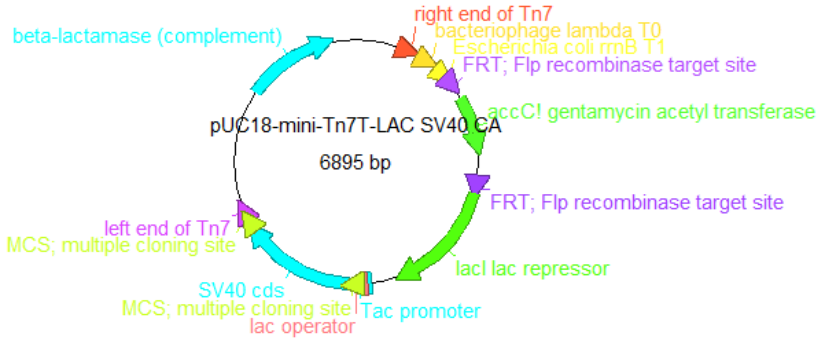
PI: Dr. Nikunj Somia

Background (General)

- “Gene therapy”: The practice of genetically modifying a patient’s cells in order to improve the health of the patient.[1]
- Gene therapy requires a gene transfer vector, the vehicle by which genetic material is transferred into a host cell.[1]
- Viral gene transfer vectors are efficient but associated with high production costs.[2]
    - Viral vectors are typically produced in non-bacterial cells such as mammalian cells and insect cells.[3]
    - Successful production of viral vectors in bacterial cells such as *E. coli* has the potential to lower production costs due to the faster division rate of *E. coli* cells and ability to generatate high protein yields.[3]
    - However, many bacteria lack post translational modification systems that are present in mammalian cells.[4]

Background (VP1)

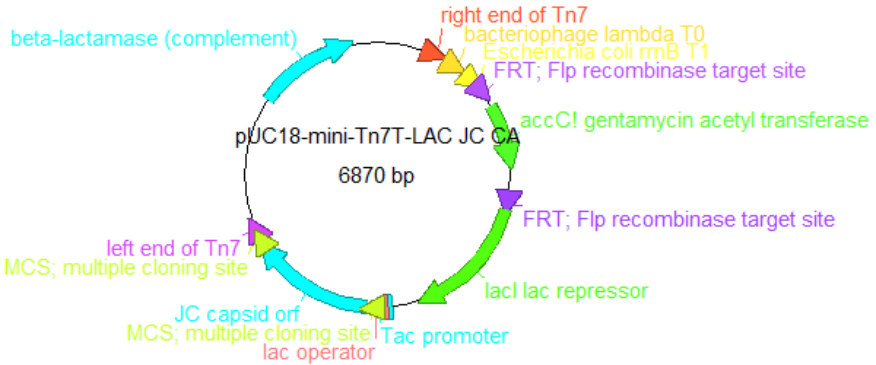
- VP1, the major capsid protein of human JC virus, has been demonstrated as a successful viral vector.[3]
- When expressed in *E. coli* cells, the VP1 protein self-assembles into capsid-like particles and retains the ability to contain, or "encapsidate," genetic material.[3]
- These "pseudocapsids" can deliver exogenous DNA into mammalian cells (specifically, human kidney cells).[3]
- As a result, a plasmid containing the VP1 gene can be considered an effective gene to code for a viral gene transfer vector.



Left: A graphic representation of a plasmid containing the VP1 gene of the SV40 virus.

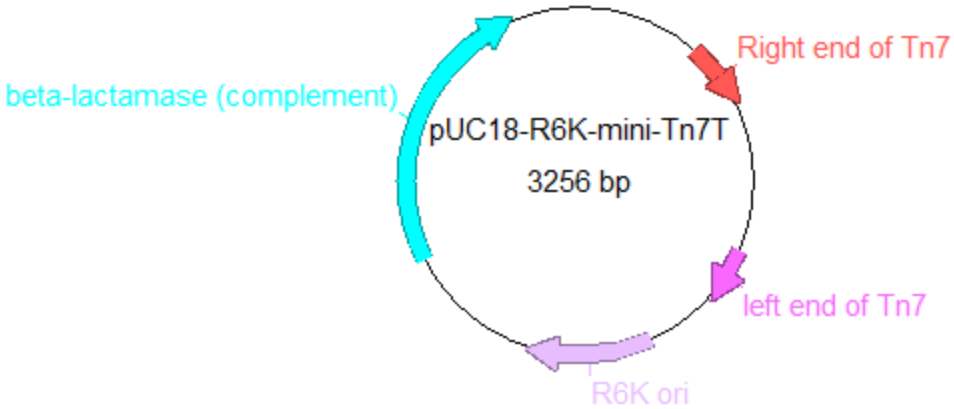
Right: A graphic representation of a plasmid containing the VP1 gene of the JC virus.

Graphics created using ApE software.



Background (Tn7)

- In order to procure a broad-range expression system, a methodology based on the Tn7 transposon has been developed.[4]
- In bacteria with a single chromosome, the introduction of a Tn7-based vector resulted in efficient transposition at the attTn7 site in the bacterial genome.[4]
- Therefore, the Tn7 system is a promising method to transfer genes for viral vector production in *E. coli*



Left: A graphic representation of an R6K vector plasmid. The Tn7 section can be seen in the image. Graphic created using ApE software.

Goal & Hypothesis

Goal: This research seeks to utilize the Tn7 system to transfer the gene to produce VP1 viral vectors in *E. coli*

Hypothesis: The production of the self-assembling VP1 capsid protein in *E. coli* cells via the transfer of the gene using the Tn7 system of transposition will result in the encapsidation of genetic material and subsequent gene transfer to mammalian cells due to the encapsidation ability of VP1 viral capsids and the ability of VP1 to act as a viral vector.

Aims

Aim 1*:* Successfully integrate the VP1-edited Tn7 vector into the *E.coli* genome. A PCR test will be performed to confirm success.

Aim 2*:* Successfully encapsidate a plasmid carrying the EGFP (green fluorescent protein) gene within the expressed viral capsids present in the *E. coli* cells. *E. coli* lysate will be treated with DNase to confirm success.

Aim 3*:* Transfer the EGFP gene from *E. coli* cells to mammalian cells via the expressed viral capsids. Mammalian cells will be observed for signs of green fluorescence in order to confirm success.

None of the aims were fully accomplished within the timespan of this UROP project. Only Aim 1 was attempted.

Methods

Detailed here are the methods used to attempt the first part of Aim 1 (cloning the VP1 capsid gene into the Tn7 transposon vector), which encompasses the entire scope of my efforts within the timespan of this UROP project.

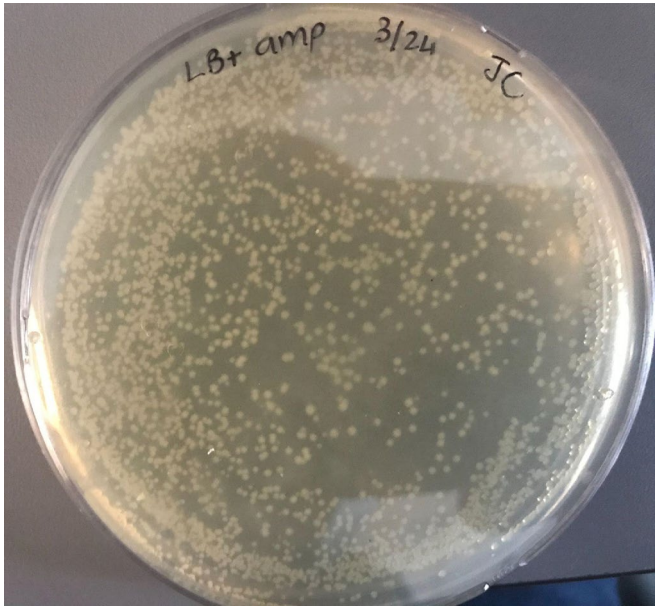
- Purify two pre-made minipreps containing SV40 and JC1 capsid genes with plasmid backbones respectively. Purify a pre-made miniprep containing R6K vector plasmids as well.
- Digest all three purified minipreps with with Sal1 and Stu1 restriction enzymes. The digest is carried out with restriction enzyme buffer.
- Run these minipreps through gel electrophoresis to confirm band sizes are accurate.
- Transform the SV40, JC1, and R6K minipreps into *E.coli* bacteria on ampicillin plates, since all three plasmids confer ampicillin resistance. Incubate all at 37 degrees Celsius overnight.
- Pick colonies from each ampicillin plate and grow each colony in an lb media liquid culture containing ampicillin. Incubate in a shaking incubator at 37 degrees Celsius overnight.

Methods

- Make minipreps from each liquid culture using P1, P2, N3, PB, and PE buffer.
- Digest all minipreps with with Sal1 and Stu1 restriction enzymes.
- Run these minipreps through gel electrophoresis and cut out the gel bands that correspond to the relevant DNA fragments.
- Melt and purify each gel band.
- Ligate each of the three DNA fragments together using T4 DNA ligase and 5x T4 DNA ligase buffer.
- Transform the control and experimental ligations into *E. coli* cells on gentamicin plates, since the SV40 and JC1 sections of the recombinant plasmid should confer gentamicin resistance as well. Incubate all at 37 degrees Celsius overnight.
- Pick colonies from the experimental plate and grow each colony in a liquid culture of lb media and gentamicin. Incubate in a shaking incubator at 37 degrees Celsius overnight.
- Make minipreps from each liquid culture.
- Digest all minipreps with with Sal1 and Stu1 restriction enzymes.
- Run these minipreps through gel electrophoresis to confirm that proper ligation occurred.

Results

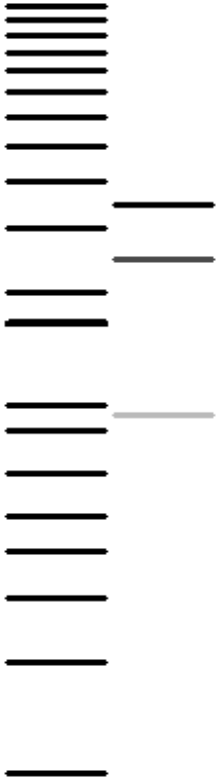
- Successfully cut SV40, JC1, and R6K plasmids with restriction enzymes.
- Successfully transformed each plasmid into *E. coli* cells on ampicillin.
- Gel electrophoresis bands for each plasmid looked accurate.
- Growth of ligated recombinant plasmid on gentamicin was inconsistent.
- Gel electrophoresis bands for recombinant plasmid were inaccurate.



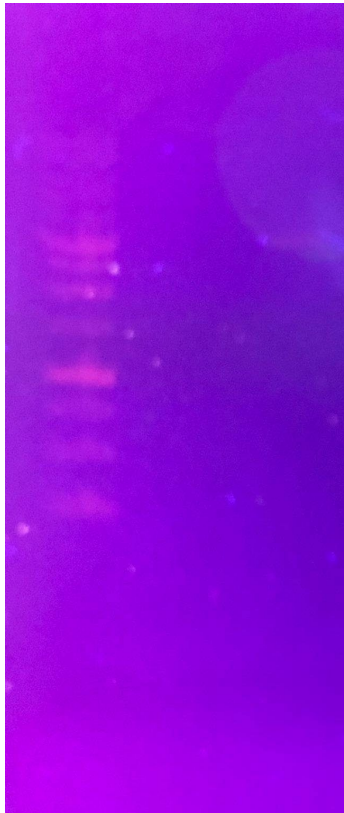
Successful JC plasmid transformed into E. coli cells on ampicillin.

Findings

- Discovered that breakthrough growth on gentamicin plates was due to old antibiotic.
- Created new gentamicin and attempted to induce gentamicin resistance in the plasmids.
- Despite attempting the following...
    - A variety of different restriction enzymes
    - Dephosphorylation of the vector plasmid
    - Using fresh antibiotics and plasmids
    - Inducing gentamicin resistance with arabinose
- ...no recombinant plasmids could be selected for.
- Conclusion: The gentamicin selectable marker is not working/induced for some reason.



Left: Expected results of R6K-SV40 recombinant plasmid digest cut with BGIII and run through an electrophoresis gel. Graphic created using ApE software.



Right: Actual results of an attempted R6K-SV40 recombinant plasmid digest cut with BGIII and run through an electrophoresis gel. Band results do not match those of the expected results.

Future Directions

- Secure contact with the authors of the Tn7 paper to acquire feedback regarding our difficulties with the Tn7 vector system.
- Successfully clone the JC1 and SV40 fragments into the Tn7 transposon vector.
- Continue the overall project by integrating the VP1-edited Tn7 vector into the *E.coli* genome.

Acknowledgements

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References

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