Team EVICT: Enacting Viral Protein in Cancer Therapy

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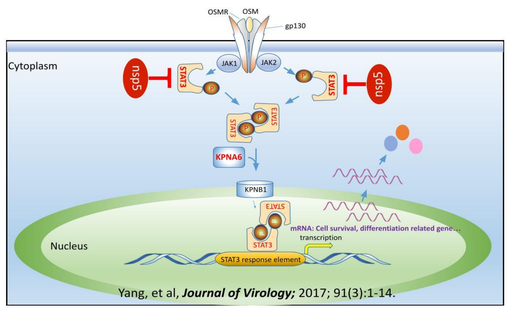
Introduction

Lymphoma

- **Seventh** most common type of cancer
- Arises from cells in the **lymphatic system**
- There is a need for more **targeted** and **effective** cancer **treatments**

STAT3

- Signal Transducer and Activator Transcription (STAT3)
- It is a **transcription factor** that is upregulated in lymphoma and contributes to cancer growth



Yang, et al, ***Journal of Virology;*** 2017; 91(3):1-14.

Jak-STAT signaling pathway. nsp5 prevents STAT3 translocation to the nucleus.

nsp5

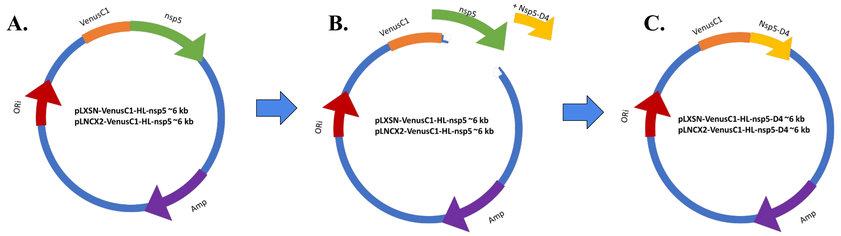
References

- Nonstructural protein 5 (nsp5)
- **Viral protein** from the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)
- Can lead to the **downregulation of STAT3**

Research Question

How can we optimize the **downregulation of STAT3** by **viral protein nsp5** in order to **induce cancer cell death**?

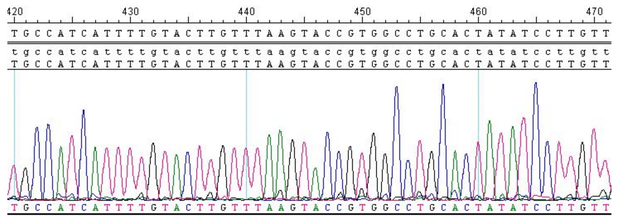
Plasmid Construction



A. **Example original plasmid** with full length nsp5, green fluorescent protein (VenusC1), origin of replication (ORi), and Ampicillin resistant gene (Amp)

B. Full length nsp5 is **cut** out of the plasmid from A using restriction enzymes.

C. nsp5-D4 **inserted** into plasmid from B



Sanger DNA sequencing chromatogram for pLXSN-VenusC1-HL-nsp5-D4 confirming that D4 was cloned into the plasmid.

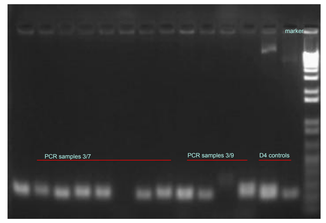
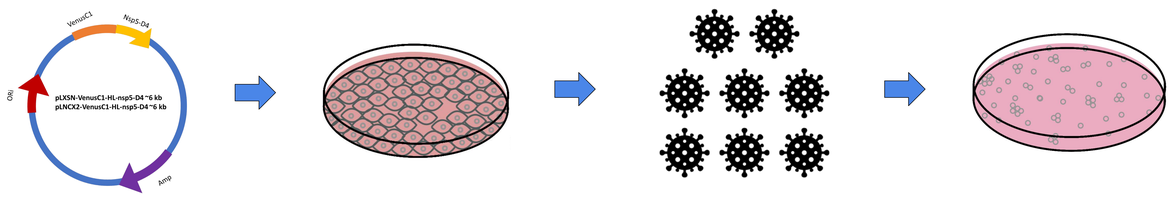


Image of Agarose Gel Electrophoresis of the pLNCX2-VenusC1-HL-nsp5-D4 vector. Expected products seen around 400 kb.

Transfection and Transduction

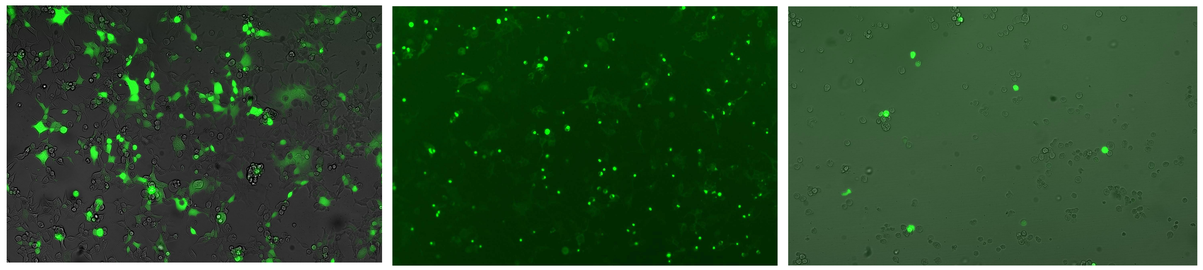


Transfection

GP2-293 packaging cells are transfected with the desired plasmids containing nsp5-D4 for production of the recombinant retroviruses.

Transduction

BCBL-1 lymphoma cells are transduced by the recombinant retroviruses to test the effect of the viral protein.



Fluorescent image: GP2-293 cells transfected with pLNCX2-VenusC1 control (left), GP2-293 cells transfected with pLNCX2-VenusC1-HL-nsp5 vector (middle), and BCBL-1 lymphoma cells transduced with pLNCX2-VenusC1 control (right).

Overall Aim

Clone **nsp5-D4** into a **vector** in order to **deliver** it to **lymphoma** cells and **inhibit** cancer cell proliferation

Discussion

Plasmid Construction

- pLXSN-VenusC1-HL-nsp5-D4: done
- pLNCX2-VenusC1-HL-nsp5-D4: construction ongoing

Transfection

- **Success** of our **transfection** assessed by green fluorescence level and intensity
- Better transfection was observed with pLNCX2 vector

Transduction

- Health of BCBL-1 cell culture maintained
- Optimization of transductions ongoing with improvements in retrovirus titers

Future Directions

- **Complete** plasmid construction
- Optimize **transfection** for higher retrovirus yield
- Optimize **transduction** for improved efficiency
- Run **western blot** to determine STAT3 reduction in BCBL-1 cells
- Determine **cell viability** after transduction