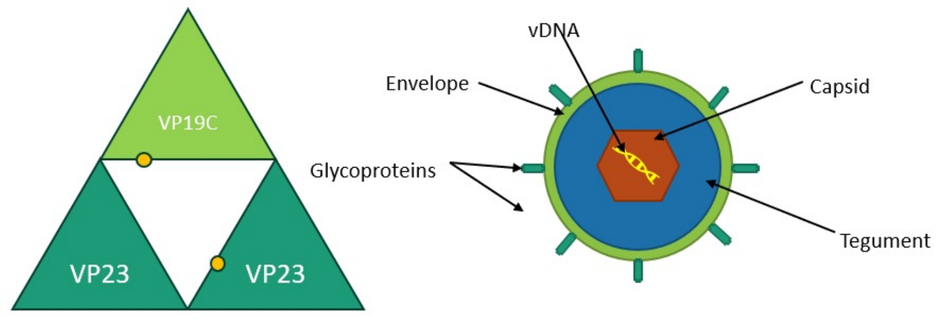
Virology for Dummies: An introduction to Herpes Simplex Virus 1 and my experience in a Virology Lab.

Introduction:

- The project explored the contribution of viral proteins in HSV1 to the overall stability of the viral capsid and therefore the ability of the virus to replicate and continue infection.
- Our research required several points of adjustment for me to produce the results we sought.
- To understand the connection between amino acids and capsid stability, we need to be able to change those amino acids at will.
- Our plan involved using mutagenic PCR to change a single codon at a specific point in the genetic sequences of VP19C and VP23 respectively[1].
- This process involves cloning, Polymerase Chain Reaction (PCR), restriction digestion and ligation into an appropriate plasmid vector which can be used to express proteins in bacterial cells.

Fig 1. An Introduction to the Triplex:

A schematic diagram of the viral structure of both the HSV-1 triplex and the virus as a whole.



Methodology

- Using the NEB Q5 DNA polymerase, we induced a mutation within VP19C from an Arginine (R) to an Alanine (A) at the 146th position (notated as R146A).
- We designed primers to both target the mutation and clone to produce a fusion with the N-terminal 10His tag in the plasmid pET16b.
- Using specific restriction enzymes, we could digest the ends of the mutated VP19C sequence so it could then be inserted into the pET16b plasmid, cut with the same restriction enzymes, for directional cloning and ligation.

Fig 2. Colony PCR of VP23-C298A

- Once the insert and vector were ligated, we used the NEB HiFi DNA assembly cloning kit and then spread the mixture on a plate of LB-Ampicillin and incubated overnight at 37℃.
- The following day, ten isolated colonies were selected and placed on a secondary plate.
- As each colony was picked, it was mixed into a PCR tube to act as a template or colony PCR with the master mix and primers flanking the insertion site of pET16b.
- These repeated PCRs, alongside a control of the parent plasmid, allowed us to see we have not simply replicated the parent plasmid. We could then pick the most suitable colonies from a replica plate to purify DNA and send off for sequencing.
- For VP19C this was colony 4 (figure 3). For VP23, we purified both colonies 1 and 4, however only sent colony 1 for sequencing.

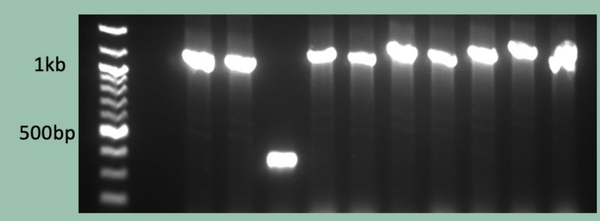


Fig 3. A capillary sequencing of a successful R146A mutation of HSV-1 VP19C.

- *The image below is a capillary sequencing of HSV-1 VP19C Colony 4 as taken from Chromas 2.6.6. We obtained a 99% identity to the subject sequence and the only change in the query sequence was the mutation we inserted (R146A).*

- We then worked on transforming our plasmids into BL21 DE3 cells so we could begin to produce proteins.
- We transformed both VP19c R146A and VP23 into BL21 DE3 cells obtained from Dr Mark Odell.
- Once this was achieved, we induced the culture overnight at 37 ℃ and then diluted it to 1/200 in LB broth.
- It was left to grow until an OD_{600} of 0.6 was reached.
- 0.4mM IPTG was added to the overnight culture, and it was incubated, with samples taken at 0, 2, 4 and 24 hours. These were pelleted and the supernatant removed.

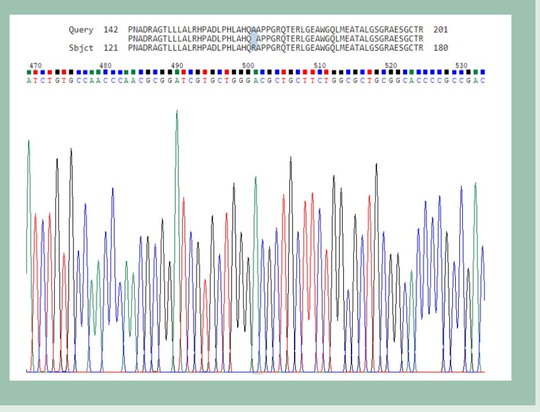
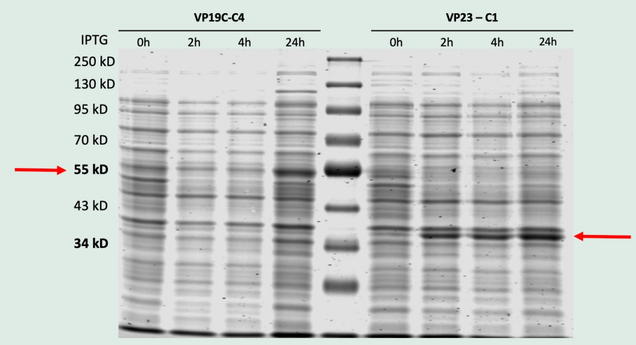


Fig 4. Gel showing the successful production of viral proteins 19C and 23.

- The resulting pellets were resuspended in TGN and protein concentrations determined by A280, to make sure they run evenly.
- We ran the protein gel (sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE)) to separate all the proteins present in the bacteria to see if our target proteins had been made.
- Gels were stained with Imperial Protein stain (Invitrogen) to visualise the total protein composition of our bacterial cultures (Figure 4).
- *A clear band at 55kDa corresponding to VP19c was seen 24 hours post IPTG application. Similarly, a clear band at 33kDa consistent with VP23 was seen in the 2-, 4- and 24-hours post IPTG treatment samples. These results showed our success.*



Conclusion:

- I now have a much better understanding of the day-to-day life in a research lab which only makes me surer of my decision to pursue further education through an M.Sc. or PhD.
- We overcame many challenges and mistakes but I now feel comfortable thinking on my feet and being able to quickly troubleshoot a solution.

References: 1. Walker, K. W., & King, J. D. (2023). Site-Directed Mutagenesis. In R. A. Bradshaw, G. W. Hart, & P. D. Stahl (Eds.), Encyclopedia of Cell Biology (Second Edition) (pp. 161–169). Academic Press.

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