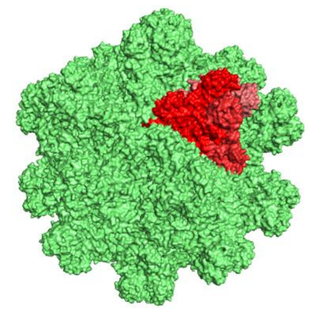
MOL231: Comparison of two variants of Infectious pancreatic necrosis virus



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Introduction

Infectious Pancreatic Necrosis Virus (IPNV) is a double stranded RNA virus which can cause the disease Infectious pancreatic necrosis in salmonid fish [1]. The IPNV genome consists of two linear RNA strands, A and B, coding for viral proteins (VPs) [2] (figure 1). VP2 is the main component of the viral capsid, and is composed of a trimer with a spike, a shell and a base. This is also the main antigenic protein in the virus, and the protein making IPNV capable of attaching and infecting host cells due to its apparent interaction with E-Cadherin and the cell surface [3], [4]. In recent years IPNV-infections have increased. A mutated variant of IPNV V-1244, Vir410/18, is thought to be responsible for this [5].

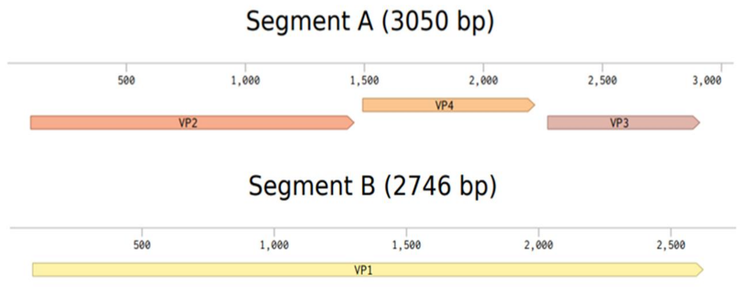

Figure 1: IPNV genome: Overview of the IPNV genome composed of segment A and B, coding for the proteins VP2, VP4, VP3 and VP1.

Aim: Identify antibodies that can be used against Vir410/18.

Materials and Methods

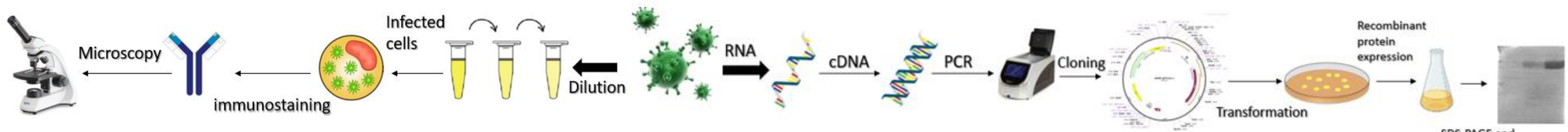

Figure 2: Workflow. The main tecniques used during the project.

Results and Discussion

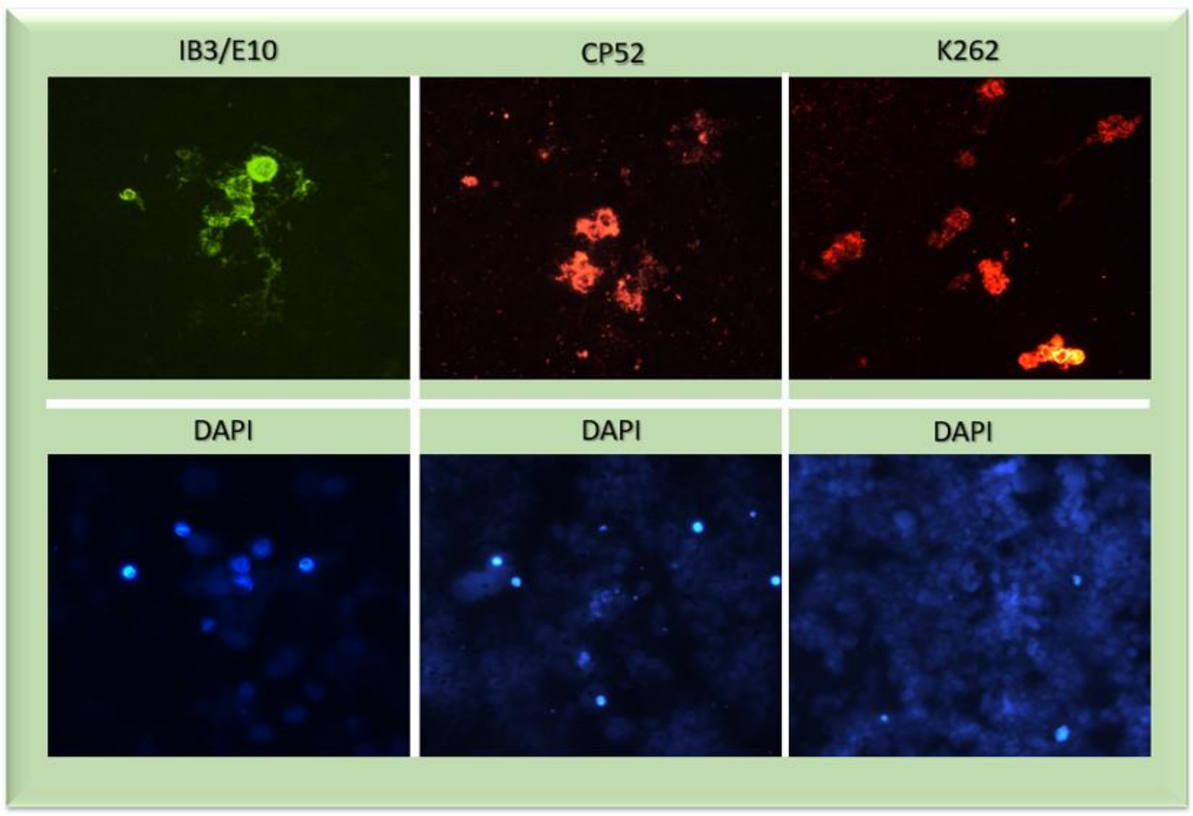

Figure 4: Immunofluorescence of infected, fixed CHSE cells. Cells infected with IPNV V-1244 and immunostained with IB3/E10, CP52 and K262. Green and red displays antibodies bound to VP2. Blue displays cell nuclei stained with DAPI.

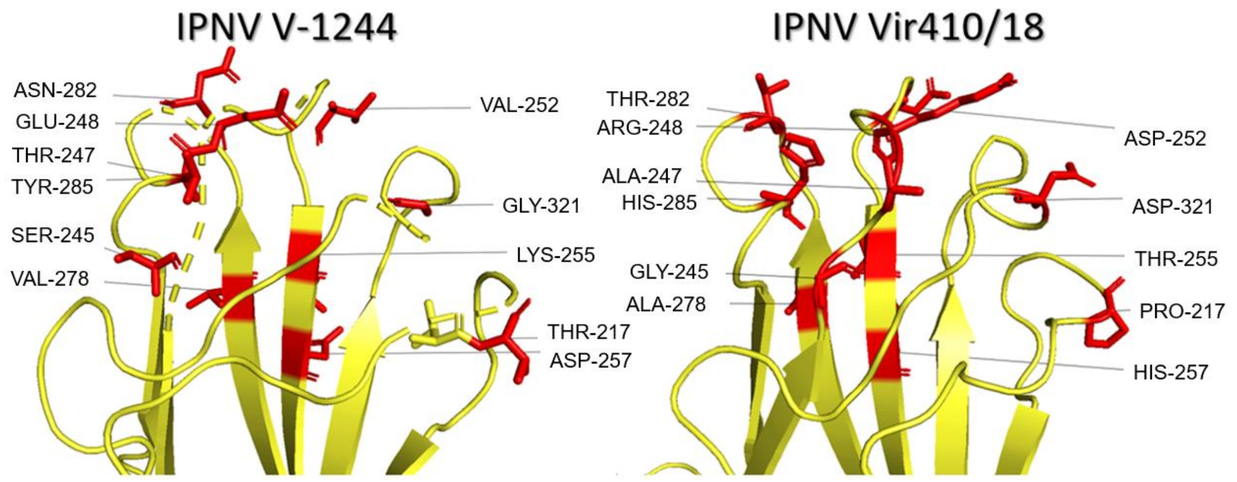

Figure 3: **Structural comparison of IPNV V-1244 and Vir410/18.** Protein structure of VP2 encoded by IPNV V-1244 and IPNV Vir410/18. Amino acids marked in red display the differences between the two proteins.

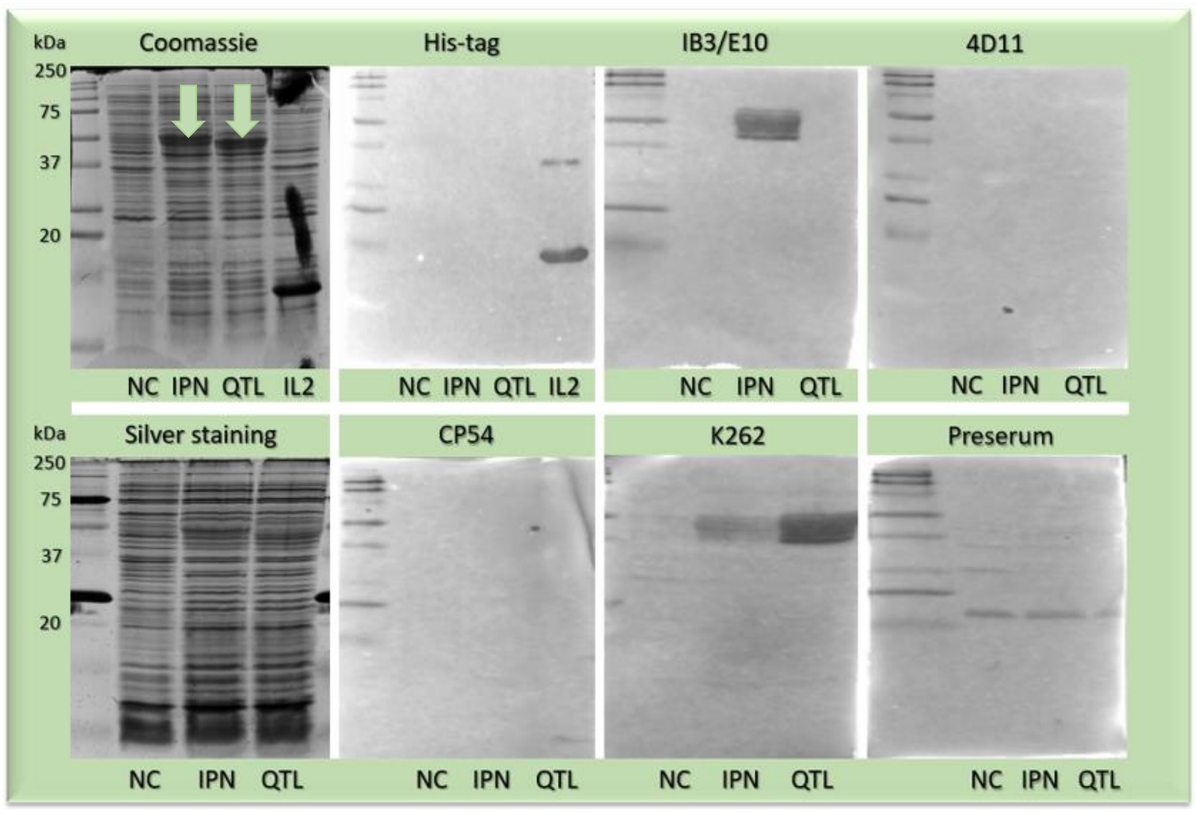

Figure 5: SDS-PAGE and Western blot of recomibnant VP2. Coomassie and silver staining protein gels and immunoblotting with His-tag, IB3/E10, CP54, K262 and 4D11 antibodies and rabbit preserum. The samples used were NC (negative control), IPN (IPNV V-1244 VP2), QTL (IPNV Vir410/18 VP2) and a positive control, IL2. α-mouse secondary antibody was used on His-Tag, IB3/E10 and 4D11 and α-rabbit secondary antibody was used on CP54, K262 and Preserum. VP2 bonds were expected at 48 kDa and IL2 bonds were expected at 15 kDa.

- Bioinformatic analysis of the protein structure of the two strains showed multiple differences in amino acids between position 217 and 321, all in the spike region of VP2 (figure 3). Position 217 and 221 have been known to be important for virulence.
- In the immunofluorescence of IPNV infected cells (figure 4), IB3/E10, CP54 and K262 were shown to bind to V-1244 VP2. No antibodies successfully bound Vir 410/18 VP2.
- VP2 from IPNV V-1244 and Vir 410/18 were immunoblotted with the antibodies known to work for IPNV; IB3/E10, CP54 and K262 (figure 5). IL2 was used as a positive control and immunoblotted with His-tag. 4D11 and Preserum were used as a negative controls known not to bind VP2. VP2 bonds were expected to be at 48 kDa. K262 showed bonds for both strains and IB3/E10 showed bonds for V-1244.
- **Conclusion: The results suggests IB3/E10 and K262 is most suited to use against V-1244 and Vir410/18 respectively.**
- Future prospects: More research into how IPNV interacts and infects the host cell should be conducted.

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References

1. Sommerset, I., Jensen, B. B., Bornø, G., Haukaas, A., & Brun, E. (2021).
2. Argot, J., & Malsberger, R. G. (1972).
3. Hillestad, B., Johannessen, S., Melingen, G. O., & Moghadam, H. K. (2021). *of Microbiology*, *18*(6).
4. Arragh, E. A., & Macdonald, R. D. (1982)
5. Endringer i IPN-viruset gjør fisken mer utsatt for sykdom (vetinst.no)