Expression of Deer Adenovirus Spike Protein

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Background

Introduction

- Adenoviruses infect wide variety of animals – Wild & Domestic
  - **Pathogenic** in Deer
  - **Symptoms of AV** are ulcers and abscesses in the mouth and throat
  - **Acute Symptoms** would be rapid breathing, diarrhea, foaming at the mouth
  - Death can occur with **3-5 days** from the time of the exposure.
  - No known cases of transferring to **humans**

- **Transmission**: direct contact, contact with bodily fluids, possibly airborne routes

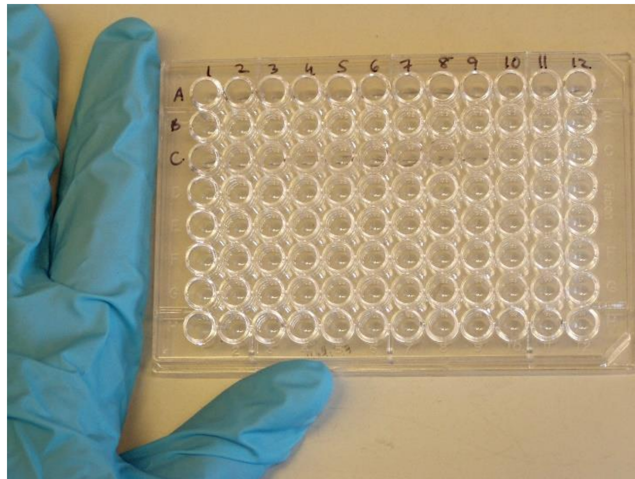
Background

Detection of DAV

- Isolation of the Virus
  - Enzyme-linked Immuno Assay (ELISA) based test is needed
  - Exam serum to see if the animal is exposed

Generate DAV spike protein

- Coat the plate with the spike protein to make ELISA plate that can capture the IgG specific to DAV
- Making monoclonal antibody to detect the virus in the tissue



Goals of the Study

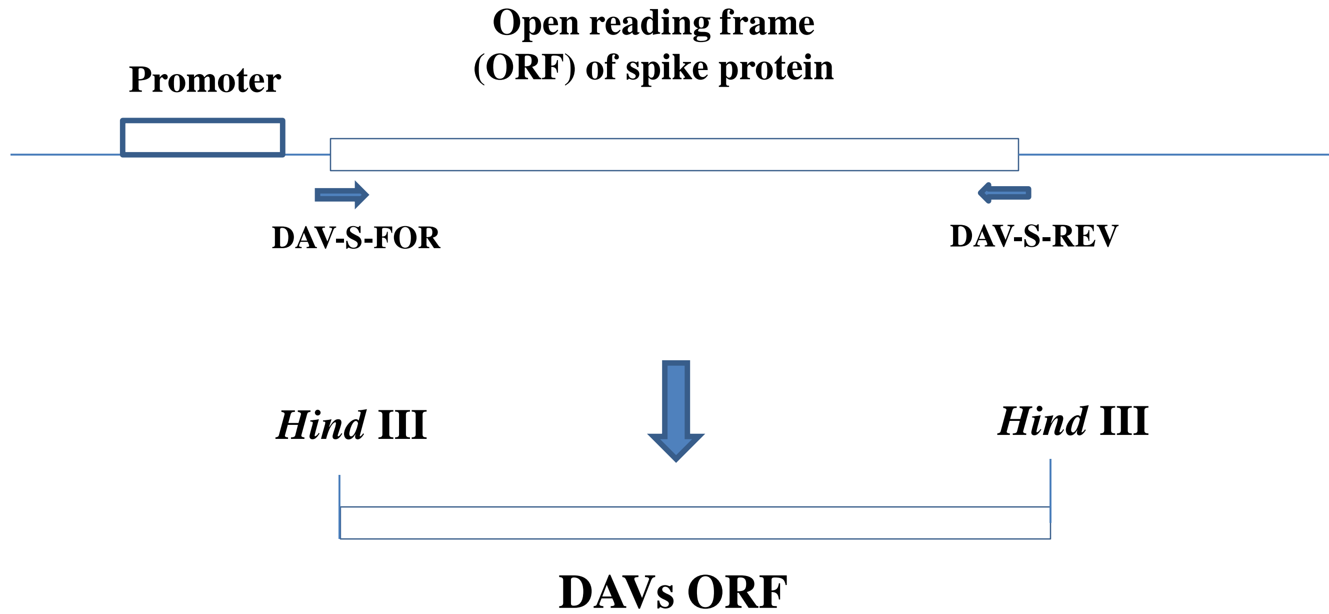
Expressing the DAV spike protein in a PET6XHN-N Vector

Being able to produce monoclonal antibody

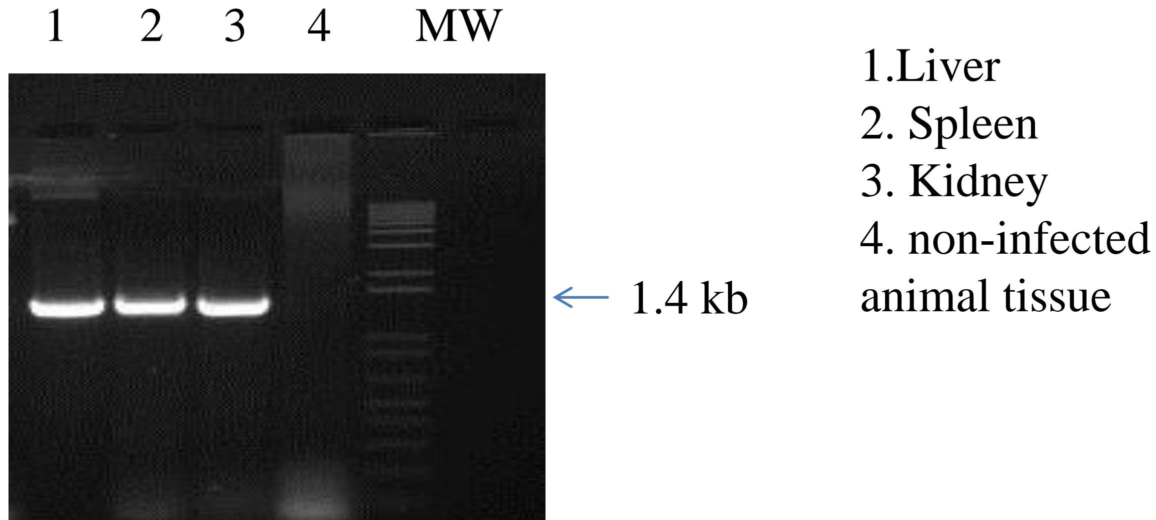
Research Outline

- **Amplify** the coding region of the spike protein by PCR using primer flanking the S protein coding region
- **Clone** the coding region into PCR-TOPO 2.1 cloning vector
- **Re-clone** the coding region into PET6XHN-N vector
- **Induce** the spike protein expression in BL-21 cells

PCR with DAV specific primers

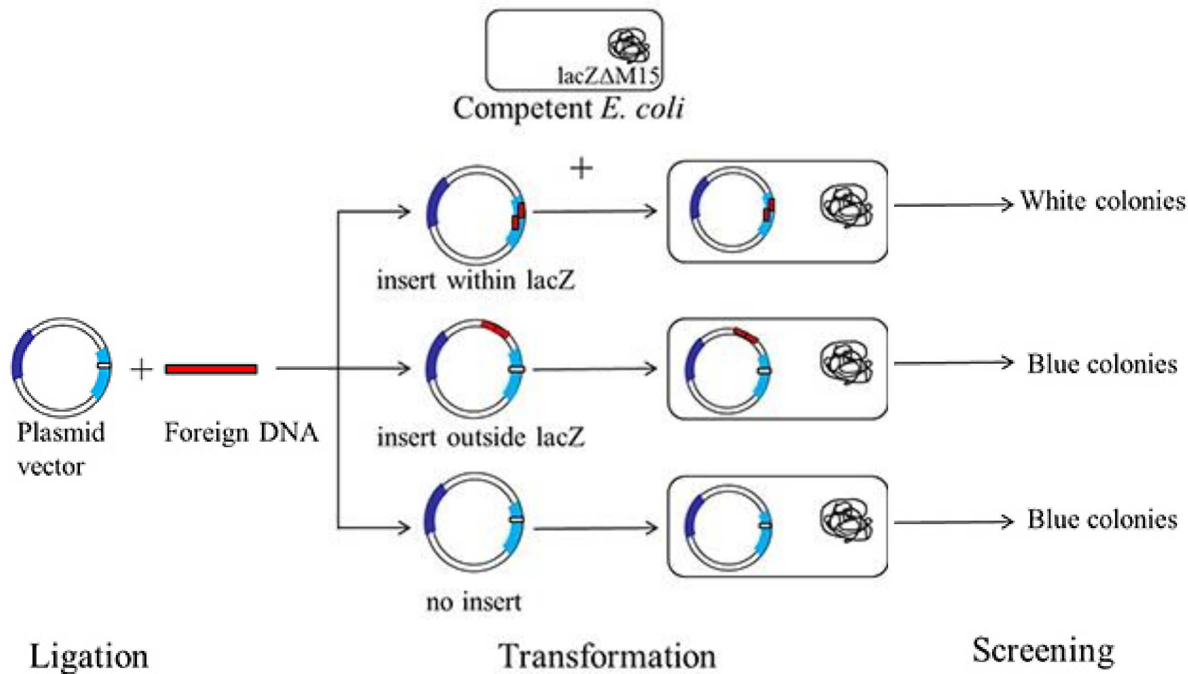


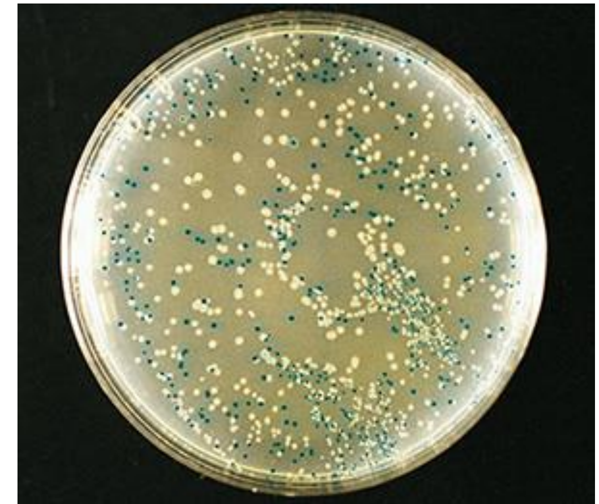
Amplification of Spike Protein Coding Region



*** 1, 2, and 3 were success in detecting the Spike Protein ***

PCR-TOPO Cloning: TOPO-DAVs



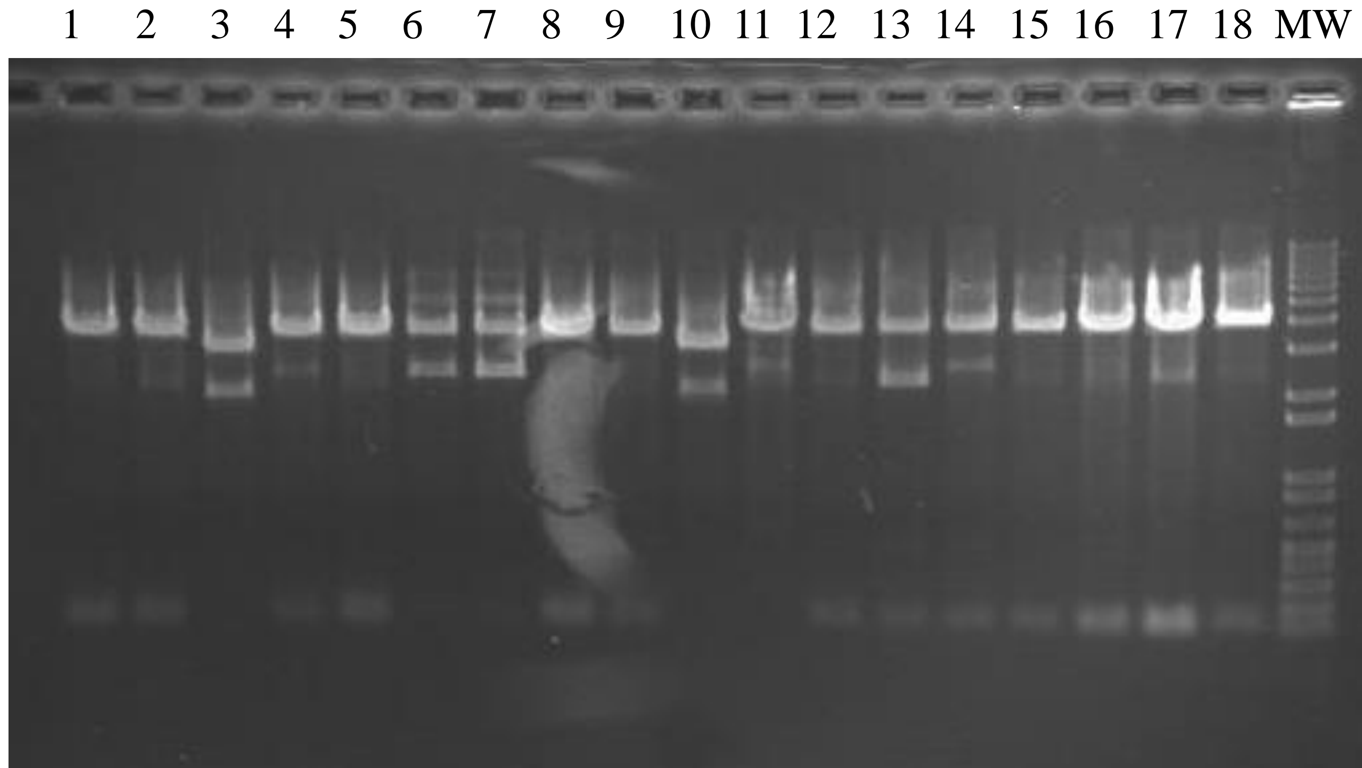


- **Ligate**
- **Transform**
- **Spread**
- **Incubate**

Screening for TOPO-DAVs

- **Select** of White Colonies in Petri Dish
    - Picking the white colony and inoculate into 2 ml LB broth with kanamycin
- **Grow** the Bacteria overnight at 37 degree C in a shaking incubator
- **Extract** the plasmid DNA by mini-prep
- **Digest** the extracted DNA with *EcoR* I
- **Run** the digested DNA in agarose gel
    - See if the insert is expressed in the gel run

Result of the Screening by EcoR I digestion

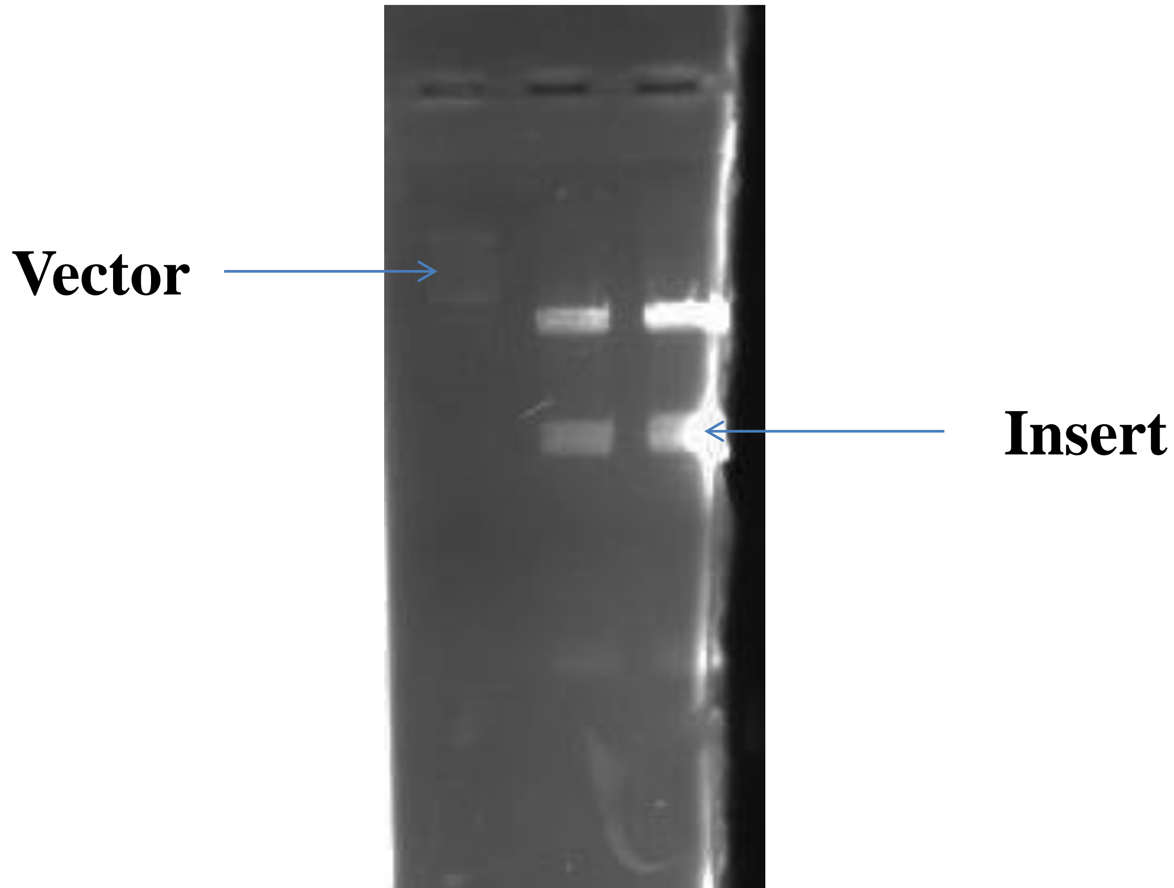


*** Positive Insert (TOPO-DAVs): 3, 10, 13 ***

Re-clone the coding region into pET6XHN-N vector

- **Digest** the TOPO-DAVs and pET6XHN-N with *Hind* III
- **Run** them on the gel
- **Cut** the DNA fragment
- **Ligate** the DAV spike sequence with pET6XHN-N at *Hind* III site
- **Transform** the ligation into DH5a *E. Coli*

Digest the DAV-S plasmid and pET6XHN-N with Hind III



Screening for pET6XHN-N-DAVs

- **Pick** 20 colonies
- **Grow** the bacteria in LB with ampicillin overnight
- **Isolate** the plasmid by mini-prep
- **Digest** the extract DNA with Hind III
- **Run** the digestion in agarose gel

Summary

- DAV-spike gene was amplified by PCR using primers flanking the coding sequence
- The PCR product was successfully cloned into TOPO vector
- Re-cloning the DAV-spike gene into the expression vector result is pending
- After successfully ligating into the expression vector, Purify the vector so that His-spike is left.
- Protein synthesis and monoclonal antibodies.