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**Cloning, Expression, Purification and Characterization of
Brucella abortus Auto-transporter Genes and Their Products**

Brucella spp.

- Gram negative cell wall structure
- 10 species
 - *B. abortus*
 - *B. melitensis*
- Major Veterinary Pathogens
- Cause *Brucellosis*
- BSL III level organisms



Brucellosis

Epidemiology

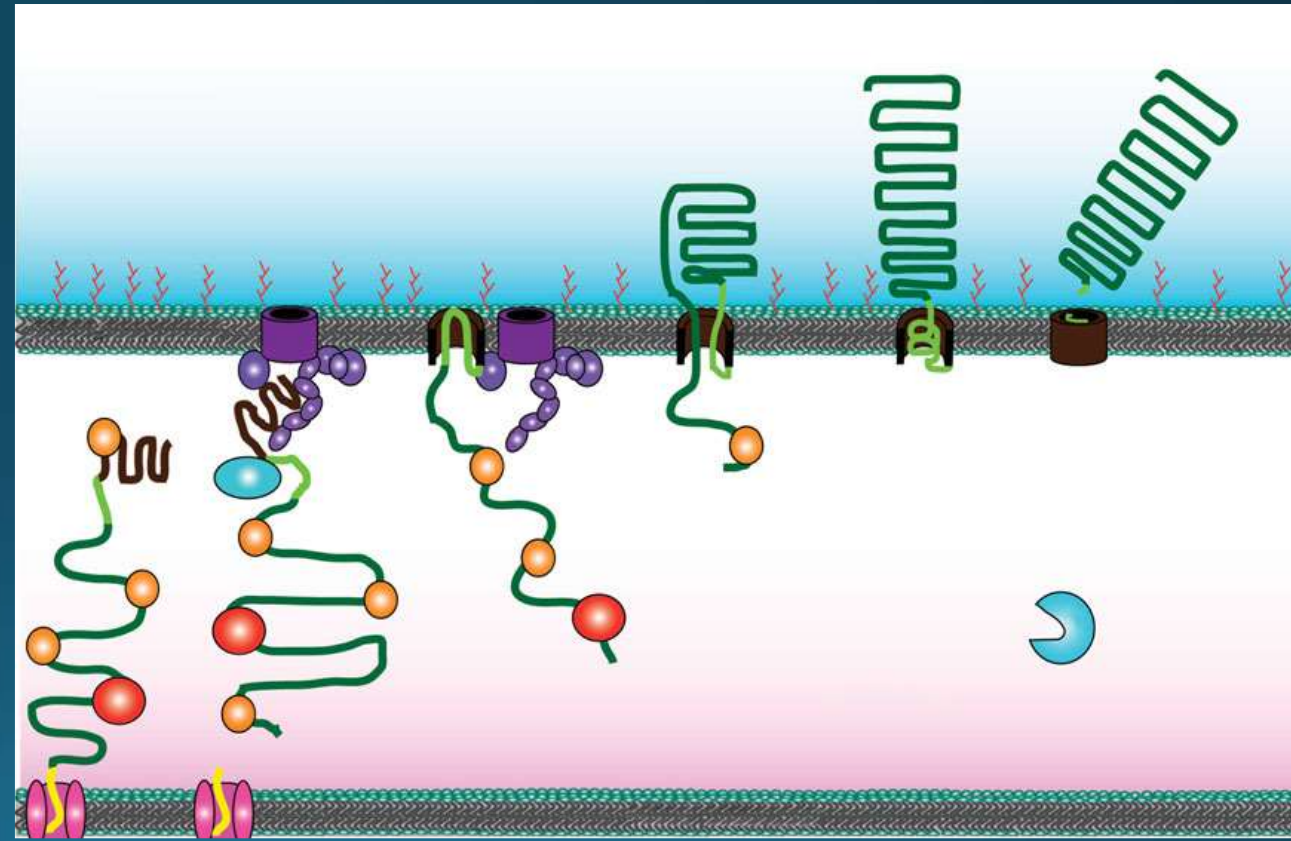
- Cattle, sheep, goats, swine
 - Wildlife: elk etc.
- *B. melitensis* most common cause
- 4 species cause disease in humans
- Risk group primarily animal handling

Pathogenesis

- Intracellular pathogen
 - Replicate in phagosome
 - Macrophages, Placental Trophoblasts
 - Survive in lymph tissues, not isolated from blood
- Complex virulence
 - LPS
 - Type IV & V secretion systems

Type V Secretion Systems

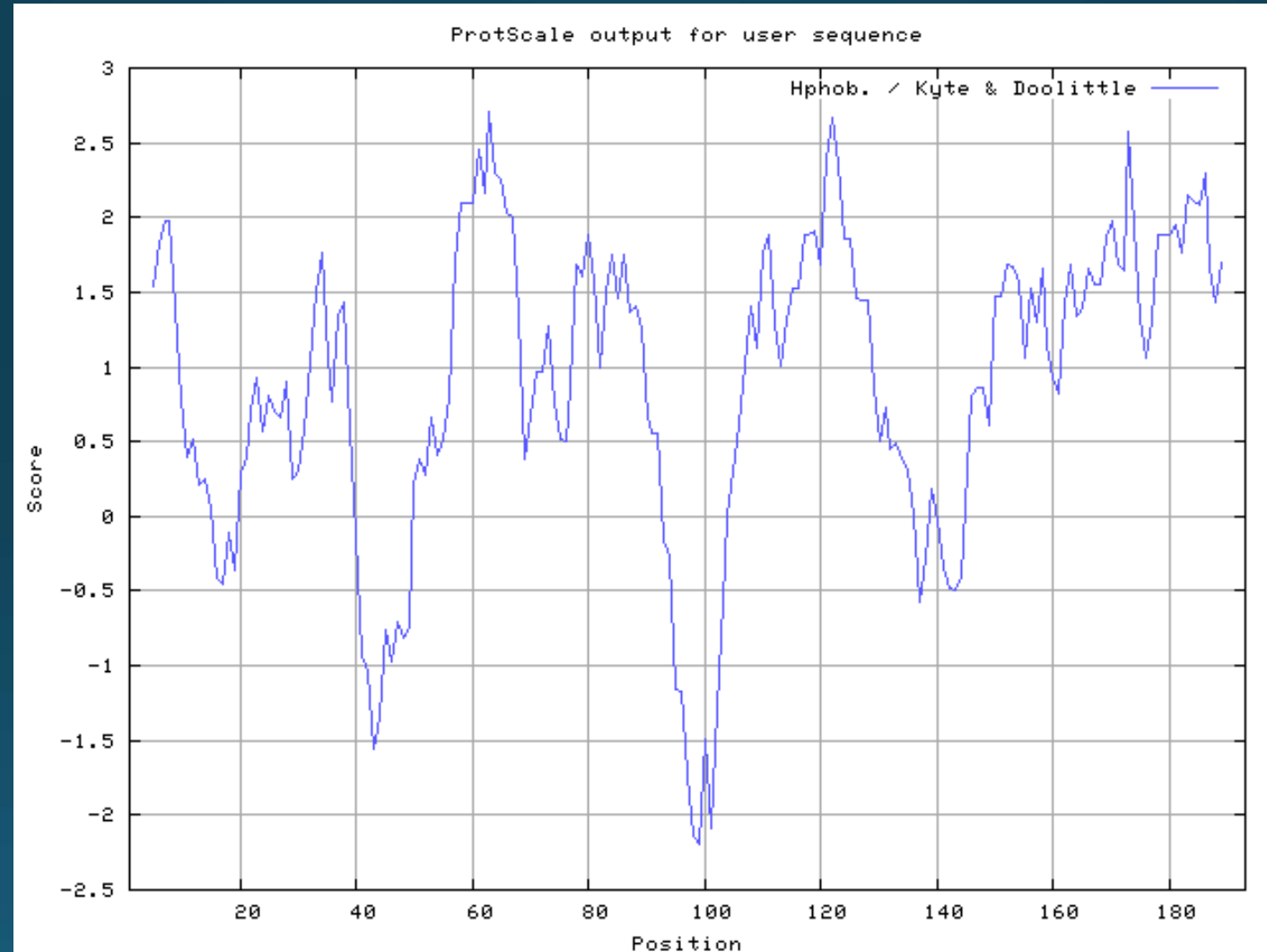
- AKA autotransporters
- Utilize the Sec pathway to cross the inner membrane
- Characterized with 2 domains
 - Passenger domain
 - Hydrophobic β -barrel transporting domain



Approach

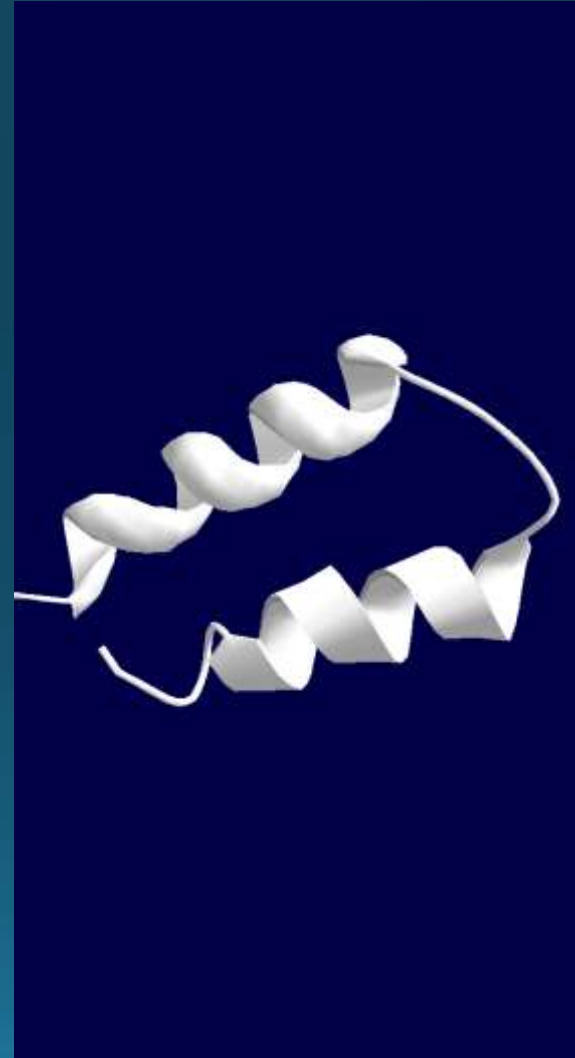
- Target genes PCR amplified from *B. abortus* RB-51 strain
- Cloned into pET-46 vector using ligation independent cloning
- Expressed in *BL21 (DE3) E. coli* competent cells
- Purified and tested for reactivity against cattle serum in Western Blots.

BruAb1_1989



BruAb1_1989

- A bioinformatic approach also suggested a structure composed of primarily α -helices
- A PSORTb analysis suggested that *BruAb1_1989* is localized to the inner membrane



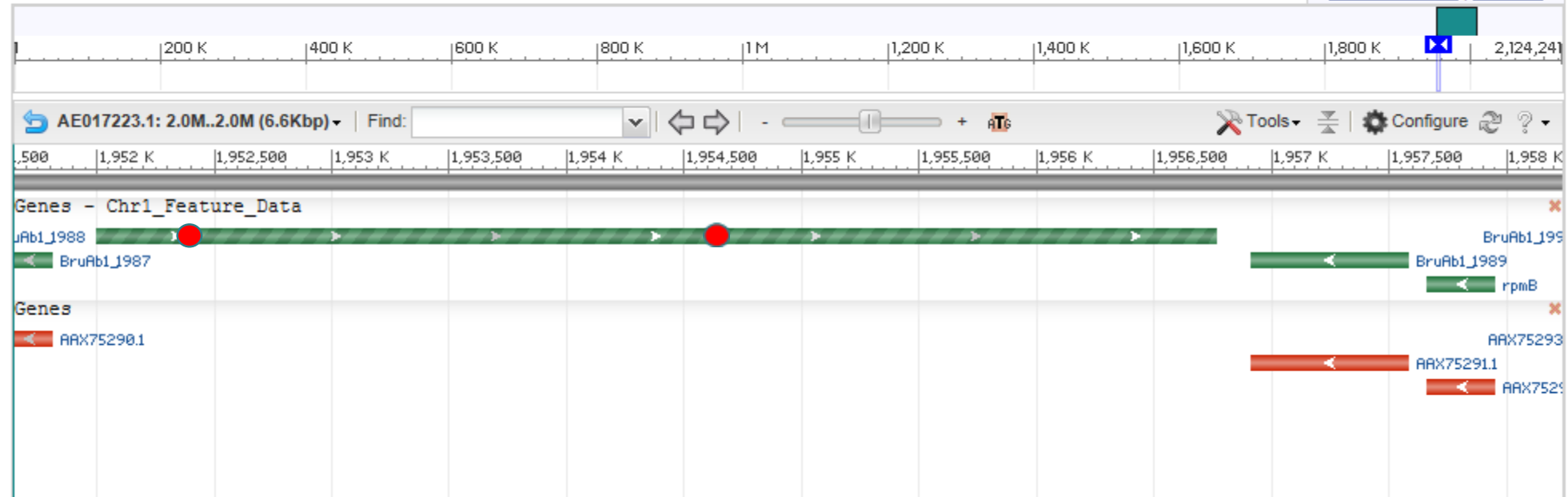
BruAb1_1988

Brucella abortus biovar 1 str. 9-941 chromosome I, complete sequence

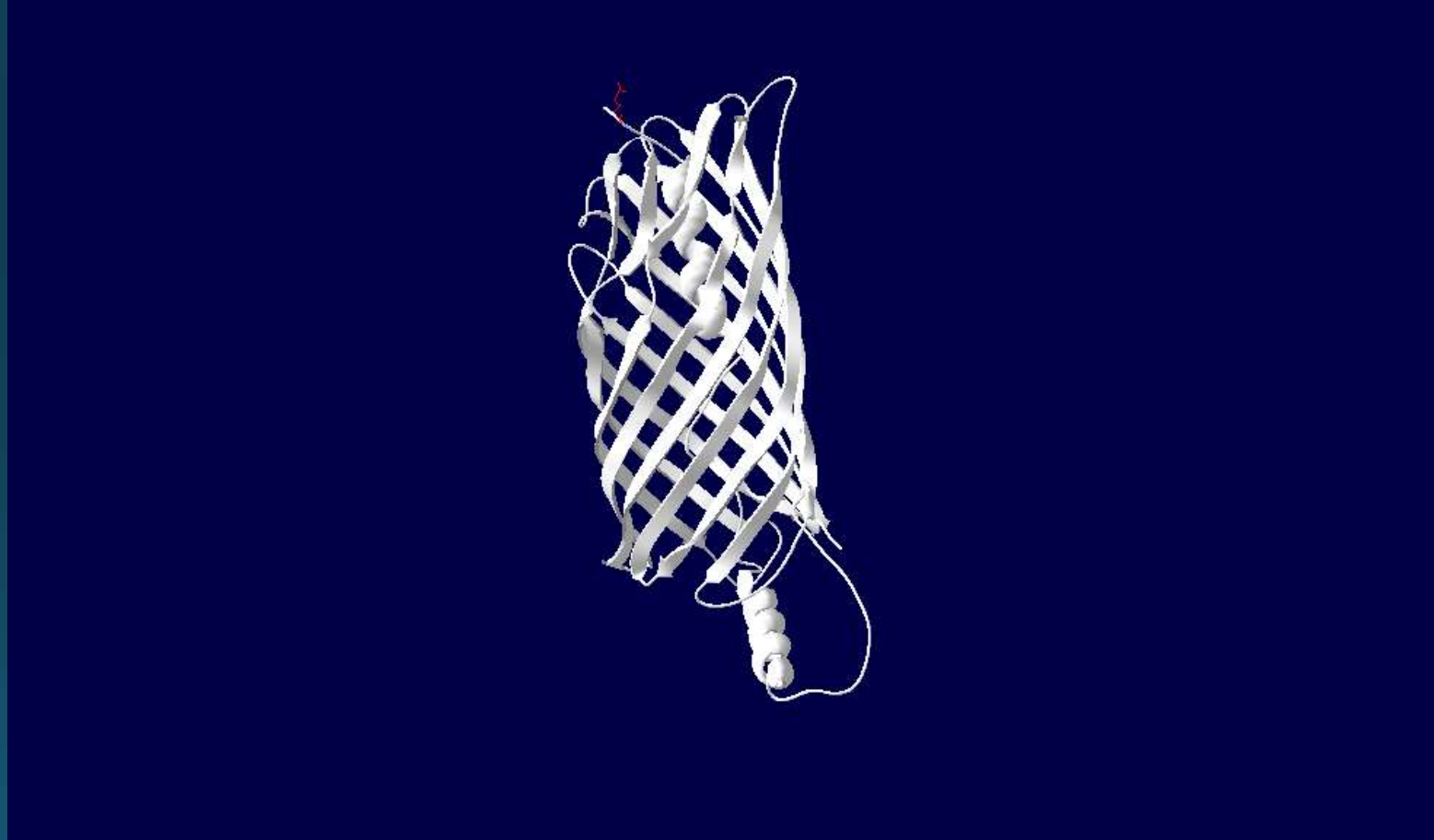
GenBank: AE017223.1

[GenBank](#) [FASTA](#)

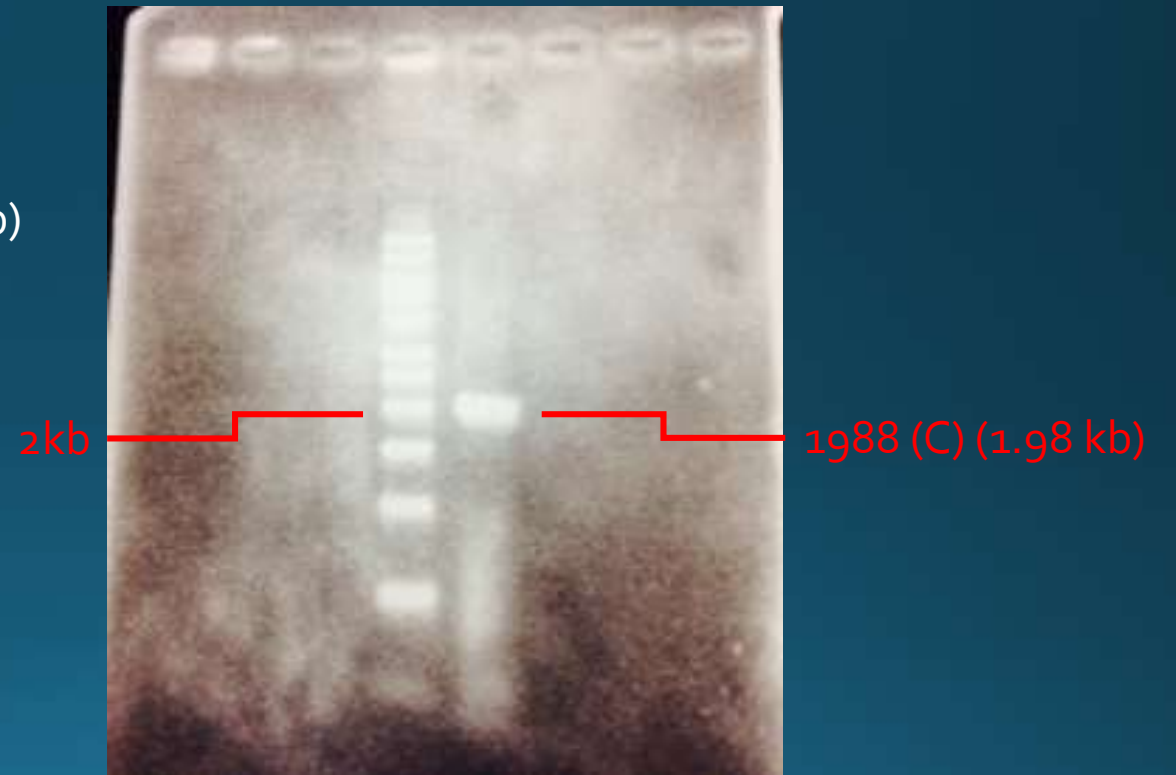
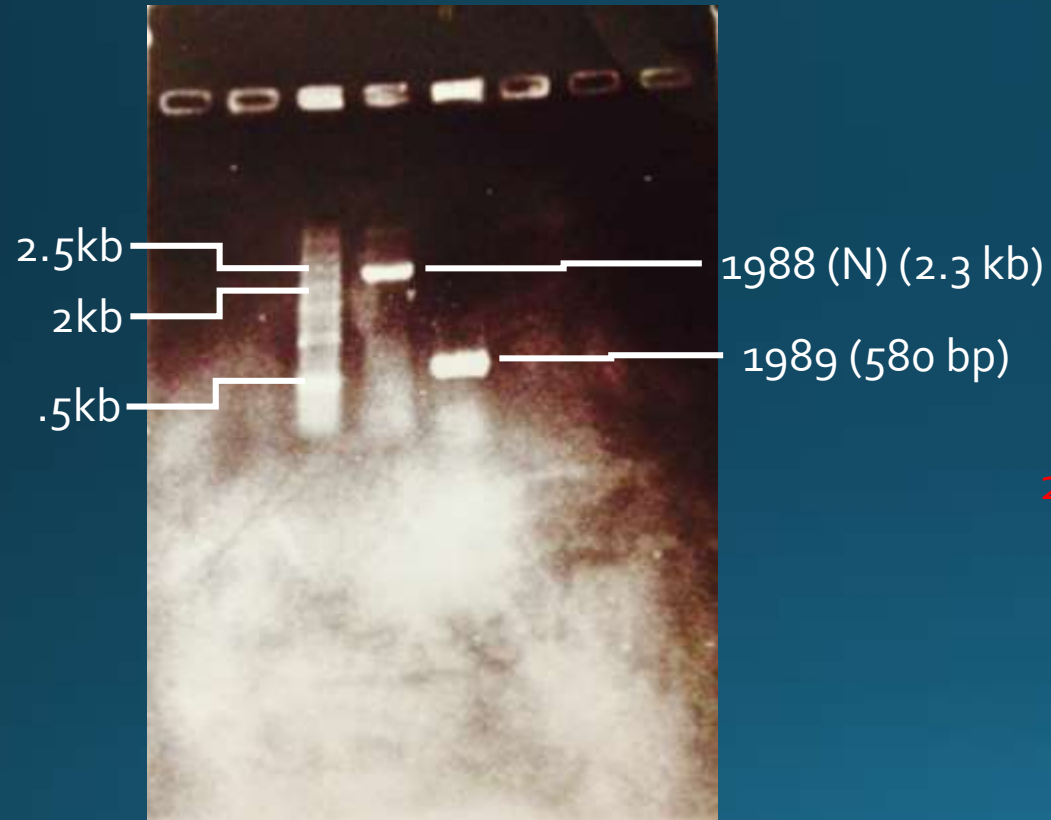
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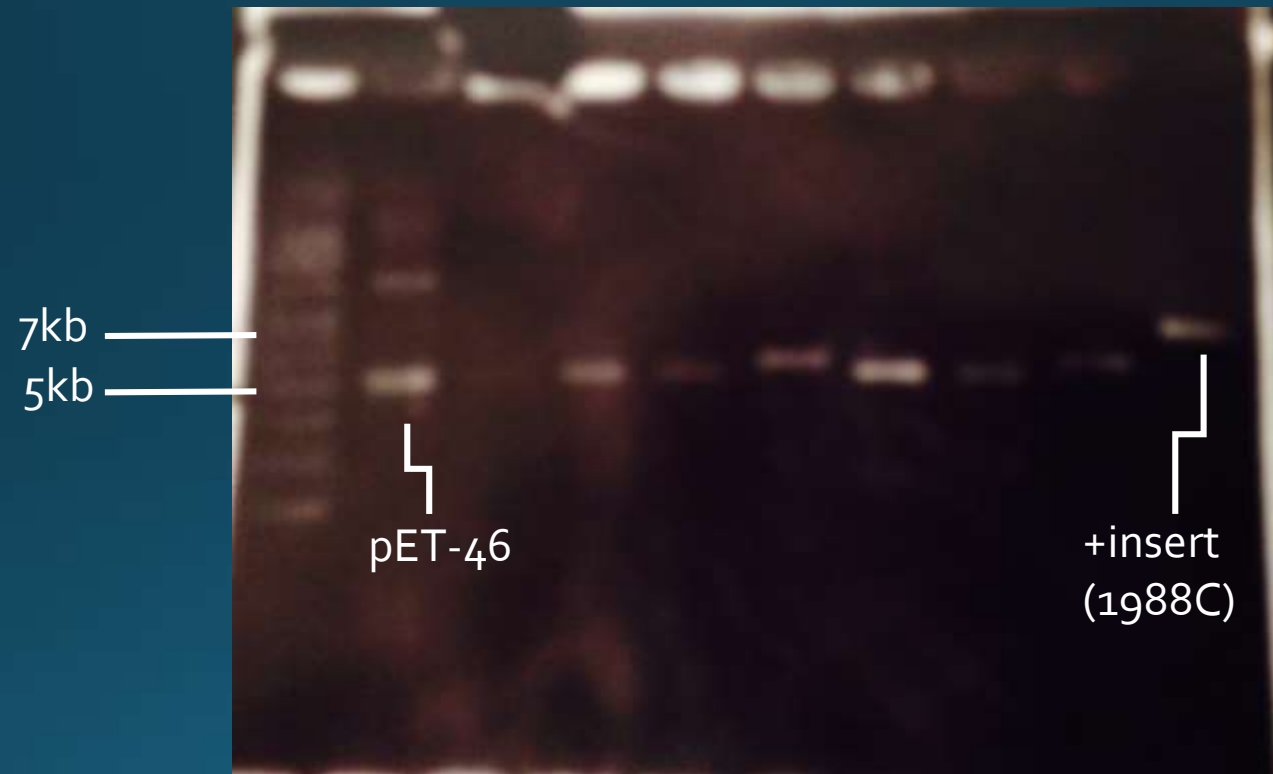
BruAb1_1988 C Terminus



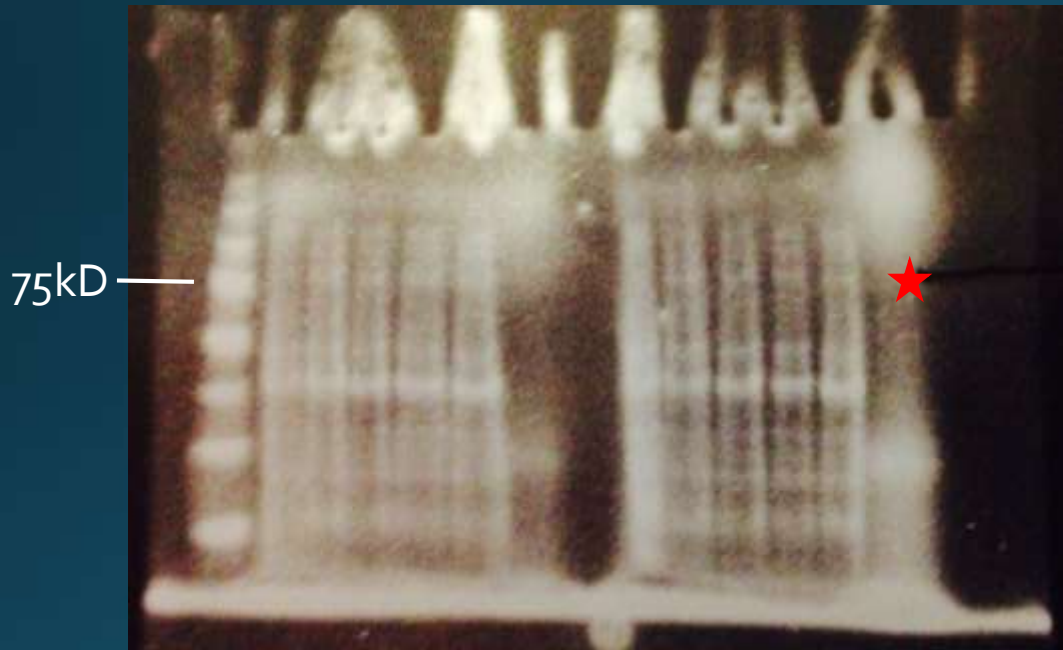
Cloning Results



Cloning Results Continued



Expression results



1988 Passenger domain expression



1988 β -Barrel C terminal expression

Western Blot Results

- Neither segment of BruAb1_1988 has showed reactivity against cattle serum.
 - Not enough data yet to be statistically significant
- This result is expected
 - Method of creating serum is unknown
 - Unknown time of expression in-vivo

Way Forward/Applications

- Perform Western Blots using serum against *B. melitensis*
- *BruAb1_1988* could provide a unique serological diagnostic to differentiate the two species
- Potential adaptation to rapid testing platform. i.e. Lateral flow Device
 - Similar to Western Blot/ELISA

Acknowledgements

- Dr. G and his Grad Students
 - Amanda Dougherty
 - Pradeep Neupane