Reference: 02/PT

Project Title: Utility of ubiquitin and degrons in African swine fever virus subunit vaccines

Supervisors: Dr Priscilla Tng and Dr Chris Netherton
Research group: African Swine Fever Vaccinology Group

Project Summary: African swine fever virus (ASFV) is a complex DNA virus that has caused many large outbreaks around the world. ASFV causes a contagious and highly fatal disease that affects domestic and wild pigs. There is no approved vaccine available and disease control relies on the quarantine and culling of infected and exposed animals. Previous studies demonstrated the importance of cellular immunity in protection against ASFV, highlighting the need to improve T-cell mediated immunity induced by vaccines in development.

The first aim of this project is to develop methods to improve activation of T-cells by viral vectored subunit vaccines against strains of ASFV that are currently circulating in Europe and Asia. This will be achieved through the construction and assessment of a panel of ubiquitin tagged luciferase expression constructs. Ubiquitin is a small regulatory protein that is used by cells to tag other proteins for degradation in the proteosome, which generates the peptides used in antigen presentation. Candidates with the best ability to increase antigen presentation for the activation of T-cells will be identified. In the second part of this study, the focus will be on incorporating the selected candidates from the panel into improved subunit vaccine viral vectors and testing these in *in vitro* assays for efficacy. The results from this study will aid the development of subunit vaccines to curb the spread of ASFV.

Further Details: Depending on progress, the successful student may undertake the following during the course of this research project:

1. Cloning of tagged luciferase expression constructs
2. Cell culture and transfection of immortalised porcine cell lines
3. Dual luciferase assays using firefly luciferase, nanoluciferase or *Renilla* luciferase
4. Quantification of protein translation through Western blot
5. Quantification of protein transcription through quantitative reverse transcription PCR
6. Cloning of subunit vaccine viral vectors
7. Measurement of immune responses in T-cell assays such as ELISPOT and flow cytometry.

References for Suggested Reading:

Netherton CL, Goatley LC, Reis AL, Portugal R, Nash RH, Morgan SB, Gault L, Nieto R, Norlin V, Gallardo C, Ho CS, Sánchez-Cordón PJ, Taylor G, Dixon LK. (2019) Identification and Immunogenicity of African Swine Fever Virus Antigens. Front Immunol. 19;10:1318. PMID: 31275307 DOI: 10.3389/fimmu.2019.01318

Takamatsu HH, Denyer MS, Lacasta A, Stirling CM, Argilaguet JM, Netherton CL, Oura CA, Martins C, Rodríguez F. (2013) Cellular immunity in ASFV responses. Virus Research 173:110 PMID: 23201582 DOI: 10.1016/j.virusres.2012.11.009

Rodriguez F, Zhang J, Whitton JL. (1997) DNA Immunization: Ubiquitination of a Viral Protein Enhances Cytotoxic T-Lymphocyte Induction and Antiviral Protection but Abrogates Antibody Induction. Journal of Virology 71:11 p. 8497 PMID: 9343207 DOI: 10.1128/JVI.71.11.8497-8503.1997

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Please email your CV (no more than two sides of A4) and a covering letter, detailing why you would like to undertake the placement and the knowledge and skills that you will bring to the Institute, to studentship@pirbright.ac.uk.

Closing date to apply: 09.00, 7th February 2022