REF: 08/LG/TT

Project Title: African swine fever virus genomics as an approach to identify variations in immunogenic proteins and test their role in protection.

Supervisor: Lynnette Goatley **Research group:** African swine fever vaccinology

Project Summary:
African swine fever virus (ASFV) is large DNA virus that encodes for at least 150 different genes and causes a lethal haemorrhagic disease in domestic swine. At present there is no vaccine or treatment for African swine fever and control is dependent on rapid diagnosis, quarantine and slaughter of affected animals. Previous work at Pirbright has included producing live attenuated viruses and viral vectored vaccines with some success. However, one limitation of these experimental vaccines is their ability to protect against genetically distinct strains of ASFV. Predicting such cross-protection is difficult due to the lack of full genome sequences. Therefore, the main objective of this project is to help develop methods to generate DNA libraries for next generation sequencing and to work with the bioinformatics department to analyse any data generated. This information will inform the second aim of the project, which is to identify which of the 150 encoded genes are required for protection and these to develop a subunit vaccine against strains of ASFV circulating in Eastern Europe.

Details:
Whole genome data of ASFV isolates from focal areas within the EU will be sourced from different times within a geographical region to allow us to quantify for the first time the local variability of the virus, and reconstruct its evolution in recent times by means of a comprehensive phylogenetic/phylogeographic analysis.

1) The student would be instrumental in helping to develop and refine a probe enrichment method to extract ASFV DNA for generating libraries for next generation sequencing. Pirbright holds a large and diverse reference collection of ASFV isolates and additional strains will be obtained from collaborators within the EU. A commercial company will generate the sequencing reads and the student will then be involved assembling and analysing the complete genomes with Paolo Ribeca's Integrated Biology group.

   Pigs immunised with attenuated strains of ASFV are protected against challenge with related virulent strains of virus that would normally kill a pig within 5 to 7 days. Analysis of samples taken from these animals show the development of both an antibody and cellular immune response to ASFV. Peripheral blood mononuclear cells (PBMCs) collected from these animals secrete interferon gamma when exposed to whole virus and to peptides. Our aim is to identify which of the 150 encoded genes are required for protection and incorporate these into a subunit vaccine against strains of ASFV circulating in Eastern Europe.

2) The student will help develop methods to investigate the immunogenicity of the genes selected for the subunit vaccines. This will include using bacterially produced proteins to look for immune responses in sera samples from previous vaccine trials. We will explore differences in protein sequences identified in objective 1 in the context of antibody, T cell and B cell responses to specific proteins.

The student will be trained in all of the techniques and methods required to complete the project. Some of this will be provided by the Institute, but most will be hands on in the lab with Lynnette Goatley. The student will be trained to work in high containment, will gain experience of next generation sequencing, bioinformatics and immunological techniques. The laboratory is based in a multi-user lab shared with the African swine fever virus group, Large DNA virus and Mucosal Immunology groups.

References for Suggested Reading:

- Phylogenomic analysis of 11 complete African swine fever virus genome sequences. (2010) de Villiers EP1, Gallardo C, Arias M, da Silva M, Upton C, Martin R, Bishop RP. Virology. 400:128-136. https://www.ncbi.nlm.nih.gov/pubmed/20171711
- Live attenuated African swine fever viruses as ideal tools to dissect the mechanisms involved in viral pathogenesis and immune protection. (2015) Lacasta A, Monteagudo PL, Jiménez-Marín Á, Accensi F, Ballester M, Argilaguet J, Galindo-Cardiel I, Segalés J, Salas ML, Domínguez J, Moreno Á, Garrido JJ, Rodríguez F. Vet Res. 46:135. https://www.ncbi.nlm.nih.gov/pubmed/26589145