Ref: 2024/07
Closing date: 26.02.24

Project Title: Genome Engineering for Gene Function Study and Improvement of HVT Vector

Supervisors: Dr Yaoyao Zhang & Dr Yongxiu Yao
Research group: Viral Oncogenesis

About The Pirbright Institute

The Pirbright Institute delivers world-leading research to understand, predict, detect and respond to viral disease outbreaks. We study viruses of livestock that are endemic and exotic to the UK, including zoonotic viruses, by using the most advanced tools and technologies to understand host-pathogen interactions in animals and arthropod vectors. Our Institute is made up of a dynamic and vibrant community of employees covering a diverse set of chosen fields, backgrounds and experience. Our outlook is always balanced by our strong sense of purpose, values and behaviours, and an unwavering commitment to a 'one Institute' approach.

Project Summary:

Herpesvirus of Turkey (HVT) is one of the most successful and widely used viral vectors for generation of recombinant vaccines that deliver protective antigens against other avian pathogens. To overcome the interference between individual recombinant HVT vaccines, we have developed triple-insert HVT-vectored vaccine candidate capable of inducing simultaneous protection against multiple avian pathogens. However, the animal experiments showed reduced replication efficiency in chickens, indicating that the insertion of foreign genes affects HVT replication in vivo, and the HVT may not be able to express more than 3 cassettes without compromising on replication thus limiting the induction of immunity.

To explore the hypothesis that knocking out the non-essential genes in the HVT vector can enhance replication enabling recombinant HVT-based vaccines to induce stronger immune responses, we have identified essential/non-essential genes of HVT using targeted CRISPR library screening approach. Following the deletion of 6 non-essential genes in the triple-insert vaccine HVT-ND-IBD-ILTV, the growth kinetics showed that deletion of specific genes leads to the improved HVT replication in vitro. In addition to further deletion of non-essential genes to streamline the HVT vector platform, the aim of this project also includes the validation of some top-ranking essential genes.

Further Details:

The student will explore the following avenues:

1. Continue the knockout of individual non-essential genes in HVT-ND-IBD-ILT using the established pipeline to generate the mutant viruses. In this part of the work, the student will learn the technique on the generation of mutant virus by CRISPR/Cas9 editing.
2. Determine in vitro replication of mutant viruses. The student will compare the growth of mutant viruses with the parental virus which involves the titration of virus stock and producing the growth curve of mutant viruses using the established protocol.

3. Validation of essential genes identified from CRISPR screening. The student will assess gene essentiality by infecting Cas9/gRNA expressing DF-1 with GFP expressing HVT and passing the cells several times. No survival virus indicates that the deleted gene is essential for virus growth.

The viral oncogenesis group has extensive experience in working with genome engineering systems using CRISPR/Cas9 editing and virology. The student will be trained in all the techniques and methods required to complete the project. They will have the opportunity to generate mutant viruses using CRISPR/Cas9 system, alongside more commonly used techniques including PCR, quantitative PCR, RT-PCR, cell culture, plaque assays and data analysis. As a member of the group, the student will attend weekly laboratory meetings to discuss the progress of the project and troubleshoot any problems. The group has a supportive contingent of students and researchers who will be available for training and support.

References for Suggested Reading:

Zhang, Y., et al., Targeted Editing of the pp38 Gene in Marek's Disease Virus-Transformed Cell Lines Using CRISPR/Cas9 System. Viruses, 2019. 11(5).

Zhang, Y., et al., Application of CRISPR/Cas9 Gene Editing System on MDV-1 Genome for the Study of Gene Function. Viruses, 2018. 10(6).

Zhang, Y.Y., et al., Marek's Disease Virus-Encoded MicroRNA 155 Ortholog Critical for the Induction of Lymphomas Is Not Essential for the Proliferation of Transformed Cell Lines. Journal of Virology, 2019. 93(17).

Tang, N., et al., Generation of A Triple Insert Live Avian Herpesvirus Vectored Vaccine Using CRISPR/Cas9-Based Gene Editing. Vaccines (Basel), 2020. 8(1).

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Closing date to apply: 26.02.24.