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Project: Using sequence space to manipulate and study the role of nucleocapsid in coronavirus evolution

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Project Summary:
The gammacoronavirus infectious bronchitis virus (IBV) causes an acute, highly contagious respiratory disease of poultry causing animal welfare issues and severe economic losses to the poultry industry worldwide. Given their high mutation rates, large population sizes and short generation times, RNA viruses such as IBV evolve rapidly producing swarms of similar, but not identical, progeny virions. Consequently, current live attenuated IBV vaccines have a high risk of reversion to a virulent phenotype. The localisation of these viral populations in sequence space generally impacts upon which mutational neighbourhoods are accessible and how these viruses evolve and It has been proposed that certain neighbourhoods will influence the potential of reaching beneficial or disadvantageous mutations that facilitate attenuation. We intend to assess the effect of shifting the virus in sequence space towards less hospitable regions that are expected to have the greatest impact on viability. The project will utilise reverse genetics to generate and characterise novel viruses, focussing on assessment of genetic stability and viral fitness.

Details:
We propose to develop tools to explore the genome function and evolution of coronaviruses, specifically infectious bronchitis virus. The student will develop and characterise viruses by rationally altering regions of the IBV genome to redirect their trajectories in sequence space to more detrimental regions i.e. to attenuate progeny virions. Specifically, we will engineer viral genomes to harbour more serine and leucine codons with nonsense mutation targets i.e. codons likely to generate stop mutations after a single nucleotide substitution. We will then use the progeny viruses to inform the impact of the changes upon the viral genome, its overall fitness and level of attenuation. We will focus upon the nucleocapsid gene within the M41 strain of IBV as the nucleocapsid gene is essential to replication of the virus. Using our well established reverse genetics system, we will insert synthetic constructs of the entire nucleocapsid gene (~1.2kb) containing synonymous changes in serine and leucine residues only (~11%), into the M41 backbone. Of the two constructs, one will contain changes that are only one step away from generating a stop mutation (1-to-STOP), with the second containing changes that are two changes away (2-to-STOP) from developing stop codons. Recombinant M41 clones containing these synthetic ORFs will be rescued and validated using Sanger sequencing. The stability and fitness of the mutations will be analysed during serial passage and the replication kinetics of these novel viruses in cell culture will be characterised using plaque assay and quantitative RT-PCR. The sensitivity of the modified viruses to mutation will be assessed by serial passage in the presence of mutagens such as base analogues or manganese.
The student will have the opportunity to generate novel viruses which can be used for further study and will learn specialist skills including IBV reverse genetics, alongside more commonly used techniques including PCR, quantitative RT-PCR, Sanger sequencing, cell culture, viral plaque assays and data analysis. The group has a supportive contingent of students and researchers that will be available for training and support.

References for Suggested Reading:
- Britton, P. *et al.* (2012) Modification of the avian coronavirus infectious bronchitis virus for vaccine development. doi.org/10.4161/bbug.18983
- Lauring, A. *et al.* (2012) Codon usage determines the mutational robustness, evolutionary capacity, and virulence of an RNA virus. Cell Host Microbe 12(5):623 – 632.