EPIZONE short-term mission scientific report

Host institute: Friedrich-Loeffler-Institut (FLI), Germany
Home institute: DTU Vet, Denmark
Period of research stay: 11.01.16 – 05.02.16
Grantee: PhD student Johanne Hadsbjerg
PhD supervisors: Professor Graham J. Belsham
& Senior Scientist Thomas B. Rasmussen

Purpose of the visit

The purpose of the visit was to become acquainted with a method called selective 2′-hydroxyl acylation analysed by primer extension (SHAPE), which took place within Dr. Dirk Höper's laboratory for NGS and Microarray Diagnostics at the Friedrich-Loeffler-Institut (FLI), Germany. This method provides structural information on RNA and interaction patterns of nucleotides within RNA molecules. This SHAPE analysis should allow a more in-depth knowledge of the secondary structure of the classical swine fever virus (CSFV) genomic RNA. Furthermore, the protocol for generating full-length CSFV RNA transcripts *in vitro* was optimised in Dr. Ilona Reimann's laboratory for molecular virus characterisation.

Work carried out during the visit

The basis for obtaining efficient information on RNA secondary structure first relies on the generation of full-length CSFV RNA transcripts. Before the visit, the *in vitro* transcription procedure did not function optimally with inconsistent generation of full-length transcripts. At the lab of Dr. Ilona Reimann, the DNA template is routinely ethanol precipitated before transcription. Hence, this precipitation was applied to full-length DNA of my constructs, which included synonymous mutations in a motif within the coding region for NS5B (the RNA-dependent RNA polymerase). After the *in vitro* transcription, the DNA template was removed by DNase treatment and the RNA transcripts were purified using magnetic beads. Performing EtOH precipitation prior to transcription increased the RNA yield 6-fold. To eliminate any smaller RNA fragments, the RNA was gel purified. However, this did not work and all the RNA was lost. Instead, it was decided to check if the purified RNA looked intact as judged by agarose gel electrophoresis. It was shown possible to obtain a good yield of the intact RNA.

The SHAPE protocol has been previously tested at the lab of Dr. Dirk Höper but only on short RNA fragments up to 600 nt. As my RNA is 12,300 nt in length, adjustments to the procedure had to be applied. First, the concentration of the SHAPE reagent N-methylisatoic anhydride (NMIA) for effective probing was tested. Three NMIA concentrations (130 nM, 97.5 nM and 65 nM) were used for the probing followed by primer extension, adapter ligation and amplification of the cDNA. The resulting DNA fragments were analysed by Agilent's Bioanalyzer but no DNA could be detected. Whether this was due to inefficient cDNA synthesis or problems with the amplification was not clear. Based on the literature, a NMIA concentration of 65 nM was

used for further experiments. An efficient protocol for SHAPE probing on long RNA fragments was established using these conditions. Two constructs were probed and made ready for next generation sequencing on an Ion Torrent platform.

Personal experiences

The short-term mission has allowed me to broaden my horizon in terms of both practical hands-on work as well as personal connections. It has been useful to work in another laboratory and seeing how they organise things. From the small differences in lab procedures to experiencing new techniques. In addition, it has been exciting to get to know new people and interact both in a lab and office setting plus in more informal surroundings after normal working hours. At has been good to discuss my project and the results with scientists that can provide new inputs and ideas. The short-term mission has given me an excellent platform for networking, and to achieve results that otherwise would have taken a lot longer to obtain.

Conclusion

The RNA secondary structure information should provide valuable data needed to answer some critical questions about CSFV RNA replication. At the moment, sequencing of the probed constructs is still pending but execution of this by staff at FLI has been agreed on in principle. During the visit, I have gained expertise in new methodology that can be adapted for future lab procedures at my home institute. Moreover, the stay has provided a great forum to strengthen my networking possibilities and allow me to collaborate with scientists at other institutes. I believe that a solid network is essential for a scientific career where co-operation between other scientists within your field is necessary and that has definitely been achieved.