DTU Library

The porcine systemic response to pleuropneumonia studied by transcriptional profiling of liver and tracheobronchial lung lymph nodes using multiplexed mRNA-Seq

Hedegaard, Jakob; Schou, Kirstine Klitgaard; Skovgaard, Kerstin; Zhan, Bujie; Panitz, Frank; Hornshøj, Henrik; Heegaard, Peter M. H.; Angen, Øystein; Boye, Mette; Bendixen, Christian

Link back to DTU Orbit

The porcine systemic response to pleuropneumonia studied by transcriptional profiling of liver and tracheobronchial lung lymph nodes using multiplexed mRNA-Seq

Jakob Hedegaard[1], Kirstine Klitgaard Schou[2], Kerstin Skovgaard[2], Bujie Zhan[1], Frank Panitz[1], Henrik Hornshøj[1], Peter M. Heegaard[2], Øystein Angen[2], Mette Boye[2], Christian Bendixen[1]

[1]Aarhus University, Faculty of Agricultural Sciences, Department of Genetics and Biotechnology, Postbox 50, DK-8830 Tjele, Denmark
[2]Technical University of Denmark, National Veterinary Institute, Division of Veterinary Diagnostics and Research, Bülowsvej 27, DK-1790 Copenhagen, Denmark
Corresponding author: Jakob.Hedegaard@agrsci.dk

Actinobacillus pleuropneumoniae (Ap) is a gram-negative bacterium that causes porcine pleuropneumonia, which is a widespread, highly contagious and often fatal respiratory disease in swine. A total of 44 pigs were experimentally inoculated with Ap serotype 2 or 6 and samples of liver and tracheobronchial lung lymph nodes were collected 6, 12, 24 and 48 hours after experimental inoculation, as well as from six non-inoculated control pigs. Transcriptional profiles of the liver samples have been generated by preparation of 12-plexed mRNA-Seq libraries followed by sequencing on an Illumina GAIIx (51+7 cycles) obtaining more than 200 million tag sequences. The 12-plexed mRNA-Seq libraries of the lung lymph node samples have presently (April 2010) been prepared and are to be sequenced. The PCR amplicons of the liver libraries were quantified using both a fluorometer and a qPCR assay, including the use of a sequence-titrated, in-house control library. The libraries were diluted to 6 pM based on the qPCR assay, except for a single library set which was duplicated and diluted based on the fluorometer measurements as well. Analysis of the obtained sequences revealed that the qPCR based quantifications reduced the cluster density variability as compared to fluorometer based quantifications. Furthermore, it was found that the fluorometer based measurements tended to deviate for dilute as well as for more concentrated libraries. Following the sequencing of the lung lymph node samples analyses are to be conducted to study the time and serotype dependent transcriptional response to Ap infection.