Conference Agenda

Session

DA: Data Analysis in qPCR & dPCR & NGS

Time: **Wednesday, 22/Mar/2023: 8:30am - 10:30am**
Session Chair: **Andreas Untergasser**, University Heidelberg, Germany
Session Chair: **Philip Day**, University of Manchester, United Kingdom

Location: **HS 15**

ZHG -- Lecture Hall 15

Presentations

Bioinformatics Tools For Ultra-sensitive Sequencing Data Using Unique Molecular Identifiers

Tobias Österlund[1,2], Stefan Filges[1], Gustav Johansson[1], Andrers Ståhlberg[1,2]

[1]University of Gothenburg, Sweden; [2]Sahlgrenska University Hospital, Sweden

Targeted sequencing using Unique Molecular Identifiers (UMIs) enables detection and quantification of rare variant alleles in challenging applications, such as cell-free DNA analysis from liquid biopsies. Standard bioinformatics pipelines for data processing and variant calling are not adapted for deep-sequencing data containing UMIs and are inflexible, require multi-step workflows or dedicated computing resources. Here, we developed UMIErrorCorrect, a bioinformatics pipeline for analyzing sequencing data containing UMIs. UMIErrorCorrect only requires fastq files as inputs and performs alignment, UMI clustering, error correction and variant calling. We also provide UMIAnalyzer, a graphical user interface, for data mining, visualization, variant interpretation and report generation. UMIAnalyzer allows the user to adjust analysis parameters and study their effect on variant calling. We demonstrated the flexibility of UMIErrorCorrect by analyzing data from four different targeted sequencing protocols and accurately quantified rare variants in standardized cell-free DNA reference material. UMIErrorCorrect outperformed existing pipelines developed for targeted UMI sequencing data in terms of variant detection sensitivity. UMIErrorCorrect and UMIAnalyzer are comprehensive and customizable bioinformatics tools that can be applied to any type of library preparation protocol and enrichment chemistry using UMIs. Access to simple, generic and open-source bioinformatics tools will facilitate the use and implementation of UMI-based sequencing approaches in basic research and clinical applications.

Enhancing transcript measurement via the Gini Index

Philip Day, Steve O'Hagan

University of Manchester, United Kingdom

A novel software permitting the assessment of the Gini Index will be described that identifies highly stably expressed genes and unique gene signatures to permit exquisite measurements of genes underpinning subtle changes that define never before seen changes that represent signatures of disease states. When used with specific dye chemistry permits both a low detection sensitivity and more rapid assessment of qPCR results than is currently obtainable.

Gene Fusion Detection - more complex than you might assume

David Langenberger

ecSeq Bioinformatics GmbH, Germany

Gene fusions, which occur when two previously separate genes become joined together, can result in the formation of abnormal proteins and have been implicated in various human diseases such as cancer. Detection of gene fusions is a crucial step in understanding the underlying biology of these diseases and in developing new diagnostic and therapeutic approaches.

Next-generation sequencing (NGS) technologies have revolutionized the field of gene fusion detection by providing a high-throughput and cost-effective method for identifying these genetic events. The split-read method takes advantage of the fact that during the sequencing process, when a fusion occurs between two genes, the resulting chimeric RNA molecule or the rearranged genomic DNA fragment is sequenced in one contiguous step. Aligning these reads, that are spanning fusion junctions, to the reference genome results in so-called split-reads where different parts map to different genomic locations.

However, this split-read method for detecting fusion events is not without limitations. It relies on the presence of at least some split-reads for each gene fusion, which may not always be the case, particularly for low-frequency or lowly expressed fusions. Furthermore, the approach can be prone to false-positive calls, which can be caused by various factors such as sequencing errors and annotation errors.

Efficiency-Corrected PCR Quantification For Identification Of Prevalence And Load Of Respiratory Disease-Causing Agents In Feedlot Cattle

Ian Marsh[1,4], Rebecca Barnewall[2,3], Thomas Williams[2,3], Paul Cusack[2,5], Narelle Sales[4], Francesca Galea[1,4], Andrew Szentirmay[1,4], Jan Ruijter[6], Maurice van den Hoff[6], Jane Quinn[2,3]

[1]D'Central, Unit 3C, Building 4, 256B New Line Road, Dural, New South Wales, 2158, Australia; [2]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, New South Wales, 2678, Australia; [3]Gulbali Institute for Agriculture, Water and the Environment, Wagga Wagga, New South Wales, 2678, Australia; [4]NSW DPI, Elizabeth Macarthur Agricultural Institute, PMB 4008, Narellan, New South Wales, 2567, Australia; [5]Australian Livestock Production Services, Cowra, New South Wales, 2794, Australia; [6]Department of Medical Biology, Amsterdam University Medical Centres, Location Academic Medical Center, Amsterdam, the Netherlands

From a veterinary diagnostic perspective, PCR is predominantly performed qualitatively, be it conventional or real-time. However, pathogen load is becoming increasingly important in disease management. Quantitative PCR aims to measure the nucleic acid concentration of a specific target based on the quantification cycle (Cq) value, derived from the sample amplification curve, plotted on a standard curve generated from purified nucleic acid standards. Acceptance of the PCR run is often made using the statistical attributes of the standard curve only whilst ignoring similar criteria from the samples. In doing so, the impact of the sample is often overlooked or underestimated. At best this approach might best be described as semi-quantitative. Typically, qPCR assumes assays are 100% efficient, that is, produce a doubling of amplified product each cycle. It could be argued this is rarely the case in a diagnostic assay.

To achieve a more meaningful quantitative result, we used the Cq value and efficiency derived from the sample amplification curve used in conjunction with the efficiency value from a single calibrator standard (with a known target concentration), a process known as efficiency corrected quantitative PCR (EC-qPCR). EC-qPCR was used in a recent study to investigate the agents involved in bovine respiratory disease on cattle at induction to the feedlot. Bovine respiratory disease (BRD) is the most prevalent disease in feedlot cattle worldwide and considered one of the most difficult and complicated cattle diseases, largely due to the number of agents involved. BRD is commonly attributed to Bovine alphaherpesvirus 1 (BoAHV1), *Histophilus somni*, *Mannheimia haemolytica*, *Mycoplasma bovis*, *Pasteurella multocida* and *Trueperella pyogenes*.

BRD disease investigation is often complicated by the fact that *H. somni*, *M. haemolytica*, *P. multocida* and *T. pyogenes* are considered normal flora of cattle and therefore their presence in the upper airways alone is not necessarily informative with respect to disease status or risk. To get a better understanding of the relationship between presence, load and disease status, we investigated these agents using EC-qPCR to accurately determine the prevalence and load in the upper respiratory tract from newly inducted cattle were compared with cattle in the hospital pen of the feedlot. EC-qPCR results and clinical data were combined to establish profiles to elucidate the combinations of agents and those animals' experiencing proliferation of the agents verses normal levels. Using technology that can produce individual amplification efficiencies for each sample and EC-qPCR to investigate, analyse and identify BRD-associated viral and bacterial agents represents a new opportunity for Australian feedlot systems to manage and treat BRD. EC-qPCR opens the scope for any disease investigation where accurate qPCR results are required.