universität ulm

Newsletter Spring 2022

New developments & techniques

Dear colleagues,

in a time of limited personal communications and the lack of direct interaction with our customers, we would like to provide you with some information on novel sequencing techniques that are available at our Genomics Core Facility and new analysis methods at the Core Unit Bioinformatics.

1. Metagenomics

In 2020, we performed our first metagenomics sequencing run for the Laboratory for Molecular Psychosomatics of Prof. S. Reber. In contrast to shotgun metagenomics where the complete genomes of the microbiome can be sequenced, in 16S RNA sequencing only specifically variable regions of the ribosomal RNA are sequenced. Therefore, the throughput for 16S RNA sequencing allows for up to 600 samples per run even on the MySeq while shotgun sequencing typically achieves around 35 samples per NextSeq run. This enables for more in-depth analysis of the material (taxonomy, phylogeny, differential abundance, diversity) at the expense of a more complex processing for demultiplexing and denoising.

2. Bacterial RNA (Mycobacterium tuberculosis)

The isolation of RNA from M.Tb is challenging due to a very robust cell wall of these bacteria. It is necessary to remove the ribosomal RNA however
most commercially available RNA depletion kits have not been tested against against M.Tb. Togehter with the group of Prof. S. Stenger we successfully established RNA sequencing for this species in Ulm. In cooperation with the group of Professor Stenger, we added M. Tb. reference and feature files (H37 Rv) to our portfolio for bioinformatic analysis (readcounting etc.)

universität ulm

3. Chromatin profiling by CUT&RUN

Together with Prof. S. Britsch and Dr. C. Wiegreffe from the Institute of Molecular and Cellular Anatomy, we are developing a new technique for chromatin profiling called CUT&RUN - cleavage under targets and release using nuclease. The method is used to analyze protein interactions with DNA. It combines antibody-targeted controlled cleavage by micrococcal nuclease with massively parallel DNA sequencing. Compared to conventional ChIP-Seq CUT&RUN has a much better signal-to-noise ratio.
Computational analysis consisting of alignments, spike-in calibration, and peak calling allows identification of sites modulating transcriptional regulatory elements and chromatin domains, investigation of the chromatin landscape and histone enrichment around genes. These profiles can also be utilized to investigate the consequences of mutant phenotypes during development.

4. miRNA Sequencing from Exosomes

Exosomes are membrane-bound extracellular vesicles (EVs) that are produced in the endosomal compartment of most eukaryotic cells and are also released in vitro by cultured cells into their growth medium. Although they are known for more than 35 years, the knowledge about their role in the etiology of disease and normal cellular physiology is still largely unknown. Mesenchymal stem cell (MSC)-derived exosomes are known to mediate tissue regeneration in a variety of diseases, and are increasingly being recognized as potential therapeutics.

A key player in these processes are miRNAs and therefore it is necessary to characterize the miRNA profile of such exosomes. After enrichment and isolation of a set of exosomes in the Section of Experimental Anesthesiology of Prof. M. Schneider we could establish first miRNA profiles by sequencing. miRNA samples from exosomes were successfully assessed using a versioned and containerized pipeline for bioinformatic analysis. We want to use differential analysis to classify groups of treated samples.

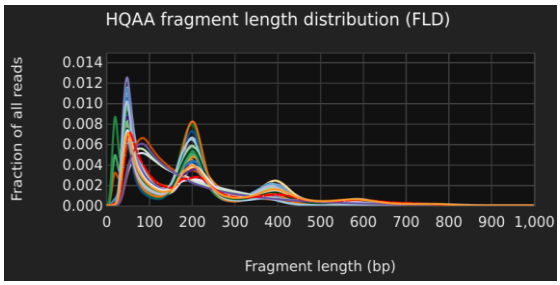
universität ulm

5. miRNA Sequencing from plasma

Because of the limited quantity of RNA in plasma, RNA sequencing is already difficult. miRNAs are even more challenging since they make up only roughly a thousandth of the total RNA composition. Despite these challenges, we were able to get enough RNA from Sus scrofa plasma for Prof. H. Schrezenmeier and Prof. M. Kalbitz (Institute of Transfusion Medicine, Department of Traumatology).
Using a standardized bioinformatics best-practice workflow for analysis of small RNA sample profiles, an initial analysis was carried out. As a result, we were able to sequence first miRNA profiles from plasma.

6. ATACSeq

ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) is used to assess genome-wide chromatin accessibility and study chromatin-accessibility signatures. We updated a pipeline for ATACSeq analysis (including tools like ataqv, MACS2, or HOMER) and provide a design file in the setup to accommodate for duplicates and dispersed samples due to the broad variety of experimental settings.



7. Development of containerized pipelines

Containerization is a technique for effortlessly packaging, versioning, and porting an application and all of its dependencies in almost any hardware environment. A minimal operating system, essential software and applications, runtime environments, and analytic processes are packed together to create a reproducible and reliable blueprint for bioinformatic pipelines. Different pipeline versions allow for replication of results, parameters allow adaptation of pipelines for different sequencing setups or genomes. This standardizes the execution of pipelines in a controlled environment, regardless of operating system or hardware resources.

Contact
Dr. Karlheinz Holzmann
Genomics Core Facility
89081 Ulm
Telefon +49 731 50044694
karlheinz.holzmann@uni-ulm.de
https://t1p.de/p9a8

Contact
Prof. Hans A. Kestler
Core Unit Bioinformatics
89081 Ulm
Telefon +49 731 50024500
cubi@lists.uni-ulm.de
https://cubi.medizin.uni-ulm.de