Graduate Program in Molecular Cell Biology and Oncology: MCBO Methods Courses

Special Lecture/Course in:
Advanced promoter analysis with ChIP-seq and methylation analysis

Lecturer: Heidelinde Fiegl, Frédéric Santer, Dietmar Rieder, Anne Krogsdam

Number: 041541

Type: VU ECTS: 1,5 (2 SSt.)

Character: Lectures and Practical Course

Time/Date: 06.-08.05.2019: Santer
09.-10.05.2019: Rieder
27.-28.05.2019: Fiegl
tba: Krogsdam

Location: Urologielabor, Gynäkologielabor, CCB

Limited number of places YES, number of places 6, registration necessary YES

For registration or questions please contact: frederic.santer@i-med.ac.at

Aim:

To conduct and evaluate an experimental setup involving identification of promoter sequences bound by the androgen receptor, and the methylation status of such promoters. –Includes data generation (wet-lab), sequencing (NGS-core), and final analysis (computer-lab)

Description/contents:

In a prostate cancer model system, Androgen receptor-bound promoter sequences will be isolated by ChIP (chromatin immune precipitation). The isolated DNA will be further analyzed for methylation status, applying the bisulfite method. Sequences and methylation status will be identified by NGS library generation and sequencing. Bioinformatic data analyses will be performed to extract relevant data and identify possible connections between promoter-binding and –methylation status.

Main points:

Performing a ChIP experiment for subsequent NGS
-Assess binding coverage of Androgen Receptor (AR) after stimulation with androgens vs. inhibition with antiandrogens
- Crosslinking of Protein/DNA
- Immunoprecipitation with anti-AR -Reverse crosslinking
- DNA purification -Quality control using qPCR prior NGS

Methylation analysis
- DNA methylation in cancer
- CpG island search
- Bisulfitemodification
- MethyLight PCR (including primer design)

Sequencing
- Library generation
- Library QC
- introduction to sequencing technology
- evaulating a sequencing run in terms of output and quality

Bioinformatics analysis of the generated NGS data will include:
- ChIPseq data analysis:
-Working with HT-sequencing data (FASTQ, SAM/BAM)
- NGS data Quality Control (FASTQC, cutadapt, flexbar)
- short read alignment and filtering (BWA, Picard, samtools)
- Peak calling (MACS2)
- Peak annotation (BEDtools, ChIPseeker, GREAT, Bioconductor)
- data comparison (ChIPseeker, ngs.plot)
- Data visualization (UCSC genome browser, IGV)
- Motif prediction/finding (MEME)

Suggested reading: