

AUTHENTICATION OF KEY RESOURCES – PROJECT 1 BIOMARKERS OF EPILEPTOGENESIS AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Plasma quality

The hemolysis can be detected by existence of miR-23a and miR-451a in the sample.

Ref: <http://www.sciencedirect.com/science/article/pii/S0003269715003681>

RNA quality: RNA extraction: RIN >7.0 (Bioanalyzer 2100, Agilent Technologies) as well as OD60/230 and OD260/280 ratio ~2.0 (NanoDrop, Thermo Scientific).

For miRNAs there is no standardized method for quality control. miRNAs are generally well-preserved are not as susceptible to RNases due to their size. The RIN number and 260/280 ratio will be checked before and after transportation as well as prior to use, if samples have been stored for a longer period of time (> one year).

miRNA-Seq: Exiqon's guidelines (miR-seq): RIN 7-10 indicates high quality RNA. It is possible to do miRNA-Seq in samples that have RIN 5-10. With RIN<5 sequencing is possible but sequencing of degraded material may contribute to results.

RT-qPCR: Housekeeping genes GAPDH for mRNA, U6 for miRNA in brain tissue, miR-425 for miRNA analysis in plasma.

Internal controls: GAPDH and U6 are commonly used. Studies in Pitkänen lab indicate that miR-425 has a stable expression over a period of two days to two months post-TBI.

Exiqon will perform the RNA isolation for microRNA-Seq. Exiqon has developed its own qPCR based quality control panel for serum and plasma RNA samples. The method is based on miRCURY LNA Universal RT microRNA PCR system and it uses four synthetic spike-ins. The degree of hemolysis will be evaluated. After library generation, Reverse Transcription (RT) and PCR pre-amplification the insert rate of the desired RNA type is evaluated using Bioanalyzer DNA high sensitivity chip. After sequencing, reads are compared to a number of reference sources (miRBase, Rfam). Before differential expression analysis results are normalized to compensate sample specific effects.