

## **Service:** Transcriptome Analysis (RNAseq)

### **Required activities – description, scope (for example number of samples, forest area), temporal aspect (requirements) etc.:**

- RNAseq analysis of delivered samples of Norway spruce RNA
- Number of samples: **64**
- Samples of sufficient quality mean samples with the following minimum parameters: sample volume  $m \geq 1\mu\text{g}$ , RNA concentration  $c \geq 20\text{ng}/\mu\text{l}$ , sample purity  $\text{OD } 260/280 \geq 1.8$ ,  $\text{OD } 260/230 \geq 1.8$ ; RNA 28S:18S  $\geq 1.0$ , RIN  $\geq 6.5$ ;
- If any of the samples does not meet quality requirements for the analysis, the contractor hereby agrees to immediately (no later than within the deadline pursuant to Article III of the Contract) inform the client who is liable to supply a new sample in the required quality without undue delay.

### **Requirements for methodological procedure:**

- Quality control of the delivered RNA samples before the analysis commencement
- mRNA fraction enrichment (polyA enrichment or ribodepletion) before cDNA library preparation
- cDNA library preparation (random-primed library type)
- Use of the most up-to-date platforms of RNA sequencing, for example HiSeq 4000 or BGI-seq 2000)
- 100 or 150 bp pair-end reads (bilateral readings across section lengths 100 bp or 150 bp)
- Minimum 30 million reads (reading frames) per sample
- Data quality requirement: Phred value at least Q30 for at least 85% of the reads (reading frames) and at the same time Phred value at least Q20 for at least 90% of the reads (reading frames)
- Data delivery in the fastQ format, the data must be generally compatible with the reference genome databases regardless the applied sequencing methodology