

Name of the service: General sequencing

Required activities - their description, scope (e.g. number of samples, forest area), time (requirements) etc.:

- RNAseq analysis of provided samples of bark beetles from the subfamily Scolytinae (total RNA)
- Number of samples: **350 pcs**
- Samples of sufficient quality mean samples with the following minimal parameters: amount of sample $\geq 1\mu\text{g}$, concentration RNA $c \geq 20\text{ ng}/\mu\text{l}$, cleanness of the sample $\text{OD } 260/280 \geq 1.8$, $\text{OD } 260/230 \geq 1.8$; RNA 28S:18S ≥ 1.0 , RIN ≥ 7 ;
- In case some of the samples shall not be assessed of quality sufficient for the analysis, the Contractor agrees to immediately inform the Client who shall additionally and without unnecessary delay provide a sample/samples of sufficient quality.
- Period for the delivery of samples to the Contractor:
- The results of the sequencing shall be delivered to the Client within 40 working days after the receipt of the samples by the Contractor.
- The samples shall be sent to analysis (by post/freight forwarding) successively (at least 24 and at most 96 samples in one group) on dry ice and according to a procedure recommended by the Contractor, at the Client's costs. The Contractor shall always inform the Client about sending the group of samples at least 2 weeks before sending the samples itself.
- the first group of samples shall be handed over by the Client to the Contractor within the period of 12 weeks after the conclusion of the contract. The contract shall be concluded for a definite period of 3 years, all samples shall be delivered and analyzed within this period.
- Data delivery (Hard disk) and sample transport under 15-20 kg of dry ice (12 times) charges need to be included in the bid.

Requirements on the methodological procedure:

- Quality check of the delivered samples RNA before the commencement of the analysis
- Enhancement of **mRNA fraction** (polyA enrichment or ribodepletion) before the preparation of cDNA library
- Preparation of cDNA library (type of the library random-primed).
- Usage of the newest platforms of RNA sequencing, e.g. **Hiseq 4000** or BGI-seq 2000 or Novaseq6000 the Client shall explicitly enable offer of equal solutions similarly
- **150 bp pair-end reads** (bilateral reading in the section length 150 bp)
- At least **30 million good quality reads** (reading frames) per replicate or sample (9G raw data).
- Requirement on the quality of data: Phred value min. **Q30 for at least 85%** reads (reading frames) and at the same time Phred value min. Q20 for at least 90% reads (reading frames)
- Recommendation of the sequencing results electronically via server / shared disc in the format fasta and fastq, generally compatible data with databases of referential genome regardless of the used technology of sequencing.
- Multiplex reading, removing of adaptors and their delivery with reports QC.
- The Client requires description of methodology of the preparation of the library, together with the delivery of sequencing results.