

**Name of the service:** Metagenomic analysis (sequencing of amplicon and data analysis)

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

- Analysis of amplicon of delivered samples of bark beetles from the subfamily Scolytinae (total DNA)
- Number of samples: **400 pcs**
- Samples of sufficient quality mean samples with the following minimal parameters: amount of sample  $\leq 250$  ng, concentration DNA  $\leq 10$  ng/ $\mu$ l, volume – 20  $\mu$ l, cleanness of the sample OD 260/280 = 1.8 – 2.0, without degradation, without contamination.
- In case some of the samples shall not be assessed of quality sufficient for the analysis, the Contractor agrees to inform the Client within 2 weeks who shall additionally and without unnecessary delay provide a sample/samples of sufficient quality.
- The first group of samples shall be sent by the Client within 6 months after the conclusion of the contract, the last group shall be sent by the end of 2021.
- The results 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 120 samples in one group, but 120 samples in one month at most) 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.
- Invoice shall be issued always 15 days after the receipt of each group of the samples by the Contractor.

**Requirements on the methodological procedure:**

- Quality check of the delivered samples of DNA before the commencement of the analysis.
- Preparation of DNA library (130-470 bp, specific primers for 16s rDNA or 18s rDNA or areas ITS (internal transcribed spacer) hub (as required).
- Usage of the newest platforms NGS, e.g. Hiseq 4000 or BGI-seq 2000).
- Sequencing strategy 250 bp pair-end reads (bilateral reading in the section length 250 bp).
- Amount of data for each sample at least 100,000 tags with Q30 > 80%.
- After the quality check, the following data analyses need to be done: OTUs (Operational Taxonomic Units) clustering and filtering; analysis *Alpha*-diversity including rarefaction curve, Chao-1 curve, Shannon curve, curve of assessment of abundance and table of alpha index; OTUs analysis and comment on the types including Krona results, analyses of phylogenetic composition, phylogenetic tree, heat map and taxonomic tree; analysis of *Beta*-diversity including unweighted UniFrac distance of heat map, PCA (principal component analysis), PCoA (principal coordinates analysis), UPGMA (unweighted pair-group method with arithmetic means) and NMDS (non-metric multidimensional scaling) analysis, multi-variational statistic analysis including LefSe (LDA effect size) analyses, metastats analyses, ANOSIM and MRPP analyses

- Sequenced files including raw reads shall be delivered by the Contractor in the format fast or fastq and the analysed data shall be delivered in the format xls and tiff (for images) or other compatible format, in electronic form via server / shared disc.
- After looking at the analysed data from the Contractor, client may recommend some minor alterations such as removal of some outliers from analysis or specific statistical comparison between selected groups of samples, etc. The Contractor needs to perform them without delay and additional charges.
- A skype consultation session at least of 1 hour for each set of analysis is compulsory to explain the client the analysis pipeline and queries (if any).