Appendix No. 1a – Technical specifications for part one

Technical specifications

Part One - RNA sequencing using Next-Generation Sequencing (NGS) Illumina technology

Objective:

RNA sequencing for transcriptome analyses (qualitative and quantitative approaches).

Biological samples for transcriptome analyses:

RNA from plant tissue: roots and leaves of the oaks *Quercus suber* (genome available) and *Quercus variabilis*. Some samples will be infected with *Phytophthora cinnamomi* (genome available). Samples will be obtained from micropropagated plants and from 2 years-old plants.

Contact persons:

Issues related to public tender and samples:

Marília Horta Jung (marilia.jung@mendelu.cz or mariliahortajung@gmail.com) and Josef Janoušek (josef.janousek@mendelu.cz or janousek.jose@gmail.com)

Issues related to sequencing results:

Marília Horta Jung (marilia.jung@mendelu.cz or mariliahortajung@gmail.com) and Tomáš Kudláček (tomas.kudlacek@mendelu.cz or Kudlak@seznam.cz)

Services required as follows:

1) Quality of the samples provided and transportation

RNA samples will be prepared by the contracting authority and the service provider will assure their collection and transportation packed on dry ice. Transport will be as fast as possible, to avoid degradation of the RNA samples. Samples will be delivered as batches of 24 or 48 or 96

samples (this will allow the service provider to prepare a pool of provided samples and sequence this pool at once).

RNA samples will be provided in 2 ml tubes containing ≥ 20 µl with a RNA concentration of at least 10 ng/µl and RIN ≥7. In case that the samples present a lower quality, the service provider will be informed.

2) Quality Control (QC) of samples

The service provider will carry out quality control (by capillary electrophoresis or equivalent method suitable for evaluation of RNA quality and concentration in each sample) and sample concentration adjustment prior to cDNA libraries preparation. In case of any unexpected issues during this work phase, (e.g. low quality of RNA), the service provider will contact Marília Horta Jung.

3) rRNA removal

An rRNA removal procedure will be applied before cDNA libraries preparation. A protocol using poly(A) enrichment or rRNA depletion will be carried upon agreement with the contact person (Marília Horta Jung) – after quality control of the RNA samples, if not specified earlier.

3a) poly(A) enrichment

Poly(A) enrichment will be performed using a poly(A) enrichment kit (specifications for this kit in point 10 of this document).

3b) rRNA depletion

rRNA depletion will be performed using a rRNA depletion kit (specifications for this kit in point 10 of this document) and with oligonucleotides which will be shipped to the service provider at the time of samples submission.

4) RNA (cDNA) libraries preparation

RNA (cDNA libraries) will be prepared using the “unique dual indexes” and using the kit for RNA (cDNA) library preparation for Ilumina sequencing (specifications for this kit in the point 10 of this document).

5) Next Generation Sequencing (NGS)

NGS will be run on an Illumina – NovaSeq 6000 instrument under the paired-end 150 bp setting (bilateral reading in the section length 150 bp). Sequencing reagents and number of lanes used are specified in the Appendix no. 2a (in function to the number of samples submitted for sequencing).

6) Expected outputs, bioinformatics analyses and QC

It is expected to obtain at least 100 million high quality reads per sample (50 M clusters read from both strands in 2x150 bp run) per sample. Lower number of reads is allowed for low quality samples. Data quality requirement: ≥ 85% of bases higher than Q30 at 2 × 150 bp.

The contracting authority requires the service provider to deliver raw data and basic pre-processed data (after de-multiplexing, removing of adaptors, estimation of index hopping, etc.).

Sequencing data should be delivered electronically, via a secured server.

7) Time-schedule for the required services

The contracting authority will send an order to the service provider who has to confirm this order within two working days (if not confirmed, it will be considered as accepted the second working day). The service provider has to organise a collection of the RNA samples within 3 working days from the day of acceptance of the order.

The contracting authority requires the service provider to carry out QC of samples and concentration adjustments prior to cDNA libraries preparation within 10 working days from the date of collection of the RNA samples.

The contracting authority requires the sequencing results within 6 weeks from the time the samples undergo quality control by the service provider.

The contracting authority requires the service provider to store the sequencing results data for a minimum of 8 weeks.

8) General requirements and expectations

The contracting authority requires that the remaining RNA samples will be stored by the service provider for a minimum of 4 weeks and up to 12 weeks from the time of delivery of the results to the contracting authority.

All services must be performed by the service provider in conformity with standard operation procedures for laboratory practices, starting with QC of input samples and ending with bioinformatics procedures.

The service provider will prove previous experience on performing the required services by contacts of previous three customers that can be used as references (see section n. 5.7. of Call to submit bids).

The contracting authority requires the service provider to present a fully detailed description of the methodologies used for preparation of NGS libraries in the form of a report including exact type of NGS library preparation kit and protocol (stating all modifications or additional steps), cDNA libraries QC (Qubit HS DNA Assay and capillary electrophoresis results), sequencing settings, processing and QC of data.

The service provider will ensure 6 hours of consulting with the contracting authority (essentially for final assessment of sequencing results - bioinformatics support - but also for discussing sample processing related issues, if necessary; online or face-to-face).

Reports, emails, online consulting, and other contacts should be in English language.

The tender price must include all necessary costs. No additional charges are allowed.

9) Summary of required outputs and subsequent invoicing

The contracting authority requires that, on the basis of each partial order, it always receives:

1) raw data (see section 6)

2) partially processed data “pre-processed data” and evaluated data (see section 6)

3) report (see section 8)

Following the delivery of the above results (raw data, pre-processed data - evaluated data and report protocol) the contracting authority will check them within 15 working days and confirm their acceptance and the possibility of invoicing. In the event that the contracting authority does not confirm the acceptance of the data within 15 working days, the supplier may invoice after this period even without prior confirmation from the contracting authority.

10) Required specifications of kits

1) poly(A) enrichment kit

- the kit has to be fully compatible with the subsequent RNA (cDNA) library preparation for NGS sequencing

- mRNA will remain intact

- kit has to be suitable for poly(A) enrichment from total RNA

2) rRNA depletion kit

- rRNA depletion kit will allow to use custom ssDNA probes

- input amount of RNA is of approximately from 10 ng – 1 µg

- kit has to be suitable also for low quality RNA samples

- principle of the kit is based on the degradation of RNA by RNase H enzyme

- ssDNA probes will be provided by the contracting authority (the probes will be designed based on the rRNA sequence data obtained from oak species)

- RNA has to be suitable for subsequent RNA (cDNA) libraries for NGS sequencing

3) kit for RNA (cDNA) library preparation for Illumina sequencing

- the kit has to be fully compatible with RNA after poly(A) enrichment and also with RNA after rRNA depletion

- the kit has to be fully compatible with NovaSeq instrument (Illumina)

- input amount of RNA approximately: 10 ng – 1 µg (i.e.: the libraries can be prepared also with the indicated low limit amount of RNA)

- kit has to allow to maintain about the strand specificity – to know from which DNA strand the transcript was obtained (strand-specific method of the library preparation)

- DNA polymerase that is part of the kit has to be at least 200x of higher fidelity than *Taq* polymerase (this characteristic specify error rate of the polymerase)