EXPLANATION OF THE PROCUREMENT DOCUMENTS

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In Prague on 30th December 2021

EXPLANATION OF THE PROCUREMENT DOCUMENETS II.

Pursuant to Section 98 of Act No. 134/2016 Coll., On Public Procurement, as amended (hereinafter referred to as the "Act"), the aforementioned contracting entity shall provide you with the following explanation / amendment or supplement to the tender documentation „**Service for metatranscriptome sequencing"**

Wording request for the explanation of the procurement documents No. 1:

This question is related to the explanation of the procurement documents No. 1: "rRNA depletion technology would individually treat each of Contracting Authorities samples, and the treatment result could be read from the library QC test. There is no risk of false-positive results from a mixed library. The whole process is complete, well-optimized and perfect. Contracting Authority has previous experience with rRNA depletion methods, which suits his bark beetle samples. So, Contracting Authority does not want to experiment with other methodologies while he rushes to publish his data. Contracting Authority already had past data, and he needs the new data to be comparable."

Unluckily, the explanation does not explicitly answer the question. "Would the Client also accept a solution based on depletion after cDNA library preparation, instead of prior cDNA synthesis?" Our solution does not carry any risk of false-positive results, as the samples/libraries are treated separately. But it seems that the Client seeks for particular rRNA depletion solution/kit to be used in the service.

Moreover, the explanation brings additional questions.

As the sample delivered to the contractor should be RNA isolate, what kit will be used for the RNA purification, especially what will be the elution buffer used for the RNA sample? Does the Client use any preservatives for RNA stabilization like the ones from, e.g. GenTegra?

Additionally, the Client mentioned, "Contracting Authority has previous experience with rRNA depletion methods, which suits his bark beetle samples. So, Contracting Authority does not want to experiment with other methodologies while he rushes to publish his data." What particular methodology has the Client in mind? There are several methodological approaches to rRNA depletion from RNA isolate represented by different products (FastSelect, probe-based, enzymatic, …). If the Client requires a specific methodology that copes with the previous data preparation, which one is it? Please, could you specify it concretely?

The explanation of the procurement documents No. 1:

The Client doesn´t accept a solution based on depletion after cDNA library preparation to maintain uniformity with Client´s previous results in the project. The Client used PureLink RNA Mini Kit (Invitrogen). The Client did not use any RNA stabilizer. Optimal rRNA removal and lib prep strategy (preferably Bead-based rRNA depletion) required for the beetle samples (as per Client´s experience):

rRNA content in RNA samples was removed using Ribo-Zero rRNA Removal Kit Human/Mouse/Rat and Ribo-Zero rRNA Removal Kit Bacteria (Illumina). Oligonucleotide probes from both types of Ribo-Zero kits were mixed and added to each sample, allowing their annealing to rRNA and subsequent rRNA removal. The removal efficiency was checked using a 2100 Bioanalyzer, and removal must be repeated when necessary. The lib preparation can be done using TruSeq Stranded Total RNA Library Prep (Illumina).

The Client points out, it will be optimal to follow Client´s published requirements as proposed because the company to company opinion varies. The Client will only pay if the service is given as per Client´s procurement without any change or compromise.

Best regards

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