DNA and RNA NGS Sequencing

Scope of work

For all samples, high-molecular-weight DNA and RNA should be captured, extracted using the Environmental RNA/DNA Isolation Kit and collected in separate tubes: one for shotgun metagenome NGS sequencing and the other for RT-PCR followed by RNASeq protocol. Samples should be sequenced on custom Illumina sequencer to 5-6M 150x150 reads per sample. IIT Madras will have the ownership of data and we expect the selected vendor to sign an NDA.

Sample collection will be done by IITM and samples will be sent directly the vendor for DNA/RNA extraction process. Each sample will be collected by IITM using a sterile and DNA/RNA free swab, following the MetaSUB sampling protocol.

Specifications

1. Metagenome DNA Seq

DNA extraction from 100 samples:
Total DNA should be isolated using Qiagen Power Soil DNA Isolation Kit.

Sample Quality Control:
Quality and quantity of extracted DNA should be analyzed using Qubit and DNA samples with >20ng/µl should be used for library preparation. QC data will have to be shared with us before proceeding for sequencing. Only after approval of QC results, sequencing should be done.

Library Preparation:
Shotgun DNA Library should be prepared using NEBnext Ultra DNA library preparation kit.

Sequencing 100 samples:
- 2x150bp Paired End reads sequenced using Illumina HiSeq X
- Minimum 5-7 million reads per sample
- Data to be delivered as de-multiplexed FASTQ files

2. RNA Seq (COVID-19)

RNA extraction from 100 samples:
Total RNA should be isolated using automated nucleic acid extraction on the QIAsymphony and the QIAmp Viral RNA mini kit. One step reverse transcription to cDNA and real-time PCR (RT-PCR) amplification of viral targets, E (Envelope) and S (Spike) genes and internal control, should be performed using a real time (qRT) Thermocycler and commercially available QIAGEN QuantiNova Kits.

Sample Quality Control:
Completed libraries should be quantified using Qubit and run on BioAnalyzer for size determination. QC data will have to be shared with us before proceeding for sequencing. Only after approval of QC results, sequencing should be done.

Library Preparation:
Samples with total nucleic acid (TNA) should be treated with DNAse-I (Zymo Research, Catalog # E1010), which cuts both double-stranded and single-stranded DNA. DNAse treated RNA should be used to prepare a library using NEBNext Ultra II RNA directional kit following rRNA depletion using Lexogen RiboCop v2 kit.

Sequencing 100 samples:
- 2x150bp paired end reads sequenced using Illumina HiSeq X
- Minimum 1 million reads per sample
- Data to be delivered as de-multiplexed FASTQ files

General Conditions
1. Delivery time for the data from the date of receiving sample from IITM, should be indicated by the vendor.
2. Vendor Eligibility Criteria

Sl. No.	Bidder Eligibility Criteria	Compliance (YES/NO)	Reference Document page no.	Remarks, if any
1.	The bidder must have supplied at least five similar services across India, preferably in IIT Madras/other IITs/ R&D labs over the last three years. Please provide a list of your service and submit a "citation (template attached)" from these institutions.			
2.	The annual turnover of the tenderer should not be less than ₹70 Lakh (Rupees Seventy Lakh only) each during the last three financial years i.e. 2017-18, 2018-19 and 2019-20. The turnover is to be supported by financial statement of accounts (including balance sheet, profit and loss account statement). (Necessary document proof should be attached).			
3.	The tenderer should submit copy of filed ITR for the last three years i.e. AY 2017-18, 2018-19 and 2019-20.			
4.	The tenderer should submit a performance certificate on the institution's letterhead as per the proforma attached with this Tender. The service provider must obtain it from the past customers, i.e., from the five customers as per the Sl. No. 1 of the eligibility criteria. Vendors who do not have an average score of >= 4.0 in both Quality of Service and Speed of Delivery will be rejected.			

3. NDA agreement will be signed between the selected vendor and all DNA and RNA sequencing data and other associated data and metadata will be the sole property of IITM.