COVIDSeq:

Our COVIDSeq protocol is an amplicon assay based on an xGen SARS-CoV-2 amplicon panel (IDT). Briefly, RNA is DNAse treated to remove residual DNA. This step is followed by cDNA synthesis and two PCR steps. First PCR utilizes SARS-CoV-2 primers to amplify the genome, followed by a bead cleanup step. Next, a second PCR step is performed to add unique dual indexes and adapter sequences necessary for sequencing. The final libraries are quantified by qPCR and pooled in equal ratios before sequencing.

FluSeq:

Our FluSeq protocol is based on a modified COVIDSeq protocol (Illumina) that uses flu-specific primers. Extracted RNA undergoes DNAse treatment followed by a combined cDNA synthesis and amplification reaction. Next, the PCR product is cleaned up and tagmented, followed by a post-tagmentation cleanup step. The tagmented amplicon is amplified by PCR using unique indexes for each sample. Individual libraries are cleaned up, quantified by qPCR, and pooled in equal ratios for sequencing.

Metagenomics:

Our metagenomics library prep protocol is based on a Nextera XT protocol (Illumina). For RNA metagenomic sequencing, extracted RNA undergoes DNAse treatment followed by a SuperScript IV Reverse Transcriptase (ThermoFisher) assay to synthesize first and second cDNA strands. A cleanup step is performed to purify the generated cDNA. For DNA metagenomic sequencing, no preliminary steps are performed. To prepare the metagenomics libraries, cDNA or DNA is subjected to a Nextera XT protocol (Illumina), which simultaneously fragments and inserts adapter sequences necessary for sequencing. The resulting libraries are then purified, quantified by qPCR, and pooled in equal ratios for sequencing.