Resource Summary Report

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A protocol for massively parallel diagnosis and genome sequencing of SARS-CoV-2

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Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.betrjem6

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Group: Coronavirus Method Development Community

Summary: Managing the current COVID-19 pandemic requires diagnostic testing at an unprecedented scale. However, the crisis has revealed severe deficiencies in our capacity to perform such testing. Here we build on the work of others (1-3) to develop a protocol that not only enables many thousand diagnostic tests to be run in parallel, but also provides near-whole genome sequencing data to facilitate phylogenetic analysis and contact tracing. One of the key features of our protocol is a magnetic bead-based strategy for RNA capture that may circumvent the need for SARS-CoV-2 RNA extraction, currently one of the major bottlenecks in both reagent supply and hands-on sample processing time. This approach also eliminates the requirement for per-sample reverse transcription, significantly reducing per-sample costs. The major steps in our wet lab workflow can be summarised as follows. First, we generate a collection of bead-bound, single-stranded DNA probes tiling the entire SARS-CoV-2 genome. Multiple uniquely barcoded probe sets are prepared, each of which are used to capture viral RNA directly from patient swab samples via DNA/RNA hybridisation. Samples are combined together at this stage, enabling cDNA synthesis to be performed in a single pooled reaction. Finally, multiplex PCR is used to generate a library of overlapping amplicons ready for Illumina sequencing. A schematic overview of the workflow is attached below, along with a more detailed figure depicting the various stages of library preparation.Referenceshttps://docs.google.com/document/d/1kP2w_uTMSep2UxTCOnUhh1TMCjW
2019-sequencing-protocol-
bbmuik6w/abstracthttps://www.biorxiv.org/content/10.1101/2020.03.20.001008v1.full.pdf

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