Docket #: S20-342

Massively Sacalable Viral Testing and Asymptomatic Surveillance

Technology Reference
CZ Biohub Ref. No. CZB-180S-PC
Stanford Ref. No. S20-342

Researchers at Stanford have developed a method called Identity Preserving Sample Multiplexing (IPSM) for the scaled up and rapid identification of SARS-CoV-2 positive subjects.

At the beginning of the 2020 COVID-19 pandemic, reliably tracking SARS-CoV-2 among patients was a critical step in quarantining, triaging, and treating positive patients. However most hospitals were initially unable to process even 1000 tests per day due to the shortage of reagents and other resources, whereas an order of magnitude or more that number of clinical tests were needed to adequately meet patients' needs. In addition, due to the high positivity rate of tested populations, standard pooling assays (where groups of samples are co-purified and patients in positive groups are re-tested individually) were not appropriate as, by mid-March 2020, up to 25% of tests in highly populated regions such as New York were positive. Therefore, a multiplexed strategy was necessary to allow the scaling up of tests while robustly preserving patient identity.

Stage of Research
The inventors have designed an approach to non-enzymatically barcode patient samples in large collection volumes and then concentrate those samples within a pooled library. Each barcode consists of three high-melting temperature oligos that anneal at adjacent positions along the viral genome and thereby also provide a position barcode. After annealing barcoding oligos to the target viral RNA, each oligo is then ligated using an RNA-splinted ligation. The ligation product is then amplified and distributed into 2n wells and the abundance of each ligated barcode is then quantified by qPCR with patient-specific primers as a proxy for viral load. Each

barcoded patient sample is also redundantly pooled in two non-overlapping patient cohorts along with positive and normalizing controls, providing replicate readouts for each patient's samples.

Stage of Development
Research – *in vitro*

Applications

- A unified testing platform for clinical laboratories to dramatically scale up community surveillance and patient diagnostics.
- Identifying positive patient samples within a pooled population without the need to exhaustively reprocess all patients within positive cohorts.

Advantages

- The assay allows testing with a ligase rather than a reverse-transcriptase enzyme, providing an alternative way to create the template for the qPCR reaction.
- The assay provides quadruple redundancy to capture measurement noise for both sample barcoding and real-time qPCR.
- The core workflow with approximately 1000 samples can be completed in ~2.5 hours by a staff of 2-3 people, thereby the inventors' conservative estimate of throughput for this method is 10,000 samples per day for each available 384-well plate qPCR machine.
- All aspects of the IPSM technology can be implemented using standard equipment and commonly available molecular biology reagents.

Patents

- Published Application: WO2022076600
- Published Application: 20230407418

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