Nanopore Sequencing SARS-CoV-2 genome in Qatar

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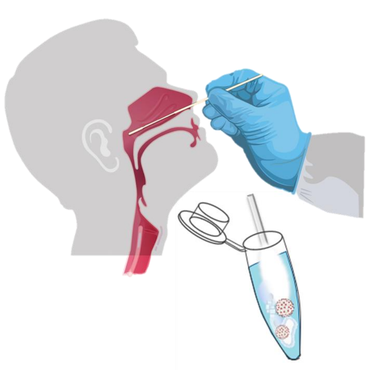
BACKGROUND

The current pandemic, Coronaviruses Disease (COVID-19), is cause by an RNA coronavirus that was recently identified as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). RNA viruses tend to have a high mutation rate; the rate is around a million times greater than that of their hosts. The mutagenic potential of the virus depends on many factors, including the fidelity of nucleic acid-replicating viral enzymes, such as SARS-CoV-2 RNA dependent RNA polymerase (RdRp). The rate of mutation drives viral evolution and genome variability, consequently allowing viruses to escape the immunity of the host and develop resistance to drugs. Therefore, the characterization of SARS-CoV-2 variants might lead to implement better therapeutics treatments, vaccines design and identify new diagnostics approaches.

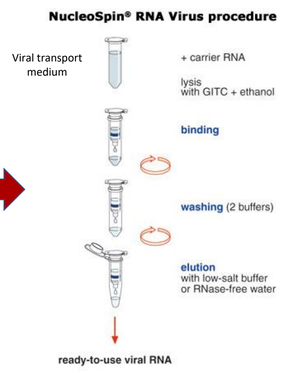
AIM

The aim of this study is to establish a fast sequencing method to identify SARS-CoV-2 mutations. This will help to assess if there are new viral variants that are spreading in Qatar.

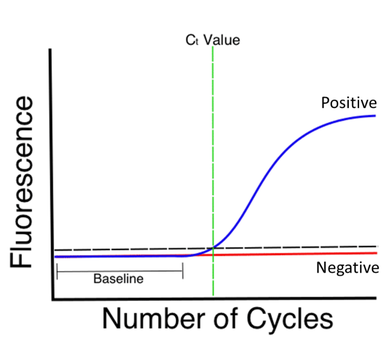
METHODS



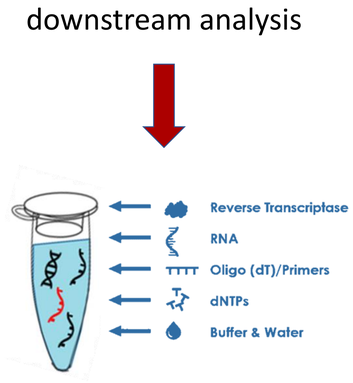
Nasopharyngeal swabs collected from COVID-19 positive patients



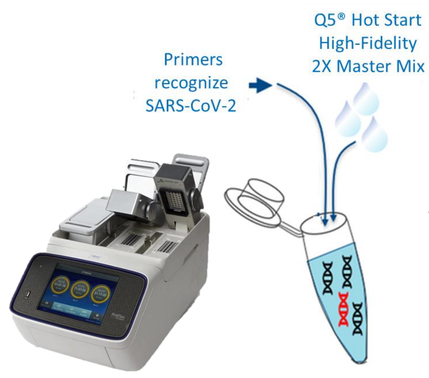
RNA Isolation using commercial kit



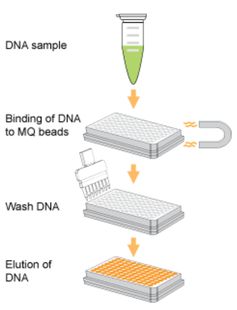
24 positive samples were selected for downstream analysis



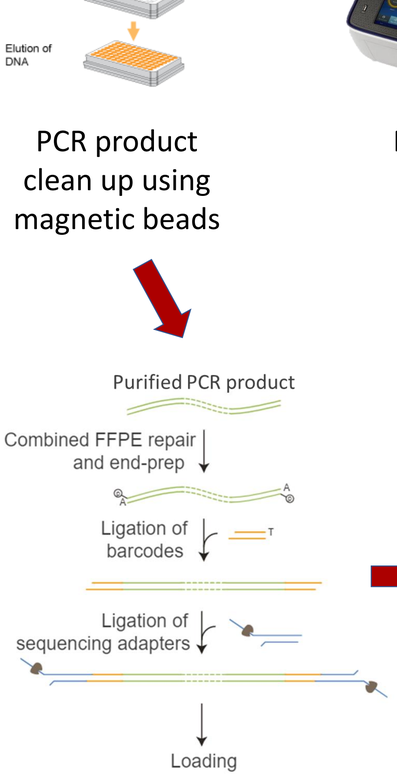
Reverse transcription using SuperScript IV reverse transcriptase



PCR using Artic network V3 primers and NEB Q5 Hot Start master mix

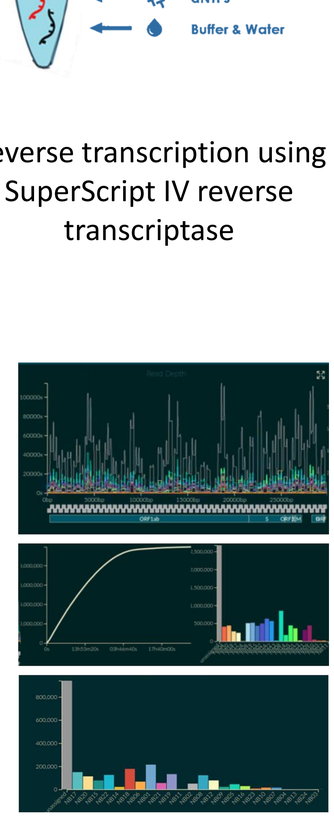


PCR product clean up using magnetic beads



Barcoding and library preparation using ONT kits

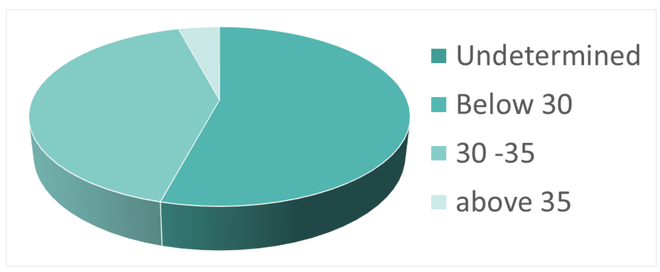
Library loaded in to a flow cell and ran on a GrilON

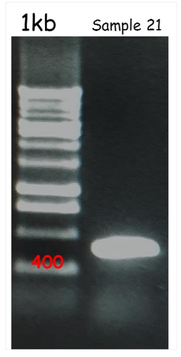


Bioinformatics Analysis according to the Artic Network

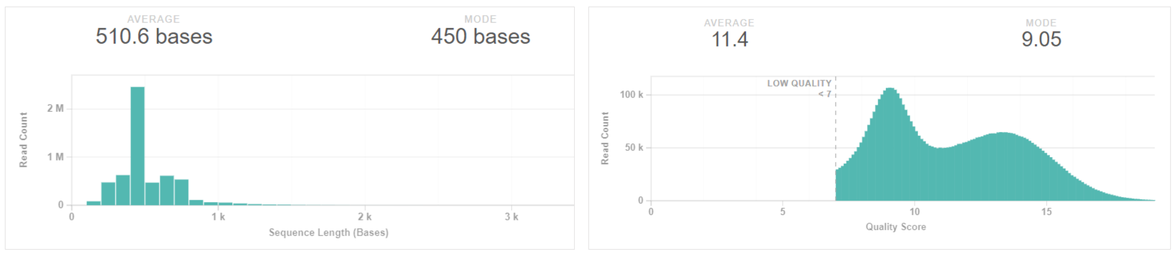
RESULTS

CT value of the 24 processed samples

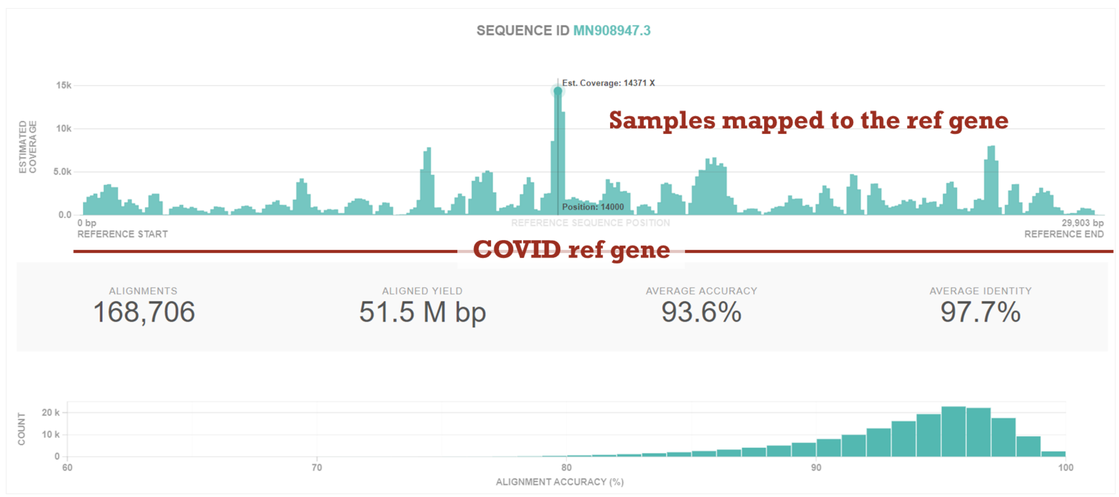




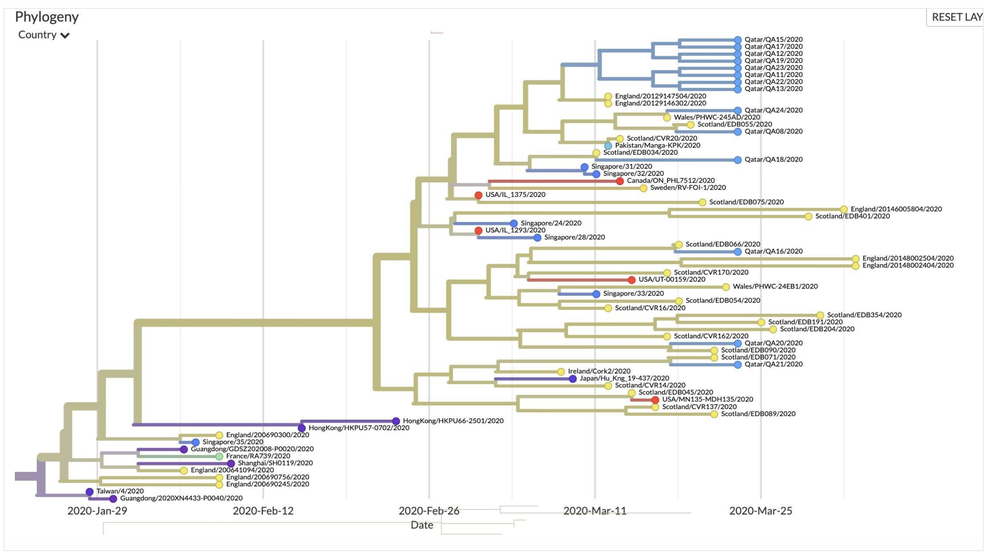
Gel picture of the PCR amplification product showing the expected fragment size at 400bp



Quality check during ONT runs showing that the average sequenced fragment size is at 510bp (400bp+adapters) as well as the average quality of the reads (11.4).



Mapped reads to the Wuhan SARS-CoV-2 genome showing the coverage percentage (97%) and depth (averaged at 400x).



GISAID phylogenetic tree showing Qatar deposited samples in blue. Samples mainly clustered with samples deposited from the United Kingdom (UK)

CONCLUSION

The use of ONT in combination with V3 primers that are recommended by the Artic Network to sequences SARA-CoV-2 generates genomes at >80% coverage, 200x depth in less than 24hours. As such it is a quick, affordable, and reliable technique to determine viral mutations. Using this technique, the first sequences from Qatar were deposited in to GISAID. This technique has now been used to sequence over 700 genomes in Qatar.

ACKNOWLEDGEMENT

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- Tyson, J.R., James, P., Stoddart, D., Sparks, N., Wickenhagen, A., Hall, G., Choi, J.H., Lapointe, H., Kamelian, K., Smith, A.D. and Prystajecky, N., 2020. Improvements to the ARTIC multiplex PCR method for SARS-CoV-2 genome sequencing using nanopore. bioRxiv.