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OPTIMIZED WORKFLOW FOR SARS-COV-2 WHOLE GENOME SEQUENCING: THREE-WAY ANALYSIS BETWEEN DNA CONCENTRATION, GEL ELECTROPHORESIS IMAGING AND SUCCESSFUL SUBMISSION OF SEQUENCES TO GENBANK

Salomon RC, Tseng AC, Qin Y, Ching LL, Yeung JW, Siu, JM, Pangelinan M, Zorilla R, Nerurkar VR

Introduction: Whole Genome sequencing (WGS) is essential for monitoring mutations and detecting the emergence of SARS-CoV-2 variants as the virus makes its way through the population. However, WGS of every specimen is not feasible due to several clinical and technical variables. Further, WGS is a time consuming and expensive technique, which requires highly trained personnel, therefore it is not practical to conduct WGS on every sample. Laboratories must select the ideal samples for WGS based on time post infection, collection media including swabs, sample quality and quantity, primers, and CT value.
Objective: The objective of this study was to correlate variables such as DNA concentration and gel electrophoresis based image analysis, prior to conducting viral cDNA library, to identify ideal samples for successful WGS and submission to GenBank.
Methods: Nasal swabs, mid-turbinate swabs, and nasopharyngeal swabs were collected from individuals confirmed to be SARS-CoV-2 RT-PCR positive, from clinical laboratories on Oahu. Total RNA from the samples was extracted and the entire SARS-CoV-2 genome was amplified using the ARTIC Network V3 primer pools and RT-PCR. Gel electrophoresis was conducted on purified PCR products to verify band size and quality followed by image analysis using GelAnalyzer. Further, DNA concentration of PCR products were measured using the Quant-iT PicoGreen dsDNA Assay. PCR products were submitted to the Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) facility at UH Manoa and sequenced using the Illumina MiSeq platform. Sequencing reads were mapped to the original Wuhan sequence (MN908947.3), assembled into whole genomes using the iVar workflow, and submitted to GenBank. To determine ideal samples for successful WGS, three-way correlation analysis was conducted using DNA concentration, an arbitrary unit assigned by the GelAnalyzer and sequences submitted to the GenBank using GraphPad Prism.
Results: Of the 149 samples submitted for WGS, 99 (66.4%) samples were successfully sequenced and submitted to the GenBank. DNA concentration (ng/μL) of samples successfully submitted to GenBank was significantly higher (median 27.24, IQR 11.73-61.36, $p<0.001$) as compared to those which were not submitted (median 0.67, IQR 0.38-1.41) to the GenBank. Similarly, GelAnalyer quantitation based on the ~400-bp PCR band to molecular weight ladder brightness ratio, among samples successfully submitted to GenBank were significantly higher (median 1.14, IQR 0.75-3.33, $p<0.001$) as compared to those which were not submitted (median 0.10, IQR 0.00-0.25) to GenBank. Further, as expected we observed positive correlation between GelAnalyzer quantitation and DNA concentration by Spearman's r (r_s = 0.91).

Conclusion: These data demonstrate that samples successfully submitted to the GenBank, have a minimum band to ladder ratio of 0.167 and/or a DNA concentration of at least 1.096 ng/μL. Beta testing of 100 samples using the techniques mentioned above is ongoing. These data will assist in determining the ideal samples for which WGS and subsequent GenBank submission will be most successful, to conserve precious reagents, personnel resources, and to cut down on sequencing time.
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