Assignment 1: abstract and introduction summary

■We want to do whole genome sequencing for (SARS-COV-2) with the use of Long - read sequencing from Oxford Nanopore

■The problem is the short read sequencing platform takes a lot of time and more costly so we want to use long read sequencing devices because it is more efficient, it's response time little and the possibility of moving it easier but we have a problem in them also and this problem is the accuracy of the sequence

■In order to solve this problem here we perform viral WGS with ONT and illumina platforms on 157 matched SARS-COV-2 positive patient specimens and synthetic RNA control enabling rigorous evaluation of analytical performance

■after we use this solution We report that, despite the elevated error rates observed in ONT sequencing reads, highly accurate consensus-level sequence determination was achieved, with single nucleotide variants (SNVs) detected at >99%sensitivity and >99% precision above a minimum ~60-fold coverage depth, thereby ensuring suitability for SARS-CoV-2 genome analysis. ONT sequencing also identified a surprising diversity of structural variation within SARS-CoV-2 specimens that were supported by evidence from short-read sequencing on matched samples.

■Now we can say that however ONT is more better than illumina but there is a problem in it like that ONT sequencing failed to accurately detect short indels and variants at low read-count frequencies. as a result of the problem in two sequencing technologies and in order to address concerns regarding ONT sequencing accuracy and evaluate its analytical validity for SARS-CoV-2 genomics, we have performed amplicon-based nanopore and short-read WGS on matched SARS-CoV-2-positive patient specimens and synthetic RNA controls, allowing rigorous evaluation of ONT performance characteristics.

■(SARS-CoV-2) is the causative pathogen for COVID-19 disease1,2. SARS-CoV-2 is a positive-sense single-stranded RNA virus with a ~30-kb poly-adenylated genome Complete genome sequences published in January 2020

■Whole-genome sequencing (WGS) of SARS-CoV-2 provides additional data to complement routine diagnostic testing. Viral WGS informs public health responses by defining the phylogenetic structure of disease outbreaks5. Integration with epidemiological data identifies transmission networks and can infer the origin of unknown cases

■WGS can be performed by 2 ways

■The first way is PCR amplification

■The second way is hybrid-capture of the reverse-transcribed SARS-CoV-2 genome sequence, followed by high-throughput sequencing. Short-read sequencing technologies (e.g., Illumina) enable accurate sequence determination and are the current standard for pathogen genomics. However, long-read sequencing devices from Oxford Nanopore Technologies (ONT) offer an alternative with several advantages. ONT devices are portable, cheap, require minimal supporting laboratory infrastructure or technical expertise for sample preparation, and can be used to perform rapid sequencing analysis with flexible scalability

■ONT has been used during Ebola, zika and other diseases outbreak

■And like what we said before ONT devices exhibit lower read - level sequencing accuracy than short - read platforms and This may have a disproportionate impact on SARS-CoV-2 analysis, due to the virus' low mutation rate (8×10^{-4} substitutions per site per year26), which ensures erroneous (false-positive) or undetected (false-negative) genetic variants have a strong confounding effect.

■and to solve this problem we have performed amplicon-based nanopore and short-read WGS on matched SARS-CoV-2-positive patient specimens and synthetic RNA controls.