

## How is SARS-CoV-2 sequencing done?

### Alt-text Figure 4 - Methods for SARS-CoV-2 genome sequencing

This is a figure about methods used for SARS-CoV-2 genome sequencing. DNA strands are represented by thick black lines. The figure is made up of three separate diagrams labelled A, B, and C. Diagram A is a workflow detailing Illumina's Nextera DNA Flex Enrichment protocol. The first step of this workflow shows a strand of DNA, the First-strand, which forms a map from which cDNA, (complementary DNA) strands will be synthesised. The second step of this workflow shows that the complementary strands are now the maps for which another set of complementary DNA strands are synthesised. This is called Second strand DNA synthesis. The third step in the workflow is called Bead-linked tagmentation. Strands of DNA are shown with small grey extensions representing molecular tags. The fourth step is called Indexing PCR, and the small grey tags are now represented in red. These are labelled: Sequencing library. The fifth step shows Eppendorf tubes containing a small volume of liquid being added together - these are Pooled samples. The sixth step shows a magnet with a probe being used to match the red-tagged DNA fragments. The seventh step shows an Eppendorf tube which is labelled: Enriched Library. The eighth step is called QC and sequence and shows that the Enriched Library can be used in an Illumina sequencing machine. Diagram B is a workflow for the ARTIC protocol. The first step of this workflow shows a strand of DNA, the First-strand, which forms a map from which cDNA strands will be synthesised. The second step of this workflow is called Multiplex PCR (2 pools), Untailed Primers. Two Eppendorf tubes are shown being added together, and are labelled: Combine pools and QC. The third step is labelled Barcode addition / NGS library preparation. Strands of DNA are shown with small red extensions and are labelled: sequencing library. The fourth step is labelled: Normalise, QC and sequence, and shows that the sequencing library can be used in Oxford Nanopore and Illumina machines. Diagram C is a workflow for the Tailed Amplicon Method. The first step of this workflow shows a strand of DNA, the First-strand, which forms a map from which cDNA strands will be synthesised. The second step of this workflow is called Multiplex PCR (2 or 4 pools), Tailed primers. Four Eppendorf tubes with small volumes of liquid are shown being combined. The third step is called indexing PCR, and strands of DNA with small red extensions are shown. These are labelled: Sequencing Library. The fourth step is called Normalise, QC and sequence, and shows that the sequencing library can be used in Illumina machines.