

Resource Summary Report

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SARS-CoV-2 McGill Nextera Flex sequencing protocol_SS_V3_LA1_5uLRT

DOI:10.17504/protocols.io.bisbkean

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.bisbkean>

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Summary: How the Nextera DNA Flex Assay Works The Nextera DNA Flex library prep kit uses a bead-based transposome complex to tagment genomic DNA, which is a process that fragments DNA and then tags the DNA with adapter sequences in one step. After it is saturated with input DNA, the bead-based transposome complex fragments a set number of DNA molecules. This fragmentation provides flexibility to use a wide DNA input range to generate normalized libraries of consistent tight fragment size distribution. Following tagmentation, a limited-cycle PCR adds Nextera DNA Flex-specific index adapter sequences to the ends of a DNA fragment. This step enables capability across all Illumina sequencing platforms. A subsequent Sample Purification Beads (SPB) cleanup step then purifies libraries for use on an Illumina sequencer. The double-stranded DNA library is denatured before hybridization of the biotin probe oligonucleotide pool. PCR Amplicons for Nextera Flex When starting with PCR amplicons, the PCR amplicon must be > 150 bp. The standard clean up protocol depletes libraries Therefore, Illumina recommends that amplicons Shorter amplicons can otherwise be lost during the library cleanup step. Tagmentation cannot add an adapter directly to the distal end of a fragment, so a drop in sequencing coverage of ~50 bp from each distal end is expected. To ensure sufficient coverage of the amplicon target region, design primers to extend beyond the target region by 50 bp per end. More information can be found here: https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera_dna_flex_dna-flex-library-prep-reference-guide-1000000025416-07.pdf

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