

Resource Summary Report

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nCoV-2019 sequencing protocol for illumina

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Type: Protocol

Proper Citation

Kentaro Itokawa, Tsuyoshi Sekizuka, Masanori Hashino, Rina Tanaka, Makoto Kuroda
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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.bnn7mdhn>

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Group: Coronavirus Method Development Community

Summary: This protocol is forked from "ARTIC amplicon sequencing protocol for MinION for nCoV-2019" by Josh Quick to adapt it to illumina sequencers. In this Version V3, the reverse-transcription step was changed to use of NEB's LunaScript RT SuperMix Kit instead of Thermo Fisher's SuperScript IV Reverse Transcriptase as adapting to the recent update of ARTIC Network's protocol (LoCost). Tyson et al., Improvements to the ARTIC multiplex PCR method for SARS-CoV-2 genome sequencing using nanopore [bioRxiv2020.09.04.283077](https://doi.org/10.1101/2020.09.04.283077);doi:<https://doi.org/10.1101/2020.09.04.283077> Because the PCR products are fragmented before ligated with adapters, this protocol is compatible to many sequencing kits with various read lengths (75PE, 150PE, etc.) in Illumina machines. While the library preparation uses QiaSeq FX by Qiagen and is basically straight forward (as per kit instruction but modified to 1/4 scale), some tweaks for much of simplicity and speed were added. Change histories (V2): The amount of cDNA input to multiplex PCR is increased (now almost same amount to the ARTIC Network's original protocol) (Step 10). This change gives better results for samples with extremely low RNA copy. Amount of adapter solution input was corrected (Step 19). Change histories (V3): RT step has been changed to use of LunaScript (Tyson et al., 2020). Corrected typos and wrong descriptions about amount of reagents. Added a link to library quantification protocol. Other minor changes. Change histories (V3): RT step has been changed to use of LunaScript (Tyson et al., 2020). Corrected typos and wrong descriptions about amount of reagents. Added a link to library quantification protocol. Other minor changes.

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