Illumina® RNA Prep with Enrichment on Eppendorf's ep*Motion*® 5075t NGS Solution

Next Generation Sequencing (NGS) is a powerful tool that is used in various scientific fields and applications. One, that is of special interest is the identification, characterization and continuous surveillance of known and emerging viruses, like SARS-CoV-2, Influenza and others.

For that purpose, Illumina's RNA prep with Enrichment kit can perfectly be combined with the Respiratory Virus Oligos Panel (RVOP). It uses a target enrichment through hybrid–capture resequencing approach which allows highly sensitive detection without requiring the high read depth needed for shotgun metagenomics sequencing. Furthermore, it provides an effective way to detect a broad range of viruses without specifically knowing the infectious agent first, and obtains reliable sequence data, enabling applications such as variant analysis for viral surveillance and evolution. The target enrichment is facilitated by

capturing genomic regions of interest by hybridization to target-specific biotinylated probes. The collection of probes used in this protocol make up the Respiratory Virus Oligo Panel v2, targeting numerous viruses, relevant for the monitoring of respiratory disease status.

Preparing samples for NGS is a multi-step process that may stretch over several days and requires high precision. Automating this procedure on the Eppendorf ep*Motion* 5075t NGS solution (Fig. 1) can increase the throughput and reproducibility while at the same time minimizing the potential for human error. Furthermore, it reduces the required hands-on time by up to 80%, freeing up time for other activities.

The method described here, was tested in conjunction with Illumina.

Protocol structure

Instrument configuration	ep*Motion* 5075t NGS solution
No. of sub-methods	8
No. of samples	24-96 (pre-enrichment) 8-32 (post-enrichment)
Total processing time*	5 h (24 samples) – 9 h (96 samples)
Hands-on time	approx. 45 min
Pooling approach	3-plex; pool by volume (2,5 µL/sample)

*w/o considering off-deck incubation times

Figure 1: ep*Motion* 5075t with MultiCon control panel.

Qualification data

> The epMotion workflow is exemplarily shown for a sample input of 25 ng Universal Human Reference RNA (UHR, Agilent®, #740000) with spiked in SARS-CoV-2 control RNA 2 (TWIST™ Bioscience, #MN908947.3) in copy numbers of 0, 10, 100 and 1,000 per sample.
> QC was performed using the Qubit™ dsDNA HS Assay (Invitrogen™ #Q32851) ahead of method 4 (prior to pooling of pre-enriched libraries). Library concentrations showed an average of 146 ng/µL. To stay within Illumina's recommendation sample concentrations were adjusted before proceeding with method 4 (pooling) to not exceed the max. input of 600 ng DNA per pool using a 3-plex pooling approach.
> The final libraries were analyzed again using the Qubit™ dsDNA HS Assay, showing concentrations of 1.39 ng/µL on average. Additional analysis was carried out with the Bioanalyzer® High Sensitivity DNA Analysis Kit (Agilent®, #5067-4626) showing an evenly distributed peak around an average fragment size of 357 bp (Fig. 2A).

Sequencing and data analysis

Sequencing was performed on Illumina's NextSeq® 550 with a starting concentration of 4 nM. For data analysis the DRAGEN® Pathogen Detection and DRAGEN Metagenomics Pipeline was used.
Data analysis is based on k-mers and alignment steps, including protein encoding transcript level detection of viruses, which increases the ability to identify novel and highly divergent viruses. The analysis showed classified reads for SARS-CoV-2 at concentrations as low as 10 copies per sample (Fig. 2C). Nearly full genome coverage was achieved at 1,000 copies with minimal coverage being observed with as low as 10 copies (Fig. 2B).

Summary

Illumina's RNA Prep with Enrichment enables researchers to identify and characterize known and emerging viruses, which is central to improving public health. Seeing the importance thereof, Eppendorf has established a qualified method for the ep*Motion* 5075t NGS solution, producing high quality sequencing libraries that are comparable to libraries from manual processing, while increasing productivity by freeing up time for other activities.

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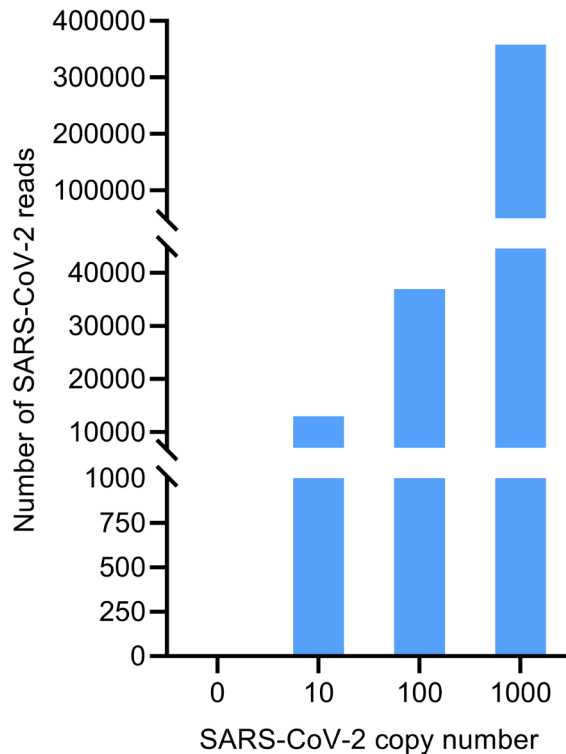
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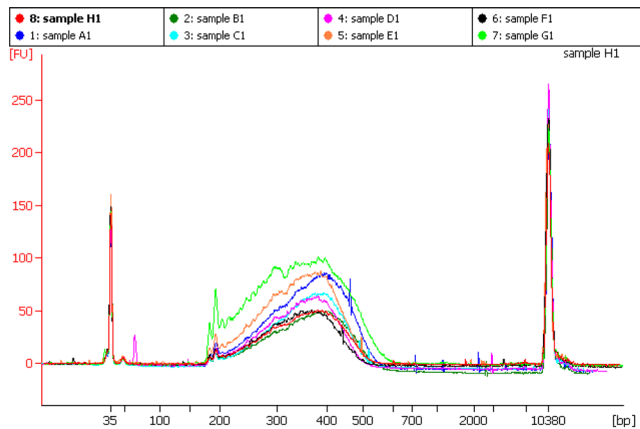
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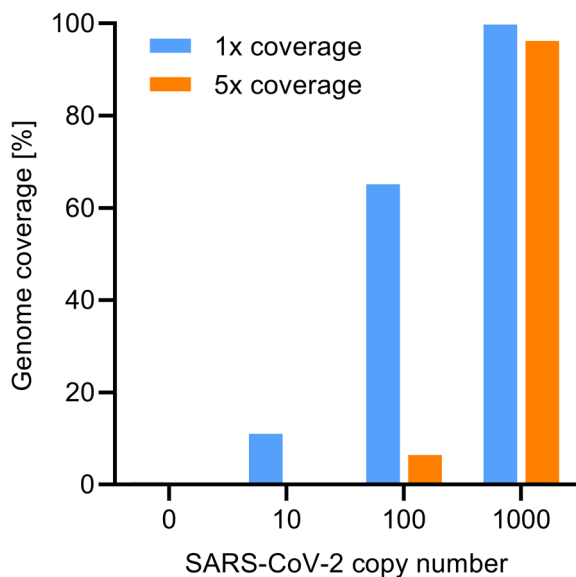
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Figure 2: Collected data of Illumina's RNA Prep with Enrichment performance on an Eppendorf ep*Motion* 5075t NGS Solution.

A: Overlay of final libraries that were analyzed with the Bioanalyzer high sensitivity DNA Assay. The electropherogram shows evenly distributed peaks around an average fragment size of 357 bp.

B: SARS-CoV-2 Genome coverages at different viral copy numbers spiked into the sample – as determined by sequencing on a NextSeq 550.

C: Number of SARS-CoV-2 reads at different viral copy numbers.

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Ordering information

Description	Quantity	Order no. international
Kit components		
Illumina® RNA Prep with Enrichment, (L) Tagmentation (96 Samples)	1	20040537
Illumina® IDT® for DNA/RNA UD Indexes Set A/B, Tagmentation (96 Indexes, 96 Samples)	1 per 96 samples	Set A: 20027213 Set B: 20027214
Illumina® Respiratory Virus Oligos Panel V2	1	20044311
Equipment and Accessories		
ep*Motion*® 5075t NGS Solution, (optional with clean cap)	1	5075 000.962
Enhanced Feature Set 1	1	5075 000.964
ReservoirRack, Module NGS	1	5075 751.917
ReservoirRack	1	5075 754.002
Eppendorf Magnetadapter, Magnum FLX®	1	5075 751.836
Thermoblock PCR, 96 OC	1	5075 751.666
Thermoadapter, for PCR	3	5075 787.008
Consumables		
epT.I.P.S.® Motion Reload, 50 µL, PCR Clean, with filter	10 per sample (based on 96 sample run)	0030 014.430
epT.I.P.S.® Motion Reload, 300 µL, PCR Clean, with filter	18 per sample (based on 96 sample run)	0030 014.472
Box for epT.I.P.S.® Motion, 10/50/300 µL Reload	28 per 96 samples	0030 014.669
Clip for epT.I.P.S.® Motion Reloads	8	5075 751.070
ep*Motion*® reservoir, 30 mL	10	0030 126.505
Eppendorf DNA Tubes, 1,5 mL	7	0030 108.051
Eppendorf DNA Tubes, 5 mL	4	0030 108.310
Eppendorf PCR Film, self-adhesive, PCR clean	various	0030 127.781
Eppendorf Storage Foil, self-adhesive, PCR clean	various	0030 127.889
Micronic, 1,10 mL, Non coded Screw Cap tubes V-bottom	40	MP32033
Eppendorf twin.tec® PCR Plate 96, semi-skirted, PCR clean	8	0030 128.575
Optional		
Eppendorf Mastercycler® X50s	1	6311 000.010
Eppendorf ThermoMixer® C	1	5382 000.015
Eppendorf SmartBlock, 15 mL	1	5366 000.021
Eppendorf HeatSealer S100/S200	1	S100; 5391 000.001 S200; 5392 000.005
Plate/Tube centrifuge (e.g. Eppendorf Centrifuge 5910 Ri)	1	5943 000.011
Eppendorf Research Plus / Reference 2	various	various