
EasySeq™

SARS-CoV-2 (novel coronavirus) Whole Genome Sequencing

NGS library prep by Reverse Complement PCR

Version: RC-COVID096-2.01

Revision date: May 7, 2021



Protocol

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Product and Company Information

Product name: EasySeq™ SARS-CoV-2 Whole Genome NGS Sequencing kit
Product Info: www.nimagen.com/covid19
Product use: Research Use Only
Company: NimaGen BV
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Product Use

Multiplex Amplicon based NGS Library preparation for sequencing the genome of SARS-CoV-2 virus. To be used to detect mutations, monitoring viral populations for epidemiology and outbreak events. This Reverse-Complement PCR based library prep contains all reagents to generate Illumina® compatible libraries in a simple, sensitive and robust method for fast and cost-effective WGS of the viral genome.

Minimum Read depth per sample needed: 100K-200K

Minimal NGS Sequencing scheme: 151-8-8-151

Kit Content.

Note: One complete kit consists of three part numbers, to be ordered separately:

NimaGen Part# RC-COV096	Box #	Content
SARS CoV2 WGS Panel A (RC-PCR probe mix, black cap)	1 (Frozen)	Tube 24 µL
SARS CoV2 WGS Panel B (RC-PCR probe mix, red cap)	1 (Frozen)	Tube 24 µL
RC-PCR Probe Dilution Buffer Plus (blue cap)	1 (Frozen)	Tube 190 µL
AmpliClean Bead solution	2 (Cooled)	Tube 1.7 mL
RC-PCR Low-TE Buffer (yellow cap)	2 (Cooled)	Tubes 1 x 2 mL

NimaGen Part# MMHS-25		Content
PCR 2x Hotstart HiFi Mastermix, compatible with RC-PCR (white cap). p/n MMHS-25	1 (Frozen)	Tubes 2 x 1100 µL

Choose one of the index plates of choice

NimaGen Part# IDX96-U0xD		Content
2 x IDX Index Primer Plates. Choose one of the 6 available Index Plate (U01D, U02D, U03D, U04D, U05D, U06D) with 96 Unique Dual, 10 bp* Indexes for Illumina®. p/n IDX96-U0xD	1 (Frozen)	2 x 96-well plates (pre-spotted with 4 µL Index Primers per well)

IDX plates are semi-skirted, ABI style PCR Plates, dividable per 24 rxns, containing 4 µL of Unique Dual Index primer pairs in each well, ready to use. Index Sequences can be downloaded from the product page, section "downloads"

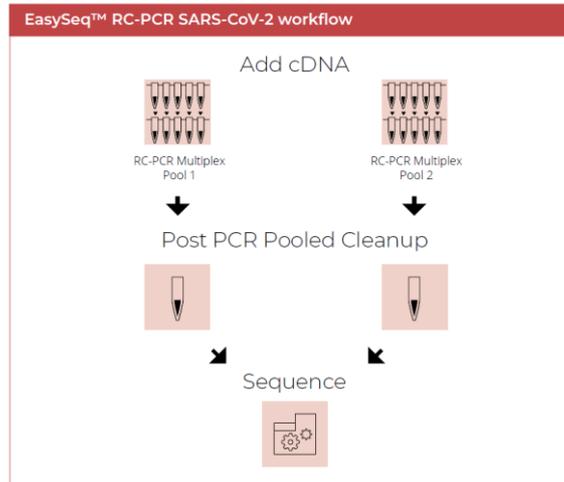
**All UDI sequences in the generated amplicons are 10 bp long. For NovaSeq, NimaGen recommends to read the full 10 bp indexes (151-10-10-151). To enable enough reagents for the needed 151 reads, for other Illumina sequencing systems (MiniSeq, NextSeq, MiSeq), it is recommended to read only 8 bp of the indexes (run scheme: 151-8-8-151). All Indexes are designed to be Unique Dual Indexes when reading the first 8 bp.*

Needed, but not included

Description	Vendor
Adjustable Pipette Set (P10, P20, P200, P1000)	Multiple Vendors
LunaScript (p/n E3010L) cDNA synthesis kit (protocol page 17)	New England Biolabs
Agarose Gel system, TapeStation, Bioanalyzer Instrument, or equivalent, incl. consumables	Agilent® or other
Ethanol absolute, mol. biol. Grade	Multiple Vendors
General plasticware, DNase free (1.5mL tubes, pipette tips w/filter)	Multiple Vendors
Ice or tabletop cooling block	Multiple Vendors
Illumina® NGS Sequencing instrument (iSeq, MiSeq, MiniSeq, NextSeq and NovaSeq)	Illumina®
Illumina® Sequencing Reagent kit (min. 2x150 bp run)	Illumina®
Mini Spinner for 1.5 mL tubes	Multiple Vendors
Plate Spinner for 96-well plates	Multiple Vendors
Magnetic Stand for 1.5 mL Eppendorf tubes	Multiple Vendors
PCR Grade Water	Multiple Vendors
Qubit™ Fluorometer including dsDNA High Sensitivity kit	Thermofisher®
Thermocycler with heated lid, (0.2 mL standard PCR tubes), compatible with semi-skirted ABI style PCR plates and option for ramp rate programming. Example: Applied Biosystems™ Veriti™, SimpliAmp or MiniAmp Thermal Cycler	Multiple Vendors
The next three items are only necessary when sequencing in-house: (not needed in case of sending samples to a core facility)	
NaOH solution (2N)	Multiple Vendors
Tris/HCl (200 mM), pH 7	Multiple Vendors
Low TE (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)	Multiple Vendors

General precautions

- Use Pre-PCR environment for setting up the RC-PCR reaction. Pooling, cleaning and library preparation should be performed in a Post-PCR environment.
- All reagents need to be thawed and centrifuged before use
- Make sure to mix reagents and reactions properly



1. Thermocycling program

Temp:	Duration:	Ramping rate: (from previous step)	Cycles
98°C	2 minutes	N/A	1 x
98°C	10 seconds	Max	1 x
80°C	1 second	Max	
58°C	10 minutes	0.1°C/sec (or 2% of max)	
72°C	1 minute	Max	
98°C	10 seconds	Max	2 x
80°C	1 second	Max	
62°C	90 minutes	0.1°C/sec (or 2% of max)	
72°C	1 minute	Max	
95°C	10 seconds	Max	40 x
80°C	1 second	Max	
62°C	2 minutes	0.5°C/sec (or 10% of max)	
72°C	1 minute	Max	

Heated lid at 105°C

Depending on the instrument, this protocol takes 6,5-7 hours to complete

NOTE: For Applied Biosystems 96-well (0.2 mL) Thermal Cyclers Veriti, SimpliAmp, MiniAmp, and Bio-Rad C1000 cyclers download the method files from the product page (section “downloads”), copy to a USB stick and import directly into your cyclers.

2. Reverse Complement PCR

The target specific RC-probes will be transformed into the functional, tailed and indexed PCR primers, followed by multiplex cDNA amplification of the target regions. Protocol to convert extracted RNA to cDNA, see page 17.

2.1 Thaw on ice:

- SARS CoV2 WGS Panel A probe mix (Black Cap)
- SARS CoV2 WGS Panel B probe mix (Red cap)
- RC-PCR Probe Dilution Buffer Plus (Blue cap)
- PCR 2x Hotstart HiFi Mastermix (White cap)

Spin down:

- SARS CoV2 WGS Panel A probe mix (Black Cap)
- SARS CoV2 WGS Panel B probe mix (Red cap)

Vortex 3 seconds and spin down:

- RC-PCR Probe Dilution Buffer Plus (Blue cap)
- PCR 2x Hotstart HiFi Mastermix (White cap)

Note: The 2x mastermix contains isostabilizers and may not freeze completely, even when stored at -15°C to -25°C. The mastermix may contain precipitates when thawed at +2°C to +8°C. Always ensure that the mastermix is fully thawed and thoroughly mixed before use.

2.2 Take the two IDX PCR plates and cut off the number of strips needed from both plates. Mark the plates with “A” and “B”.

Note: Register the indexes used (IDX set / and well position for each sample). Download the index details for setting up Illumina® samplesheets at the download section of <https://www.nimagen.com/covid19>

Note: For each sample, two PCR reactions are needed (pool A and pool B). Use always the same strip and well position for the same sample, in order to have identical indexes for the sample in both pools. Example: For setting up 24 samples, cut off strip 1-3 from both IDX plates and store the remaining of the plates immediately at -20°C.

2.3 Prepare in a fresh 1.5 mL Eppendorf tube the Probe-Polymerase premix A, by combining

- 0.2 µL panel **A** probe mix per reaction (black cap)
- 0.8 µL RC-PCR Probe Dilution Buffer Plus per reaction (blue cap)
- 10 µL RC-PCR 2x mastermix per reaction (white cap)

2.4 Prepare in a fresh 1.5 mL Eppendorf tube the Probe-Polymerase premix B, by combining:

- 0.2 µL panel **B** probe mix per reaction (red cap)
- 0.8 µL RC-PCR Probe Dilution Buffer Plus per reaction (blue cap)
- 10 µL RC-PCR 2x mastermix per reaction (white cap)

It is recommended to allow for a 10% extra when preparing the mastermix to correct for pipetting loss. The kit contains extra reagent for this.

Example: 48 samples + 10% extra volume

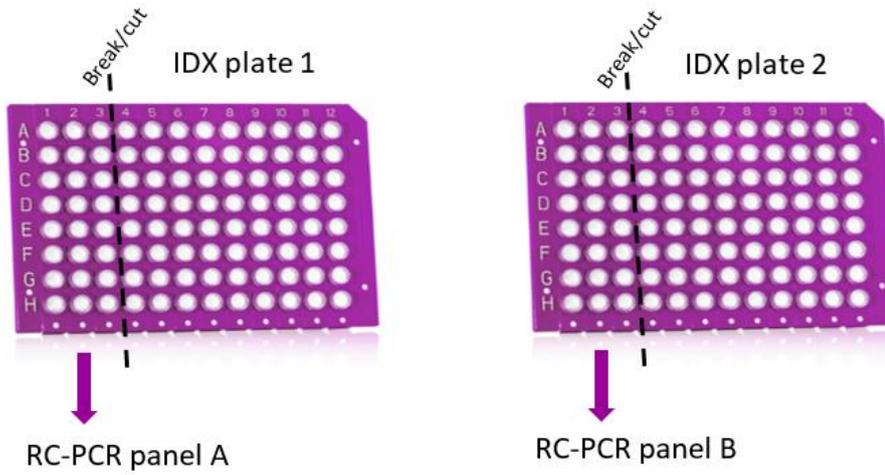
Tube A:

- 10.56 µL Panel A probe mix (black cap)
- 42.24 µL RC-PCR Probe Dilution Buffer Plus (blue cap)
- 528 µL RC-PCR 2x mastermix (white cap)

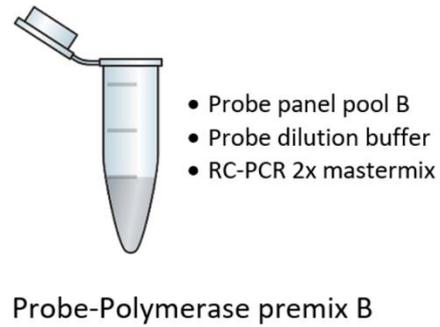
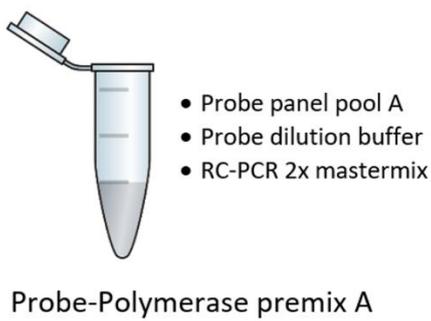
Tube B:

- 10.56 µL Panel A probe mix (red cap)
- 42.24 µL RC-PCR Probe Dilution Buffer Plus (blue cap)
- 528 µL RC-PCR 2x mastermix (white cap)

Step 2.2 Take the two IDX PCR plates and cut off the number of strips needed.



Step 2.3 Prepare in two 1.5 mL eppendorf tubes the Probe-Polymerase premix



2.5 Quick-spin the PCR plates (A and B), and remove seals carefully.

2.6 Add to each tube of plate A: 11 μ L of the Probe-Polymerase premix A.

2.7 Add to each tube of plate B: 11 μ L of the Probe-Polymerase premix B.

2.8 Add to the same well position of both plate/strips A and B: 5 μ L cDNA.

Example: Add 5 μ L of cDNA from sample 1 to both wells A1 of plate A and B. Total cDNA per sample needed is 10 μ L.

2.9 Close the tube strips carefully with caps (included in the kit) and mix by **vortexing** 3 seconds.

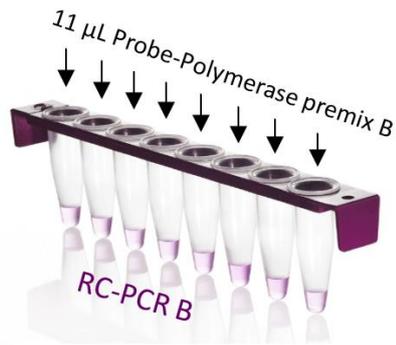
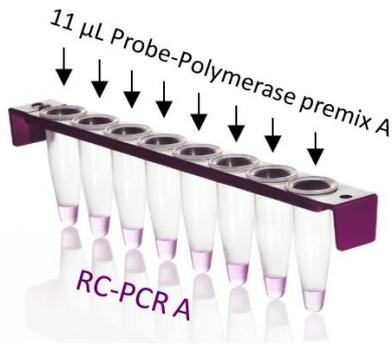
2.10 Spin the plates to make sure all liquid is in the bottom of the wells

2.11 Start the RC-PCR program in the thermal cycler(s) and place samples in the cycler when the block is between 60°C and 98°C, close the lid.

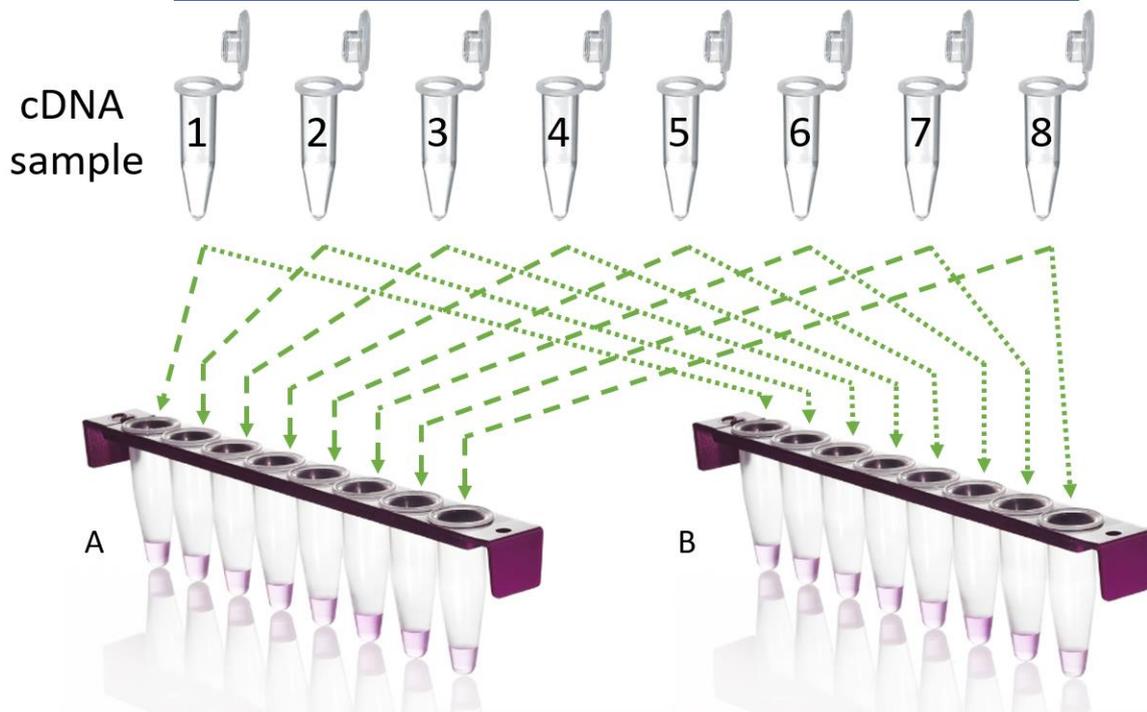
Note: When running >48 samples in a run, two PCR cyclers are needed.



Safe stopping point after RC-PCR. Store at 4°C for max. 48H



- 5 μL cDNA from each sample to plate/strip A
- 5 μL cDNA from each sample to plate/strip B



! It is very important to make sure always to transfer each cDNA to the same strip and well position in both A and B

The samples have now been amplified and tagged with sample specific indexes and sequencing tails. From this point, PCR products will be pooled together in two tubes, as their corresponding pool A and B, purified by a “2x” AmpliClean purification to remove primers, primer dimers and salts. In order to decrease read depth variation between samples with low and high viral loads, it is recommended to follow a pooling strategy based on the Ct values from the Real-Time PCR assay the samples were detected Covid19 positive with (see table at 3.3).

3. Pool, Purify and Sequence

Note: Before pooling, optionally check 3 µL of the unpurified PCR products on agarose (2%).

- 3.1 Bring the AmpliClean™ beads solution (Brown Cap) to Room Temperature.
- 3.2 Mark 2 x 1.5 mL Eppendorf tubes with resp. “A” and “B”.
- 3.3 Create, in the tubes A and B, two pools, by combining RC-PCR products from all the wells in each of the two plates (except negative controls), according to the table below.

Ct value from qPCR	RC-PCR volume
< 20 (very high viral load)	2 µL
20-24 (high viral load)	4 µL
25-28 (medium viral load)	8 µL
> 28 (low viral load)	16 µL

*Note: Keep pool A and B separated during the complete cleanup procedure in two tubes.
Note: If Ct values are unknown, pool 5 µL of each PCR product.*

- 3.4 Mix, spin down and transfer 40 µL of both pools to two new 1.5 mL Eppendorf tubes (marked “A2”/“B2”).
- 3.5 Add 60 µL RC-PCR Low TE buffer (yellow cap) to both tubes A2 and B2, mix and spin down (total volumes are now 100 µL each).

3.6 Double AmpliClean™ beads purification:

Purification #1

- a. Vortex beads thoroughly to resuspend.
- b. Add 85 μ L beads solution to the two 100 μ L pools A2 and B2 (from step 3.5) and mix well immediately by pipetting up and down 5 times.
- c. Incubate for 5 minutes, off magnet.
- d. Place both tubes on magnet for 3 minutes or for the solutions to be fully cleared.
- e. Remove and discard liquid carefully without disturbing the beads.
- f. Add 200 μ L (freshly prepared) 75% ethanol, without disturbing the beads.
- g. Wait for 1 minute.
- h. Repeat steps e., f. and g. for a second ethanol wash step
- i. Carefully remove all liquid without leaving traces of ethanol. (Optionally a quick spin can be performed, then place tube back on magnet and remove excess ethanol)
- j. Dry with open cap for 2-3 minutes at Room Temperature. **Do not over-dry.**
- k. On Magnet: Add 110 μ L RC-PCR Low TE buffer (Yellow cap).
- l. Off Magnet: Re-suspend the beads by pipetting up and down or flicking.
- m. Incubate for 2 minutes, off magnet.
- n. Put on magnet and wait for 3-5 minutes or for the solution to be fully cleared.
- o. Carefully bring 100 μ L of the clear solution to two new 1.5 ml Eppendorf tubes (marked "A3" and "B3") making sure not to transfer any of the beads.

Purification #2

- p. Add 85 μ L resuspended beads solution to the two 100 μ L pools A3 and B3 and mix well immediately by pipetting up and down 5 times
- q. Incubate for 5 minutes, off magnet.
- r. Place both tubes on magnet for 3 minutes or for the solutions to be fully cleared.
- s. Remove and discard liquid carefully without disturbing the beads.
- t. Add 200 μ L (freshly prepared) 75% ethanol, without disturbing the beads.
- u. Wait for 1 minute.
- v. Repeat steps s., t. and u. for a second ethanol wash step
- w. Carefully remove all liquid without leaving traces of ethanol. (Optionally a quick spin can be performed, then place tube back on magnet and remove excess ethanol)
- x. Dry with open cap for 2-3 minutes at Room Temperature. **Do not over-dry.**

3.7 Elution

- a. On Magnet: Add 50 μ L RC-PCR Low TE buffer to the two tubes A3 and B3
- b. Off Magnet: Re-suspend the beads by flicking or short vortexing.
- c. Incubate for 2 minutes, off magnet.
- d. Put on magnet and wait for 3-5 minutes or for the solutions to be fully cleared.
- e. Carefully bring 40 μ L of the clear solution to two new 1.5 ml Eppendorf tubes (marked A4 and B4) making sure not to transfer any of the beads.



Safe stopping point. Store at -20°C if not continuing same day.

Libraries are now ready for quantification and qualification.

3.8 Determine final concentration of both A4 and B4 by a duplo Qubit (HS) measurement according to manufacturer's manual. Use the dedicated SARS-CoV-2 WGS calculator at the download section of www.nimagen.com/covid19

SARS-CoV-2 Calculator

Step 3.8: Download the SARS-CoV-2 WGS calculator and just fill in (at the green fields, step 1.) the concentration of both A4 and B4, in ng/μL. The sheet automatically calculates the pipetting schemes for creating 2 nM libraries (step 2), followed by the pipetting scheme for the denaturation (step 3) and creating the final library for the different Illumina instruments / kits (step 4).

SARS-CoV-2 WGS Calculator

1. Qubit Concentration calculation

Calculated Qubit Readout:	
pool A: 15.00 ng/μL	= 51.1 nM
pool B: 12.00 ng/μL	= 40.9 nM

2. Dilution of both pools to 50 μL of 2 nM

	pool volume (μL)	lowTE volume
pool A:	2.0	48.0
pool B:	2.4	47.6

3. Denaturation pipetting scheme

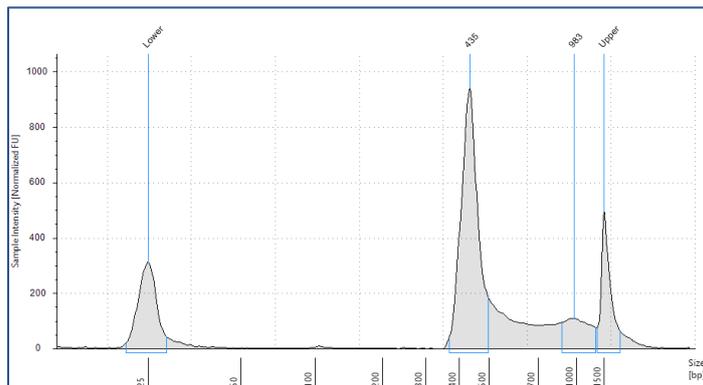
Pool A (2 nM)	5 μL
Pool B (2 nM)	5 μL
NaOH (0.2 N)	10 μL
<i>incubate 5 minutes at room temp</i>	
Tris-HCl (200 mM)	10 μL
Ice Cold Buffer HT1	970 μL
Total (20 pM)	1000 μL

4. Final Library pipetting scheme

	Loading concentration (pM)	end volume (μL)	Denaturated pool (20 pM) volume (μL)	HT1 volume (μL)	20 pM PhiX control (μL)
MiniSeq	0.8	500	20	479	1
MiSeq v2	9	600	270	325	5
MiSeq v3	15	600	450	140	10
NextSeq	0.9	1500	67.5	1429.5	3

3.9 Verify library on TapeStation or Bioanalyzer, according to the manufacturer's protocol. If needed, dilute pool before loading on the electrophoresis system. Example: For TapeStation High Sensitivity kit, dilute to ~2 ng/μL

Example of a clean library on TapeStation:



3.10 Perform Sequencing on an Illumina® NGS platform, according to the manufacturer's manual.

NOTE: Download Index Sequences from www.nimagen.com/covid19, section "downloads" to create a sample sheet. For technical assistance contact our technical support at techsupport@nimagen.com.

APPENDIX 1:

ILLUMINA® SEQUENCER PROTOCOLS

Note: When using an iSeq or NovaSeq instrument, please contact our support at info@nimagen.com

A: Illumina® MiSeq protocol

Use Illumina® MiSeq v2 or v3 kit for 300 cycles (2x150 bp run).

Ref: MiSeq System Denature and Dilute Libraries Guide #15039740 v03.

1 Thaw DNA sample/library, buffer HT1, and MiSeq cartridge.

NOTE: MiSeq cartridge should be thawed by submerging it in (but not covering it completely with) water at room temperature. (thawing takes ~ 1.5hr, do not use warm water as it degrades the enzymes). Store other component of MiSeq kit in 4°C refrigerator until ready to start the MiSeq run.

2 Prepare sample sheet. Workflow: Generate FASTQ. Use the following adapter sequences for trimming in the sample sheet:

Adapter: GCGAATTTTCGACGATCGTTGCATTAAGCTCGCGAA

AdapterRead2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

3 Dilute DNA to 2 nM using LowTE (yellow cap)

4 Prepare fresh 0.2 N NaOH.

5 mix 10 µL of 2 nM DNA with 10 µL of 0.2N NaOH, vortex, spin down.

6 Incubate for 5 minutes at Room Temperature.

7 Add 10 µL of 200 mM Tris-HCl pH 7.0 to hydrolyse the NaOH.

8 Add 970 µL Ice Cold HT1 to DNA/NaOH mix to dilute DNA to 20 pM.

9 Dilute the 20 pM library with Ice Cold HT1 to 9 pM (for v2 kits) or 15 pM (for v3 kits) in a new tube:

a. Dilute 275 of library (20 pM) with 725 µL of HT1
(Total volume is now 1000 µL of 5.5 pM loading concentration)

b. Invert to mix and then pulse centrifuge.

10 Mix the MiSeq cartridge by inverting 10x, make sure the reagents do not contain ice.

After mixing, tap the cartridge on the bench 2-3 times to dislodge any air trapped in the bottom of the tubes.

11 Load MiSeq cartridge:

a. Optional but recommended, use PhiX control: Add 3% of PhiX control to the library

b. Load 600 µL of **the library** to the **Load Samples** well.

12 Set up and start MiSeq run: Clean flow cell according to instructions, follow on-screen instructions to load and start instrument.

B: Illumina® NextSeq protocol

Use Illumina® NextSeq v2 kit for 300 cycles (2x150 bp run)

1. Thaw DNA sample/library, buffer HT1, and NextSeq cartridge

NOTE: Nextseq cartridge should be thawed by submerging it in (but not covering it completely with) water at room temperature. (thawing takes ~ 1.5hr, do not use warm water as it degrades the enzymes)

2. Prepare sample sheet. Workflow: Generate FASTQ. Use the following adapter sequences for trimming in the sample sheet:

Adapter: GCGAATTTTCGACGATCGTTGCATTAAGTCGCGAA

AdapterRead2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

3. Dilute DNA to 2 nM using LowTE (yellow cap)
4. Prepare fresh 0.2 N NaOH
5. Mix 10 µL of 2 nM DNA with 10 µL of 0.2N NaOH, vortex, spin down
6. Incubate for 5 minutes at Room Temperature
7. Add 10 µL of 200mM Tris-HCl pH7.0 to hydrolyze the NaOH
8. Add 970 µL Ice Cold HT1 to DNA/NaOH mix to dilute DNA to 20 pM
9. Dilute the 20 pM library with Ice Cold HT1 to 0.9 pM in a new tube
 - a. Dilute 67.5 µL of library (20pM) with 1432.5 µL of HT1
 1. (This is now 1500µL of 0.9pM loading concentration)
 - b. Invert to mix and then pulse centrifuge.
10. Mix the NextSeq cartridge by inverting 10x, make sure the reagents do not contain ice. After mixing, tap the cartridge on the bench 2-3 times to dislodge any air trapped in the bottom of the tubes.
11. Load NextSeq cartridge:
 - a. Optional but recommended, use PhiX control: Add 3% of PhiX control to the library
 - b. Load 1300 µL of **the library** to the **Load Library Here (10)** well
12. Set up and start Nextseq run: Clean flow cell according to instructions, follow on-screen instructions to load and start instrument

C: Illumina® MiniSeq protocol

Use Illumina® MiniSeq kit for 300 cycles (2x150 bp run)

1. Thaw DNA sample/library, buffer HT1, and MiniSeq cartridge

NOTE: MiniSeq cartridge should be thawed by submerging it in (but not covering it completely with) water at room temperature. (thawing takes 2-3 hour, do not use warm water as it degrades the enzymes)

2. Prepare sample sheet. Workflow: Generate FASTQ. Use the following adapter sequences for trimming in the sample sheet:

Adapter: GCGAATTTTCGACGATCGTTGCATTAAGTCGCGAA

AdapterRead2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

3. Dilute DNA to 2 nM using lowTE (yellow cap).
4. Prepare fresh 0.1N NaOH.
5. Mix 10 µL of 2 nM DNA with 10 µL of 0.1N NaOH, vortex, spin down.
6. Incubate for 5 minutes at room temperature.
7. Add 10 µL of 200 mM Tris-HCl pH 7.0 to hydrolyze the NaOH.
8. Add 970 µL Ice Cold HT1 to DNA/NaOH mix to dilute DNA to 20 pM.
9. Dilute the 20 pM library with Ice Cold HT1 to 0.8 pM in a new tube.
 - a. Dilute 40 µL of library (20 pM) with 960 µL of HT1
 - b. (This is now 1000 µL of 0.8 pM loading concentration)
 - c. Invert to mix and then pulse centrifuge.
10. Mix the MiniSeq cartridge by inverting 10x, make sure the reagents do not contain ice. After mixing, tap the cartridge on the bench 2-3 times to dislodge any air trapped in the bottom of the tubes.
11. Load MiniSeq cartridge:
 - a. Optional but recommended, use PhiX control: Add 3% of PhiX control to the library
 - b. Load 500 µL of **the library** to the **Load Library Here (16)** well
12. Set up and start MiniSeq run: Clean flow cell according to instructions, follow on-screen instructions to load and start instrument

APPENDIX 2

Recommended cDNA synthesis with LunaScript RT Supermix 5x (New England Biolabs p/n E3010S/L):

- 2.4 µl Lunascript RT SuperMix 5x
- 3.6 µl RNase-free water
- 6.0 µl sample-RNA
- Mix by vortexing 3-5 seconds
- Short spin
- Thermo cycler program:
 - o 2min 25°C
 - o 20-45 min 55°C
 - o 1 min 95°C

Software tools:

JSI – Virseak

<https://www.jsi-medisys.de/company/news-detail/article/find-known-and-new-variants-of-sars-cov-2-with-our-new-surveillance-system-virseak/>

QIAGEN - CLC Bio

<https://digitalinsights.qiagen.com/resources/science/sars-cov-2-resources/>

Ridom – SeqSphere

<https://www.ridom.de/seqsphere/>

Open Source GITHUB

– co-development Nimagen & Radboud University Medical Center

Department of Medical Microbiology - Dr Jordy Coolen

https://github.com/JordyCoolen/easyseq_covid19

Illumina® Systems Reference Guides

iSeq100 System Guide

MiSeq System Guide

MiSeq Denature and Dilute Libraries Guide

MiniSeq System Guide

MiniSeq Denature and Dilute Libraries Guide

Nextseq 550 System Guide

NextSeq System Denature and Dilute Libraries Guide

Illumina® experiment manager



Support:

<https://www.nimagen.com/covid19#product-information>

techsupport@nimagen.com

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Reference:

Wolters et al., 2020: "Novel SARS-CoV-2 Whole-genome sequencing technique using Reverse Complement PCR enables fast and accurate outbreak analysis".

doi: <https://doi.org/10.1101/2020.10.29.360578>

Legal Notice:

RC-PCR is patent protected (PCT/GB2016/050558, WO2016146968A1) and exclusively licensed to NimaGen B.V. Nijmegen

Version tracking:

Version	change	Date
V1.5 -> v1.6	Miseq v2 loading changed from 5.5 to 9pM MiSeq v3 loading changed from 10 to 15pM EBT buffer → LowTE	19jan2021, WvdV
V1.6 -> v1.7	iSeq100 kit Safe stopping points added	20jan2021, wvdv
V1.7 -> v1.8	Reverse Transcription kit recommendation, safe stopping points info, tube labelling	19feb2021, jth
V1.8 -> v1.9	safe stopping points info, tube labelling	02mar2021, jth
V1.9 -> v1.10	Naming of Probe Dilution Buffer Plus Thaw, mix and centrifuge of reagents before use	02Apr2021, WvdV
V1.10 -> v2.01	Vortexing of complete 20µl RCPCR mixture prior to thermo-profiling. Updated picture of library calculator Addition of index plate UDI04D Addition of Lunascript protocol	09Apr2021, WvdV

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