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# Miniaturized and Automatized Whole-Genome Amplification of SARS-CoV 2 Virus using Illumina CovidSeq reagents for Next-Generation Sequencing V.1 V.1

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GENEPII Sequencing Pla...

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

Since December 2019, the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) around the world has necessitated to drastically scale-up the viral genomic surveillance to track the emergence of variant of concern. To scope with large-scale surveillance studies, various Next Generation Sequencing (NGS) methods have been reported and one of the main limitation has been the complexity of workflow in terms of turnaround time and cost effectiveness, limiting yield. By optimizing the Artic v4.1 sequencing strategy, we set-up automatization and miniaturization of SARS-CoV-2 whole genome sequencing protocol for monitoring emergence of new variants, applicable to a large workflow in a rapid and cost-effective manner.

## Materials

Illumina CovidSeq Assay **Illumina, Inc.**

MGI Nucleic Acid Extraction Kit **MGI Tech Co Catalog #**1000021043

## Abstract

1 Since December 2019, the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) worlwide has necessitated to drastically scale-up the viral genomic surveillance to track the emergence of variant of concern.

The limitations of NGS workflow for increasing the genomic surveillance includes the cost effectivennes and the lack of automation of the technics used. By optimizing the Artic v4.1 sequencing strategy, we developped automatization and miniaturization of SARS-CoV-2 whole genome sequencing protocol for monitoring emergence of new variants, applicable to a large workflow in a rapid and cost-effective manner.

RNA extraction from nasopharyngeal swab was performed with the MGISP-960 workstation using MGI Easy Magnetic Beads Virus DNA/RNA Extraction Kit (MGI Tech, Marupe, Latvia).

The automatized and miniaturized method (Pre and Post PCR part) was performed with the Mosquito HV and Dragonfly® Discovery platforms (SPT Labtech, Melbourn, UK). Each reagent distribution being performed with the Dragonfly and each pipetting step with the Mosquito HV excepted for the last Library Clean Up part of the process.

2 This protocol is suitable for miniaturized and automatized CovidSeq Illumina protocol on 384 samples (SARS-CoV 2). All reagents used are parts of Illumina COVIDSeq Assay (RUO) Kit excepted Amplification Primers (IDT ARTIC V4.1), UDI Indexes (IDT Indexes Illumina compatible).


## Nucleic acids extraction 3h

3 Clinical SARS-CoV2 positive samples were extracted using MGI Nucleic Acid Extraction Kit (Catalog #1000021043) on MGISP 960 following manufacturer recommandations. It will provided a workflow of up to 192 samples in 1 hour and 20 minutes resulting in four 96 well RNA plates in 2 hours and 40 minutes. 3h

## Pre-PCR - Anneling - cDNA Synthesis - Amplification 5h

4 Pre-PCR was prepared using Illumina COVIDSeq Assay (RUO) kit with a miniturized and automatized protocol using SPT Labtech automatons (Mosquito and Dragonfly).

Equipment

| | |
|---|---|
| Mosquito HV | NAME |
| High Volume 16-Channel Robotic Liquid Handler | TYPE |
| SPT LabTech | BRAND |
| 3097-01057 | SKU |
| https://www.sptlabtech.com/products/liquid-handling/mosquito-hv/ | LINK |

Equipment

| | |
|---|---|
| Dragonfly | NAME |
| Low volume liquid dispensing automaton | TYPE |
| SPT Labtech | BRAND |
| SU-0185 | SKU |
| https://www.sptlabtech.com/products/dragonfly/dragonfly-discovery | LINK |

5 **PRE-PCR ASSAY**

Prepare following mixes for each steps of Pre-PCR part (Annealing, RT, Amplification). Volumes are edited for 384 samples including reagent overgage required by SPT automatons.

| | Reagents | Volume for 384 samples (µL) |
|---|---|---|
| | EPH3 HT | 1500 |

Premix 1 - Annealing

| | Reagents | Volume for 384 samples (µL) |
|---|---|---|
| | FSM HT | 1890 |
| | RVT HT | 210 |
| | **TOTAL** | **2100** |

Premix 2 - RT

| | Reagents | Volume for 384 samples (µL) |
|---|---|---|
| | IPM HT | 3600 |
| | Primer Pool 1 or 2 (1/10) | 1032 |
| | NUCLEASE FREE WATER | 1128 |
| | **TOTAL** | **5760** |

Premix 3 - Amplification ( Do twice for each primer pool)

## 5.1 Annealing - Preparation

- Using Dragonfly, distribute 3.57µL of EPH3 per well of an "Annealing" Eppendorf LoBind 384 Well Plate.

3.57 µL EPH3

## 5.2 RNA Extract Pool

- Using Mosquito HV 9mm, pool the four 96 Well RNA Plates into the "Annealing" Eppendorf LoBind 384 Well Plate.

3.57 µL RNA ***per Well***

## 5.3 Annealing

- Seal, brielfly vortex the "Annealing" Eppendorf LoBind 384 Well Plate and centrifuge.
- Place the plate in a thermocycler and run the following program :

| | Temperature | Duration |
|---|---|---|
| | 65°C | 5 min |
| | 4°C | Infinite |

Annealing Program - Indicate 7 µL as volume and heat lid at 75ºC.

## 5.4 RT-PCR - Preparation

- When the thermal cycler program RT displays 5 min remaining, engage *Premix 3 - RT* distribution using the Dragonfly into a new Eppendorf LoBind 384 Well Plate ("RT" Eppendorf LoBind 384 Well Plate).

4.706 µL RT Premix ***per Well***

- When the thermal cycler program Annealing is done, briefly centrifuge the plate. Proceed to Mosquito HV 9mm transfer of Annealed RNA into the "RT" Eppendorf LoBind 384 Well Plate.

5 µL Annealed RNA ***per Well***

## 5.5 RT-PCR

- Seal, brielfly vortex the "RT" Eppendorf LoBind 384 Well Plate and centrifuge.
- Place the plate in a thermocycler and run the following program :

| | Temperature | Duration |
|---|---|---|
| | 25°C | 5 min |
| | 50°C | 10 min |
| | 80°C | 5 min |
| | 4°C | Infinite |

RT Program - Indicate 10 µL as volume and heat lid at 99ºC.

## 5.6 Amplification - Preparation

- When the thermal cycler program RT displays 1 min remaining, engage *Premix 2 - Amplification* distribution for Primer Pool 1 and 2 using the Dragonfly into two new Eppendorf LoBind 384 Well Plate identifiate :

- "POOL 1" Eppendorf LoBind 384 Well Plate
- "POOL 2" Eppendorf LoBind 384 Well Plate.

14 µL Amplification Premix ***per Well for Primer Pool 1 and 2***

- When the thermal cycler program RT is done, briefly centrifuge the plate.
- Proceed to Mosquito HV 9mm transfer of cDNA into the two Amplification Eppendorf LoBind 384 Well Plate.

3.5 µL cDNA ***per Well for the two amplification plates***

5.7 **Amplification**

- Seal, brielfly vortex the two Amplification Eppendorf LoBind 384 Well Plates and centrifuge.
- Place the plate in a thermocycler and run the following program :

| | Temperature | Duration | Cycles |
|---|---|---|---|
| | 98°C | 3 min | - |
| | 98°C | 15 sec | 32 |
| | 63°C | 5 min | |
| | 4°C | Infinite | - |

Amplification Program - Indicate 17.5 µL as volume and heat lid at 100°C.

## Post-PCR - Library Preparation

3h

6 Sequencing library was prepared using Illumina COVIDSeq Assay (RUO) kit with a miniturized and automatized protocol using SPT Labtech automatons (Mosquito and Dragonfly).

Equipment

| | |
|---|---|
| Mosquito HV | NAME |
| High Volume 16-Channel Robotic Liquid Handler | TYPE |
| SPT LabTech | BRAND |
| 3097-01057 | SKU |
| https://www.sptlabtech.com/products/liquid-handling/mosquito-hv/ | LINK |

Equipment

| | |
|---|---|
| Dragonfly | NAME |
| Low volume liquid dispensing automaton | TYPE |
| SPT Labtech | BRAND |
| SU-0185 | SKU |
| https://www.sptlabtech.com/products/dragonfly/dragonfly-discovery | LINK |

## 7 POST-PCR ASSAY

- Prepare following mixes for each steps of Post-PCR part (Tagmentation, Indexing PCR).

Volumes are edited for 384 samples including reagent overgage required by SPT automatons.

- Washes and Tagmentation Stop steps reagents will be distributed directly into an new LVSD 384 well Plate using Dragonfly.

| | Reagents | Volume for 384 samples (µL) |
|---|---|---|
| | ELBTs | 160.4 |
| | TB1 | 481.3 |
| | NUCLEASE FREE WATER | 802.1 |
| | **TOTAL** | **1443.8** |

Premix 1 - Tagmentation

| | Reagents | Volume for 384 samples (µL) |
|---|---|---|
| | EPM | 1200 |
| | NUCLEASE FREE WATER | 1200 |
| | **TOTAL** | **2400** |

Premix 2 - Amplification / Indexing PCR

## 7.1 Amplification POOL 1 and POOL 2 Eppendorf LoBind 384 Well Plates Pool

- Following Amplification program, brielfly centrifuge the two Amplification POOL 1 and POOL 2 Eppendorf LoBind 384 Well Plates and place them on Mosquito HV 4.5mm board in order to proceed to Pool step :
- Identify a new Eppendorf LoBind 384 Well Plates as " Library Pool Plate" and place it on Moquito HV 4.5mm board with the two Amplification Eppendorf LoBind 384 Well Plates.
- Proceed to transfer :

5 µL from each Pool plates (POOL 1 and 2) **into the *Library Pool Plate***

- It will result of **10µL POOL 1/2 per well for 384 samples.**

## 7.2 Tagmentation

- Prepare two different new plates for mix distribution :

1. Eppendorf LoBind 384 Well Plates labelled **"Tagmentation Plate"**
2. LVSD 384 well Plate labelled "**Reagent / Trash Plate"** (Half of this plate will be used for distribution of TWB and ST2 reagents; the other half will be used as trash for

washes steps)

- Using Dragonfly, proceed to :

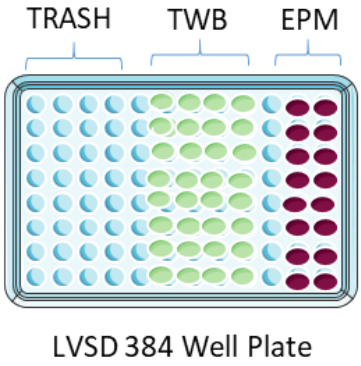
1- Premix 1 distribution on **"Tagmentation Plate"**

3 µL of ELBTs per well

2- Premix 2 distribution on "**Reagent / Trash Plate"** organized as follow :

50 µL per well for TWB **on green columns**

40 µL per well for EPM **on red columns**




"Reagent / Trash Plate" Organization

When distribution is done, place Plates on Mosquito HV 4.5mm board and proceed to transfer of Amplicons from "Library Pool Plate" to the "Tagmentation Plate".

2 µL of SARS-CoV2 amplicons **into *Tagmentation Plate***

## 7.3 Tagmentation incubation / Stop

5m

- When Mosquito tranfer is done, directly proceed to Tagmentation step.
- Place the plate in a thermocycler and run the following program :

| | Temperature | Duration |
|---|---|---|
| | 55°C | 5 min |

| | Temperature | Duration |
|---|---|---|
| | 10°C | Infinite |

Tagmentation Program - Indicate 5 µL as volume and heat lid at 80°C - Proceed to thermal cycler pre heat befrore to launch.

- When Tagmentation is done, directly proceed to Stop step using Dragonfly :

1 µL of ST2 per well **directly into the Tagmentation Plate**

- Seal, briefly vortex and centrifuge the plate. Incubate :

00:05:00 Room Temperature

## 7.4 Post Tagmentation Clean Up

2m

- When Tagmentation is stopped, proceed to washes steps using Mosquito :

- Place "Tagmentation Plate" on Mosquito 384 Magnetic Block
- Incubate 384 Magnetic Block

00:02:00 384 Magnetic Block

- Remove supernatant and transfer it to "Reagent / Trash Plate"

4 µL removed from "Tagmentation Plate" and transfer to "Reagent / Trash Plate"

- Add TWB in each well of the "Tagmentation Plate"

5 µL of TWB into "Tagmentation Plate"

- Seal the plate, vortex and brilefly centrifuge.
- **Proceed to Wash Step once again until last supernatant removing**

## 7.5 Amplify and Index Tagmented Amplicons

When washes steps are done :

- Removed supernatant
- Proceed to Mosquito transfer of Premix 2 (EPM) AND indexes :

4 µL of Premix 2 into "Tagmentation Plate"

1 µL of Indexes into "Tagmentation Plate"

- Seal, briefly vortex and centrifuge the plate.
- Place the plate in a thermocycler and run the following program :

| | Temperature | Duration | Cycles |
|---|---|---|---|
| | 72°C | 3 min | - |
| | 98°C | 3 min | - |
| | 98°C | 20 sec | 7 |
| | 60°C | 30 sec | |
| | 72°C | 1 min | |
| | 72°C | 3 min | - |
| | 10°C | Infinite | - |

Amplification / Indexing Program - Indicate 7 µL as volume and heat lid at 100°C.

## 7.6 Library Pool and Manual Clean Up

7m

- When Amplification/Indexing Program is done, proceed to Pool of the 384 wells of "Tagmentation Plate" into a new Eppendorf LoBind 384 Well Plate labelled "Library Pool Plate" :

1 µL of the 384 wells into **one column** of the new "Library Pool Plate"

- Pool **12 µL of the 16 wells** of the "Library Pool Plate" column into one Eppendorf LoBind 1.5mL Tube. It will result of a final volume :

192 µL Pool Library

- Proceed to Manual Clean Up using Illumina Tune Beads (ITB) at 0.9x. Add :

172.8 µL of ITB into the Eppendorf LoBind 1.5mL containing the 192µL Final Pool.

**Be careful to hardly vortex ITB before use.**

- Vortex to mix and incubate :

00:05:00 at Room Temperature

- Centrifugre briefly and place the Tube on magnetic stand until liquid is clear.
- Remove supernatant and proceed to two EtOH 80% washes steps (30 sec contact, remove supernatant X2)
- Let beads dry 1min after last superntaant removing
- Add RSB to proceed to Elution :

55 µL of RSB

- Vortex and centrifuge briefly. Incubate :

00:02:00 at Room Temperature

- Place the Tube on magnetic stand until liquid is clear.
- Transfer 50µL of supernatant into a new  Eppendorf LoBind 1.5mL labelled "Final Library Pool"

Store on ice or at +4°C until Quantification Process following Illumina COVIDSeq Assay (RUO) Kit recommandations.

## Protocol references

https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/Illumina-COVIDSeq-Test/illumina-covidseq-ruo-kits-reference-guide-1000000126053-08.pdf