



Mar 15, 2023

Version 2

# 🌐 Pathogen-Oriented Low-cost Assembly & Re-sequencing on Opentrons (POLARtron): An automation-friendly highly sensitive and high-throughput SARS-CoV-2 diagnostic based on whole genome sequencing V.2



DOI

[dx.doi.org/10.17504/protocols.io.81wgbp8jqvpk/v2](https://dx.doi.org/10.17504/protocols.io.81wgbp8jqvpk/v2)

Per A. Adastra<sup>1,2,3</sup>, Neva C. Durand<sup>1,2,3,4</sup>, Namita Mitra<sup>1,2,3</sup>, Saul Godinez<sup>1,2,3</sup>, Ragini Mahajan<sup>1,3,5</sup>, Alyssa Blackburn<sup>1,2,3</sup>, Zane Colaric<sup>1,2,3</sup>, Joshua W. M. Theisen<sup>1,3</sup>, David Weisz<sup>1,2,3</sup>, Olga Dudchenko<sup>1,2,3</sup>, Andreas Gnirke<sup>1,4</sup>, Suhas S.P. Rao<sup>1,2,3,6</sup>, Parwinder Kaur<sup>7</sup>, Erez Lieberman Aiden<sup>1,2,3,8</sup>, Aviva Presser Aiden<sup>1,2,9,10</sup>

<sup>1</sup>The Center for Genome Architecture, Baylor College of Medicine, Houston, TX 77030, USA;

<sup>2</sup>Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX;

<sup>3</sup>Center for Theoretical Biological Physics, Rice University, Houston, TX 77030, USA;

<sup>4</sup>Broad Institute of MIT and Harvard, Cambridge, MA 02139, USA;

<sup>5</sup>Department of Biosciences, Rice University, Houston, TX 77030, USA;

<sup>6</sup>Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305, USA;

<sup>7</sup>UWA School of Agriculture and Environment, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia;

<sup>8</sup>Departments of Computer Science and Computational and Applied Mathematics, Rice University, Houston, TX 77030, USA;

<sup>9</sup>Department of Bioengineering, Rice University, Houston, TX, USA;

<sup>10</sup>Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305, USA

XPRIZE Rapid Covid Tes...

**Per A. Adastra**

Baylor College of Medicine

## Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



**DOI:** <https://dx.doi.org/10.17504/protocols.io.81wgbp8jqvpk/v2>

**Protocol Citation:** Per A. Adastra, Neva C. Durand, Namita Mitra, Saul Godinez, Ragini Mahajan, Alyssa Blackburn, Zane Colaric, Joshua W. M. Theisen, David Weisz, Olga Dudchenko, Andreas Gnirke, Suhas S.P. Rao, Parwinder Kaur, Erez Lieberman Aiden, Aviva Presser Aiden 2023. Pathogen-Oriented Low-cost Assembly & Re-sequencing on Opentrons (POLARtron): An automation-friendly highly sensitive and high-throughput SARS-CoV-2 diagnostic based on whole genome sequencing . **protocols.io** <https://dx.doi.org/10.17504/protocols.io.81wgbp8jqvpk/v2> Version created by **Per A. Adastra**

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** In development

**We are still developing and optimizing this protocol**

**Created:** March 15, 2023

**Last Modified:** March 15, 2023

**Protocol Integer ID:** 78863

**Keywords:** polartron, pathogen, throughput sar, genome, whole genome, sar, friendly variant of polar, opentron



## Disclaimer

### DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

## Abstract

An automation-friendly variant of POLAR.

## Guidelines

### SARS-CoV-2 Specific Primer Set

The ARTIC Network designed and tested<sup>1</sup> the primer set used in this protocol and must be custom ordered prior to experiments. Details on their primer set can be found on their Github page <https://github.com/artic-network/artic-ncov2019>.

1. Artic Network. <https://artic.network/resources/ncov/ncov-amplicon-v3.pdf>.

### The World Health Organization: Dos and Don'ts for Molecular Testing

(<https://www.who.int/malaria/areas/diagnosis/molecular-testing-dos-donts/en/>)

Molecular detection methods have the ability to produce a large volume of nucleic acid through the amplification of trace quantities found in samples. While this is beneficial for enabling sensitive detection, it also introduces the possibility of contamination through the spreading of amplicon aerosols in the laboratory environment. When conducting experiments, measures can be undertaken to avoid the contamination of reagents, laboratory equipment, and bench space, as such contamination may generate false-positive (or false-negative) results. To help reduce the likelihood of contamination, Good Laboratory Practice should be exercised at all times. Specifically, precautions should be taken regarding the following points:

#### ***Handling reagents***

- Briefly centrifuge reagent tubes before opening to avoid the generation of aerosols.
- Aliquot reagents to avoid multiple freeze-thaw and the contamination of master stocks.
- Clearly label and date all reagent and reaction tubes and maintain logs of reagent lot and batch numbers used in all experiments.
- Pipette all reagents and samples using filter tips. Prior to purchase, it is advisable to confirm with the manufacturer that the filter tips fit the brand of the pipette to be used.

#### ***Organization of workspace and equipment***

*The workspace should be organized to ensure that the flow of work occurs in one direction, from clean areas (pre-PCR) to dirty areas (post-PCR). The following general precautions will help to reduce the chance of contamination.*

Have separate designated rooms, or at minimum physically separate areas, for:

1. master mix preparation,
2. nucleic acid extraction and DNA template addition

In some settings, having 4 separate rooms is difficult. A possible but less desirable option is to do the master mix preparation in a containment area, e.g. a laminar flow cabinet. In the case of nested PCR amplification, the

preparation of the master mix for the second round reaction should be prepared in the 'clean' area for master mix preparation, but the inoculation with the primary PCR product should be done in the amplification room, and if possible in a dedicated containment area (e.g. a laminar flow cabinet).

Each room/area needs a separate set of clearly labeled pipettes, filter tips, tube racks, vortexes, centrifuges (if relevant), pens, generic lab reagents, lab coats, and boxes of gloves that will remain at their respective workstations.

Hands must be washed and gloves and lab coats changed when moving between the designated areas. Reagents and equipment should not be moved from a dirty area to a clean area. Should an extreme case arise where a reagent or piece of equipment needs to be moved backward, it must first be decontaminated with 10% sodium hypochlorite, followed by a wipe down with sterile water

Ideally, staff should abide by the unidirectional workflow ethos and not go from dirty areas (post-PCR) back to clean areas (pre-PCR) on the same day. However, there may be occasions when this is unavoidable. When such occasion arises, personnel must take care to thoroughly wash hands, change gloves, use the designated lab coat and not introduce any equipment they will want to take out of the room again, such as lab books. Such control measures should be emphasized in staff training on molecular methods.

After use, bench spaces should be cleaned with 10% sodium hypochlorite (followed by sterile water to remove residual bleach), 70% ethanol, or a validated commercially available DNA-destroying decontaminant. Ideally, ultra-violet (UV) lamps should be fitted to enable decontamination by irradiation. However, the use of UV lamps should be restricted to closed working areas, e.g. safety cabinets, in order to limit the laboratory staff's UV exposure. Please abide by manufacturer instructions for UV lamp care, ventilation, and cleaning in order to ensure that lamps remain effective.

If manufacturer instructions permit it, pipettes should be routinely sterilized by autoclave. If pipettes cannot be autoclaved, it should suffice to clean them with 10% sodium hypochlorite (followed by a thorough wipe down with sterile water) or with a commercial DNA-destroying decontaminant followed by UV exposure.

All equipment needs to be calibrated regularly according to the manufacturer-recommended schedule. A designated person should be in charge of ensuring that the calibration schedule is adhered to, detailed logs are maintained, and service labels are clearly displayed on equipment.

### ***Use and cleaning advice for the designated molecular space***

#### **■ Pre-PCR: Reagent aliquoting / mastermix preparation**

This should be the cleanest of all spaces used for the preparation of molecular experiments and should ideally be a designated laminar flow cabinet equipped with a UV light.

Samples, extracted nucleic acid, and amplified PCR products must not be handled in this area.

Amplification reagents should be kept in a freezer (or refrigerator, as per manufacturer recommendations) in the same designated space, ideally next to the laminar flow cabinet or pre-PCR area.

Gloves should be changed each time upon entering the pre-PCR area or laminar flow cabinet.

The pre-PCR area or laminar flow cabinet should be cleaned before and after use as follows: Wipe down all items in the cabinet, e.g. pipettes, tip boxes, vortex, centrifuge, tube racks, pens, etc. with 70% ethanol or a commercial DNA-destroying decontaminant, and allow to dry. In the case of a closed working area, e.g. a laminar flow cabinet, expose the hood to UV light for 30 minutes.

#### ■ **Pre-PCR: Nucleic acid extraction/template addition**

Nucleic acid must be extracted and handled in a second designated area, using a separate set of pipettes, filter tips, tube racks, fresh gloves, lab coats, and other equipment.

This area is also for the addition of template, controls, and trendlines to the master mix tubes or plates. To avoid contamination of the extracted nucleic acid samples that are being analyzed, it is recommended to change gloves prior to handling positive controls or standards and to use a separate set of pipettes.

PCR reagents and amplified products must not be pipetted in this area.

Samples should be stored in designated fridges or freezers in the same area.

The sample workspace should be cleaned in the same way as the master mix space.

#### ■ **Post-PCR: Amplification and handling of the amplified product**

This designated space is for post-amplification processes and should be physically separate from the pre-PCR areas. It usually contains thermocyclers and real-time platforms, and ideally should have a laminar flow cabinet for adding the round 1 PCR product to the round 2 reaction, if nested PCR is being performed.














PCR reagents and extracted nucleic acid must not be handled in this area since the risk of contamination are high. This area should have a separate set of gloves, lab coats, plate and tube racks, pipettes, filter tips, bins, and other equipment.

Tubes must be centrifuged before opening.










The sample workspace should be cleaned in the same way as the master mix space.

## Materials

### MATERIALS

-  Nuclease-Free Water, 150ml **Promega Catalog #P1195**
-  Luna Universal Probe One-Step RT-qPCR Kit - 2,500 rxns **New England Biolabs Catalog #E3006E**
-  isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W292907**
-  2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**
-  Proteinase K **New England Biolabs Catalog #P8107S**
-  200 Proof Ethanol pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023**
-  Random Hexamer Primer **Thermo Fisher Catalog #SO142**
-  NEBNext Q5U Master Mix – 50 rxns **New England Biolabs Catalog #M0597S**
-  Magnesium Chloride Solution 1 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1028**
-  sparQ PureMag Beads **Quantabio Catalog #95196-060**
-  Nextera DNA Flex Library Prep Kit **Illumina, Inc.**
-  NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-1L**
-  TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
-  Corning 10% SDS (Sodium Dodecyl Sulfate) **Fisher Scientific Catalog #MT-46040CI**
-  IDT for Illumina DNA/RNA UD Indexes **Illumina, Inc. Catalog #20027213**
-  Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
-  NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-100ML**
-  EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**
-  NaCl - 1Kg **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S3014-1KG**

### STEP MATERIALS

-  200 Proof Ethanol pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023**
-  Mineral Oil **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M5904**
-  Corning 10% SDS (Sodium Dodecyl Sulfate) **Fisher Scientific Catalog #MT-46040CI**
-  Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
-  Nextera DNA Flex Library Prep Kit **Illumina, Inc.**
-  NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-100ML**
-  Magnesium Chloride Solution 1 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1028**
-  Poly(ethylene glycol) 8000 **Merck MilliporeSigma (Sigma-Aldrich)**
-  EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**



- ✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
- ✕ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**
- ✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
- ✕ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**
- ✕ Poly Ethylene Glycol (PEG) 8000 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #89510-250G-F**
- ✕ NaCl - 1Kg **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S3014-1KG**
- ✕ Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
- ✕ Nuclease-Free Water, 150ml **Promega Catalog #P1195**
- ✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
- ✕ Luna Universal Probe One-Step RT-qPCR Kit - 2,500 rxns **New England Biolabs Catalog #E3006E**
- ✕ Random Hexamer **Thermo Fisher Scientific Catalog ##SO142**
- ✕ sparQ PureMag Beads **Quantabio Catalog #95196-060**
- ✕ IDT for Illumina DNA/RNA UD Indexes **Illumina, Inc. Catalog #20027213**
- ✕ NEBNext Q5U Master Mix – 50 rxns **New England Biolabs Catalog #M0597S**
- ✕ sparQ PureMag Beads **Quantabio Catalog #95196-060**
- ✕ Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**
- ✕ Bio-rad Hard-shell low-profile 96 well skirted PCR plates **Catalog #HSP9601**
- ✕ Proteinase K **New England Biolabs Catalog #P8107S**
- ✕ Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**
- ✕ 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**





## Protocol materials

- ⊗ 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**
- ⊗ Nuclease-Free Water, 150ml **Promega Catalog #P1195**
- ⊗ Luna Universal Probe One-Step RT-qPCR Kit - 2,500 rxns **New England Biolabs Catalog #E3006E**
- ⊗ NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-100ML**
- ⊗ 200 Proof Ethanol pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023**
- ⊗ Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
- ⊗ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**
- ⊗ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**
- ⊗ Luna Universal Probe One-Step RT-qPCR Kit - 2,500 rxns **New England Biolabs Catalog #E3006E**
- ⊗ IDT for Illumina DNA/RNA UD Indexes **Illumina, Inc. Catalog #20027213**
- ⊗ sparQ PureMag Beads **Quantabio Catalog #95196-060**
- ⊗ Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**
- ⊗ Poly(ethylene glycol) 8000 **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
- ⊗ Nextera DNA Flex Library Prep Kit **Illumina, Inc.**
- ⊗ IDT for Illumina DNA/RNA UD Indexes **Illumina, Inc. Catalog #20027213**
- ⊗ Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
- ⊗ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**
- ⊗ Mineral Oil **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M5904**
- ⊗ Corning 10% SDS (Sodium Dodecyl Sulfate) **Fisher Scientific Catalog #MT-46040CI**
- ⊗ Random Hexamer Primer **Thermo Fisher Catalog #SO142**
- ⊗ Nuclease-Free Water, 150ml **Promega Catalog #P1195**
- ⊗ Poly Ethylene Glycol (PEG) 8000 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #89510-250G-F**
- ⊗ sparQ PureMag Beads **Quantabio Catalog #95196-060**
- ⊗ Proteinase K **New England Biolabs Catalog #P8107S**
- ⊗ Bio-rad Hard-shell low-profile 96 well skirted PCR plates **Catalog #HSP9601**
- ⊗ Proteinase K **New England Biolabs Catalog #P8107S**
- ⊗ 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**
- ⊗ Magnesium Chloride Solution 1 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1028**
- ⊗ NaCl - 1Kg **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S3014-1KG**



- ✕ Nextera DNA Flex Library Prep Kit **Illumina, Inc.**
- ✕ Magnesium Chloride Solution 1 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1028**
- ✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
- ✕ Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
- ✕ Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**
- ✕ Corning 10% SDS (Sodium Dodecyl Sulfate) **Fisher Scientific Catalog #MT-46040CI**
- ✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
- ✕ 200 Proof Ethanol pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023**
- ✕ isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W292907**
- ✕ NaCl - 1Kg **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S3014-1KG**
- ✕ NEBNext Q5U Master Mix – 50 rxns **New England Biolabs Catalog #M0597S**
- ✕ NEBNext Q5U Master Mix – 50 rxns **New England Biolabs Catalog #M0597S**
- ✕ sparQ PureMag Beads **Quantabio Catalog #95196-060**
- ✕ Random Hexamer **Thermo Fisher Scientific Catalog ##SO142**
- ✕ NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-1L**
- ✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**
- ✕ NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-100ML**
- ✕ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**
- ✕ Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**
- ✕ Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**
- ✕ Bio-rad Hard-shell low-profile 96 well skirted PCR plates **Catalog #HSP9601**
- ✕ Proteinase K **New England Biolabs Catalog #P8107S**
- ✕ 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**
- ✕ Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
- ✕ Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**
- ✕ 200 Proof Ethanol pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023**
- ✕ Nuclease-Free Water, 150ml **Promega Catalog #P1195**
- ✕ Luna Universal Probe One-Step RT-qPCR Kit - 2,500 rxns **New England Biolabs Catalog #E3006E**
- ✕ Random Hexamer **Thermo Fisher Scientific Catalog ##SO142**
- ✕ Mineral Oil **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M5904**
- ✕ sparQ PureMag Beads **Quantabio Catalog #95196-060**



✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**

✕ Nextera DNA Flex Library Prep Kit **Illumina, Inc.**

✕ NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-100ML**

✕ Magnesium Chloride Solution 1 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1028**

✕ Corning 10% SDS (Sodium Dodecyl Sulfate) **Fisher Scientific Catalog #MT-46040CI**

✕ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**

✕ Poly(ethylene glycol) 8000 **Merck MilliporeSigma (Sigma-Aldrich)**

✕ Poly Ethylene Glycol (PEG) 8000 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #89510-250G-F**

✕ NaCl - 1Kg **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S3014-1KG**

✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**

✕ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**

✕ NEBNext Q5U Master Mix – 50 rxns **New England Biolabs Catalog #M0597S**

✕ IDT for Illumina DNA/RNA UD Indexes **Illumina, Inc. Catalog #20027213**

✕ sparQ PureMag Beads **Quantabio Catalog #95196-060**

✕ TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**

✕ EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**

## Troubleshooting

### Before start


- Add 500 µl beta-mercaptoethanol per 100 ml Viral DNA/RNA Buffer (final concentration of 0.5% (v/v)) from the Quick-DNA/RNA Viral MagBead.
- Add 80 ml (R2141) of isopropanol to the MagBead DNA/RNA Wash 1 concentrate from the Quick-DNA/RNA Viral MagBead.
- Add 120 ml (R2141) of isopropanol to the MagBead DNA/RNA Wash 2 concentrate from the Quick-DNA/RNA Viral MagBead.


## RNA Extraction


1h

- 1 For each saliva sample recieved add equal volume saliva and 2X DNA/RNA Shield from the Quick-DNA/RNA Viral magbead kit and vortex. Centrifuge the samples at



 500 rpm, 00:05:00 to bring down debris.





 Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**

- 2 Without disturbing the pellet, transfer  25  $\mu$ L of saliva sample and 1X DNA/RNA to the bottom of a well in a 96-well deep-plate of each sample to a new tube

 Quick-DNA/RNA Viral MagBead **Zymo Research Catalog #R2141**

 Bio-rad Hard-shell low-profile 96 well skirted PCR plates **Catalog #HSP9601**

- 3 Add  25  $\mu$ L 1X DNA/RNA Shield supplemented with  2.5  $\mu$ L of Proteinase K (20mg/mL) to each sample.

Briefly mix by using a plate shaker at  1300 rpm, 25°C for  00:00:10 and incubate  00:15:00 at  Room temperature .

### Equipment

ThermoMixer® C

NAME

Eppendorf

BRAND

Catalog No. 2231000680

SKU




<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

LINK







 Proteinase K **New England Biolabs Catalog #P8107S**



- 4 Remove  5  $\mu\text{L}$  of MagBinding Beads per sample of the stock and place on a magnet stand. Incubate for  00:02:00 or until the beads have pelleted and the supernatant is completely clear. Then while avoiding the bead pellet, carefully remove the clear supernatant. Resuspend the beads in  100  $\mu\text{L}$  of Viral DNA/RNA Buffer (2-Mercaptoethanol 0.5% (v/v)) per sample and vortex to fully resuspend

#### Safety information


2-Mercaptoethanol is toxic, causing irritation to the nasal passageways and respiratory tract upon inhalation, irritation to the skin, vomiting and stomach pain through ingestion, and potentially death if severe exposure occurs.

- 5 Add  100  $\mu\text{L}$  of the Viral DNA/RNA Buffer (2-Mercaptoethanol 0.5% (v/v)) and MagBinding Beads mixture to each  50  $\mu\text{L}$  sample in 1X DNA/RNA Shield. Mix by using a plate shaker at  1300 rpm, 25°C for  00:10:00 .

#### Safety information

2-Mercaptoethanol is toxic, causing irritation to the nasal passageways and respiratory tract upon inhalation, irritation to the skin, vomiting and stomach pain through ingestion, and potentially death if severe exposure occurs.

 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**

- 6 Pellet the beads onto the side of the sample tube using a magnet stand. Incubate for  00:02:00 or until the beads have pelleted and the supernatant is completely clear. Then, while avoiding the bead pellet, carefully remove the clear supernatant.

1m





## Equipment

SPRIPlate 96R Ring Super Magnet Plate

NAME

96-well Magnet Plate

TYPE

Agencourt




BRAND

A32782


SKU


<https://www.beckman.com/supplies/plates/a32782>

LINK

- 7 Add  100  $\mu$ L MagBead DNA/RNA Wash 1 and mix by using a plate shaker at  1300 rpm, 25°C for  00:02:00 .




10m

 Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**


- 8 Pellet the beads onto the side of the sample tube using a magnet stand. Incubate for  00:02:00 or until the beads have pelleted and the supernatant is completely clear. Then, while avoiding the bead pellet, carefully remove the clear supernatant.

1m



- 9 Add  100  $\mu$ L MagBead DNA/RNA Wash 2 and mix by using a plate shaker at  1300 rpm, 25°C for  00:02:00 .

10m

 Quick-DNA/RNA Viral Magbead **Zymo Research Catalog #R2141**

## Equipment

ThermoMixer® C

NAME

Eppendorf

BRAND


Catalog No. 2231000680

SKU

<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

LINK



- 10 Pellet the beads onto the side of the sample tube using a magnet stand. Incubate for  00:02:00 or until the beads have pelleted and the supernatant is completely clear. Then, while avoiding the bead pellet, carefully remove the clear supernatant.

1m



## Equipment

SPRIPlate 96R Ring Super Magnet Plate

NAME

96-well Magnet Plate

TYPE

Agencourt




BRAND

A32782

SKU

<https://www.beckman.com/supplies/plates/a32782>

LINK


- 11 Add  150  $\mu$ L 95-100% ethanol and mix by using a plate shaker at  1300 rpm, 25°C for  00:02:00 .

10m



 200 Proof Ethanol pure **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023**



- 12 Pellet the beads onto the side of the sample tube using a magnet stand. Incubate for  00:02:00 or until the beads have pelleted and the supernatant is completely clear. Then, while avoiding the bead pellet, carefully remove the clear supernatant.

1m



#### Equipment

SPRIPlate 96R Ring Super Magnet Plate

NAME

96-well Magnet Plate

TYPE

Agencourt

BRAND


A32782

SKU

<https://www.beckman.com/supplies/plates/a32782>

LINK

- 13  and repeat once.

- 14 To elute DNA/RNA from the beads, add  9  $\mu$ L DNase/RNase-Free Water and mix using a plate shaker at

5m



 1300 rpm, 25°C for  00:01:00

 Nuclease-Free Water, 150ml **Promega Catalog #P1195**





## Equipment

ThermoMixer® C

NAME

Eppendorf

BRAND



Catalog No. 2231000680

SKU

<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

LINK



- 15 Pellet the beads and transfer  7  $\mu\text{L}$  of supernatant into a new tube. The eluted DNA/RNA can be used immediately or stored frozen at   $-80\text{ }^{\circ}\text{C}$  .






II

## RT-PCR


2h

- 16 Combine the following components into a thin-walled PCR tube.


1m

-  10  $\mu\text{L}$  Luna Universal Probe One-Step Reaction Mix (2X)
-  1  $\mu\text{L}$  Luna WarmStart RT Enzyme Mix (20X)
-  1  $\mu\text{L}$  hCoV-2019/nCoV-2019 (V3) Primer Set mix (Primer pool 1 & 2) (100 $\mu\text{m}$ )
-  1  $\mu\text{L}$  Random Hexamers (100  $\mu\text{M}$ )
-  7  $\mu\text{L}$  Viral RNA/DNA extract




 Luna Universal Probe One-Step RT-qPCR Kit - 2,500 rxns **New England Biolabs Catalog #E3006E**

 Random Hexamer **Thermo Fisher Scientific Catalog ##SO142**

- 17 Add  20  $\mu\text{L}$  of mineral oil to each RT-PCR reaction to avoid evaporation and subsequent reaction failure.



 Mineral Oil **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M5904**



18 Set up and run the following RT-PCR program.

1h 30m



1. Reverse transcription: 00:10:00 at 55 °C
2. Initial PCR activation: 00:01:00 at 95 °C
3. 2-step PCR cycling (25X):
  - Denaturation: 00:00:15 at 95 °C
  - Annealing & Extension: 00:03:00 at 63 °C
4. Hold: 4 °C

#### Equipment

Veriti 96-Well Thermal Cycler

NAME

Applied Biosystems

BRAND

4375786

SKU

<https://www.thermofisher.com/order/catalog/product/4375786#/4375786><sup>LINK</sup>

19 Add 0.7x volume of sparQ PureMag beads to each sample well in RT-PCR plate and mix gently by either flicking or pipetting. For example, add 14 µL sparQ PureMag beads to a 20 µL reaction. Note that the volume of oil is not taken into consideration given the oil is effectively inert. Then pulse centrifuge to collect all liquid at the bottom of the tube.

1m



sparQ PureMag Beads **Quantabio Catalog #95196-060**

20 Incubate for 00:05:00 at Room temperature .

5m



21 Pellet the beads onto the side of the sample tube using a magnet stand. Incubate for 00:02:00 or until the beads have pelleted and the supernatant is completely clear.

2m





Then, while avoiding the bead pellet, carefully remove the clear supernatant.

#### Equipment

SPRIPlate 96R Ring Super Magnet Plate

NAME

96-well Magnet Plate

TYPE

Agencourt





BRAND

A32782

SKU


<https://www.beckman.com/supplies/plates/a32782>

LINK

22 Keeping on the magnetic plate, add  150  $\mu$ L of  Room temperature freshly made  80 % (v/v) ethanol to the side of the wall opposite to the pellet and let sit for  00:00:30 .


30s






23 Avoid disturbing the bead pellet, carefully remove and discard ethanol. Wait for  00:00:10 then remove any remaining ethanol.

10s



24  and repeat once.

25 Add 11  $\mu$ L of  10 mM Tris-HCl (Ph 8.0) and pipette to mix well. Incubate for  00:01:00 at  37  $^{\circ}$ C .

1m



 TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**



### Equipment

ThermoMixer® C

NAME

Eppendorf

BRAND


Catalog No. 2231000680

SKU

<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

LINK



- 26 Separate beads on the Agencourt SPRIPlate Super Magnet Plate for  00:02:00 or until the beads have pelleted.

2m



### Equipment

SPRIPlate 96R Ring Super Magnet Plate

NAME

96-well Magnet Plate

TYPE

Agencourt


BRAND

A32782

SKU

<https://www.beckman.com/supplies/plates/a32782>

LINK

- 27 Pellet the beads and transfer 10 µl of supernatant containing SARS-CoV-2 amplicons into a new tube. The eluted DNA can be used immediately or stored frozen at  -20 °C .



## Hackflex Library Preparation

2h

- 28 Combine the following components into a thin-walled PCR tube.





🧪 4  $\mu$ L 5X Hacklfex Buffer (20mM Tris, 20 mM MgCl, 50% DMF)

🧪 5.5  $\mu$ L Nuclease-Free Water

🧪 0.5  $\mu$ L Enrichment Bead-Linked Transposomes (eBLT)

🧪 10  $\mu$ L SARS-CoV-2 amplicons

🧬 Nextera DNA Flex Library Prep Kit **Illumina, Inc.**

🧬 NN-Dimethylformamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #227056-100ML**

🧬 Magnesium Chloride Solution 1 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1028**

29 Set up and run the following thermocycler program.

15m

1. Tagmentation: ⌚ 00:05:00 at 🌡 55 °C

2. Hold: 🌡 10 °C



#### Equipment

ThermoMixer® C

NAME

Eppendorf

BRAND

Catalog No. 2231000680

SKU

<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

LINK



30 Add 🧪 5  $\mu$ L of Hackflex Stop Buffer (0.2% SDS) to the sample.

1m





Corning 10% SDS (Sodium Dodecyl Sulfate) **Fisher**  
Scientific Catalog #MT-46040CI

31 Set up and run the following thermocycler program.

15m

1. Tagmentation: 00:05:00 at 25 °C

2. Hold: 10 °C



#### Equipment

ThermoMixer® C

NAME

Eppendorf

BRAND

Catalog No. 2231000680

SKU

<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

LINK



32 Place the plates on the Agencourt SPRIPlate Super Magnet Plate and incubate for 00:05:00 or until the beads have pelleted and the supernatant is completely clear. Then, while avoiding the bead pellet, carefully remove the clear supernatant.





## Equipment

SPRIPlate 96R Ring Super Magnet Plate

NAME

96-well Magnet Plate

TYPE

Agencourt







BRAND

A32782


SKU

<https://www.beckman.com/supplies/plates/a32782>

LINK

- 33 Keeping on the magnetic plate, add  30  $\mu\text{L}$  of  Room temperature Hackflex 30s  
Wash Buffer (10% PEG 8000, 0.25 M NaCl, 10mM Tris-HCL pH 8.0, 0.1mM EDTA) gently     
mix and then let sit for  00:00:30 .

 Poly(ethylene glycol) 8000 Merck MilliporeSigma (Sigma-Aldrich) EDTA 100mL Thermo Fisher Scientific Catalog #AM9260G

- 34 Separate beads on the Agencourt SPRIPlate Super Magnet Plate for  00:02:00 or 2m  
until the beads have pelleted.




## Equipment


SPRIPlate 96R Ring Super Magnet Plate	NAME
96-well Magnet Plate	TYPE
Agencourt	BRAND
A32782	SKU
<a href="https://www.beckman.com/supplies/plates/a32782">https://www.beckman.com/supplies/plates/a32782</a>	LINK

- 35 Avoid disturbing the bead pellet, carefully remove and discard HWB (10% PEG 8000, 0.25 M NaCl, 10mM Tris-HCL pH 8.0, 0.1mM EDTA).

30s



 Poly Ethylene Glycol (PEG) 8000 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #89510-250G-F**

 NaCl - 1Kg **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S3014-1KG**

 TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**


 EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**


- 36  and repeat once.


- 37 Resuspend beads in the following  20  $\mu$ L Indexing-PCR Master Mix

1m



 10  $\mu$ L NEBNext Q5U Master Mix (2X)

 1  $\mu$ L IDT for Illumina DNA/RNA UD Indexes

 9  $\mu$ L Nuclease-free Water

 NEBNext Q5U Master Mix – 50 rxns **New England Biolabs Catalog #M0597S**














 IDT for Illumina DNA/RNA UD Indexes **Illumina, Inc. Catalog #20027213**

38 Set up and run the following Indexing-PCR program.

20m



1. Initial Denaturation:  00:01:00 at  98 °C
2. 3-step PCR cycling (6X):
  - Denaturation:  00:00:15 at  98 °C
  - Annealing:  00:00:30 at  62 °C
  - Extension:  00:00:30 at  65 °C
3. Final Extension:  00:01:00 at  65 °C
4. Hold:  4 °C

#### Equipment

Veriti 96-Well Thermal Cycler

NAME


Applied Biosystems

BRAND



4375786

SKU

<https://www.thermofisher.com/order/catalog/product/4375786#/4375786><sup>LINK</sup>


39 After PCR, pool  10 µL of each sample into a single tube and vortex to mix.





40 Add an equal volume (1:1) of sparQ PureMag beads to the library pool and mix gently by either flicking or pipetting. For example, add  25 µL sparQ PureMag beads to a  25 µL reaction. Then pulse centrifuge to collect all liquid at the bottom of the tube.

1m




 sparQ PureMag Beads **Quantabio Catalog #95196-060**

41 Incubate for  00:05:00 at  Room temperature .

5m



42 Place the pool on a magnet and incubate for  00:02:00 or until the beads have pelleted and the supernatant is completely clear. Then, while avoiding the bead pellet, carefully remove the clear supernatant.

2m

### Equipment

#### Magnetic Stand

NAME

Magnetic Stand

TYPE

Thermo Scientific

BRAND




MR02

SKU

<https://www.thermofisher.com/order/catalog/product/MR02><sup>LINK</sup>


Any magnetic rack that fits your tubes will suffice.<sup>SPECIFICATIONS</sup>



43 Keeping the pool on the magnet, add  200  $\mu$ L of  Room temperature freshly made **[M]** 80 % (v/v) ethanol to the side of the wall opposite to the pellet and let sit for  00:00:30 .


30s



44 Avoid disturbing the bead pellet, carefully remove and discard ethanol. Wait for  00:00:10 then remove any remaining ethanol.

10s



45  and repeat once.

46

Add 25 µl of 

1M

 10 mM TE Buffer (10mM Tris-HCL pH 8.0, 0.1mM EDTA) and pipette to mix well. Incubate for 


00:01:00


 at 

37 °C

 .

1m



 TRIS 1M pH 8.0 **VWR International (Avantor) Catalog #E199-500mL**

 EDTA 100mL **Thermo Fisher Scientific Catalog #AM9260G**

## Quantifying Pool and Sequencing

47

Quantify final pool with adapted libraries. Load onto sequencer using platform appropriate dilutions.

Equipment	
MiSeq	NAME
Sequencer	TYPE
illumina	BRAND
SY-410-1003	SKU
<a href="https://www.illumina.com/systems/sequencing-platforms/miseq/order-miseq.html">https://www.illumina.com/systems/sequencing-platforms/miseq/order-miseq.html</a>	LINK
	