

21120.10517 SARS-CoV-2 NGS Whole Genome Sequencing (WGS) Procedure

Copy of version 3.0 (approved and current)

**Last Approval or
Periodic Review Completed** 07-Apr-2021

Controlled Copy ID 308480

**Next Periodic Review
Needed On or Before** 07-Apr-2022

Location client request

Effective Date 12-Apr-2021

Organization Eurofins - Viracor

Comments for version 3.0

Added Analysis instructions

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	(b) (6)	07-Apr-2021 8:55	3.0	(b) (6)	
				(b) (6)	
Approval	(b) (6) Approval	06-Apr-2021 12:30	3.0	(b) (6)	
				(b) (6)	
Approval	(b) (6) Approval	05-Apr-2021 17:12	3.0	(b) (6)	
				(b) (6)	
Approval	(b) (6)	24-Mar-2021 8:39	2.0	(b) (6)	
				(b) (6)	
Approval	(b) (6) Approval	23-Mar-2021 19:45	2.0	(b) (6)	

(b) (6)

Approval (b) (6) Approval 23-Mar-2021 10:10 2.0

(b) (6)

(b) (6)

Approval (b) (6) 17-Mar-2021 13:09 1.0

(b) (6)

(b) (6)

Approval (b) (6) Approval 17-Mar-2021 1:13 1.0

(b) (6)

(b) (6)

Approval (b) (6) Approval 15-Mar-2021 12:12 1.0

(b) (6)

(b) (6)

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
3.0	Approved and Current	Major revision	05-Apr-2021	12-Apr-2021	Indefinite
2.0	Retired	Major revision	22-Mar-2021	29-Mar-2021	12-Apr-2021
1.0	Retired	Initial version	14-Mar-2021	22-Mar-2021	29-Mar-2021

BioPharma Procedure for the SARS-CoV-2 Next-Generation Sequencing Assay for NP Swab Specimens in UTM

Table of Contents

INTENDED USE.....	2
TEST INFORMATION.....	2
METHOD PRINCIPLE.....	2
SPECIMEN REQUIREMENTS.....	2
REAGENTS AND MATERIALS.....	3
QUALITY CONTROL.....	3
EQUIPMENT AND SUPPLIES.....	4
PROCEDURE.....	5
ANALYSIS.....	16
PERFORMANCE SPECIFICATIONS.....	17
SAFETY.....	17
RELATED DOCUMENTS.....	18
REFERENCES.....	18

INTENDED USE

The intended use of the assay is to provide SARS-CoV-2 nucleotide sequence determination by Whole Genome Sequencing.

TEST INFORMATION

Assay name: SARS-CoV-2 WGS

Date test initially placed into service: 22-Mar-2021

METHOD PRINCIPLE

Whole Genome Sequencing

This assay uses Next Generation Sequencing to determine the nucleotide sequence of SARS-CoV-2. Nucleic acid extraction is performed followed by cDNA synthesis and ARTIC primer PCR. The samples are then pooled and purified using magnetic beads. After purification, samples are quantified with the Qubit Flex and diluted. Library Preparation and indexing is performed using the NEB Next Ultra II-FS Library Prep Kit and the Agilent Bravo. (b) (4)

(b) (4) the quality of the samples are checked with the Qubit Flex and concentrations are obtained for dilution to (b) (4). Libraries are then pooled and the base pair size determined with the Agilent 4200 TapeStation. Following base pair size determination, samples are denatured and loaded onto a sequencing reagent cartridge for processing on the Illumina NextSeq 550 instrument. After run completion, raw sequencing data is demultiplexed and analyzed with the (b) (4)

SPECIMEN REQUIREMENTS

Patient Preparation:

- No preparation is required.
- Specimen Collection and Transport

See SOP 21120.435 *Specimen Collection and Transport* for procedures for collecting the proper specimens and how to prepare the specimens for transportation to the laboratory.

Specimen Type and Handling

- NP Swab specimens received in UTM/VTM from clients/sites/central lab are the acceptable specimen types for this assay.
- NP Swab specimens in UTM/VTM specimens¹ are (b) (4)

(b) (4)

Extracted RNA should be stored frozen immediately following extraction if not preparing for RT-PCR within (b) (4)

(b) (4)

(b) (4)

REAGENTS AND MATERIALS

Table 3. Reagents and Materials			
Description	Source	Part/Cat No	Storage
NovaPrime (b) (4) RNA Extraction Kit	GSD	(b) (4)	Ambient
ARTIC PCR primer pools	(b) (4)		
(b) (4)			
(b) (4) ARTIC SARS-CoV-2	(b) (4)		
(b) (4)			
NEBNext ULTRA II FS DNA	New England BioLabs	E7805L	-15°C to -35°C
Purification Beads	Beckman Coulter	(b) (4)	
(b) (4)			
(b) (4)			
TapeStation (b) (4) tapes	Agilent	(b) (4)	
TapeStation (b) (4) reagents	Agilent	(b) (4)	
RNase-, DNase-free water	(b) (4)		
(b) (4) pipette tips	(b) (4)		
(b) (4) plates	(b) (4)		
(b) (4) PCR plates	(b) (4)		
(b) (4) Reagent Cartridge	(b) (4)		
(b) (4) Flow Cell	(b) (4)		
Buffer Cartridge	(b) (4)		
(b) (4) buffer	(b) (4)		
(b) (4)			

QUALITY CONTROL

The quality control program for this test is established in accordance with SOP 21120.517 *Analytical Quality Control: Quality Control Procedures*.

Quality control samples are prepared, and control ranges are established and maintained in accordance with SOP 21120.517 *Analytical Quality Control: Quality Control Procedures*.

Extraction Controls

Description	Source	Part/Cat No.	Storage / Expiration*
SARS-CoV-2 Whole Virus Positive Control	(b) (4)		
(b) (4)			
Twist RNA positive control	(b) (4)		

*: See SOP 21120.380 *Expiration Dating of Laboratory Materials* for proper expiration dating assignment.

Control Procedure

SARS-CoV-2 Positive and Negative controls are included on (b) (4)

SARS Twist RNA is used as a positive control during sequencing.

Whole Virus Positive Control: This control has an approximate concentration of (b) (4) copies/mL.

Negative control:

(b) (4)

Quality Control Acceptance Criteria/Repeat Criteria

Thoroughly document all QC failures or repeat testing on the SARS-CoV-2 Whole Genome Sequencing Test Record 21120.10518.

Proficiency Testing

Refer to SOP 21120.384 *Proficiency Testing Program* for information on proficiency testing.

EQUIPMENT AND SUPPLIES

Description
Biological Safety Cabinet, Class II
Fixed-angle benchtop centrifuge (with rotor for 2mL tubes)
Dead Air Box
2mL non-skirted (b) (4) tubes
Thermo Fisher KingFisher Flex Instrument
(b) (4) Thermal Cycler
(b) (4) PCR tubes or (b) (4) Optical 96-well Reaction Plate (standard plate)
(b) (4) caps or Optical Adhesive Covers
(b) (4) (b) (4) deep-well swing-bucket centrifuge
Agilent Bravo
Qubit (b) (4) Flex
Agilent 2200 or 4200 TapeStation and associated components and software
(b) (4) Optical Tube Strips (8x)
(b) (4) Optical Tube Strip Caps (8x)
(b) (4) vortexer
PCR strip tube mini-centrifuge
(b) (4) 96-well (b) (4) plate magnetic stand
(b) (4) High-speed micro-plate shaker
96-well conical-bottom (b) (4) plate
Illumina NextSeq Sequencer
Refrigerator capable of sustaining 2-8°C
Freezer capable of sustaining -15°C to -35°C
Freezer capable of sustaining -64°C to -90°C

PROCEDURE

Analytical

Print a blank copy of the 21120.10518 SARS-CoV-2 Whole Genome Next-Generation Sequencing Assay Test Record.

Print a copy of COV2 wgNGS Workbook Template from media lab or use the digital version which is located on

(b) (4)

(b) (4)

(b) (4)

the steps as specified below.

The plate maps will be used in

Record date and Run # (format: MMDDYY-SARS WGS).

Nucleic Acid Extractions are performed using the one of the following SOPs:

- A. Refer to 21120.9718 *KingFisher NovaPrime Nucleic Acid Extration* for all instructions for nucleic acid isolations.

(b) (4)

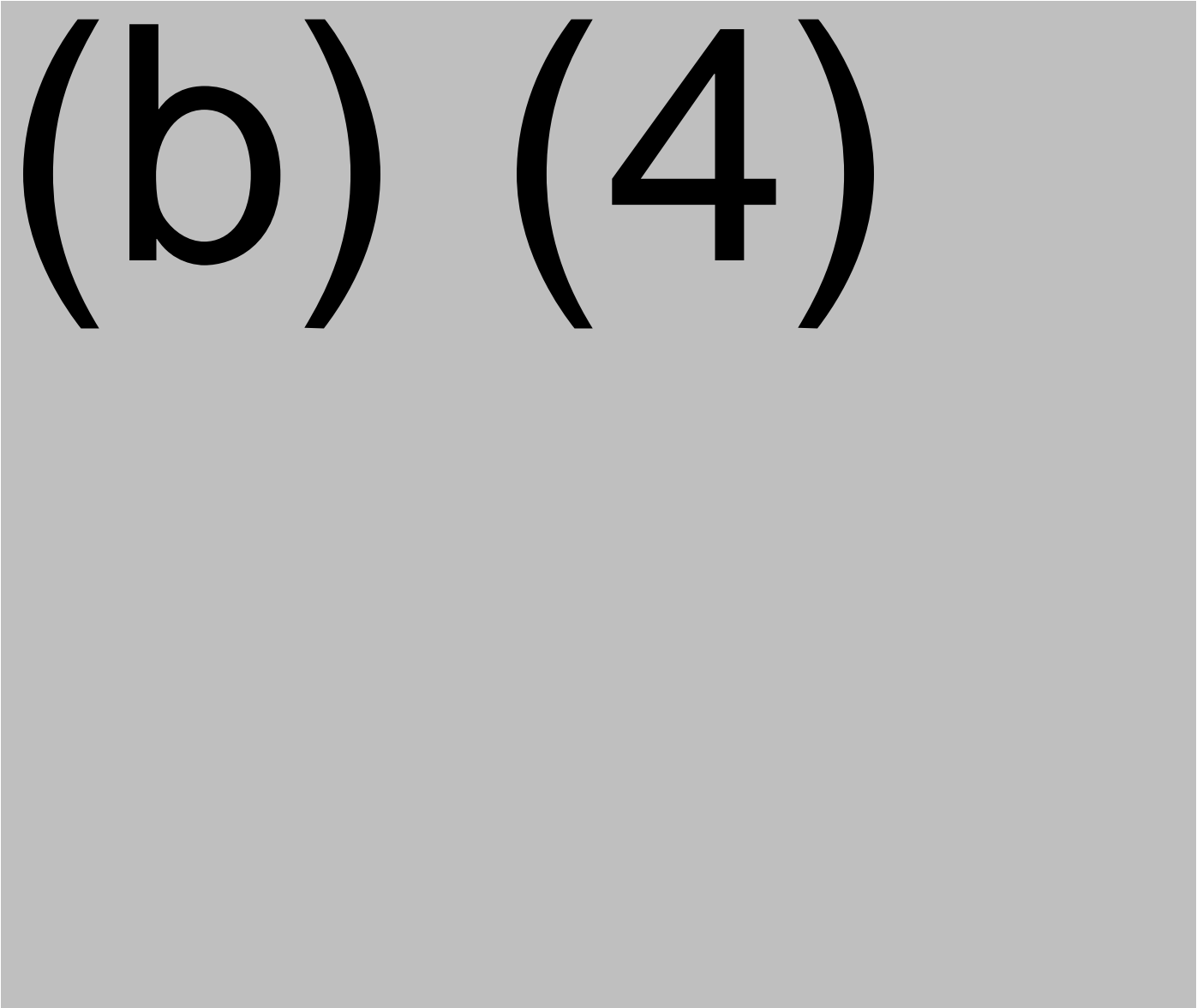
cDNA Synthesis

(b) (4)

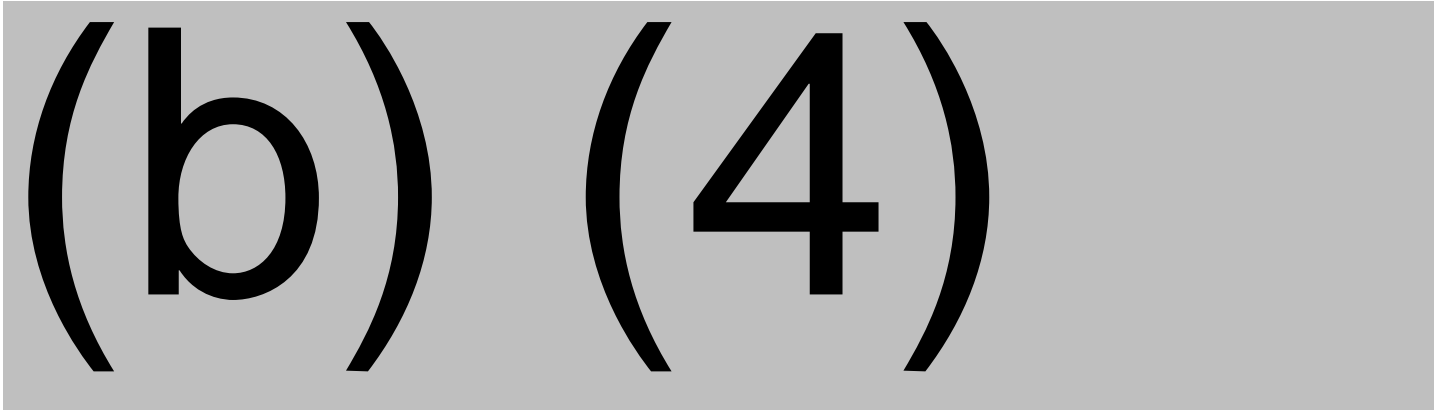
Print the report from the thermal cycler for the data packet.

ARTIC PCR

Use the ARTIC PCR plate maps.



Magnetic Bead Cleanup ^{(b) (4)}



(b) (4)

B. Manual Magnetic Bead Cleanup

(b) (4)

Qubit Quantification Procedure

(b) (4)

(b) (4)

(b) (4)

B. Manual preparation

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Library Pooling

(b) (4)

Library QC (Size Distribution QC and Normalization)

(b) (4)

TapeStation

(b) (4)

(b) (4)

Figure 1. TapeStation (b) (4)

(b) (4)

Denature and Dilute Libraries Procedure

15

(b) (4)

(b) (4)

(b) (4)

Analysis – SARS-CoV-2 Analysis

SARS CoV-2 WGS sequencing results will be analyzed with (b) (4) for the (b) (4) are summarized below (Figure 1). (b) (4)

Instructions

(b) (4)

Figure 2. (b) (4)

(b) (4)

SARS CoV-2 WGS Acceptance Criteria

Acceptance criteria for WGS will be positive samples must (b) (4) (b) (4)

General Acceptance Criteria for Controls

(b) (4)

PERFORMANCE SPECIFICATIONS

Not established

SAFETY

The following personal protective equipment will be applied as directed in currently effective Safety SOPs, including but not limited to, gloves, masks, lab coats, face shields and eye protection.

Personnel executing these procedures must be trained on effective Safety SOPs as listed in 21120.265 *Safety Program*.

This procedure requires the use of blood derived from human patients. Follow provisions of SOP 21120.238 *Bloodborne Pathogen Exposure Control Plan* when working with human blood products and disposing of potentially contaminated items.

Avoid contact of reagents with eyes, skin and clothing and follow the procedure in SOP 21120.237 *Chemical Hygiene Plan*.

See Safety Data Sheet (SDS) manual for further details regarding all reagents in the kit(s).

RELATED DOCUMENTS

21120.369 *Analytical Quality Control: Quality Control Procedures*

21120.370 *Analytical Quality Control: Preparation of Control Samples and Establishment of Control Ranges*

21120.384 *Proficiency Testing Program*

SOP 21120.9152 (b) (4) *Viral Pathogen Nucleic Acid Isolation*

SOP 21120.5983 *Agilent Tapestation Operation, Maintenance, and Calibration*

SOP 21120.7524 *Illumina NextSeq Operation, Maintenance, and Calibration*

21120.443 *Thermal Cycler Preventative Maintenance Operation, Maintenance and Calibration of the* (b) (4) *Thermal Cycler*

SOP 21120.269 *Records Management*

SOP 21120.9806 *NGS Data Retention*

21120.595 *Specimen Processing Guide*

21120.572 *Verification and Qualification of Critical Laboratory Materials*

21120.764 *Preparation and QC of Oligonucleotides*

21120.10121 *SARS-CoV-2 NGS Test Record*

21120.10181 *SARS-CoV-2 Spike NGS Reagent Log*

21120.6292 *SARS-CoV-2 Sequencing Assay easyMAG Run Map*

REFERENCES

None