Oct 18, 2021

UW Virology Swift SNAPv2 protocol

This protocol is a draft, published without a DOI.

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Protocol Citation: Lasata Shrestha, Hong Xie, Shah A. Mohamed Bakhash, Robert J. Livingston, Meei-Li Huang, Alexander L. Greninger, Pavitra Roychoudhury 2021. UW Virology Swift SNAPv2 protocol. **protocols.io https://protocols.io/view/uw-virology-swift-snapv2-protocol-byw4pxgw**

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

Created: October 09, 2021

Last Modified: October 18, 2021

Protocol Integer ID: 53948

Keywords: whole genome sequencing, SARS-CoV-2, SWIFT Biosciences, PerkinElmer, automation, genome, protocol viral whole genome sequencing, multiplex amplicon method, amplicon, amplifies 345 amplicon, complete genome, multiplex amplicon panel, instrumental in outbreak investigation, outbreak investigation, vaccine, evaluation of vaccine

Abstract

Viral whole genome sequencing (WGS) has been instrumental in outbreak investigations, deployment of public health interventions, development as well as evaluation of vaccines and therapeutics. While multiple methods are commercially available for WGS, multiplex amplicon method has proven to be faster, more efficient, scalable, and more cost-effective compared to other methods. Here, we describe the automation of a multiplex amplicon panel for WGS of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the causative agent of the current COVID-19 pandemic. The SWIFT Biosciences' primer set amplifies 345 amplicons designed against the SARS-CoV-2 Wuhan-Hu-1 complete genome (NC_045512.2), in a single tube to cover the ~30 kb SARS-CoV-2 genome in less than three hours.

Materials

- SuperScript IV Kit **Kit content**
- RNaseOUT™ Recombinant Ribonuclease Inhibitor **Kit details**
- SNAP SARS-CoV-2 Kit **Kit content Page: 4**
- SPRIselect beads https://www.beckman.com/reagents/genomic/cleanup-and-size-selection/size-selection
- 200 Proof Ethanol
- Molecular-grade water
- Qubit®, Nanodrop, or other similar input RNA quantification assay
- qPCR-, electrophoretic-, or fluorometric-based library quantification assay for Illumina® libraries
- Microcentrifuge
- Vortex
- Programmable thermocycler
- Aerosol-resistant tips and pipettes ranging from 1 to 1000 µL
- Pipette tips (e.g., 8-channel or 12-channel), 8-tube strips, an un-skirted 96 well plate, or plate puncher for pre-piercing the foil seal if using single-use UD indexing plates.
- Qubit dsDNA HS Assay Kit https://www.thermofisher.com/order/catalog/product/Q32851#/Q32851
- TapeStation DNA ScreenTape & Reagents https://www.agilent.com/en/product/automated-electrophoresis/tapestation-systems/tapestation-dna-screentape-reagents/dna-screentape-analysis-228260
- 80 uL - Barrier Sterile 96 Rack Tips https://www.perkinelmer.com/product/80ul-art-sterile-96-rack-10-racks-111624
- 150 uL - Barrier Sterile 96 Rack Tips https://www.perkinelmer.com/product/150ul-96-art-tip-box-10-racks-111426
- Polypropylene 384-well microplate, U-Bottom, case of 50 https://www.perkinelmer.com/product/pp-microplate-384-35ul-u-bottom-50-6008890
- Polypropylene 96-well Microplate, Deep Well U-bottom, 2 mL https://www.perkinelmer.com/product/pp-microplate-96-2ml-v-bottom-25-6008880
- HARDSHELL PCR PLATE-96, BLUE/ 50 https://www.perkinelmer.com/product/hardshell-pcr-plate-96-blue-50-6008870
- StorPlate-96V, PP, 96 well, V-bottom, (V), 450µL, 200/box https://www.perkinelmer.com/product/storplate-96-v-450-l-200-6008299
- PP RESERVOIR, DW, V, 12 COL, 21mL /25 https://www.perkinelmer.com/product/pp-reservoir-dw-v-12-col-21ml-25-6008700

Before start

The critical steps are tagged. Steps 5-108 are optional (for troubleshooting).

Single-strand cDNA synthesis Reagent Set-up

1 Prepare reagents for required number of samples as per the manufacturer's instructions (including 10% overage) linked below.
https://assets.thermofisher.com/TFS-Assets/LSG/manuals/SSIV_Reverse_Transcriptase_UG.pdf

Single-strand cDNA (sscDNA) synthesis Reagent Sciclone Set-up

2 Select the correct protocol on the liquid handler (Perkin Elmer Sciclone) and load the Sciclone as instructed.

This program will perform steps corresponding to steps 3 to 20 in the manual protocol. The code can be obtained from PerkinElmer.

Note

The SuperScript RT IV protocol was automated and optimized by Perkin Elmer personnel following ThermoFisher's commercially available protocol linked below.
SuperScript RT IV

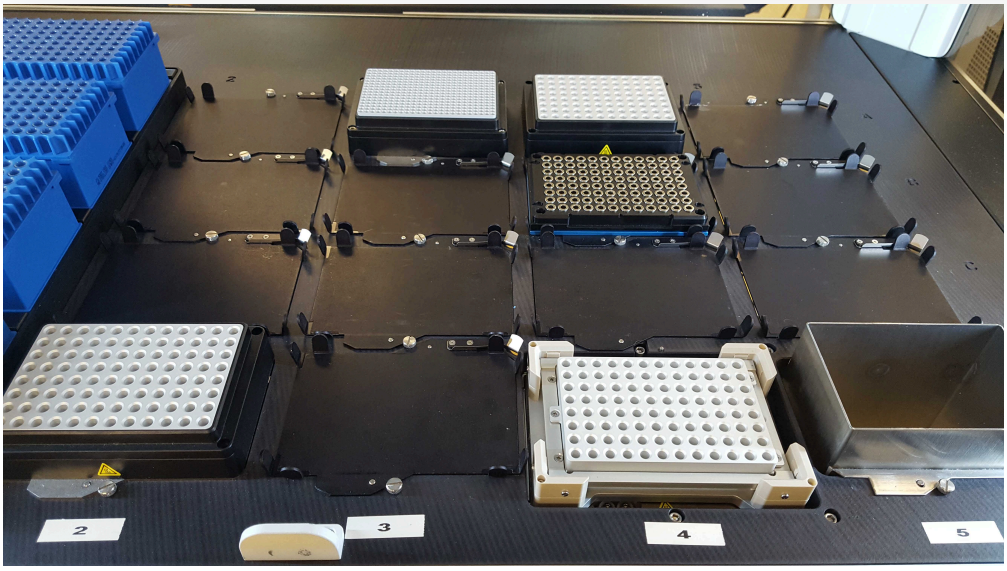

Fig. 01: Empty Sciclone deck.

Equipment

Sciclone G3 NGSx iQ Workstation	NAME
Automated Liquid Handling	TYPE
Perkin Elmer	BRAND
CLS145321	SKU
https://www.perkinelmer.com/category/automation-liquid-handling-instruments	LINK

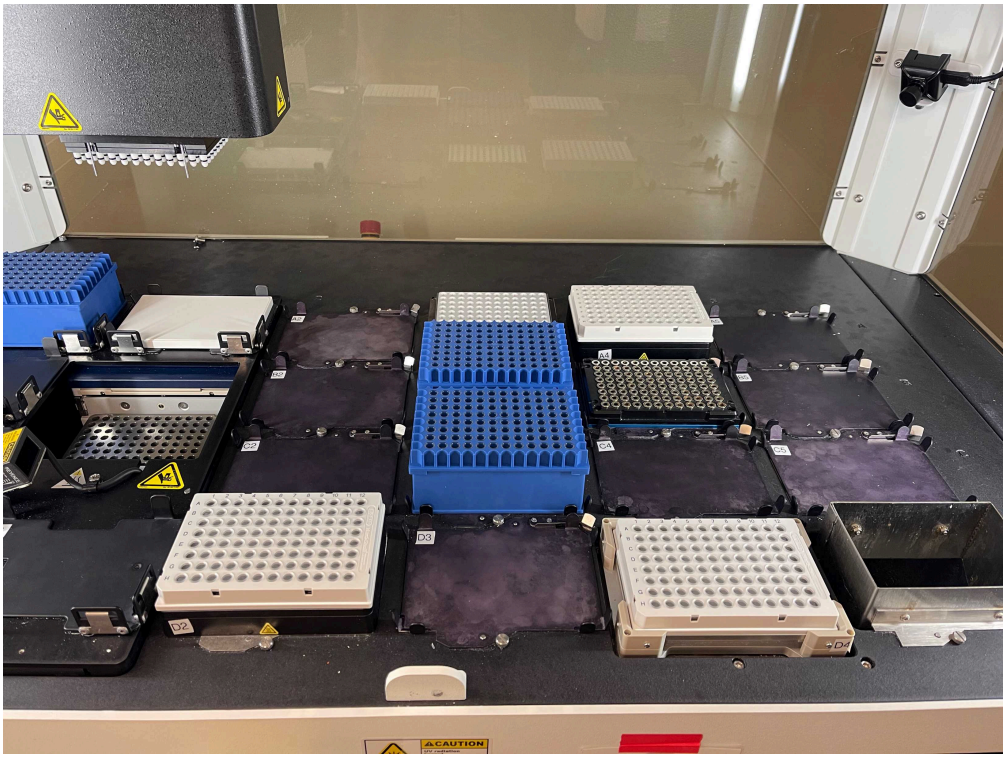

Fig. 02 Final (fully set-up) Sciclone desck set-up for sscDNA synthesis.

Sciclone movement: sscDNA synthesis

3 Moved Plate from D4 to B4 (magnet with no spacer)

4 Moved Plate from D2 to D4

5 Aspirate 11 µL of RNA from B4 and Dispense to plate on D4

6 Pipette mix and shake for 00:01:00 1m

6.1 Plate on B4 moved to C0 concurrently

6.2 Plate on C0 discarded

7 Moved plate from D4 to onboard thermocycler

8 Move ODTC lid from A1 to thermocycler

9 Thermocycler door closes and runs 00:07:40 7m 40s

10 Thermocycler door opens

11 ODTC lid moved from Thermocycler to A1

12 Moved plate from Thermocycler to D4

12.1 Empty tip box discarded. Fresh tip box placed onto C3

13 Load new tips

14 Aspirate 7 µL of PCR master mix from A4 and dispense into plate on D4

14.1 Pipette mix and shake for 00:01:00 1m

15 Move plate from D4 to onboard thermocycler

16 Move ODTC lid from A1 to thermocycler

17 Thermocycler door closes and runs for 00:53:04 53m 4s

18 Thermocycler door opens

19 Plate moved from thermocycler to D4

20 End product of 20 µL

Swift SNAP v2 Reagent Set-up

21 Prepare reagents for required number of samples as per the manufacturer's instructions (including 10% overage) linked below.
Swift Normalase™ Amplicon Panels (SNAP) SARS-CoV-2, Additional Genome Coverage, and SARS-CoV-2 S Gene Panel

Swift SNAP v2 Sciclone Set-up

22 Select the correct protocol on the liquid handler (Perkin Elmer Sciclone) and load the Sciclone as instructed.

The selected program will perform steps corresponding to steps 23 to 127. The code can be obtained from PerkinElmer.

Note

The SWIFT SNAP v2 protocol was automated and optimized by Perkin Elmer personnel following SWIFT Bioscience's commercially available protocol linked below.
SWIFT SNAP v2

Insert labeled photo of the deck without components (deck only)

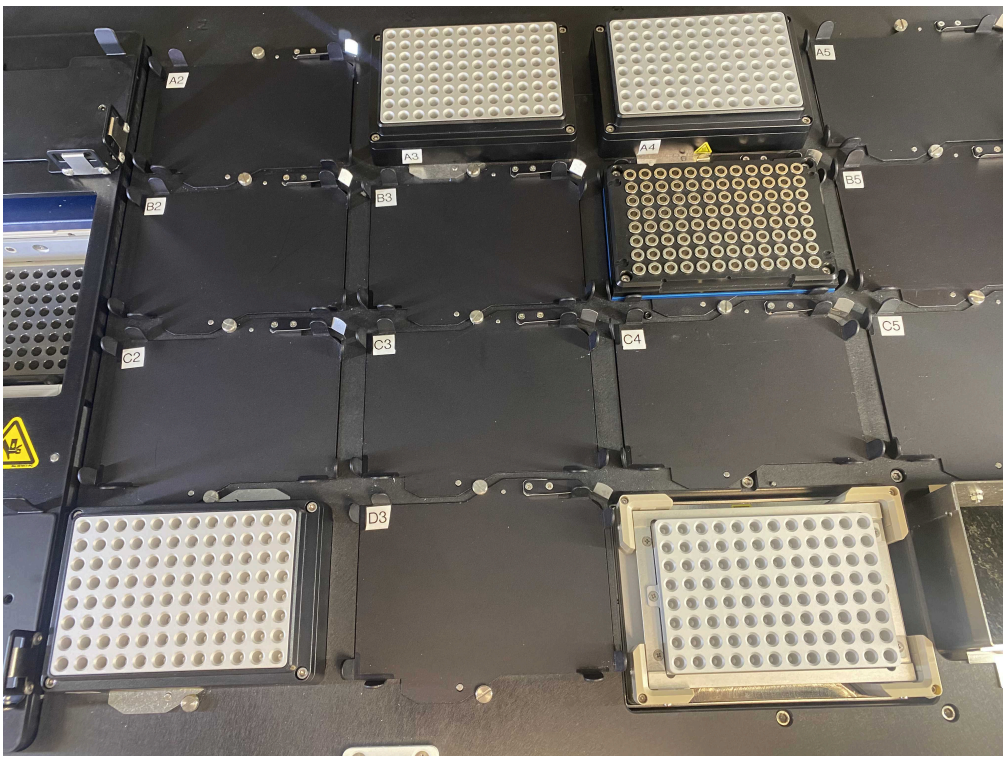

Fig. 03. Empty Sciclone deck.

Insert labeled photo of the deck with labeled components and volume information

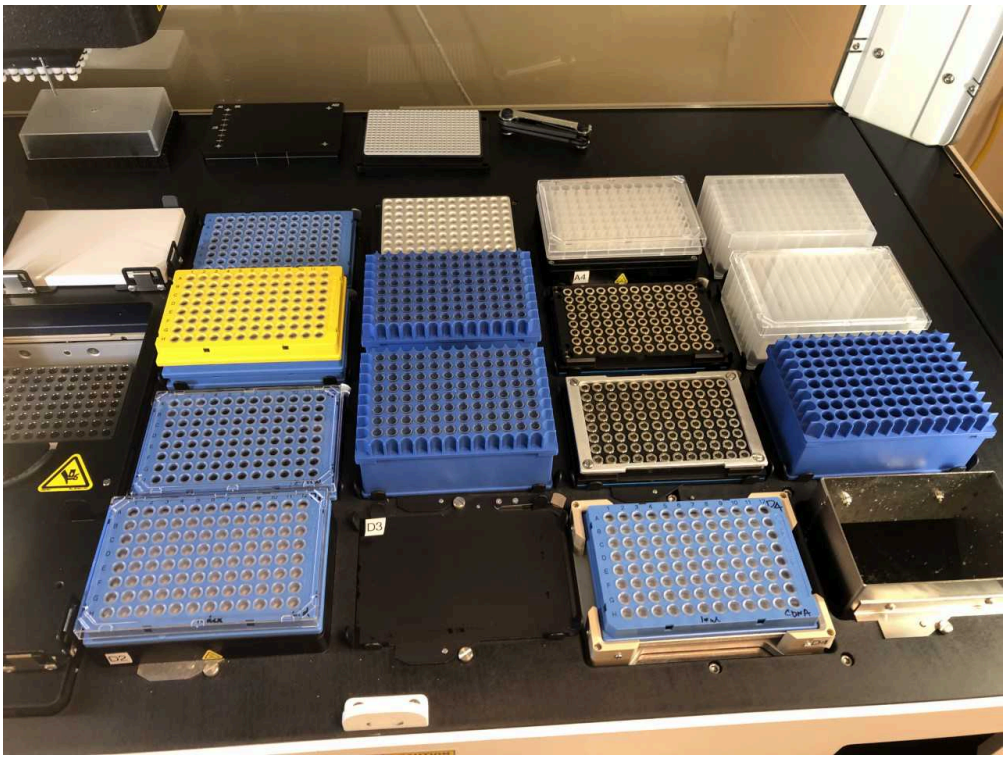

Fig. 04. Final (fully-loaded) deck set-up for SWIFT SNAP v2 protocol on the Sicclone.

Equipment	
Sicclone G3 NGSx iQ Workstation	NAME
Automated Library Preparation	TYPE
Perkin Elmer	BRAND
********	SKU
https://www.perkinelmer.com/category/automation-liquid-handling-instruments	LINK

Sicclone Movements: Swift SNAP v2 Multiplex PCR

1h 20m

23

Note

Steps 23-127 include detailed Sciclone deck movements for troubleshooting.

24 Moved Lid from A4 to A2

25 Pipette multiplex master-mix (A4 to samples on D4)

26 Dispose off tips

27 Lid from A2 to A4

28 Mix samples on D4

29 Tips disposed

30 Shake mix of D4 (Noted: 30uL)

31 D4 to Thermocycler

32 ODTC lid placed

33 Thermo-cycler cover closed 01:15:00 1h 15m

34 PCR door opened

35 ODTC lid removed

36 The PCR plate was moved to D4

37 Moved plate from B2 to A3

38 Mix beads in B2, 10 X

39 Picked up 30 uL Beads from B2 to D4 30 µL Beads

40 Mixed D4 wells, 10 X

41 Shake mix D4 for several minutes

42 Plate moved from D4 to B4

43 Allow beads to sediment 00:05:00 5m

44 Supernatant discarded

45 Tips discarded

46 Alcohol cover B5 removed to C5

47 Fresh tips picked.

48 Picked up 80%ETOH 150 µL 80% Ethanol

49 Dispensed to D2

50 Supernatant removed to Wash plate

51 Tips disposed

52 Fresh tips picked

53 Alcohol (150uL) dispensed to plate B4 150 µL

54 Removed supernatant from B4

55 Tips discarded

56 Fresh tips picked

57 Alcohol transferred to B4 150 µL 80% EtOH

58 Supernatant collected and disposed

59 Tips discarded

60 Alcohol lid replaced back

61 Sample plate moved to D4

62 New tips

63 TE buffer lid

64 TE buffer added 17.4 µL NEED TO CHECK

65 Mixed

66 Tips discarded

67 Plate D4 shake mixed

68 Lid from A4 lifted

69 Transfer master mix A4 to A3 28.9 µL Indexing PCR Reaction Mix

70 Tips disposed

Sciclone Movements: SWIFT SNAP v2 Indexing PCR

1h 20m

71 Pipette Index from D2 to A3 (3.7 uL of indexes)

72 Pipette master mix plus indexes from A3 to D4

73 Shake mixed D4

74 Lid of A2 to A4

75 Fresh tips

76 D4 mixed by pipetting

77 Tips disposal

78 D4 shake mixed

79 D4 plate moved to thermocycler

80 21 min PCR run 00:21:00 21m

81 Incubation 26 min 00:26:00 26m

82 Thermocycler door open

83 OPTC lid removed

84 Sample plate moved to D4

85 Beads plate moved from B2 to D3

86 Pipetted PEG NaCl from plate on B2 to plate on D4 32.5 µL PEG NaCl (ratio: 0.65)

87 Mixing in D4 by pipetting (10 times)

88 Shake mixed D4, for 4 min 35 sec 00:04:35 4m 35s

89 Plate moved from D4 to C4 (on the magnet)

90 Incubation at room temperature 00:05:00 5m

91 Supernatant from C4 discarded

92 Tips discarded

93 Alcohol lid removed

94 New tips picked

95 Picked up alcohol 150 µL 80% EtOH

96 Transferred alcohol to C4

97 1 min wait

98 Supernatant discarded

99 Tips dropped off

100 New tips picked up

101 Picked up alcohol 150 µL 80% EtOH

102 Dispensed to C4

103 1 min wait

104 Supernatant discarded

105 Tips discarded

106 Moved Tip container from C3 to D0

107 New tip container picked up from A0

108 New tips picked up

109 Alcohol picked up 150 µL 80% EtOH

110 Dispensed alcohol to C4

111 1 min wait

112 Supernatant from C4 pipetted up and discarded

113 Tips discarded

114 Alcohol cover replaced

115 2 min wait (incubate at room temperature until residual alcohol evaporates)

116 Moved Plate from C4 to D4

117 Picked up TE

118 Transfer TE to D4 20 µL TE

119 Mixing

120 Tips discarded

121 Mix by pipetting and shaking in plate D4

122 Moved plate D4 to B4

123 Moved empty Plate A2 to D4

124 Tips picked up

125 Transfer from B4 to D4 20 µL Eluate

126 Tips discarded

127 Plate lid from A4 to D4

Library Quality Control I

128

Following the manufacturer's instructions for library quality control, quantify (Qubit or other fluorometric instruments) and determine the size (TapeStation or other electrophoretic instruments) of the library.

Equipment

4200 TapeStation System

NAME

Electrophoresis tool for DNA and RNA sample quality control.

TYPE

TapeStation Instruments

BRAND

G2991AA

SKU

https://www.agilent.com/en/product/automated-electrophoresis/tapestation-systems/tapestation-instruments/4200-tapestation-system-228263

LINK

Equipment

Invitrogen™ Qubit™ 3 Fluorometer

NAME

Accurately measures DNA, RNA, and protein using the highly sensitive fluorescence-based Qubit quantitation assays

TYPE

Invitrogen™ Q33216

BRAND

Q33216

SKU

https://www.fishersci.co.uk/shop/products/qubit-3-0-fluorometer/15387293

LINK

Normalase

129 Following manufacturer's instructions as linked below, normalize the libraries using SWIFT's proprietary enzymatic normalization.
Normalase Pages: 9-12

Pooled Library Quality Control II

130 Determine the library concentration for final library quality control before loading on the sequencer.
Quality Control Page: 12

Equipment

Invitrogen™ Qubit™ 3 Fluorometer	NAME
Accurately measures DNA, RNA, and protein using the highly sensitive fluorescence-based Qubit quantitation assays	TYPE
Invitrogen™ Q33216	BRAND
Q33216	SKU
https://www.fishersci.co.uk/shop/products/qubit-3-0-fluorometer/15387293	LINK

Sequencing

131 Following manufacturer's recommendation, load the pooled library for sequencing on the selected/preferred sequencer.
Sequencing Page: 13

Equipment

NextSeq 500 System	NAME
Sequencer	TYPE
Illumina	BRAND
*********	SKU
https://www.illumina.com/systems/sequencing-platforms/nextseq.html	LINK