Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel

Tecan™ Fluent™ 780 Automation Workstation

Catalog Number A51305

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IMPORTANT! This user bulletin is designed for experienced users of the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay and Tecan™ Fluent™ 780 Automation Workstation. For additional information, see the *Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay User Guide* (Pub. No. MAN0024915) and *FluentControl Manual* (Pub. No. BG/N 30135092.04).

Note: For safety and biohazard guidelines, see the “Safety” appendix in the FluentControl Manual (Pub. No. BG/N 30135092.04). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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Description

This user bulletin describes how to prepare Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GS Manual libraries using Tecan™ Fluent™ 780 Automation Workstation. The workflow for library preparation described in this bulletin is similar to the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay workflow. Additional steps to set up the Tecan™ Fluent™ 780 Automation Workstation and import and run the scripts are described.

For more information about the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay, see the *Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay User Guide* (Pub. No. MAN0024915).

For detailed instructions for using the Tecan™ Fluent™ 780 Automation Workstation, see the *FluentControl Manual* (Pub. No. BG/N 30135092.04), available by contacting the Tecan Group at **https://lifesciences.tecan.com/**.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GS Manual	A51305
Ion Torrent™ NGS Reverse Transcription Kit	A45003
Instruments and Equipment	
Tecan™ Fluent™ 780 Automation Workstation	**http://tecan.com**
One of the following thermal cyclers: • GeneAmp™ PCR System 9700[1] or GeneAmp™ PCR System 9700 96-Well[1] • 2720 Thermal Cycler[1] • Veriti™ 96-Well Thermal Cycler • ProFlex™ 96-well PCR System	See web product pages
MicroAmp™ Splash-Free 96-Well Base	4312063
MicroAmp™ Optical Film Compression Pad	4312639
Alpaqua™ 96S Super Magnet	A001322 (Alpaqua™)
96-well plate centrifuge	MLS
Reagents and consumables	
150 µL MCA Disposable Tips	30180837 (Tecan)
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483354, 4483352
MicroAmp™ Clear Adhesive Film	4306311

(continued)

Item	Source
Agencourt™ AMPure™ XP Reagent	A63880, A63881, or A63882 (Beckman Coulter™)
Nuclease-free water	AM9932
70% v/v Ethanol solution	PB82011
Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate, Sterile	3960 (Corning™)
Agilent™ 300 mL Reservoir	201244100 (Agilent™)
One or more of the following kits for nucleic acid isolation and quantification	
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
PureLink™ Genomic DNA Mini Kit	K1820-00
(Recommended for DNA quantification) TaqMan™ RNase P Detection Reagents Kit	4316831
(Recommended for RNA quantification) Qubit™ RNA HS Assay Kit	Q32852 or Q32855
Barcodes	
Ion Xpress™ Barcode Adapters Kit	Various
IonCode™ Barcode Adapters 1–384 Kit	A29751
Ion Torrent™ Dual Barcode Kit 1–96	A39360
One or more of the following kits for quantification	
Ion Library TaqMan™ Quantitation Kit	4468802
Ion Library Equalizer™ Kit	4482298
If you are not using the Ion Library Equalizer™ Kit for library normalization, select one of the following kits:	
Qubit™ Fluorometer[2] and Qubit™ dsDNA HS Assay Kit	Q33238, Q32851 or Q32854
Agilent™ 2100 Bioanalyzer™ and Agilent™ High Sensitivity DNA Kit	G2939BA, 5067-4626 (Agilent™)

[1] Supported but no longer available for purchase.

[2] Qubit™ 2.0 Fluorometer or later

Recommended materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Recommended for SARS-CoV-2 Quantification	
One of the following: • TaqMan™ 2019-nCoV Assay Kit v1 • TaqPath™ COVID-19 Combo Kit • TaqPath™ COVID-19 CE-IVD RT-PCR Kit	• A47532 • A47814 • A51738
Recommended for RNA dilution	
THE RNA Storage Solution	AM7000

Before first use—Import script

Scripts are instructions for the Tecan™ Fluent™ 780 Automation Workstation. You must download the appropriate script for preparing Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel libraries.

1. Open the Tecan™ FluentControl™ Software.
2. Select **Database ▸ Import...**.
3. Select **AmpliSeq SARS-CoV-2 Insight Research Assay 780.zeia**.
4. Press **Add all ▸ Import**, remove any detected conflicts, then, if prompted, select **Yes** to import without the missing referenced files.

Sample preparation guidelines

Guidelines for sample quality, viral copy number, and variant calling

- The amount of viral RNA among samples should be approximately equivalent so that the target amplification conditions you select are optimal for all samples.
- Ensure that RNA samples were quantified using one of the following kits.
  - TaqPath™ COVID-19 Combo Kit (Cat. No. A47814)
  - TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Cat. No. A51738)
  - TaqMan™ 2019-nCoV Assay Kit v1 (Cat. No. A47532)

- A sample containing as little as 50 copies of viral RNA after isolation (25 copies per target amplification reaction) can be used to prepare an Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel library. For optimal results, we recommend a viral copy number in the 100 to 200,000 range, or an amount of *total* RNA between 1–10 ng.

Table 1 Sample quality and viral copy number

Viral copy number	Recommendations and guidelines
200 to 200,000 copies	Recommended range for optimal results.
50 to 200 copies	Only for high-quality samples without degradation. We recommend sequencing and variant detection with a minimum allele frequency of 20%. For more information about the minimum allele frequency, see the Torrent Suite™ Software Help.

- To reliably sequence low quality samples, the samples must have a viral copy number ≥200 copies per reaction. For partially degraded samples, which likely includes low titer samples, the effective copy number that can be amplified by the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel is lower than the viral copy number detected by qPCR because the qPCR products are shorter than the 250 bp fragments generated by the panel.
- Even for samples with viral titers >200 copies per reaction, you may observe reverse transcription-derived false positives if you decrease the minimum allele frequency cutoff below 0.2 (20%). Reverse transcription-related errors occur randomly across the genome. To minimize calling false-positives, be certain to amplify a sufficient number of RNA molecules and set the minimum allele frequency to at least 20%.
- See the *Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay User Guide* (Pub. No. MAN0024915) for recommended RNA isolation and quantification kits.

Copy number determination by qPCR

Note:

- If your qPCR data give a different relationship between C_t and copy number, this is likely a result of differences in the baseline or threshold selected. Determine the copy number of a sample according to the known copy number in control reactions.
- We recommend basing copy number on the N Protein C_t value.
- If the N Protein C_t value is not accurate, use the S Protein or ORF1ab C_t values to determine copy number.
- The copy number is only an estimate.

Table 2 Approximate copy number to C_t conversion—TaqMan™ 2019-nCoV Assay Kit v1

Tier	Viral copy number	TaqMan™ C_t		
		N Protein	S Protein	ORF1ab
Low	50–1500	26–31	28–33	29–34
Medium	1500–50000	21–26	23–28	24–29
High	50000–1500000	16–21	18–23	19–24

Table 3 Approximate copy number to C_t conversion—TaqPath™ COVID-19 RT-PCR kits

Tier	Viral copy number	TaqPath™ C_t		
		N Protein	S Protein	ORF1ab
Low	50–1500	25–29	24–29	24–29
Medium	1500–50000	20–25	19–24	19–24
High	50000–1500000	15–20	14–19	15–19

Guidelines for RNA isolation, quantification, and input

- For recommended kits for isolating RNA, see “Required materials” on page 2.
- Each reverse transcription reaction requires 1–100 ng of DNase-treated RNA (≥0.14 ng/µL), prepared from normal or formaldehyde- or paraformaldehyde-fixed paraffin-embedded (FFPE) tissue.
- For quantifying RNA, we recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32852 or Q32855).
- In general, the library yield from high-quality RNA is greater than from degraded samples. Library yield is not indicative of sequencing performance.
- Having more RNA starting material generally results in higher quality libraries. However, if RNA is not degraded, high-quality libraries can be generated from as little as 1 ng starting material.

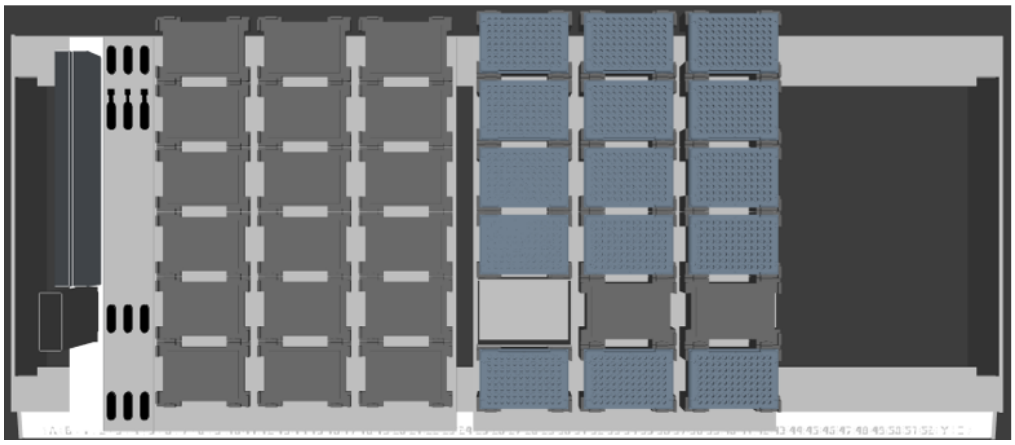
Prepare Instrument

Guidelines for Tecan Fluent

The procedure described in this user bulletin has many steps based on a specific configuration of a Tecan™ Fluent™ 780 Automation Workstation. If you are using any other configuration you must confirm performance through simulated runs and wet runs using mock solutions before testing real samples.

Sets of sample plates are grouped according to standard Tecan nomenclature such that Labware ending in the same [xxx] is part of the same sample set. For example, RNA[001] is processed in RT[001] and RNA[002] is processed in RT[002]. The worktable has been setup to have sample groups placed within the same site position on neighboring grid segments.

Note: For detailed information about grid locations and configurations, see the *FluentControl Manual* or contact support.



Segment description	Grid location
Waste Thru Trough 8x100ml RL 00	1
(Optional) Segment Deck 3 Grids	3
6 Landscape 7mm Nest	6
6 Landscape 7mm Nest	12
6 Landscape 7mm Nest	18
Empty	24
4 Landscape 61mm Nest Thru Deck Waste (with additional 61mm Nest at Site 1)	25
6 Landscape 61mm Nest	31
6 Landscape 61mm Nest	37

Prepare reagent master plates

Aliquot Ion AmpliSeq™ reagents into MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates.

For details, see “Minimum fill volumes required” on page 15.

Recommended reagent substitutions for calibration

Use the following reagent substitutions during initial setup of the Tecan™ Fluent™ 780 Automation Workstation for calibration. You can also use the recommended reagent substitutions for troubleshooting of any performance problems and inconsistencies.

Reagent	Recommended substitution
DNA	Water
Primers	
Ion Torrent™ NGS 10X RT Enzyme Mix	50% glycerol solution in water
Ion Torrent™ NGS 5X Reaction Buffer	
FuPa Reagent	
DNA Ligase	
5X Ion AmpliSeq™ HiFi Mix	40% glycerol solution in water
Switch Solution	20% Polyethylene Glycol 8000 (PEG-8000) solution in water
Agencourt™ AMPure™ XP Reagent	

Tip handling

The worktable is setup with 18 MCA tip boxes and is sufficient to complete all scripts without intervention. The MCA tips do not need to be replaced between run and the script prompts if a tip refresh is required for the next run.

Start the Tecan Fluent

1. Power on the Tecan™ Fluent™ 780 Automation Workstation.
2. Open Tecan™ FluentControl™ Software.
3. If prompted, enter your username and password.
4. Initialize the instrument by selecting **Run ▸ Initialize Instrument**.

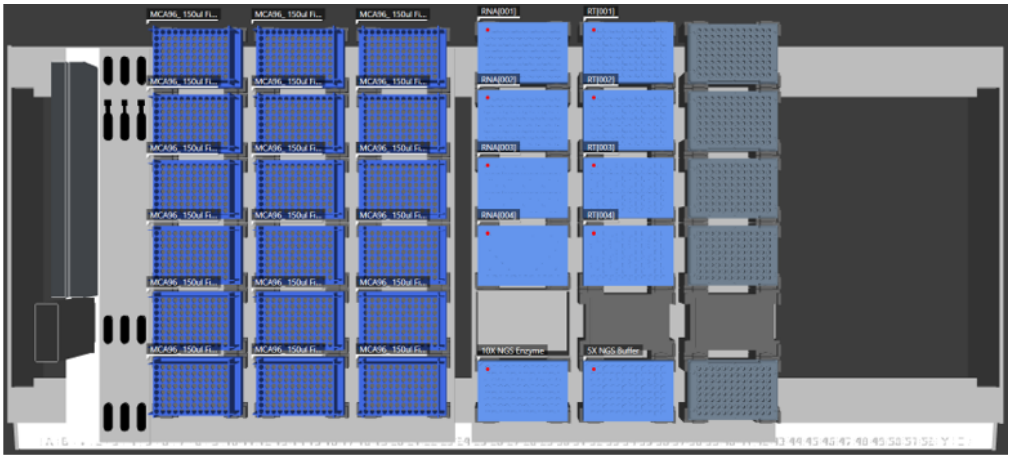
Reverse transcribe RNA with the Ion Torrent™ NGS Reverse Transcription Kit

1. Follow the alternate reverse transcription protocol in the *Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay User Guide* (Pub. No. MAN0024915).

2. In the Tecan™ FluentControl™ Software, touch , then select **AmpliSeq SARS-CoV-2 Reverse Transcribe** to start the run. Alternatively, the method can be started by selecting the method within the Tecan™ FluentControl™ Software.

3. Using the touch screen, select the number of plates to run. Follow the prompts for placement of the plates and minimum required fill volumes for reagents. The setup and volumes are matched to the number of plates being processed.

   The estimated run time for 4 plates is 7 minutes.



Perform thermal cycling using conditions in the following table.

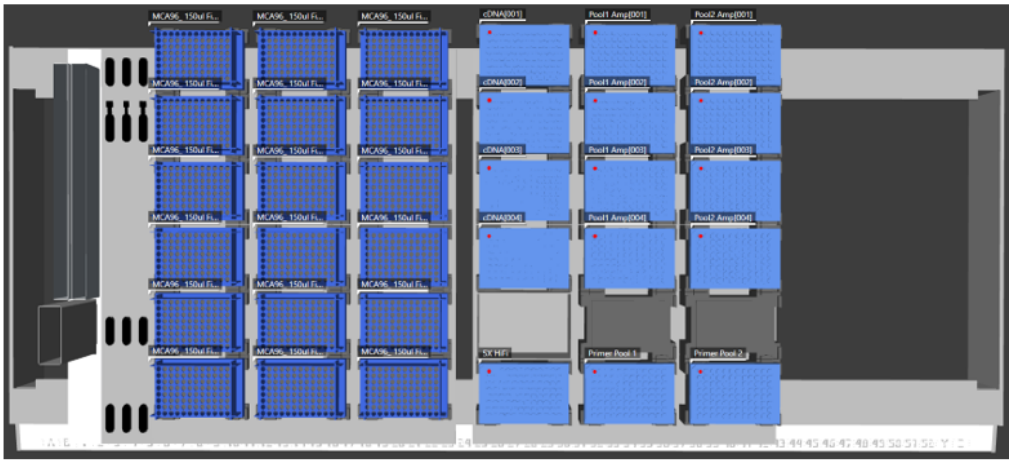
Temperature	Time
25°C	10 minutes
50°C	10 minutes
85°C	5 minutes
10°C	Hold

Prepare cDNA target amplification reactions

1. Touch , then select **AmpliSeq SARS-CoV-2 Amplify cDNA** to start the run. Alternatively, the method can be started by selecting the method within Tecan™ FluentControl™ Software.

2. Using the touch screen, select the number of plates to run. Follow the prompts for placement of the plates and minimum required fill volumes for reagents. The setup and volumes are matched to the number of plates being processed.

   The estimated run time for 4 plates is 13 minutes.



3. Run the following program to amplify the target regions.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	98°C	2 min
Cycle; set number according to Table 4	Denature	98°C	15 sec
	Anneal and extend	60°C	4 min
Hold	—	10°C	Hold

Table 4 Recommended cycle number

Tier	Viral copy number	Number of amplification cycles
Low	50–1,500	26
Medium	1,500–50,000	20
High[1]	50,000–1,500,000	15

[1] If titers are above 1,500,000 copies, samples can be diluted.

Cycle number recommendations in the preceding table are based on qPCR quantification of viral copy number. Without qPCR quantification, use the following guidelines to determine optimal cycle number empirically.

- Low viral load suspected: 26 cycles.
- High viral load suspected: 20 cycles.
- Isolates or enriched viral particles: ~15 cycles for 2 ng input.

If you are working with samples with **unknown viral load**, and cannot quantify using qPCR, use 20 target amplification cycles as a starting point for manual library preparation.

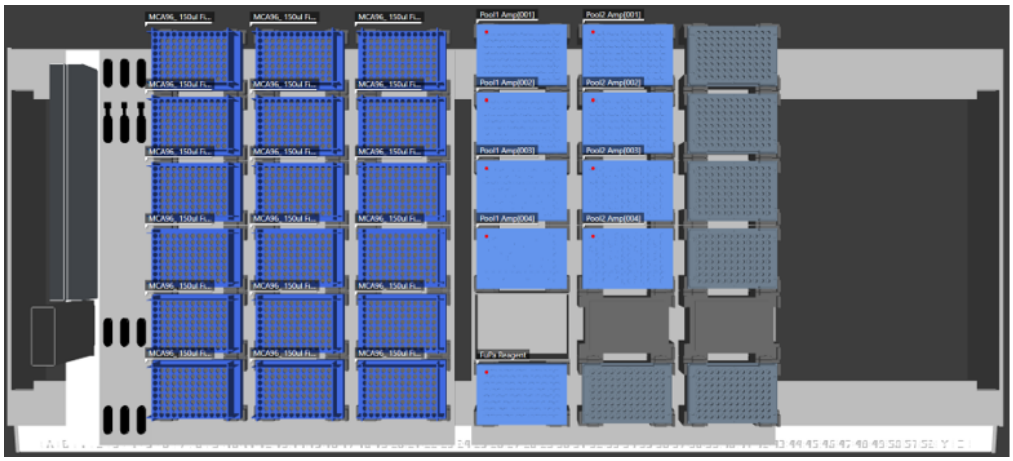
STOPPING POINT Target amplification reactions can be stored at 10°C overnight on the thermal cycler. For longer periods, store at –20°C.

Partially digest amplicons

1. Touch , then select **AmpliSeq SARS-CoV-2 Partially Digest** to start the run. Alternatively, the method can be started by selecting the method within Tecan™ FluentControl™ Software.

2. Using the touch screen, select the number of plates to run. Follow the prompts for placement of the plates and minimum required fill volumes for reagents. The setup and volumes are matched to the number of plates being processed.

   The estimated run time for 4 plates is 7 minutes.



3. Run the following program to amplify the target regions.

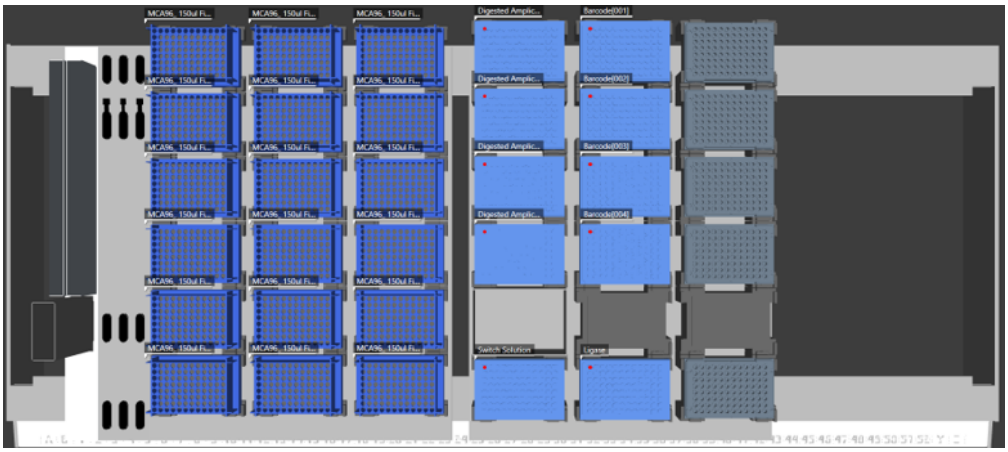
Temperature	Time
50°C	10 min
55°C	10 min
60°C	20 min
10°C	Hold (for up to 1 hour)

Ligate adapters

1. Touch , then select **AmpliSeq SARS-CoV-2 Ligate Adapters** to start the run. Alternatively, the method can be started by selecting the method within Tecan™ FluentControl™ Software.

2. Using the touch screen, select the number of plates to run. Follow the prompts for placement of the plates and minimum required fill volumes for reagents. The setup and volumes are matched to the number of plates being processed.

   The estimated run time for 4 plates is 12 minutes.



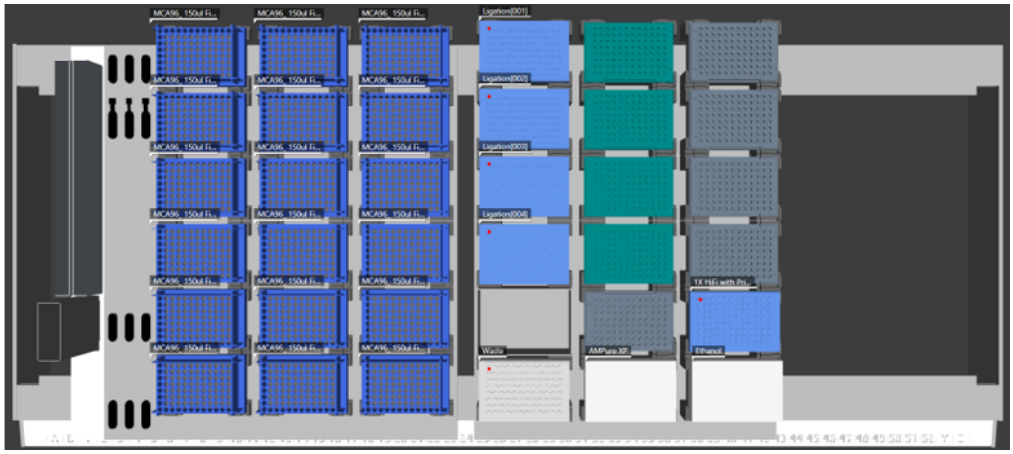
Temperature	Time
22°C	30 minutes
68°C	5 minutes
72°C	5 minutes
10°C	Hold (for up to 24 hours)

Purify the unamplified library

1. Touch , then select **AmpliSeq SARS-CoV-2 Purify & Amplify** to start the run. Alternatively, the method can be started by selecting the method within Tecan™ FluentControl™ Software.

2. Using the touch screen, select the number of plates to run. Follow the prompts for placement of the plates and minimum required fill volumes for reagents. The setup and volumes are matched to the number of plates being processed.

   The approximate run time for 4 plates is 32 minutes.



3. Run the following program to amplify the target regions.

Stage	Temperature	Time
Hold	98°C	2 minutes
9 cycles	98°C	15 seconds
	64°C	1 minute
Hold	10°C	Hold (up to 1 hour)

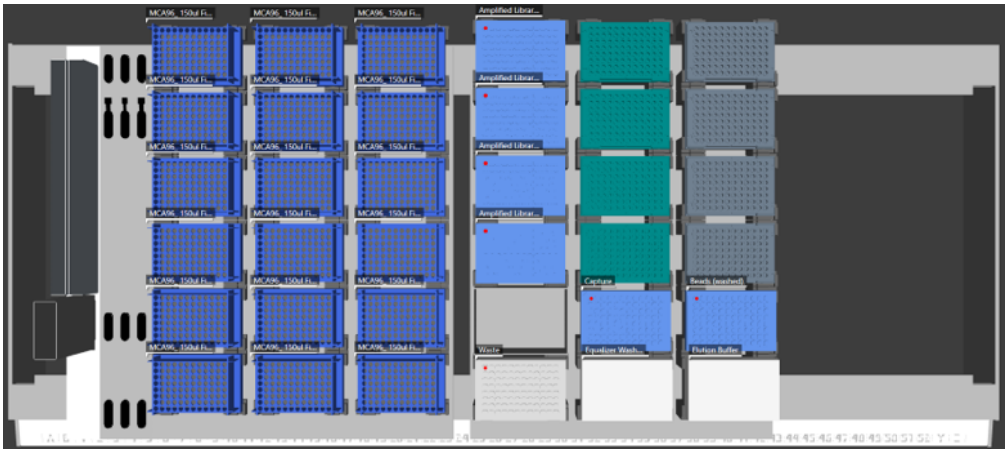
Equalize the library

1. If not previously performed, wash the Equalizer™ Beads. For instructions, see *Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay User Guide* (Pub. No. MAN0024915).

2. Touch , then select **AmpliSeq SARS-CoV-2 Equalize Library** to start the run. Alternatively, the method can be started by selecting the method within Tecan™ FluentControl™ Software.

3. Using the touch screen, select the number of plates to run. Follow the prompts for placement of the plates and minimum required fill volumes for reagents. The setup and volumes are matched to the number of plates being processed.

   The approximate run time for 4 plates is 36 minutes.



4. Perform thermal cycling using the conditions in the following table.

Stage	Temperature	Time
Hold	32°C	5 minutes
Hold	10°C	Hold (up to 1 hour)

The supernatant contains the Equalized library at ~100 pM, which can be stored with beads for up to 1 month at 4–8°C.

5. Dilute library to the appropriate concentration.

Chip	Concentration
Ion 530™ Chip	30 pM
Ion 540™ Chip	50 pM

Proceed to templating and sequencing.

Store libraries

Libraries may be stored at 4–8°C for up to 1 month. For longer term storage, store at -20°C.

Guidelines for templating and sequencing

Proceed to template preparation and sequencing using the following kits.

Chip	Maximum libraries/chip	Kit	User Guide
Ion 530™ Chip[1,2]	• 16[3] • 32[4]	Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. No. A34461)	*Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854)
Ion 540™ Chip[1,2]	• 64[3] • 128[4]	Ion 540™ Kit – Chef (Cat. No. A30011)	*Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)

[1] Template system: Ion Chef™ System
[2] Sequencer: Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer
[3] 1,000,000 reads
[4] 500,000 reads

Supplemetal information

Minimum fill volumes required

Minimum fill volumes required for each run configuration. The script prompts these fill values when starting a run.

Component	Required volume (µL per well)			
	1 plate	2 plates	3 plates	4 plates
Reverse transcription reagents				
Ion Torrent™ NGS 5X Reaction Buffer	8	11	14	17
Ion Torrent™ NGS 10X RT Enzyme Mix	6.5	8	9.5	11
cDNA target amplification reagents				
5X Ion AmpliSeq™ HiFi Mix	9	13	17	21
Ion AmpliSeq™ 5X primer pool	7	9	11	13
Amplicon digestion reagents				
FuPa Reagent	7	9	11	13
Ligation reagents				
DNA Ligase	7	9	11	13

(continued)

Component	Required volume (µL per well)			
	1 plate	2 plates	3 plates	4 plates
Switch Solution	9	13	17	21
IonCode™, Ion Xpress™, or Ion Torrent™ Dual Barcodes	6	6	6	6
Library purification reagents				
AMPure™ beads	18.5	22	25.5	29
70% Ethanol	45	75	105	135
Equalization reagents				
1X Ion AmpliSeq™ HiFi + primers	75	130	185	240
Equalizer™ Capture	15	25	35	45
Equalizer™ Beads, washed	11	17	23	29
Equalizer™ Wash Buffer	45	75	105	135
Equalizer™ Elution Buffer	25	35	45	55

Recommended fill volumes for reagent master plates (high throughput applications)

To minimize dead volume loss, we recommend filling reagent master plates to support up to 12 sample plates (1,152 total samples). If accessing the plates multiple times, ensure the total number of uses is tracked to avoid dry wells. The estimated uses per plate is 12.

Reagent	Dead volume (µL)	Recommended fill volume per well (µL)
5X Reaction Buffer	5	41
10X RT Enzyme Mix	5	23
5X Ion AmpliSeq™ HiFi Mix	5	53
Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel Pool[1]	5	29
FuPa Reagent	5	29

(continued)

Reagent	Dead volume (µL)	Recommended fill volume per well (µL)
DNA Ligase	5	29
Switch Solution	10	58
1X Library Amplification Mix + Equalizer primers[2]	20	680
Equalizer™ Capture	5	125
Washed Equalizer™ Beads	5	71

[1] You must prepare a plate for each primer.

[2] Use the Thermo Scientific™ Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates.

Documentation and support

Related documentation

Document	Publication number
Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay User Guide	MAN0024915
Ion AmpliSeq™ Library Kit Plus User Guide	MAN0017003
FluentControl Manual	BG/N 30135092.04 (**https://lifesciences.tecan.com/**)

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  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision history: Pub. No. MAN0025599

Revision	Date	Description
A.0	30 June 2022	New instructions for preparing Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel libraries using the Tecan™ Fluent™ 780 Automation Workstation.

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