Ion AmpliSeq™ SARS-CoV-2 Research Panel

Instructions for use on an Ion GeneStudio™ S5 Series System

Pub. No. MAN0019277 Rev. B.0

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/ support**.

This quick reference provides guidelines and instructions for using the Ion AmpliSeq™ SARS-CoV-2 Research Panel to prepare Ion AmpliSeq™ libraries from SARS-CoV-2 samples, sequence the libraries on an Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, or Ion GeneStudio™ S5 Prime System, then analyze the sequencing results with Torrent Suite™ Software.

Product description

The Ion AmpliSeq™ SARS-CoV-2 Research Panel consists of two 5X primer pools that target 237 amplicons specific to the SARS-CoV-2 (the virus that causes COVID-19) and 5 human expression controls. With an amplicon length range of 125–275 bp, the panel provides >99% coverage of the SARS-CoV-2 genome (~30 kb), and covers all potential serotypes. The panel is a community Ion AmpliSeq™ panel available for order through **AmpliSeq.com**.

When used in conjunction with the Ion Chef™ System and an Ion GeneStudio™ S5 Series System, the Ion AmpliSeq™ SARS-CoV-2 Research Panel offers high sensitivity, high throughput (up to 80 samples per sequencing run), fast turnaround time, and minimal hands-on time in SARS-CoV-2 research studies.

Ordering instructions

To order the Ion AmpliSeq™ SARS-CoV-2 Research Panel, follow these steps.

1. Go to **AmpliSeq.com** and sign in, or register for a new account.

2. In the navigation bar, go to the **Fixed Panels** dropdown menu, then select **Community Panels**.

3. In the **Research Area** navigation pane on the left side of the screen, select the **Infectious Disease** checkbox to filter the list. Find the Ion AmpliSeq™ SARS-CoV-2 Research Panel in the filtered list, then click **Preview Order**.

   Note: Alternatively, enter **SARS-CoV-2** in the search field at the top of the screen to find the panel page.

4. In the **Order options** window, select **GeneStudio** in the **Choose instrument** section, then click **Next**.

5. In the **Format option** window, select **Manual** or **Ion Chef**, then click **Next**.

6. In the **Order summary** window, review the order, then select **Proceed to cart**. As an option, select the **List recommended consumables** checkbox, then click **Preview** to see a list of additional products that you may need. Select the items, then click **Add all to cart**.

7. Click **Proceed to checkout** to complete the order at **thermofisher.com**.

   Unless otherwise indicated, all other materials listed in this quick reference are available at **thermofisher.com**.

Isolate and quantify viral RNA

Guidelines for RNA isolation and sample normalization

- A sample containing as little as 20 copies of viral RNA (10 copies per target amplification reaction) can be used to prepare an Ion AmpliSeq™ SARS-CoV-2 Research Panel library. For optimal results, we recommend a viral copy number in the 200 to 200,000 range, or an amount of *total* RNA between 1–10 ng. For more information, see "Guidelines for sample quality, viral copy number, and variant calling" on page 9.

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- The amount of viral RNA among samples should be approximately equivalent so that the target amplification conditions you select are optimal for all samples.
- See "Recommended materials for isolation and quantification" on page 2 for recommended Thermo Fisher Scientific kits and master mix.

Recommended materials for isolation and quantification

We recommend the following Thermo Fisher Scientific kits and master mix for the isolation and quantification of SARS-CoV-2 RNA.

Item	Cat. No.
Isolation	
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	A42352 or A48310
Quantification	
TaqMan™ 2019-nCoV Assay Kit v1	A47532
TaqMan™ 2019-nCoV Control Kit v1	A47533
TaqPath™ 1-Step RT-qPCR Master Mix, CG	A15299 or A15300

Additional positive controls are available at the BEI Resources Repository at **https://www.beiresources.org**, or through other commercial providers.

Isolate viral RNA

The MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit can be used in either a manual or a high-throughput automated mode using the MagMAX™ Express Magnetic Particle Processor or KingFisher™ Purification System. Follow these basic steps to isolate SARS-CoV-2 RNA using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (manual extraction). For detailed information on how to use the kit, and required materials not supplied, see the following user guides, which are available for download at **thermofisher.com**.

- *MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (manual extraction) User Guide* (Pub. No. MAN0018072) or the
- *MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide* (Pub. No. MAN0018073)

1. Digest 200–400 µL of each sample with Proteinase K in a deep-well 96-well plate, then bind RNA to Nucleic Acid Binding Beads.

2. Wash the Nucleic Acid Binding Beads.

3. Elute the RNA from the Nucleic Acid Binding Beads.

Use 1–10 ng *total* RNA in library target amplification reactions. We recommend quantifying viral copy number by real-time PCR, described in "Quantify by real-time PCR".

Quantify by real-time PCR

To determine the optimal number of target amplification cycles to use in library preparation, quantify viral RNA copy number in your SARS-CoV-2 samples using the TaqMan™ 2019-nCoV Assay Kit v1, the TaqMan™ 2019-nCoV Control Kit v1, and the TaqPath™ 1-Step RT-qPCR Master Mix, CG. For more information on reaction set up, see the *TaqMan™ 2019-nCoV Assay Kit v1 Product Information Sheet* (Pub. No. MAN0019096).

If you are unable to quantify viral RNA copy number in your samples, start with 16 target amplification cycles for manual library preparation, or 17 target amplification cycles for library preparation on the Ion Chef™ Instrument, then optimize if needed. For more information, see "Prepare libraries manually" on page 4, or "Prepare libraries on the Ion Chef™ Instrument" on page 5.

1. For each 2019-nCoV assay (N Protein, S Protein, and ORF1ab), combine the following components per reaction to make a reaction mix for the total number of reactions, plus 10% overage.

Component	Volume per reaction
TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X)	6.25 µL
2019-nCoV assay (20X; N Protein, S Protein, or ORF1ab)	1.25 µL
RNAse P assay (20X)	1.25 µL
RT-PCR Grade Water	11.25 µL
Total reaction mix volume	**20.0 µL**

2. For each reaction, combine the following components in a MicroAmp™ Optical 96-Well Reaction Plate (0.2-mL) well.

Component	Volume per well
Reaction mix (from step 1)	20.0 µL
- Nucleic acid research sample *or* 1 µL 2019-nCoV Control v1 + 4 µL RT-PCR Grade Water *or* - NTC	5.0 µL
Total reaction volume	**25.0 µL**

3. Set up and run the reactions on a real-time PCR instrument using the following settings:
   - Analysis method: Comparative C_t

     Note: You must use Comparative C_t to analyze 2019-nCoV assay data using QuantStudio™ Design and Analysis Software v2 and ExpressionSuite™ Software.
   - Cycling mode: Standard
   - Thermal cycling protocol:

Stage	Step	Temperature	Time
Hold	UNG incubation[1]	25°C	2 minutes
Hold	Reverse transcription	50°C	15 minutes
Hold	Activation[2]	95°C	2 minutes
Cycling (40 cycles)	Denaturation	95°C	3 seconds
	Anneal/Extension	60°C	30 seconds

[1] Heat-labile UNG in TaqPath™ 1-Step RT-qPCR Master Mix, CG is completely inactivated during the first ramp to 95°C.

[2] Required for RT inactivation, first denaturation, and activation of the DNA polymerase.

Use the C_t result for each 2019-nCoV assay to estimate copy number in your sample. See example data in the table on page 3.

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.

Copy number determination of SARS-CoV-2 with TaqMan™ 2019-nCoV Assay Kit v1 and TaqMan™ 2019-nCoV Control Kit v1

C_t of N Protein	C_t of S Protein	C_t of ORF1ab	Copies in qPCR reaction
34	36	37	5
33	35	36	10
32	34	35	20
31	33	34	39
30	32	33	78
29	31	32	156
28	30	31	312
27	29	30	625
26	28	29	1,250
25	27	28	2,500
24	26	27	5,000
23	25	26	10,000
22	24	25	20,000
21	23	24	40,000
20	22	23	80,000
19	21	22	160,000
18	20	21	320,000
17	19	20	640,000

Prepare libraries with the Ion AmpliSeq™ SARS-CoV-2 Research Panel

After reverse transcription, two options are available for preparing libraries using the Ion AmpliSeq™ SARS-CoV-2 Research Panel:

- manual preparation using the Ion AmpliSeq™ Library Kit Plus (Cat. Nos. 4488990, A35907, or A38875)—follow steps listed in "Prepare libraries manually" on page 4
- automated preparation on the Ion Chef™ Instrument using the Ion AmpliSeq™ Kit for Chef DL8 (Cat. No. A29024)—follow steps listed in "Prepare libraries on the Ion Chef™ Instrument" on page 5

For detailed information on using these kits, see the *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003), or the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432), available for download at **thermofisher.com**.

Reverse transcribe RNA with the SuperScript™ VILO™ cDNA Synthesis Kit

Use the SuperScript™ VILO™ cDNA Synthesis Kit (Cat. No. 11754050) to reverse transcribe SARS-CoV-2 RNA for both manual library preparation and automated library preparation on the Ion Chef™ Instrument.

1. Warm the 5X VILO™ Reaction Mix to room temperature for at least 20 minutes, then vortex to mix.

   Note: If there is visible precipitate in the 5X VILO™ Reaction Mix, vortex further until the mix is completely dissolved.

2. Combine the following components per reaction to make a master mix for the total number of reactions, plus 10% overage. Up to 8 sample libraries can be prepared in one Ion Chef™ Instrument run.

Component	Volume	
	Manual	Ion Chef™ Instrument
5X VILO™ Reaction Mix (blue cap)	2 µL	3 µL
10X SuperScript III™ Enzyme Blend (black cap)	1 µL	1.5 µL
Total volume per well	**3 µL**	**4.5 µL**

3. For each reaction, combine the following components in a MicroAmp™ Optical 96-Well Reaction Plate (0.2-mL) well for manual library preparation, or wells A1 to H1 of an IonCode™ 96 Well PCR Plate from the Ion AmpliSeq™ Kit for Chef DL8 for library preparation on the Ion Chef™ Instrument.

Component	Volume	
	Manual	Ion Chef™ Instrument
Master mix (from step 2)	3 µL	4.5 µL
RNA (1–10 ng)	7 µL	10.5 µL
Total volume per well	**10 µL**	**15 µL**

4. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Load the plate in a thermal cycler, place a MicroAmp™ Compression Pad on the plate, then run the following program to synthesize cDNA.

Temperature	Time
42°C	30 minutes
85°C	5 minutes
10°C	Hold

STOPPING POINT Samples can be stored at 10°C for up to 16 hours in the thermal cycler. For longer term, store at −30°C to −10°C.

Prepare libraries manually

See the *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003) for detailed instructions for amplifying targets and completing the manual library preparation workflow for a 2-pool RNA panel using the Ion AmpliSeq™ Library Kit Plus. Abbreviated steps and panel-specific guidelines are provided here.

1. Set up an amplification master mix for each sample using the entire volume of the reverse transcription reaction.

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4.5 μL
cDNA (from previous step)	10 μL
Nuclease-free Water	3.5 μL
Total volume	**18 μL**

2. Mix by pipetting up and down 5 times, then transfer 8 μL of each sample-specific master mix into 2 wells of a 96-well PCR plate.

3. Add 2 μL of Ion AmpliSeq™ SARS-CoV-2 Research Panel primer pool 1 into the first well, and 2 μL of primer pool 2 to the second well for a total of 10 μL in each well.

4. Seal the plate with a MicroAmp™ Clear Adhesive Film, then place a MicroAmp™ Compression Pad on the plate.

5. To amplify targets, run the following program.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	98°C	2 minutes
Cycle; set number according to the following table	Denature	98°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold

Viral copy number	Number of amplification cycles	
	Manual	Ion Chef™ Instrument
10	26	27
20	25	26
39	24	25
78	23	24
156	22	23
312	21	22
625	20	21
1,250	19	20
2,500	18	19
5,000	17	18
10,000	16	17
20,000	15	16
40,000	14	15
80,000	13	14
160,000	12	13
320,000	11	12
640,000	10	11

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: 24-27 cycles
- High viral load suspected: 14-21 cycles
- Isolates or enriched viral particles: ~12 cycles for 2 ng input

If you are working with samples with **unknown viral load**, and cannot quantify using qPCR, use 16 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 term, store at −30°C to −10°C.

6. See the *Ion AmpliSeq™ Library Kit Plus User Guide* to complete the remaining steps in library preparation:
   a. Combine primer pool 1 and 2 target amplification reactions.
   b. Partially digest amplicons.
   c. Ligate barcode adapters and purify.

      Note: We recommend the Ion Xpress™ Barcode Adapters 1–96 Kit (Cat. No. 4474517) or the IonCode™ Barcode Adapters 1–384 Kit (Cat. No. A29751).

   d. Quantify libraries (using the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802), or by Qubit™ fluorometry).

You can store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at −30°C to −10°C.

Prepare libraries on the Ion Chef™ Instrument

See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for detailed instructions for setting up the Ion Chef™ Instrument for library preparation for a 2-pool 5X RNA panel using the Ion AmpliSeq™ Kit for Chef DL8. Abbreviated steps and panel-specific guidelines are provided here.

1. Uncap all 4 tubes in positions A, B, C, and D in the Ion AmpliSeq™ Chef Reagents DL8 cartridge. Save the caps.
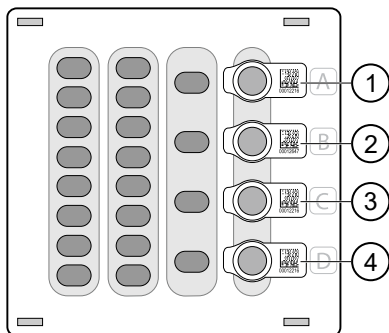
   ① Position A (150 μL Primer Pool 1 at 2X concentration)
   ② Position B (150 μL Primer Pool 2 at 2X concentration)
   ③ Position C (Empty tube)
   ④ Position D (Output tube)

2. Pipet 60 μL of the 5X Ion AmpliSeq™ SARS-CoV-2 Research Panel primer pool 1 into the Position A tube, and 60 μL of the 5X primer pool 2 into the Position B tube, then add 90 μL of Nuclease-free Water to each tube to dilute primers to 2X. Using a new tip for each tube, pipet up and down 5 times to mix.

3. After adding the primer pools, load the Ion AmpliSeq™ Chef Reagents DL8 cartridge, with uncapped tubes, in the Ion Chef™ Instrument, then load the following components:
   - Ion AmpliSeq™ Chef Solutions DL8 cartridge
   - Ion AmpliSeq™ Tip Cartridge L8
   - Enrichment Cartridge
   - IonCode™ 96 Well PCR Plate in which you prepared cDNA ("Reverse transcribe RNA with the SuperScript™ VILO™ cDNA Synthesis Kit" on page 3)—remove the seal from the plate.
   - PCR Frame Seal

4. Set up the run on the Ion Chef™ Instrument following the instructions in *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide*. On the **AmpliSeq Workflow Options** screen, make the following selections:
   - **# of primer pools:** 2
   - **Target amplification cycles:** select the number of cycles recommended for library preparation on the Ion Chef™ Instrument in step 5 of "Prepare libraries manually"
   - **Anneal & extension time:** 4 minutes

   If you are working with samples with **unknown viral load**, and cannot quantify using qPCR, use 17 target amplification

cycles as a starting point for library preparation on the Ion Chef™ Instrument.

5. After run setup, tap **Start Run** on the instrument screen.

   At completion of the run, the tube in Position D of the Ion AmpliSeq™ Chef Reagents DL8 cartridge contains 700 μL of combined barcoded libraries. We recommend that you quantify the concentration by qPCR using the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802).

You can store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at –30°C to –10°C.

Install plugins and import panel files

You can analyze the sequencing results in Torrent Suite™ Software with SARS-CoV-2 plugins.

Install the SARS-CoV-2 Research Plug-in Package

On Connect, you can download the SARS-CoV-2 Research Plug-in Package. Then, in Torrent Suite™ Software, an administrator can install the plugin package.

1. Sign in to **apps.thermofisher.com**.

2. Click ⠿ **(AppConnect)**.

3. Under **Resource Libraries**, click **Plugins**.

4. Click **SARS-CoV-2 Research Plug-in Package**.

5. Click **Download Plugin**.
   A compressed file that contains the plugin package is downloaded to your local machine.

6. Extract the downloaded package file.
   The SARS-CoV-2 Research Plug-in Package contains four plugins: COVID19AnnotateSnpEff, IRMAreport, AssemblerTrinity, and SARS_CoV_2_coverageAnalysis.

7. In Torrent Suite™ Software, click ⚙ **(Settings)** ▸ **Plugins** ▸ **Install or Upgrade Plugin**.

8. For each of the four plugins, perform the following steps. Repeat these installation steps for each of the four plugins.
   a. Click **Select File**, browse to the file location, select a plugin file, then click **Open**.
   b. In the **Install or Upgrade Plugin** dialog box, click **Upload and Install**.

Configure the AssemblerTrinity Plugin

You must complete these steps before you run the AssemblerTrinity plugin.

1. In a command prompt window on the Ion Torrent™ Server, enter this command:

   ```
   $ cd /results/plugins/AssemblerTrinity
   ```

2. In a command prompt window, enter one of these commands depending on the operating system and Torrent Suite™ Software version.

   - For servers with Ubuntu™ operating system 18.04 and Torrent Suite™ Software version 5.12, run this command in a command prompt window:

     ```
     $ sudo dpkg -i Singularity/Ubuntu18.04/
     ion- singularity_3.5.3-2_amd64.deb
     ```

   - For servers with Ubuntu™ operating system 14.04 and all Torrent Suite™ Software versions from 5.4 and later, including 5.12, run this command in a command prompt window:

     ```
     $ sudo dpkg -i Singularity/ Ubuntu14.04/
     ion- singularity_3.5.3-2_amd64.deb
     ```

Import the SARS-CoV-2 reference sequence and target region files

Your Ion Torrent™ Server account must be linked to **AmpliSeq.com** before you download the Ion AmpliSeq™ SARS-CoV-2 Research Panel reference sequence and target region files from **AmpliSeq.com**. For more information, see the *Torrent Suite™ Software 5.14 User Guide* (Pub. No. MAN0019144).

Note: You can also download panel files from the panel page on **AmpliSeq.com**, then upload to the Ion Torrent™ Server from your drive.

1. In Torrent Suite™ Software, click ⚙ **(Settings) ▸ Reference Sequences**.

2. Click **Import Preloaded Reference Sequences**.

3. Click **Import** in the **Ion AmpliSeq™ SARS-CoV-2 Reference** row.

4. When the **Status** is **Successfully Completed**, in the **Short Name** column, click **ion_ampliseq_sars-cov2**.

5. Scroll to the **Available Target Regions and Hotspot Files** section and ensure that the **Ion_AmpliSeq_SARS-CoV-2.2020323.Designed.bed** file is available.

Create a Planned Run for the Ion AmpliSeq™ SARS-CoV-2 Research Panel

These instructions include the specific settings and selections required for a Planned Run that includes the Ion AmpliSeq™ SARS-CoV-2 Research Panel. For more information, see the *Torrent Suite™ Software 5.14 User Guide* (Pub. No. MAN0019144). Alternatively, you can use one of the preconfigured Planned Run templates in the panel file set imported from **AmpliSeq.com**. A template is provided for each chip type. If you use a Planned Run template, you need only confirm that correct settings are selected.

1. In Torrent Suite™ Software, in the **Plan** tab, in the **Templates** screen, click **Plan New Run**.

2. In the **Ion Reporter** step, click **Next**.

3. In the **Research Application** step:

   a. In **Research Application**, select **DNA**.

   b. In **Target Technique**, select **AmpliSeq DNA**.

   c. Click **Next**.

4. In the **Kits** step, make the following selections.

   a. In **Chip Type**, select an appropriate chip.

   Compatible chips (ordered separately) include the Ion 510™ Chip, Ion 520™ Chip, Ion 530™ Chip, and the Ion 540™ Chip.

   b. In **Barcode Set (optional)**, select the appropriate barcode.

   Compatible barcode sets are **IonXpress** and **IonCode**.

   c. In **Template Kit**, select the **IonChef** radio button, then select either **Ion 510 & Ion 520 & Ion 530 Kit-Chef**, or **Ion 540 Kit-Chef**.

   d. In **Flows**, set the number to **550**.

   e. Click **Next**.

5. In the **Plugins** step, select the following plugins.

   - **coverageAnalysis**
   - **variantCaller**. Next to variantCaller, click **Configure**. In **Parameter settings** ensure that the default setting, **Germ Line - Low Stringency**, is selected, then click **Save Changes**.
   - **COVID19AnnotateSnpEff**
   - **IRMAreport**
   - **SARS_CoV_2_coverageAnalysis**
   - *(Optional)* **AssemblerTrinity**

6. In the **Projects** step, click **Next**.

7. Complete the **Plan** step.

   a. Enter a name for the plan.

   b. In **Reference Library**, select **ion_ampliseq_sars-cov2(AmpliSeq SARS-CoV-2 Reference)**.

   c. In **Target Regions**, select **Ion_Ampliseq_SARS-CoV-2.2020323.Designed.bed**.

We recommend that you allow 1 M reads per sample for the Ion AmpliSeq™ SARS-CoV-2 Research Panel. Use the following chip capacity table to set the maximum number of sample libraries loaded per chip.

Chip type	Reads per chip
Ion 510™ Chip	2–3 M
Ion 520™ Chip	4–6 M
Ion 530™ Chip	15–20 M
Ion 540™ Chip	60–80 M

8. Review the **Summary** pane, then click **Plan Run**.

   Note: You need to create a Planned Run for each chip that you load in an Ion Chef™ templating run.

Prepare template and load chips on the Ion Chef™ Instrument

After you have created a Planned Run, see the one of the following user guides for detailed information on setting up and running the Ion Chef™ Instrument to prepare template and load chips, depending on the Ion Torrent™ chip you are using. The user guides are available for download at **thermofisher.com**.

- *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854)

- *Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)

Use the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. No. A34461), or the Ion 540™ Kit – Chef (Cat. No. A30011), and the Ion Chef™ Instrument to template Ion Sphere™ Particles with Ion AmpliSeq™ SARS-CoV-2 Research Panel libraries that you prepared, and load one or two Ion 510™, Ion 520™, Ion 530™, or Ion 540™ Chips. Follow the guidelines in the user guides for combining and diluting libraries. The kits also contain the reagents and components required for sequencing loaded chips on an Ion GeneStudio™ S5 Series Sequencer.

Recommended library input concentration (library quantification using the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802)

Chip	Library conc.	Volume	Total molecules
Ion 510™ Chip	30 pM	25 µL	450×10^6
Ion 520™ Chip			
Ion 530™ Chip			
Ion 540™ Chip	70 pM		1050×10^6

Note:
- If the library concentration is lower than the concentration recommended in the table, the volume of input library can be increased to 50 µL to reach the number of total molecules.
- If your lab has been using other library quantification methods such the Qubit™ dsDNA HS Assay Kit, and has an established protocol for templating, follow that protocol.
- If needed, use polyclonality and low-quality filter results from a sequencing run performed with ISPs templated at the starting concentration, then titrate up or down to achieve optimal concentrations.

Start a sequencing run

Use the sequencing components in the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef, or the Ion 540™ Kit – Chef to set up and start a sequencing run with a loaded Ion 510™, Ion 520™, Ion 530™, or Ion 540™ Chip on an Ion GeneStudio™ S5 Series Sequencer. Continue using the appropriate user guide listed above for detailed information.

Analyze sequencing data from SARS-CoV-2 samples

You can annotate variants, create consensus sequences, and generate a *de novo* full-length transcriptome reconstruction with SARS-CoV-2 plugins in Torrent Suite™ Software. Results from plugins that are added to a planned run are displayed in the report summary. For more information, see "Create a Planned Run for the Ion AmpliSeq™ SARS-CoV-2 Research Panel" on page 6.

Review COVID19AnnotateSnpEff plugin results

The COVID19AnnotateSnpEff plugin generates an annotated list of variants. You can use this plugin to identify and annotate variants with public or private databases and perform multi-sample comparisons.

1. In the **Data** tab, click **Completed Runs & Reports**, then in the **Report Name** column, click the report name link for the completed sequencing run.

2. In the left navigation menu, click **COVID19AnnotateSnpEff**.

3. *(Optional)* Click **Download PDF ▸ Plugins PDF** to download a plugin report summary when the status for the plugin is **Completed**.

   The PDF contains the information that is shown on the screen. Hyperlinks to the data are not active in the PDF.

4. Click the **COVID19AnnotateSnpEff.zip** link to download a ZIP file.

   The ZIP file contains annotated variants in VCF output files for each barcode.

Review IRMAreport plugin results

The IRMAreport plugin generates FASTA files that contain the consensus sequence for each barcode. The Iterative Refinement Meta-Assembler (IRMA) plugin identifies low-frequency variants for highly variable RNA viruses.

1. In the **Data** tab, click **Completed Runs & Reports**, then in the **Report Name** column, click the report name link for the completed sequencing run.

2. In the left navigation menu, click **IRMAreport**.

3. *(Optional)* Click **Download PDF ▸ Plugins PDF** to download a plugin report summary when the status for the plugin is **Completed**.

4. Click the **IRMAreport.html** link to open the **IRMA Assembled Report**.

5. Click the **FASTA** link to view the consensus sequence for each sample.

Review AssemblerTrinity plugin results

The AssemblerTrinity plugin generates a genome-guided or *de novo* viral sequence. The AssemblerTrinity plugin uses inchworm, chrysalis, and butterfly software modules sequentially, then builds de Bruijn graphs to resolve alternatively spliced isoforms and transcripts derived from paralogous genes to generate contig sequences.

1. In the **Data** tab, click **Completed Runs & Reports**, then in the **Report Name** column, click the report name link for the completed sequencing run.

2. In the left navigation menu, click **AssemblerTrinity**.

3. *(Optional)* Click **Download PDF ▸ Plugins PDF** to download a plugin report summary when the status for the plugin is **Completed**.

4. Click the **Trinity_report.html** link to open the **Trinity De novo Assembly Report**.

5. Click the **FASTA** link to view the individual contigs and the longest contig for each sample.

6. *(Optional)* Change the configuration settings and run the plugin again. For more information, see "Run the AssemblerTrinity plugin" on page 9.

Review SARS_CoV_2_coverageAnalysis plugin results

The SARS_CoV_2_coverageAnalysis plugin generates statistics, downloadable data files, and interactive visualization of coverage over targeted regions of the SARS-CoV-2 reference genome. Reads mapped to human endogenous controls (specified by the target regions) are filtered out and ignored. The report summary lists:

- the barcodes
- the samples
- the number of mapped reads
- the percentage of filtered reads
- the percentage of on target reads
- mean base coverage depth
- base coverage uniformity

Additional details regarding read coverage are also provided on a per-barcode basis.

1. In the **Data** tab, click **Completed Runs & Reports**, then in the **Report Name** column, click the report name link for the completed sequencing run.

2. In the left navigation menu, click **SARS_CoV_2_coverageAnalysis**.

3. In the SARS_CoV_2_coverageAnalysis barcode summary, in the **Barcode Name** column, click a barcoded sample link to open a detailed **SARS-CoV-2 Analysis Report** for that sample.

4. In the **SARS-CoV-2 Analysis Report**, review the plugin results. Click the links at the bottom of the **SARS-CoV-2 Analysis Report** to download associated statistics and summary files for each barcoded sample in the run.

(Optional) Re-run the SARS-CoV-2 plugins after the sequencing run is complete

After the sequencing run is complete, you may want to reconfigure the plugin settings and run the COVID19AnnotateSnpEff, IRMAreport, AssemblerTrinity, and the SARS_CoV_2_coverageAnalysis plugins again to perform a new analysis.
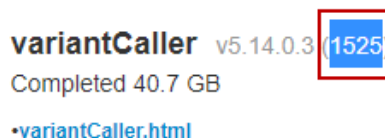
Run the COVID19AnnotateSnpEff plugin

Follow these steps if you want to run the COVID19AnnotateSnpEff plugin after the sequencing run is complete.

The variantCaller plugin provides input for the COVID19AnnotateSnpEff plugin. The variantCaller plugin analysis must be complete before you run the COVID19AnnotateSnpEff plugin.

1. In the report summary for the completed sequencing run, in the left navigation menu, click **variantCaller**.

2. Copy the **variantCaller ID**, which is the number in parentheses next to the variantCaller version.

    For example, the **variantCaller ID** in this image is **1525**.

3. Click **Plugins ▸ Select Plugins to Run**, then click **COVID19AnnotateSnpEff**.

4. In the **Torrent COVID19AnnotateSnpEff Plugin** dialog box, in **ID of VariantCaller results**, paste or enter the **variantCaller ID**.

5. Click **Submit**.

Run the IRMAreport plugin

Follow these steps if you want to run the IRMAreport plugin after the sequencing run is complete. You can select some barcodes for a new analysis and change the configuration settings, if desired.

1. In the report summary for the completed sequencing run, click **Plugins ▸ Select Plugins to Run**, then click **IRMAreport**.

2. *(Optional)* In the **Configure Plugin** dialog box, make custom configuration changes.

    a. Click **Barcode Sample Settings ₊**, then deselect the checkbox in the row of a **Barcode** to remove it from analysis.

       All selected barcodes will be included in the analysis.

    b. Click **Show advanced options**, then customize advanced options.

       You can adjust plugin parameters. For example, one of the default parameters that can be adjusted is **Skip barcodes with fewer than 1000 reads**.

3. Click **Submit**.

Run the AssemblerTrinity plugin

Follow these steps if you want to run the AssemblerTrinity plugin after the sequencing run is complete. You can change configuration settings and perform an analysis that is genome-guided or an analysis that is based on *de novo* viral sequence.

1. In the report summary for the completed sequencing run, click **Plugins ▸ Select Plugins to Run**, click **AssemblerTrinity**.

2. In the **Genome-Guided** dropdown list:
   a. Keep the **Yes** selection to run the plugin with reads aligned to the reference genome or select **No** to run the plugin genome-free.
   b. *(Optional)* Click **Show Advanced Options** to view default settings.
      For optimal performance, the **Min count of K-mers to be assembled** is set to **2**.

3. Click **Submit**.

Run the SARS_CoV_2_coverageAnalysis plugin

Follow these steps if you want to run the SARS_CoV_2_coverageAnalysis plugin after the sequencing run is complete.

1. In the report summary for the completed sequencing run, click **Plugins ▸ Select Plugins to Run**, click **SARS_CoV_2_coverageAnalysis**.

2. Click **Show Advanced Options** to view default settings.

3. Confirm or edit the **Tier 1 Coverage Depth**, **Tier 2 Coverage Depth**, and the **Tier 3 Coverage Depth**.

4. Click **Submit**.

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

Sample quality and viral copy number

Viral copy number	Recommendations and guidelines
200 to 200,000 copies	Recommended range for optimal results
120 to 199 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 5.14 User Guide* (Pub. No. MAN0019144).
20 to 119 copies	Only for high-quality samples without degradation that contain a very small quantity of human RNA. Low-frequency, random, false-positives are likely due to errors occurring in reverse transcription. We recommend ignoring heterozygous variant calls from samples in this range.

- 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 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 titer >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%.

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The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0019277

Revision	Date	Description
B.0	8 October 2020	• Updated ordering instructions for **AmpliSeq.com**. • Added new guidance for sample quality, viral copy number, and variant calling. • Added instructions to run and view results related to the SARS_CoV_2_coverageAnalysis plugin.
A.0	24 April 2020	New quick reference for using the Ion AmpliSeq™ SARS-CoV-2 Research Panel on an Ion GeneStudio™ S5 Series System.

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