OSPHL 756 Virology / Immunology Ion Torrent™ Ion Chef™ and Ion GeneStudio™ S5 Plus System Procedure

Copy of version 1.0 (approved and current)

Last Approval or Periodic Review Completed	3/28/2021
Next Periodic Review Needed On or Before	3/28/2023
Effective Date	3/28/2021

Uncontrolled Copy printed on 5/20/2021 10:42 AM

Printed By	Eugene Yeboah
Organization	Oregon State Public Health Laboratory

Author
Eugene Yeboah, Rafia Razzaque, Vanda Makris

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	3/28/2021	1.0	John Fontana John Fontana	
Approval	Quality Review	3/26/2021	1.0	Sheri Hearn Sheri Hearn	

Signatures from prior revisions are not listed.

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.0	Approved and Current	Initial version	3/26/2021	3/28/2021	Indefinite

Approved: 3/28/2021; Last reviewed: 3/28/2021

Ion Torrent™ Ion Chef™ and Ion GeneStudio™ S5 Plus System Procedure

Oregon State Public Health Laboratory

Table of Contents

I. PURPOSE:

The purpose of this procedure is to provide guided instructions for how to use the Ion Torrent™ Ion Chef™ and Ion Torrent™ Ion GeneStudio™ S5 Plus automated systems for run plan creation, template preparation, chip loading, and next-generation sequencing.

The Ion Chef™ provides automated library preparation, template preparation, and chip loading with minimal hands-on time after library preparation. The Ion Chef System allows for reproducible chip loading by automating multiple manual chip-loading steps designed to help reduce user-introduced error and variability.

The Ion GeneStudio™ S5 Plus System is a semiconductor-based next-generation (NGS) system that allows for simple targeted sequencing workflows. The Ion GeneStudio S5 Plus System uses cartridge-based reagents allowing for the flexibility to support a broad range of high throughput sequencing applications.

II. SCOPE / SPECIMEN ACCEPTANCE CRITERIA:

Specimen acceptance criteria for templating on Ion Chef™ System:

- The amplified library should be barcoded individually, purified, and normalized to ~100pM
- The normalized library can be combined and stored at 4-8ºC for up to a month. For longer lengths of time, store at -10ºC to -30ºC.

III. DEFINITIONS:

Ion Chef™ System: Automated instrument for library preparation, template preparation and chip loading for one or two chips.

Ion GeneStudio™ S5 Plus System: Semiconductor-based next-generation sequencing (NGS) system.

Amplified Library: A collection of similarly sized DNA fragments with known adapter sequences added to the 5' and 3' ends.

IV. ROLES AND RESPONSIBILITIES:

Microbiologist 2 is responsible for:

- Performing accurate and timely sequencing runs of specimens using approved procedures.
- Performing quality control and quality assurance activities for the assay.
- Entering test data, approving results, and generating test reports for submitters.
- Performing troubleshooting procedures as necessary

Microbiologist 3 or **Section Manager** or a designee is responsible for:

- Assisting with troubleshooting and other testing issues as needed.

- Monthly review of maintenance, bench and other logs.
- Monthly review of quality control.
- Review new lot quality control data.

V. EQUIPMENT / SUPPLIES / REAGENTS:

Ion Chef™ Instrument: Catalog # 4484177
Ion GeneStudio™ S5 Plus System: Catalog # A38195
Torrent Suite™ Software on the **S5 Torrent Server VM**

The various types of Ion chips, reagents and supplies are summarized below:

Ion Chips	**Cat No.**
Ion 510/520/530 Kit-Chef 2R/I 1 Kit	A34461
Ion 530 Chip Kit each	A27764
Ion 540 Chip Kit each	A27766
Ion 540 Kit-Chef (2/Init) 8 rxns	A30011

Ion Chef™ reagents and materials:

Components	**Amount/ box**	**storage**
Ion S5™ Chef Supplies (Part No. A27755)		
Chip Adapter	2	15°C to 30°C
Enrichment Cartridge v2	1	
Tip Cartridge v2	1	
PCR Plate	1	
Frame Seal v2	1	
Recovery Station Disposable Lid v2	2	
Recovery Tube v2	12	
Ion S5™ Chef Solutions (Part No. A27754)		
Ion S5™ Chef Solutions	4 cartridges	15°C to 30°C
Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents (Part No. A34018)		
Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents	4 cartridges	–10°C to –30°C

Ion S5™ sequencing reagents and materials:

Contents	**Amount/ box**	**storage**
Ion S5™ Sequencing Solutions (Part No. A27767)		
Ion S5™ Wash Solution	4 × 1.5 L	15°C to 30°C
Ion S5™ Cleaning Solution	250 mL	
Ion S5™ Sequencing Reagents (Part No. A27768)		
Ion S5™ Sequencing Reagents	4 cartridges	–10°C to –30°C

VI. HAZARDOUS SUBSTANCES / SAFETY PRECAUTIONS:

A. Lab coats and gloves must be worn when performing this procedure.

B. Ultraviolet (UV) Safety: The Ion Chef™ System uses a UV lamp which emits light at 254 nm. Under normal operating conditions, the UV lamp is powered on when performing the cleaning protocol. Safety interlocks are used to ensure that the UV lamp is not powered when the door is open.

C. Biohazardous materials are disposed in the biohazard bin or liquid waste containers.

VII. HAZARDOUS CHEMICAL WASTE DISPOSAL:

A. The IonChef deposits chemical waste into a primary container located in the instrument. After each run, the primary waste container emptied into a secondary waste container prior to disposal.
B. Chemical waste from the secondary container must be disposed of by Metro. Please refer to the Hazardous Chemical Waste Disposal procedure for more information about this process.
C. Ethanol waste can be disposed of in the lab sink under running water. Ensure that gloves, gowns are worn whenever using ethanol. Eye protection may be used as well.

VIII. INSTRUMENT START-UP / MAINTENANCE:

A. Prepare the Ion Chef™ Instrument for use:

Ensure that the Ion Chef™ Instrument has been cleaned following the previous run. If not, clean the instrument before loading it with consumables.

1. Inspect the empty compartments of the Reagents and Solutions stations for condensation. Before loading consumables into the instrument, wipe the compartments dry with a laboratory wipe or absorbent cloth, if needed.

2. Ensure that the Ion Chef™ Instrument has a connection to the Ion Torrent™ Server. On the instrument home touchscreen, select “Settings” then select “Torrent server” to view the connection status.

3. Thaw the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge at room temperature for **45 minutes** before use.

For more information on the cleaning procedure, see Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef USER GUIDE MAN0016854.

B. Prepare the Ion GeneStudio™ S5 Plus for use:

1. Remove the Ion S5™ Sequencing Reagents from the freezer and thaw at room temperature for **45 minutes** before initializing.

2. On the instrument touchscreen Main Menu, tap “Initialize”. The door, chip, and reagent cartridge clamps will unlock.

3. When prompted, remove the Ion S5™ Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir. Reinstall the empty waste reservoir.

4. Replace the expended Ion S5™ Sequencing Reagents cartridge with the new cartridge equilibrated to room temperature.

5. Ensure the new Ion S5™ Wash Solution bottle is thoroughly mixed by inverting several times and swirling the bottle gently. **Do not mix vigorously.** Remove the red cap and install.

6. Ensure that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.

7. If necessary, install a new Ion S5™ Cleaning Solution bottle. The Ion S5™ Cleaning Solution bottle contains sufficient reagent to complete 4 cleanings.

8. Close the door, then tap “Next”. The instrument confirms that the consumables and chip are properly installed and that the Ion S5™ Cleaning Solution contains sufficient reagent to perform the post-run clean. Follow all on-screen recommendations to ensure proper installation of required consumables.

9. When initialization is complete (~50 minutes), tap Home. The instrument is now ready for a sequencing run.

C. Cleaning and decontamination:

1. For Ion Chef™ Instrument, the cleaning routine is initiated from instrument touchscreen and is designed to minimize potential contamination. The instrument irradiates the deck with ultraviolet light for 1 minute after all consumables have been removed from the instrument.

2. The Ion GeneStudio™ S5 Sequencer requires that a cleaning be performed before initialization. Cleaning is normally performed automatically at the completion of the previous sequencing run. When two sequencing runs are performed on a single initialization, the post-run cleaning is performed after the second sequencing run.

NOTE: *If the sequencer is initialized and a sequencing run is not started within 24 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. An instrument reset run is required before reinitialization. For more information, see “Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge”.*

IX. CALIBRATION:

A. The Ion Chef™ Instrument verifies all aspects of the consumable setup before beginning each run. The instrument will give an error if a wrong consumable is installed in a wrong position during the desk scan.

B. The Ion GeneStudio™ S5 Plus sequencer has internal automated built-in calibration procedure to ensure proper installation of reagents, cartridges, and chips. Perform initialization before your 1st sequencing run of the day. The initialization is good for 24 hours.

X. QUALITY CONTROL:

A. A positive SC2 control (i.e. OSPHL SC2 Seq. PC) and a negative template control (NTC) will be used with each sequencing run. Other QC checks, including chip check, reagent check, and bottle leak checks are controlled by the instrument automatically.

XI. PROCEDURE:

A. Create a Planned Run

1. Sign into the Torrent Suite™ Software to create a Planned Run for the Ion AmpliSeq™ SARS-CoV-2 Research Panel by using the preconfigured Planned Run template named COVID-19_SEQ.

NOTE: All the specifics for templating the chips using the prepared library and downstream sequence analysis plug-ins are added to the COVID-19_SEQ template for ease of use. It includes 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).

2. Select the "Plan" tab and on the Templates screen, click COVID-19_SEQ. Enter a name for the plan as "SC2_xxxxxx(date)_Chip #"

3. Make sure the Analysis Parameters are set at Default as follows.

Research Application:	DNA
Research Category:	
Target Technique:	AmpliSeq DNA
Ion Reporter:	None
Sample Grouping:	
Instrument:	Ion GeneStudio S5 System
Chip Type:	Ion 530™ Chip

Sample Preparation Kit:	
Control Sequence:	
Library Kit Type:	Ion AmpliSeq Library Kit Plus
Barcode Set:	IonXpress
Template Kit:	Ion 510 & Ion 520 & Ion 530 Kit-Chef
Sequencing Kit:	Ion S5 Sequencing Kit
Library Read Length:	--
Flows:	550
Mark as Duplicate Reads:	False
Enable Realignment:	False
Plugins:	COVID19AnnotateSnpEff, IRMAreport, SARS_CoV_2_coverageAnalysis, variantCaller (germline_low_stringency_510_520_530)
Projects:	
Reference Library:	Ion_AmpliSeq_SARS-CoV-2(Ion_AmpliSeq_SARS-CoV-2)
Target Regions:	Ion_AmpliSeq_SARS-CoV-2.2020323_Designed.bed
Hotspot Regions:	
Bead Loading (%):	30
Key Signal (1-100):	30
Usable Sequence (%):	30
Advanced Settings	
Templating Protocol:	
Base Calibration Mode:	standard_recal
Forward Library Key:	Ion TCAG (TCAG)
Forward 3' Adapter:	Ion P1B (ATCACCGACTGCCCATAGAGAGGCTGAGAC)
Test Fragment Key:	ATCG
Flow Order:	Use Instrument Default

4. Add the total number of barcodes that are being used in the run for each chip. A table will pop up as soon as you enter the number of barcodes.

5. Add the name of specimens in the table. Include controls if used in the chip.

6. Complete the Plan step by selecting “Plan Run.”

Variable parameters in the run:

a. In Chip Type, you can select an appropriate chip that you are using. Compatible chips include the Ion 510™ Chip, Ion 520™ Chip, Ion 530™ Chip, and the Ion 540™ Chip.
b. In Template Kit, select the IonChef radio button, then select either Ion 510 & Ion 520 & Ion 530 Kit-Chef, or Ion 540 Kit-Chef.
c. The Barcode Set is optional, you can select the appropriate barcode. Compatible barcode sets are IonXpress and IonCode.
d. In Flows, set the number to 550.
e. In the Plugins step, you can select the following plugins: • coverage Analysis • variant Caller. Next to variant Caller, click Configure.
f. In Parameter settings ensure that the default setting, Germ Line - Low Stringency, is selected, then click Save Changes.
    • COVID19 Annotate SnpEff
    • IRMAreport
    • SARS_CoV_2_coverageAnalysis
    • AssemblerTrinity (Optional)

B. Load Ion Chef™ System

1. Unbox the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge 45 minutes before use and allow it to warm to room temperature.

2. Remove all cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.

3. Before use, gently tap the Reagents and Solutions cartridges on the bench to force the reagents to the bottoms of the tubes. Briefly centrifuge the tube of NaOH (Reagents cartridge, position C) to collect the contents.

4. Turn on the Instrument.

5. Tap (Open Door) on the instrument touchscreen to open the instrument door, then wait for the latch to open.

6. Lift the instrument door to the top of the travel until the latch mechanism engages.

7. Make sure an empty pipette tip rack in the Used (waste) Pipette Tip Position. To prevent contamination, change gloves immediately after moving the empty pipette tips rack.

8. Unwrap a new Tip Cartridge v2 and remove the cover to expose the pipette tips. Slide the catch forward to allow the locking bracket to pivot upward. Load the Tip Cartridge v2 into the *New* Pipette Tip Position, pull the bracket downward, then push

the catch backward to lock the bracket and cartridge in place.

9. Load a new PCR plate into the thermal cycler sample block, then slide a new Frame Seal v2 under the automated heated cover.

10. Load the Chef Solution cartridge into the Solutions station so that it snaps into place and is level on the deck.

11. Gently tap the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes. If bubbles are present below the surface of the liquid, repeat the step.

12. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

13. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.

14. Add the recovery tubes into the centrifuge buckets and seal with the provided recovery station disposable lids.

15. Add the enrichment cartridge into its assigned location on the instrument deck.

16. Loading the Chip-loading centrifuge:

    a. Load each chip that you will use for templating and sequencing into a centrifuge bucket, then attach a Chip Adapter to the assembly.

    b. Place the chip in the chip-loading bucket with the keyed corners of the chip and bucket aligned, then align the wells of the Chip Adapter to the wells of the chip, orienting the adapter onto the chip so that the chip barcode is visible.

    c. Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.

    d. Gently squeeze the flexible tabs at the other end of the adapter into the bucket slots until the adapter locks into place.

    e. Ensure that the tabs at all four corners of the adapter are fitted into the slots in the centrifuge bucket. *Loading can fail if the adapter is not attached securely.*

    f. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.

    g. Make a mark on top of each chip adapter to distinguish between the two chips. *This is important for downstream applications.*

    h. Ensure that the centrifuge is load-balanced, and the chip buckets are securely

seated and oriented correctly in the centrifuge so that they pivot 90° outwards when touched. Then close the lid of the chip-loading centrifuge.

Single chip loading workflow

You can set up an Ion Chef™ run to load a single chip instead of two, using the Ion Chef™ S5 Series Chip Balance loaded opposite to the sequencing chip in the Chip-loading centrifuge. The Ion Chef™ S5 Series Chip Balance is provided in the Ion S5™ Installation Kit.

Load the Ion Chef™ Instrument as you would normally load the system. For single chip loading, perform the following steps:

a. Add the single diluted library or combined library to an Ion Chef™ Library Sample Tube, then load the tube into Position A of the Reagents cartridge.
b. Load an empty Ion Chef™ Library Sample Tube into Position B of the Reagents cartridge. Uncap both tubes.
c. Load the chip in Position 1 and the Ion Chef™ S5 Series Chip Balance in Position 2 of the chip-loading centrifuge.
d. Resume the normal workflow in "Load the Chip-loading centrifuge." The Ion Chef™ Instrument detects the presence of the single chip during Deck Scan before the run starts.

C. Add diluted libraries to the Ion Chef™ Library Sample Tube

1. Pipet **25 µl** of each diluted combined library (~100 pM) to the bottom of the appropriate Ion Chef™ Library Sample Tube (barcoded tubes), then load the two Library Sample Tubes (Pool 1 and Pool 2) into Positions A and B on the Reagents cartridge.

2. *Cap, then store the sample tubes on ice until you are ready to load them onto the Ion Chef™ Instrument.*

3. Press down on each Library Sample Tube to ensure that they are firmly seated in the cartridge. Ensure that **all tubes are uncapped**, including the tube at Position D.

D. Start the Ion Chef™ run

1. On the Ion Chef™ Instrument home touchscreen, select the "Set Up Run" button.

2. Choose "Step by Step" to have the instrument lead you through the instrument setup or tap "Quick start" to skip the instrument setup screens and proceed to Deck Scan.

NOTE: It is useful to use the "step by step" feature the first couple times operating the instrument.

3. Choose "Templating" on the Run Options screen.

4. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engages.

5. When prompted, select "Start check" to start the Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to ensure their presence and compatibility.

6. When Deck Scan is complete, tap "Next" to display the Data Destination screen.

7. Ensure that the instrument displays the correct kit types, chip positions (mixed-chip run), chip type, chip barcodes, and Planned Run.

NOTE: If the correct Planned Runs do not display, tap the dropdown list to select the Planned Run for each chip, then tap "Next."

8. On the Run Options screen, tap the appropriate option to complete the run, then enter the desired time of run completion if needed.

9. On the Run Options screen, select "Start run" to start the run.

Note: *If you must stop the run for any reason, tap Cancel, then tap Yes to confirm the cancellation. If the Ion Chef™ Instrument encounters a problem during the run, it aborts the run and displays the error on the instrument touchscreen. If a run fails, perform the following tasks:*

1. *Remove the consumables from the deck, then clean the instrument. If possible, retain the consumables for troubleshooting.*
2. *Reset, then attempt to start the run again. If the run fails again, contact Technical Support to troubleshoot the problem. Record the error message for reference.*

E. Initialize the Ion GeneStudio™ sequencer

1. Initialize the sequencer at least 50 minutes before the Ion Chef™ System finishes chip loading. For more information, see section VIII. B. "Prepare the Ion GeneStudio™ S5 Plus for use."
2. Initialization of the instrument can be performed up to 24 hours before starting a sequencing run. If the intent is to perform two sequencing runs per initialization, the first run must be completed, and the second run must be started within the 24-hour period.
3. By initializing the sequencer before completion of chip loading, you ensure that the chips can be sequenced as soon as possible after loading is complete.

F. Unload the chips from Ion Chef™ for sequencing

1. Open the instrument door using the instrument touchscreen, tap (Open Door), then wait for the latch to open.

2. Lift the instrument door to the top of the travel until the latch mechanism engages.

3. Open the lid of the Chip-loading centrifuge, then unload both adapter/chip/bucket assemblies from the instrument.

4. Unload each chip from the adapter/chip/bucket assembly.

5. Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.

6. Grasp the chip by its edges, carefully lift the chip out of the bucket, then set it aside on a clean, static-free surface. Return the bucket to the Chip-loading centrifuge.

7. Load chip 1 onto a sequencer, then promptly start the sequencing run or runs, keep the chip 2 at 4°C until ready for sequencing.

Note: If you cannot sequence a loaded chip immediately or plan to sequence two chips per initialization, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6–8 hours maximum).

IMPORTANT! Liquid may be present in chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells. If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

8. Remove all consumables and waste from the Ion Chef™ and dispose in an appropriate biohazard container.

9. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

IMPORTANT! Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.

10. Select “clean” to initiate deck scan and UV decontamination of the instrument.

11. Turn off the instrument decontamination is complete.

G. Start the sequencing run

It is recommended that you start a sequencing run as soon as possible after chip loading and instrument initialization are complete. However, successful sequencing runs can be started up to 24 hours after instrument initialization.

1. After completion of initialization, tap Run in the instrument touchscreen. The door and chip clamp unlock.

2. Remove the used sequencing chip, then place the appropriate loaded chip (template-positive Ion Sphere™ Particles) in the chip clamp with the chip notch in the bottom-front corner.

NOTE: Do not remove the chip from the chip clamp until completion of the run. Removing and reinserting the chip risks introducing air bubbles in the chip.

DO NOT force the chip into the clamp. If the chip does not fit easily in the clamp, ensure that the notch is oriented correctly.

3. Slide the metal tab in fully to engage the clamp, then close the instrument door.

4. Confirm that the correct Planned Run has auto populated. Confirm that the remaining pre-populated settings are correct, or tap Edit to make changes if needed.

5. If this run is the first of two sequencing runs on this initialization, deselect the Enable post-run clean checkbox, then tap Review.

IMPORTANT! Failure to deselect the checkbox results in a cleaning performed automatically after the first run. A second run is not available. When starting the second sequencing run on a single initialization, ensure that the Enable post-run clean checkbox is selected so that the post-run cleaning is performed automatically.

6. Confirm that the instrument door is closed, then tap Start run to begin the sequencing run.

IMPORTANT! During a run, do not open the instrument door, and avoid touching the instrument. Touching the instrument during the sequencing run can reduce the quality of the measurements. Do not press the power button during a run. Interrupting power to the instrument during a run can result in sequencing run failure and loss of sample.

XII. QC ACCEPTANCE CRITERIA:

A. The correct Planned Run MUST be selected on the chip, check the run parameters (XI, A, 2).
B. The chip must NOT have significantly large bubbles, which would indicate failed loading. Do not remove the chip from the chip clamp until completion of the run. Removing and reinserting the chip risks introducing air bubbles in the chip.
C. It is recommended that you verify the status of the Planned Run before inserting the loaded chip on the sequencer. If your Planned Run does not advance to the Planned status in Torrent Suite™ Software after successful completion of an Ion Chef™ run, see “Planned Run status does not advance to "Planned” on the user guide.

XIII. RESULT REPORTING AND INTERPRETATION:

The results are processed and reported following the Ion AmpliSeq™ Ion Reporter

procedure.

XIV. TROUBLESHOOTING:

Visit the online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, contact technical support in case of repeated failure.

Observation	Possible cause	Recommended action
Chip Check fails	The chip clamp was not closed. • The chip was not properly seated. • The chip was damaged	1. Open the chip clamp, remove the chip, and look for signs of water outside the flow cell. 2. If the chip appears damaged, replace it with a new one. 3. Close the clamp, then repeat the Chip Check. 4. If the chip passes, click Next. If the chip fails, replace it with a new chip, then press Chip Check.
Reagent Check fails	A chip failure was detected.	1. Replace the used sequencing chip used during initialization with a different used chip. IMPORTANT! If you are using Torrent Suite™ Software version 5.12.1 or earlier and if a used Ion 550™ Chip was used for initialization, replace the used chip with a used Ion 510™ Chip, Ion 520™ Chip, Ion 530™ Chip, or Ion 540™ Chip for the initialization. Using an Ion 550™ Chip causes an error. 2. Press Retry. 3. If the initialization completes without failure, touch Home, then continue with your sequencing run.
	A wash failure was detected.	1. Perform a manual cleaning of the sequencer, see “Perform a manual cleaning of the sequencer” 2. Replace the Reagents cartridge, Wash Solution bottle, and Cleaning Solution bottle, then repeat initialization of the sequencer. 3. If the initialization completes without failure, press Home, then continue with your sequencing run.
Bottle leak check fails	• Bottle seal was not tight. • Bottle was damaged or defective.	1. Finger-tighten the bottles. 2. If the bottle continues to leak, replace the bottle. 3. If leak check continues to fail, contact Technical Support.
Connectivity failure with Ion Torrent Server VM	Chef is not connecting with the server	Refresh the plan, you may have to restart the Chef run.

XV. REFERENCES:

Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef USER GUIDE, publication Number MAN0016854, revision F.0, revised 29 October 2020