Oncomine[™] cfDNA Assays Part II: Plan a Run, Template Preparation, and Sequencing USER GUIDE

for use with Ion Chef[™] and Ion S5[™] Systems

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C.0	14 November 2016	Update to include Oncomine [™] Breast cfDNA Assay.
B.0	31 October 2016	Update to include Oncomine [™] Colon cfDNA Assay.
A.0	27 June 2016	Oncomine [™] Lung cfDNA Assay Part II: Plan a Run, Templating, and Sequencing User Guide. Provides instructions to sequence Oncomine [™] Lung cfDNA libraries using the Ion 520 [™] & Ion 530 [™] Kit – Chef on the Ion Chef [™] and Ion S5 [™] or Ion S5 [™] XL Instrument systems.

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Required materials not provided

$\operatorname{Ion} 520^{^{\scriptscriptstyle{ inymatrix}}}$ & $\operatorname{Ion} 530^{^{\scriptscriptstyle{ inymatrix}}}$ Kit – Chef

IMPORTANT! Do not substitute components from any other Ion sequencing kits. We have verified this protocol using these specific materials. Substitution may adversely affect system performance.

IMPORTANT! Store all consumables and cartridges under the recommended conditions and in an upright position. Do NOT store the Ion S5[™] Sequencing Reagents (Cat. No. A27768) on dry ice or in a closed environment where dry ice is present.

Each Ion 520^{TM} & Ion 530^{TM} Kit – Chef contains all materials that are required to prepare and sequence 8 Ion 530^{TM} Chips.

- Catalog No. A27757 supports 8 initializations
- Catalog No. A30010 supports 4 initializations (2 × 200 base-read sequencing runs per initialization)

On arrival, inspect all consumables and contact Technical Support if any of the products have been damaged during shipping.

Component	Do nt No	Quantity per kit	
Component	Part No.	A30010	A27757
lon S5 [™] Chef Supplies	A27755	4 boxes	4 boxes
lon S5 [™] Chef Solutions	A27754	1 box	1 box
lon 520 [™] & Ion 530 [™] Chef Reagents	A27756	1 box	1 box
Ion S5 [™] Sequencing Solutions	A27767	1 box	2 boxes
Ion S5 [™] Sequencing Reagents	A27768	1 box	2 boxes

Kit summary

Kit contents and storage

Contents	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 and Frame Seal v2	1 each	



Contents	Amount / box	Storage	
Recovery Station Disposable Lid v2	2	15°C to 30°C	
Recovery Tube v2	12		
Ion S5 [™] Chef Solutions (Part No. A27754)			
Ion S5 [™] Chef Solutions	4 cartridges	15°C to 30°C	
Ion 520 [™] & Ion 530 [™] Chef Reagents (Part No.	A27756)		
lon 520 [™] & Ion 530 [™] Chef Reagents	4 cartridges	–30°C to –10°C	
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	–30°C to –10°C	
(Cartridges ship at 2°C to 8°C. Store as indicated, do not store on dry ice.)			

IMPORTANT! Do not store the Ion S5[™] Sequencing Reagents (Part No. A27768) on dry ice or in a closed environment containing dry ice.

Ion 530[™] Chip Kit

Description	Catalog No.	Quantity	Storage
Ion 530 [™] Chip Kit (2 × 4-pack)	A27764	8 chips	15°C to 30°C



Required laboratory supplies

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Non-interruptible Power Supply (UPS) ^[1]	MLS
Thermal cycler with a heated lid	MLS
Microcentrifuge ^[2]	MLS
P2, P10, P20, P200, P1000 μL pipettor set and filtered tips	MLS
0.2-mL Thin wall PCR tubes, flat cap (do not use polystyrene tubes)	Axygen PCR-02-L-C BioExpress T-3035-1
1.5-mL or 1.7-mL Microcentrifuge tubes	MLS
Vortex mixer with a rubber platform	MLS
Gloves, powder-free nitrile	MLS
Ice buckets and ice	_
Nuclease-free water molecular biology grade	MLS
Isopropanol, 70% solution	MLS
Wipes, disposable lint-free	MLS

^[1] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use a non-interruptible power supply that is compatible with 2500 W output or higher.

^[2] Must fit standard 0.2- and 1.5-mL microcentrifuge tubes and generate 21,000 × g.

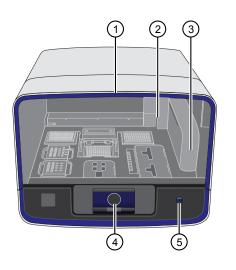


About the Ion Chef[™] System

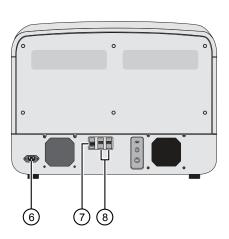
The Ion Chef[™] System provides automated, high-throughput template preparation and chip loading for use with the Ion S5[™] or Ion S5[™] XL Sequencer. The system includes a complete set of cartridge-based consumables and reagents that enable a user to load two Ion 530[™] Chips in approximately 13 hours with less than 15 minutes of hands-on time. The Ion Chef[™] System features network integration with the Torrent Suite[™] Software to enable sample and reagent traceability throughout the chip preparation workflow.

lon Chef[™] Instrument components

The following figure illustrates the major external and internal features of the Ion Chef^{TM} Instrument.



- Door Provides access to the interior of the instrument. The door is locked in the closed position during operation.
- ② Micropipettor A mechanical positivedisplacement pipettor that performs all fluid transfers during sample and chip preparation.
- ③ Robotic arm Performs fluid transfer during chip loading. The arm also contains an optical sensor that reads the barcodes of instrument reagents and consumables.
- (4) Touchscreen Provides access to all instrument functions for operation, maintenance, and troubleshooting.

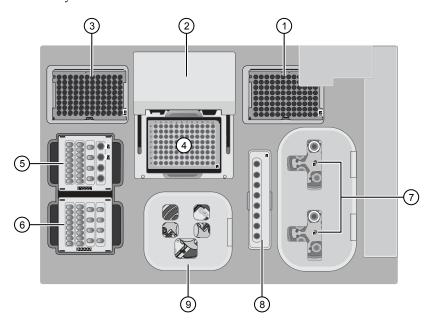


- (5) Power button Power switch for the Ion Chef[™] Instrument, where the states are on (illuminated) and off.
- 6 Power port A 100-240 VAC port that provides power to the Ion Chef[™] Instrument.
- ⑦ Ethernet port An RJ45 port that provides Ethernet (100 Mbit) communication with the lon Chef[™] Instrument.
- (8) USB port Provides USB communication with the Ion Chef[™] Instrument. Used to update the instrument firmware and to transfer data during service or maintenance.



Interior hardware and consumables

The following figure illustrates the interior of the Ion $\text{Chef}^{^{\text{TM}}}$ Instrument and describes the stations involved in the preparation of chips for sequencing on the Ion S5^{$^{\text{TM}}$} or Ion S5^{$^{\text{TM}}$} XL System.



- (1) Waste pipette tip rack The position of the rack containing waste (used) pipette tips.
- 2 Automated heated cover Transfers the plate cover to the PCR reaction plate within the sample block. During thermal cycling, the heated cover applies compression to seal the reaction plate and heats the cover to prevent condensation.
- ③ **New pipette tips** The position of the rack containing unused pipette tips.
- Thermal cycler sample block Performs thermal cycling of the sequencing reactions on a 96-well PCR reaction plate.
- (5) Reagents station The position on the instrument deck of the diluted libraries, NaOH, and the Ion 520[™] & Ion 530[™] Chef Reagents cartridge.

- (6) Solutions station The position on the instrument deck of the lon S5[™] Chef Solutions cartridge, which is maintained at room temperature.
- ⑦ Recovery centrifuges Twin stations that perform centrifugation of the ISPs during the recovery phase of template preparation.
- (8) Enrichment station The position of the rack containing consumables for the enrichment of the template-positive ISPs.
- Ohip-loading centrifuge Performs centrifugation of sequencing chips that have been mounted to chip-loading adapters and loaded with templatepositive ISPs.

About the Ion Chef[™] System touchscreen interface The Ion $\text{Chef}^{\mathbb{M}}$ System features a simple interface for loading chips, cleaning the instrument, and performing system maintenance and configuration tasks.



- Set up Run button Set up the Ion Chef[™] template preparation and chip-loading routine. Choose Step by Step to have the instrument lead you stepwise through installation of reagents and consumables, or choose Quick Start to proceed if you have already installed the consumables.
- **Open Door** button.
- ③ Notifications button View notifications about instrument status during and between runs.
- Quick Start button Proceed directly to the Quick Start instrument setup mode. User verifies the loading of a new pipette tip cartridge and an empty pipette tip rack to hold waste tips generated during the run, before proceeding to Deck Scan.

- (5) Settings button Advance to a screen providing the following options:
 - Notifications: view notifications about instrument status during and between runs
 - Instrument Settings: view current settings and network configuration, set instrument name, adjust time zone
 - Service tools: access screens for service-related maintenance and instrument diagnostics
 - Torrent Server: add and manage Torrent Server connections
 - Clean Ion Chef: proceed directly to the instrument cleaning routine
 - Check for updates: check availability of system software updates



Guidelines for using Ion Chef[™] reagents and consumables

To ensure the proper function of the Ion $\text{Chef}^{\mathbb{T}}$ System, handle the associated consumables and reagents according to the following guidelines:

- Store all Ion Chef[™] System consumables and cartridges under the recommended conditions and in an upright position.
- Inspect all Ion Chef[™] System consumables and cartridges for damage on arrival and again before use.
- Hold sequencing chips by gently gripping them by their edges.
- When the Ion Chef[™] System is not in use, remove all consumables and reagents from the deck and close the instrument door.
- Except for the *New* Pipette Tip Cartridge v2, do not reuse any of the Ion Chef[™] System consumables or reagents. After each run, the empty Pipette Tip Cartridge v2 is transferred to the waste tip station.

IMPORTANT! All Ion Chef[™] Instrument components are single-use only.

- Use only Ion Torrent[™] kits and supplies with the Ion Chef[™] Instrument. The use of third-party reagents and supplies can adversely affect the performance of the Ion Chef[™] Instrument and chips prepared.
- Always load two of the same model of sequencing chips into the Ion Chef[™] System. The instrument cannot load different models of sequencing chips during the same run.
- Remove and sequence chips within 1 hour after the Ion Chef[™] System finishes loading them. If you cannot sequence a loaded chip immediately, store it in a chip storage container at 4°C until you are ready to run it (up to 6–8 hours maximum).

Note: If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the chip storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

Precautions

Avoid nucleic acid contamination	IMPORTANT! A primary source of contamination is spurious DNA fragments from previous sample processing steps. Do not introduce amplified DNA into the library preparation laboratory or work area.
Avoid chip damage	IMPORTANT! To avoid possible damage to the chip due to electrostatic discharge, ground yourself before picking up a chip or placing a chip on a surface such as a lab bench. For example, touch the metal trim on the chip compartment before inserting or removing a chip from the chip clamp.



Before you begin

Workflow

The following workflow illustrates how to prepare and load samples using the Ion 520[™] & Ion 530[™] Kit – Chef, and Ion Chef[™] System for sequencing on the Ion S5[™] or Ion S5[™] XL Sequencer. For robust detection of low frequency somatic variants from liquid biopsy samples we recommend to multiplex up to:

- 8 Oncomine[™] Lung cfDNA Assay libraries on a single Ion 530[™] chip.
- 6 Oncomine[™] Colon cfDNA Assay libraries on a single Ion 530[™] chip.
- 12 Oncomine[™] Breast cfDNA Assay libraries on a single Ion 530[™] chip.

Create a Planned Run within the Torrent Suite[™] Software and set the run parameters. Dilute the library samples, and thaw the reagents. Load the lon Chef[™] Instrument with sequencing chips, consumables, reagents, and libraries. Start the lon Chef[™] Instrument Unload the lon Chef[™] Instrument. Load the prepared chip into the lon S5[™] or lon S5[™] XL Sequencer and

begin the sequencing run.

Create a Planned Run

About planned runs

Planned Runs contain all the settings used in a sequencing run, including number of flows, kit types, barcodes, sample information, and reference files (if any). Planned Runs are used to track samples, chips, and reagents throughout the sequencing workflow, from template preparation on the Ion Chef[™] Instrument through sequencing on the Ion S5[™] or Ion S5[™] XL Sequencer and subsequent data analysis. Each chip prepared in an Ion Chef[™] run requires its own Planned Run.

In Torrent SuiteTM Software, the Planned Run templates to be used with the OncomineTM cfDNA Assays are listed in the following table:

Application	Torrent Suite [™] Software template	Description
Oncology - Liquid Biopsy	 Oncomine[™] Lung Liquid Biopsy DNA Oncomine[™] Colon Liquid Biopsy DNA Oncomine[™] Breast Liquid Biopsy DNA 	Planned run template for use with cell-free DNA (cfDNA) research samples. Analysis parameters are optimized for the sensitive and specific detection of rare somatic variants (SNPs, InDels) present at 0.1% frequency in cfDNA.
	 Oncomine[™] Lung Tumor DNA Oncomine[™] Colon Tumor DNA Oncomine[™] Breast Tumor DNA 	Planned run template for use with solid tumor research samples from FFPE as well as fresh frozen tumor tissue. Analysis parameters are optimized for the sensitive and specific detection of rare somatic variants (SNPs, InDels) present at 0.5% frequency. Analysis parameters are optimized to eliminate false positives due to DNA damage resulting from formalin fixation.



Create a custom Planned Run template

IMPORTANT! Before creating a template file the target region and hotspot BED files must be uploaded to the Torrent Server. Required files can be found on the Ion Community Oncology – Liquid Biopsy page. If using TS v5.0, additional instructions to create a planned run are also available. See Appendix B, "Supplemental procedures" for more information.

We recommend setting up a customized Planned Run template for reuse when the same conditions will be used for multiple runs. For more information about creating Planned Runs manually or from the generic application template, see the *Torrent Suite User Documentation* available through the **Help > Software Help** menu in the Torrent Browser software.

- 1. Log in to the Torrent Browser for the Torrent Server connected to your Ion Chef[™] System.
- 2. Click the Plan tab > Templates, then click Oncology Liquid Biopsy in the left navigation menu.
- **3.** Find the appropriate Oncomine[™] Liquid Biopsy DNA (Lung, Breast, or Colon) template in the Oncology Liquid Biopsy list, click **‡** then select **Copy**.

Note: Select the appropriate Oncomine[™] Tumor DNA (Lung, Breast, or Colon) template from the list if creating a solid tumor run template.

The copy template wizard will open to the Save tab.

Field	Action
Template Name	Enter a name for the Planned Run template.
Reference Library	Select hg19(Human (hg19)).
Target Regions	 Select the appropriate Target Region file: Oncomine_Lung_cfDNA.06012016.Designed.bed Oncomine_Colon_cfDNA.10242016.Designed.bed Oncomine_Breast_cfDNA.08032016.Designed.bed
Hotspot Regions	 Select the appropriate Hotspot Region file: Oncomine_Lung_cfDNA.06012016.Hotspots.bed Oncomine_Colon_cfDNA.10242016.Hotspots.bed Oncomine_Breast_cfDNA.08032016.Hotspots.bed

4. Enter or select the required information in each field:

- **5.** Click the Ion Reporter tab:
 - To export files for analysis using Ion Reporter, select your Ion Reporter Account, select **Automatically upload to Ion Reporter after run completion** as Sample Grouping, then click **Next**.
 - If performing analysis using only the variantCaller plugin of the Torrent Suite[™] Software, ensure **None** and **Self** are selected for the Ion Reporter Account and Sample Grouping respectively, then click **Next**.

- 6. In the Application tab, verify that **Oncology–Liquid Biopsy** and **Tag Sequencing** are selected for Application and Target Technique respectively, then click **Next**.
- **7.** In the Kits page, verify the **Ion Chef** Template Kit radio button is selected, and the following fields are completed:

Field	Selection
Instrument	lon S5 [™] System
Library Kit Type	Oncomine cfDNA Assay
Read Length	200
Template Kit	lon 520 [™] & Ion 530 [™] Kit – Chef
Sequencing Kit	lon S5 [™] Sequencing Kit
Base Calibration Mode	Default Calibration
Chip Type	lon 530 [™] Chip
Barcode Set	IonCode [™] - TagSequencing
Flows	500

- **8**. Select or edit the optional information fields appropriately for your run, then click **Next**.
- 9. In the Plugins tab, ensure variantCaller is selected, then click **Configure**.
- 10. In the Plugin Configuration dialog choose the following Parameter Settings:

If setting up an	Select the Parameter Setting
Oncomine [™] Liquid Biopsy DNA template	Oncomine [™] Liquid Biopsy DNA - PGM (318) or S5/S5XL (5xx)
Oncomine [™] Tumor DNA template	Oncomine [™] Tumor DNA - PGM (318) or S5/S5XL (5xx)

- 11. Review the Projects tab and make selections appropriate to your run, then click **Next**.
- 12. In the Save tab, click **Copy Template** to save the new run template.

The customized template is now available in the Oncology - Liquid Biopsy page.

Create a Planned Run

- Log in to the Torrent Browser for the Torrent Server connected to your Ion Chef[™] System.
 - 2. Click the **Plan tab** > **Templates**, then click **Oncology Liquid Biopsy** in the left navigation menu.
 - In the Oncology Liquid Biopsy list, click on your customized Planned Run template name or the provided Oncomine[™] Liquid Biopsy DNA template, alternatively click then select **Plan Run**. The create plan wizard will open to the Plan tab.
 - 4. Enter a Run Plan Name.

- **5.** Verify the Use same reference & BED files for all barcodes radio button is selected.
- 6. In the Number of barcodes field, enter the number of barcodes that will be used in this run, then click the check mark 🖉 button to the right of this field.
- **7.** In the Sample Tube Label field(s), enter or scan the barcode of the Ion Chef[™] Library Sample Tube that will be used in the run.
- **8**. For each sample select the Barcode that will identify it, then enter or select from the available dropdown list the appropriate information for each field (all fields are required except Sample Description and Sample ID).

IMPORTANT!

- Sample Names must be unique to each sample. Do not duplicate sample names.
- Set the **IR Set ID** to the same value for related samples. In Ion Reporter[™] Software, samples with the same Set ID are launched in the same analysis.

1 IonCodeTag_0101 (CTAAGGTAAC) * Sample 1 Upload Only * Setf * Image: Transmission of transmissin of transmission of transmissinterimation of transmission of t	#	Barcode	Sample (required)	Sample Description	Sample ID	Ion Reporter Workflow		Relation	Gender		O IR ID	Set
3 lonCodeTag_0103 (AGAGAGGATTC) * Sample 3 Upload Only * Self * 1 4 lonCodeTag_0104 (TACCAAGATC) * Sample 4 Upload Only * Self * 1 5 lonCodeTag_0105 (CAGAAGGAACC) * Sample 5 Upload Only * Self * 1 6 lonCodeTag_0105 (CAGAAGGAAC) * Sample 6 Upload Only * Self * 1 7 lonCodeTag_0107 (TTCGTGATTC) * Sample 7 Upload Only * Self * 1	1	IonCodeTag_0101 (CTAAGGTAAC) 🔻	Sample 1			Upload Only	٣	Self	Ŧ	0	1	*
4 lonCodeTag_0104 (TACCAAGATC) * Sample 4 Upload Only * Self * * 1 5 lonCodeTag_0105 (CACAAGGAAC) * Sample 5 Upload Only * Self * * 1 6 lonCodeTag_0106 (CTGCAAGTTC) * Sample 6 Upload Only * Self * * 1 7 lonCodeTag_0107 (TICGTGATTC) * Sample 7 Upload Only * Self * * 1	2	IonCodeTag_0102 (TAAGGAGAAC) 🔻	Sample 2			Upload Only	*	Self	Ψ	*	1	\bigcirc
Intercentag_Discretation Computer Compu	3	IonCodeTag_0103 (AAGAGGATTC) 🔻	Sample 3			Upload Only	۳	Self	*	Ŧ	1	
6 IonCodeTag_0105 (CTGCAAGTTC) * Sample 6 Upload Only * Self * 1 7 IonCodeTag_0107 (TTCGTGATTC) * Sample 7 Upload Only * Self * 1	4	IonCodeTag_0104 (TACCAAGATC) *	Sample 4			Upload Only	*	Self	*	٣	1	
7 IonCodeTag_0107 (TTCGTGATTC) * Sample 7 Upload Only * Self * * 1	5	IonCodeTag_0105 (CAGAAGGAAC) 🔻	Sample 5			Upload Only	۳	Self	Ψ	*	1	
	6	IonCodeTag_0106 (CTGCAAGTTC) 🔻	Sample 6			Upload Only	٣	Self	*	٣	1	
8 IonCodeTag_0108 (TTCCGATAAC) * Sample 8 Upload Only * Self * * 1	7	IonCodeTag_0107 (TTCGTGATTC) *	Sample 7			Upload Only	*	Self	*	*	1	
	8	IonCodeTag_0108 (TTCCGATAAC) 🔻	Sample 8			Upload Only	٣	Self	*	٣	1	

9. For each sample select the Barcode that will identify it, then enter or select from the available dropdown list the appropriate information for each field (all fields are required except Sample Description and Sample ID).

IMPORTANT! Sample Names must be unique to each sample. Do not duplicate sample names.

10. Click Plan Run.

The run is listed in the Planned Run List page under the name that you specified and is automatically used by the Ion $\text{Chef}^{^{\text{TM}}}$ System when the associated Ion $\text{Chef}^{^{\text{TM}}}$ Library Sample Tube is loaded.

Dilute the library

Dilute libraries according to the following table. Then use polyclonality and low quality filter results from a sequencing run performed with ISPs templated at the starting concentration and titrate up or down to achieve optimal concentrations, if necessary. The quality of your sequencing data relies greatly upon achieving the correct concentration of starting library.

IMPORTANT! The recommendations in parentheses represent optimal input concentrations for control libraries.

Library read length	Recommended concentration ^[1]	Molecules per 25-µL input volume		
200 bp	50 pM (40–60 pM)	$600-900 \times 10^{6}$		

[1] Recommendations are based on qPCR quantification. If libraries are quantified with a 2100 Bioanalyzer[™] instrument, a higher calculated concentration may need to be used for equivalent input.

Note: Prepare a fresh dilution of each library before use with the Ion Chef[™] System, and use the library dilutions within 48 hours.

Prepare the Ion $\mathsf{Chef}^{^{\mathrm{TM}}}$ System for use

Before you use the Ion Chef[™] Instrument:

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

Note: For more information on the cleaning procedure, see Chapter 6, "Clean the Ion Chef[™] System".

- Inspect the empty compartments of the Reagents and Solutions stations for condensation. Condensate can collect in these compartments, depending on temperature and humidity conditions. Before loading consumables into the instrument, wipe the compartments dry with a laboratory wipe or absorbent cloth, if needed.
- Thaw the Ion 520[™] & Ion 530[™] Chef Reagents cartridge at room temperature for 45 minutes before use.
- Ensure that the Ion Chef[™] Instrument has a connection to your Torrent Server. On the Ion Chef[™] home touchscreen, touch **Settings**, then **Torrent Server** to view the connection status of your instrument.

Note: If the instrument is not connected, see Appendix C in the $Ion 520^{\mathbb{M}} & Ion 530^{\mathbb{M}} Kit - Chef User Guide (Pub. No. MAN0010846) for instructions on how to configure a direct or indirect network connection of the Ion Chef^M Instrument to a Torrent Server.$



Run the Ion Chef[™] System

This chapter describes:

- Setting up the Ion Chef[™] Instrument for use by diluting the two Ion libraries, and loading the instrument with all of the required reagents and consumables
- Starting an Ion Chef[™] run
- Unloading the Ion Chef[™] Instrument

Materials required

- Ion 520[™] & Ion 530[™] Kit Chef (Cat. Nos. A27757, A30010)
- Ion 530[™] Chip (2)
- Molecular-biology grade nuclease-free water
- P200 pipettor and filtered tips

Prepare the libraries and consumables

- 1. Unbox the Ion 520[™] & Ion 530[™] Chef Reagents Cartridge at least 45 minutes before use and allow it equilibrate to room temperature.
- 2. Pipet 25 μL of each 50-pM library (see "Dilute the library" on page 18) to the bottom of the appropriate Ion Chef[™] Library Sample Tube (flagged tubes).
- **3.** Cap, then store the two diluted libraries on ice until you are ready to load them onto the Ion Chef[™] Instrument.
- Remove all cartridges and consumables from their wrappings and boxes, then place them on the bench next to the Ion Chef[™] Instrument.
 Prepare the following:
 - Chip Adapter (2)
 - Enrichment Cartridge v2
 - Tip Cartridge v2
 - PCR Plate and Frame Seal v2
 - Recovery Station Disposable Lid v2 (2)
 - Recovery Tube v2 (12)
 - Ion S5[™] Chef Solutions Cartridge
 - Ion 520[™] & Ion 530[™] Chef Reagents Cartridge (from Step 1)

IMPORTANT! Before use, gently tap the solutions and reagents cartridges on the bench to force the reagents to the bottoms of the tubes.

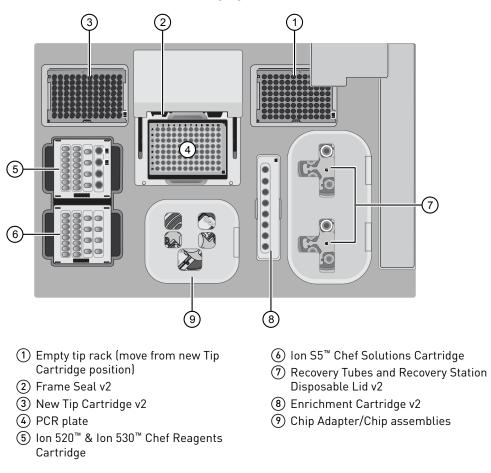
Note: When stored under normal conditions, a precipitate can form in some tubes of the Ion 520[™] & Ion 530[™] Chef Reagents Cartridge. If present, load the cartridge as directed – the precipitate will dissolve when the reagents are mixed during instrument operation.

Load the lon $\mathsf{Chef}^{^{\mathrm{M}}}$ System

IMPORTANT!

- Rated centrifuge speeds are only intended for operation with the provided buckets and approved consumable chips, tubes, and sample preparation reagents.
- The Chip-loading centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chips, and adapters. The centrifuge must be load-balanced. Proper care must be taken to load the buckets properly. If excessive vibrations arise, check that items are installed properly and rotors are load-balanced.
- Use only the materials supplied in the Ion 520[™] & Ion 530[™] Kit Chef to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets before each use to assure normal operation.
- Confirm that the instrument is powered ON and has been cleaned following the last use.
- Ensure all components are clean and dry before loading them onto the Ion Chef[™] Instrument.
- Ensure the Reagents and Solutions station compartments are free of condensate before loading components.

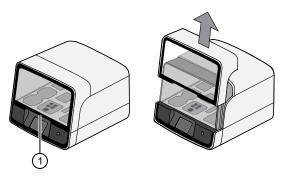
Follow the procedure below to load the Ion Chef[™] Instrument. A completely loaded instrument is shown in the following figure:



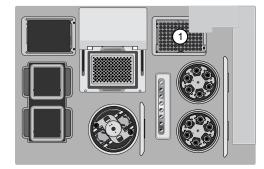


Load the pipette tip racks and PCR plate

- 1. Touch (a) (Open Door) in the instrument touchscreen to open the instrument door, then wait for the latch to open.
- **2.** Lift the instrument door to the top of the travel until the latch mechanism engages.



- 1 Hold here, then lift
- **3.** Load an empty pipette tip rack to the *Used* (Waste) Pipette Tip Position, then change gloves.



(1) Used Pipette Tip Position

IMPORTANT!

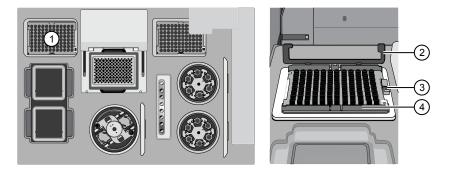
- Confirm that the pipette tip rack in the *Used* (Waste) Pipette Tip Position does not contain any tips. The instrument will abort the run if tips are present in the *used* position.
- To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used* (Waste) Pipette Tip Position.

Note: A small amount of dried residue may be present in the tub of the empty pipette tip rack after a run. This will not affect the next run in the Used Pipette Tip Position.

4. Unwrap a new Tip Cartridge v2 and remove the cover to expose the pipette tips, then load it in the *New* Pipette Tip Position.

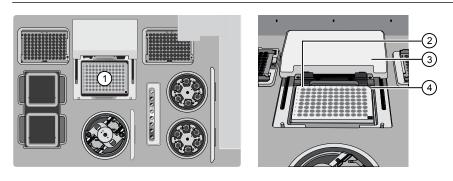
Note: Two Ion Chef[™] Piercing Tips are pre-loaded into tip positions G7 and H7 on the Tip Cartridge v2.

5. Pull the catch forward, then pivot the locking bracket upwards. Load the assembled Tip Cartridge v2 into the *New* Pipette Tip Position, then pull the bracket downwards, then push the catch backwards to lock the cartridge in place.



- (1) New Pipette Tip Position
- Bracket
- ③ Catch
- (4) New Tip Cartridge
- **6.** Load a new PCR plate into the thermal cycler sample block, then slide a new Frame Seal v2 underneath the automated heated cover.

IMPORTANT! When the Frame Seal v2 is positioned correctly, its tabs project upward and contact the heated cover.



- 1 Thermal cycler sample block
- 2 Well A1
- 3 Cover
- (4) Keyed corner

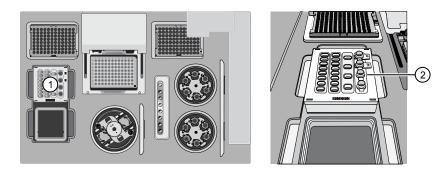


Load the Reagents and Solutions cartridges

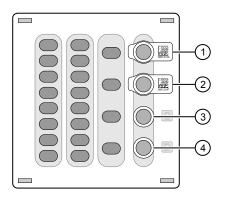
IMPORTANT! Thaw the Reagents cartridge at room temperature for 45 minutes before use.

- 1. Gently tap the Ion 520[™] & Ion 530[™] Chef Reagents Cartridge on the bench to force the reagents to the bottoms of the tubes.
- **2.** Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

IMPORTANT! Do not force the Ion Chef[™] cartridges into place. Each cartridge fits only one location on the deck and in one orientation. If a cartridge does not fit, confirm that you are loading the correct cartridge in the correct orientation.



- (1) Reagents station (4°C)
- (2) Ion 520[™] & Ion 530[™] Chef Reagents Cartridge
- **3.** Uncap, then load the two Library Sample Tubes, each containing 25μ L of diluted library, into Positions A and B on the Reagents cartridge.



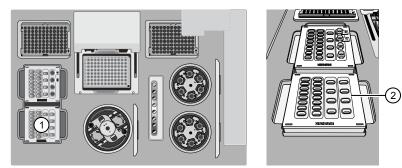
- 1 Position A (Library)
- (2) Position B (Library)
- 3 Position C (NaOH)
- (4) Position D (Empty tube)

IMPORTANT!

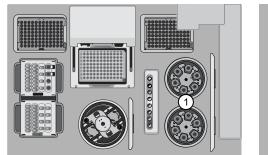
- Make sure to orient the sample tubes so that the barcodes are visible and oriented to the right.
- Make sure to remove the caps to the Library Sample Tubes before proceeding.
- Because 200- and 400-base-read libraries require different run lengths, do not load a 200-base-read library and 400-base-read library in a single Ion Chef[™] run. Both libraries loaded in a run must have a similar read length.
- **4.** Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.

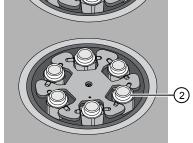
IMPORTANT! When the Reagents cartridge is loaded:

- Press down on the Library Sample Tubes to ensure that they are firmly seated in the cartridge.
- Confirm that *all* tubes are uncapped, including the tube at Position D.
- **5.** Gently tap the Ion S5[™] Chef Solutions Cartridge on the bench to force the reagents to the bottoms of the tubes.
- **6.** Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.



- (1) Solutions station (room temperature)
- ② Ion S5[™] Chef Solutions Cartridge
- 1. Load six Recovery Tubes (v2) into each Recovery centrifuge.





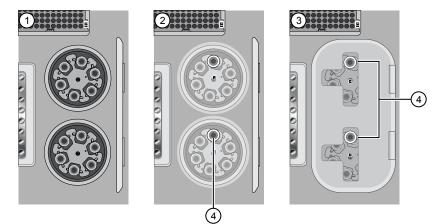
Recovery centrifuges
 Recovery Tube

Load the Recovery Tubes and Enrichment Cartridge v2 Before sealing each centrifuge, confirm that:

• The centrifuge is load-balanced with all required consumables.

IMPORTANT! The centrifuge must be load-balanced.

- The buckets are securely seated in the centrifuge rotors.
- The buckets are oriented correctly in the centrifuge so that they pivot outwards.
- **2.** Place a Recovery Station Disposable Lid v2 over each centrifuge with the port oriented toward the rear as shown in the figure below, then press down. Make certain the lids snap completely into place by applying firm downward pressure along the lid perimeter.
- **3.** Close the hinged cover of the Recovery centrifuges. Confirm that the port of each disposable lid is positioned toward the rear of the instrument.



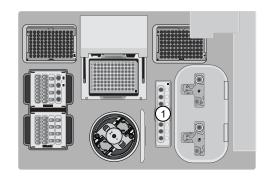
- (1) Recovery Tubes installed
- ② Recovery Station Disposable Lids installed
- (3) Recovery centrifuge cover closed
- 4 Port

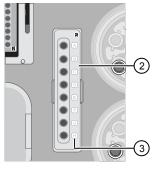
IMPORTANT!

- Do not obstruct or place any object on top of the Recovery centrifuge cover.
- Use only the supplied materials, including buckets and disposables, to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets to assure normal operation before each use.

4. Load the Enrichment Cartridge v2, then press down on the cartridge to ensure that it is level with the instrument deck.

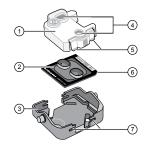
IMPORTANT! Confirm that the Enrichment Cartridge v2 is loaded so that the lettering on the cartridge is right-side-up.





- (1) Enrichment station (2) Enrichment Cartridge v2 (3) Lettering
- Load the Chiploading centrifuge
- 1. Load each prepared sequencing chip into a centrifuge bucket, then attach a Chip Adapter to the assembly.
 - **a**. Place the chip in the chiploading bucket, 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.
 - **b.** Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.

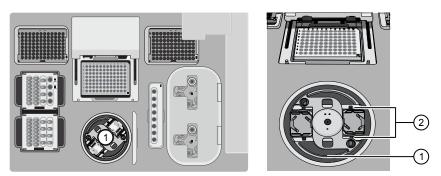
c. Gently squeeze the flexible tabs



- ① Chip Adapter
- 2 Ion chip
- ③ Bucket
- ④ Ports (align with chip)
- 5 Flexible tabs
- 6 Keyed corner (align with bucket)
- ⑦ Slots
- at the other end of the adapter into the bucket slots until the adapter locks into place.
- **d**. Confirm 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.

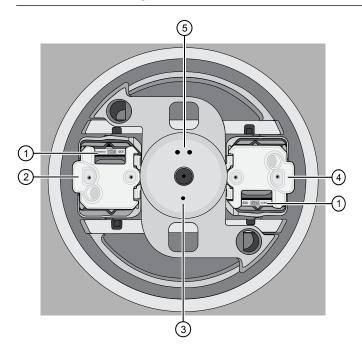
Note: If desired, you can label the tops of chips to distinguish them. Do not obstruct or overwrite the chip barcode with your label.

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



- (1) Chip-loading centrifuge
- Mounting grooves

IMPORTANT! When the Chip-loading centrifuge is loaded, confirm that each Chip Adapter is firmly attached to a bucket, and that the buckets are securely seated in the centrifuge rotors.



- 1 Chip barcode
- 2 Chip position 1
- 3 Position 1 marker hole
- (4) Chip position 2
- (5) Position 2 marker hole

Note: Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest. The chip loaded in Position 1 will be loaded with ISPs prepared from the DNA library in the Library Sample Tube loaded in Position A of the Reagents cartridge. The chip loaded in Position 2 of the centrifuge will be loaded with ISPs prepared from the DNA library in the Library Sample Tube loaded in Position B of the Reagents cartridge. **3.** Ensure 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.

IMPORTANT! Do not obstruct or place any object on top of the lid.

Confirm that consumables are correctly installed

• Confirm that each cartridge is at the correct location and in the correct orientation.

- Press down on all cartridges to confirm that they are firmly pressed into place.
- Confirm that all tubes on the Ion 520[™] & Ion 530[™] Chef Reagents Cartridge are uncapped and firmly pressed into place.
- Confirm that the centrifuge lids are installed correctly so that the port is oriented toward the rear of the instrument.
- Confirm that the tube and chip buckets are seated securely in the rotor arms of the Chip-loading and Recovery centrifuges, and that the consumables they contain are correctly installed.

CAUTION! To ensure correct and safe instrument operation, you must confirm that all consumables are installed correctly to the deck before you start a run. The Ion Chef[™] Instrument does not verify all aspects of the consumable setup prior to beginning each run.

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.

Before continuing:

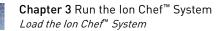
Load the Ion Chef[™] Instrument as you would normally load the system. For single chip loading, perform the following steps:



Ion Chef[™] S5 Series Chip Balance

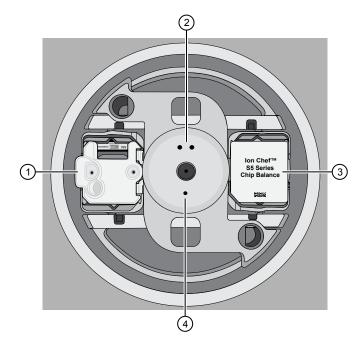
- Add the single diluted library to an Ion Chef[™] Library Sample Tube, then load the tube into Position A of the Reagents cartridge.
- **2.** Load an empty Ion Chef[™] Library Sample Tube into Position B of the Reagents cartridge. Uncap both tubes.
- **3.** Load the chip in Position 1 and the Ion Chef[™] S5 Series Chip Balance in Position 2 of the Chip-loading centrifuge.

IMPORTANT! Do not use Ion ChefTM 314, 316/318, or P-Series versions of the chip balance with the Ion 520^{TM} or Ion 530^{TM} Chip. Each chip balance is weight-matched to the chip (and corresponding chip adapter) specified on the chip balance label.

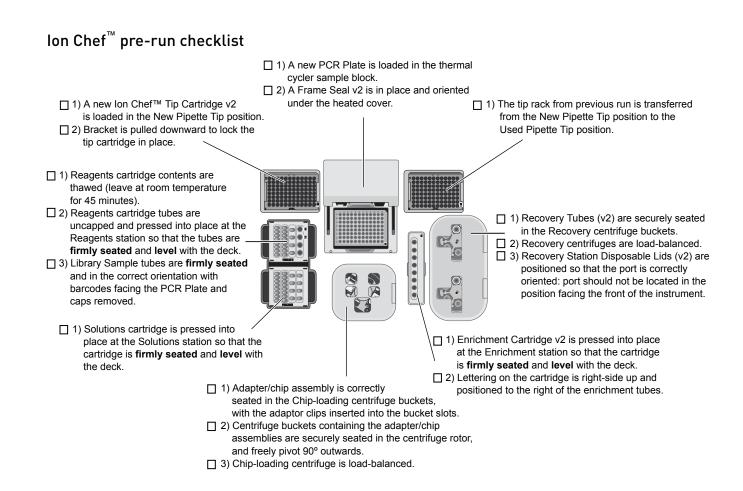


3

Note: Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest.



- 1 Position 1 (chip)
- 2 Position 2 marker holes
- (3) Position 2 (Chip Balance)
- ④ Position 1 marker holes
- Resume the normal workflow in "Load the Chip-loading centrifuge" at step 3. The Ion Chef[™] Instrument detects the presence of the single chip during Deck Scan before the run starts.



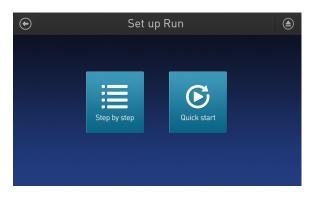


Start the Ion ${\sf Chef}^{^{\rm T}}$ run

- 1. Confirm that you have loaded the instrument with all kits and consumables.
- **2.** On the Ion Chef[™] Instrument home touchscreen, touch **Set up run**.

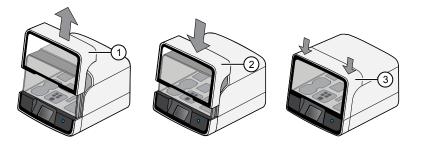


3. Touch **Step by Step** to have the instrument lead you through the instrument setup, or touch **Quick Start** to skip the instrument setup screens.



- 3
- **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 engage. After the door closes, the instrument vision system activates.

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 both sides of the door are locked after closing it.



- Lift door first
 Lower
- ③ Press down to lock
- **5.** When prompted, touch **Start check** to start Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to confirm their presence and compatibility.

During Deck Scan, the touchscreen displays warnings if the Ion Chef[™] Instrument detects missing or incompatible consumables. You must address all warnings before the run can start. After you address each condition, touch **Yes** to continue.

IMPORTANT! The Deck Scan function is not a substitute for manual inspection of the reagents and consumables on the Ion Chef[™] Instrument before starting a run. To ensure proper and safe instrument operation, confirm that all consumables are installed correctly before you continue.

- Ion Chef Deck Scan Complete Load Details €
- 6. When Deck Scan is complete, touch Next to display the Data Destination screen.

7. Confirm that the instrument displays the correct kit type, chip type, chip barcodes, and Planned Run. If the correct Planned Runs do not display, touch the dropdown menu w to select the Planned Run for each chip, then touch Next.

Data Des	stination
Kit Type Ion Chef S520 V1	Chip Type: 520
Server 10.45.1 Chip ID: XX0000194	9.226 Chip ID: XX0000191
Sample ID: X0000051	Sample ID: 0000052
ITK4W - TC1_FER-200 🗸	MIYV8 - mt22_fr_prom 💙
Template size 200	Template size 200
Refresh Plans	Cancel Next

IMPORTANT! If the kit and chip type are not correct, confirm that you are using the correct kit and chip. If you are using the correct kit and chip, and an incorrect kit or chip type is displayed on the screen, contact Technical Support.

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

\odot	Run Options				
	٤	Time Choose the approximate time you want your run to end.	05:38PM PST Run ends: Tuesday Current time: 5:38pm PST		
	Ū	Pause Run will pause at the time shown you to analyze templating efficien	05:38PM PST		
			Cancel Start run		

The Ion Chef[™] Instrument provides two options for obtaining quality control (QC) samples that can be used to evaluate templating efficiency. Depending on your selection, the QC samples are made available either during or after the run. In either case, you can obtain unenriched samples from the corresponding Library Sample Tubes at Positions A and B on the Reagents cartridge, or enriched samples from Positions A and E on the Enrichment Cartridge v2.

By selecting You can obtain the QC samples		
Time	immediately after the run ends, at the time you specify (12 hours, 45 minutes after run start).	
Pause	when the instrument pauses operation before the chip loading step (about 11 hours into the run).	

Note: The library in the Library Sample Tube loaded in Position A will be templated onto ISPs that can be sampled in Position E of Enrichment Cartridge v2 after a run. The library in the Library Sample Tube loaded in Position B is templated onto ISPs that can be sampled in Position A of Enrichment Cartridge v2.

9. On the Run Options screen, touch Start run to begin the run.

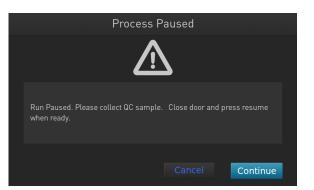
Note: If you need to stop the run for any reason, touch **Cancel**, then touch **Yes** to confirm the cancellation.

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:

- **a.** Remove the consumables from the deck, then clean the instrument. If possible, retain the consumables for troubleshooting.
- **b.** Reset, then reattempt the run. If the run fails again, contact Technical Support to troubleshoot the problem.
- **10.** Initialize the Ion S5[™] or Ion S5[™] XL Sequencer at least 40 minutes before the Ion Chef[™] System finishes chip loading.

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 started within the 24-hour period. 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.

If you chose to pause the run to analyze the templating efficiency, remove the samples for testing when prompted to do so by the Ion Chef[™] Instrument (approximately 11 hours after the start of the run).

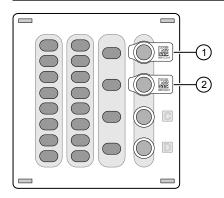


a. When prompted to remove the QC sample, open the instrument door.

IMPORTANT! If you unintentionally close the instrument door before you obtain the QC samples, you must wait until the end of the run before you can collect them. You cannot pause the run or open the door after it has been closed.

b. Transfer the QC samples (entire volume) from Positions A and B of the Ion 520[™] & Ion 530[™] Chef Reagents Cartridge on the instrument deck to two new labeled microcentrifuge tubes.

IMPORTANT! Do not remove the Library Sample Tubes from the Ion 520^{TM} & Ion 530^{TM} Chef Reagents Cartridge.



- Position A (QC Sample)
 Position B (QC Sample)
- c. Analyze the QC samples.
- d. Close the instrument door, then touch Continue to complete the run.
- **12.** When the run is complete, unload the Ion Chef[™] Instrument and sequence the chips immediately.

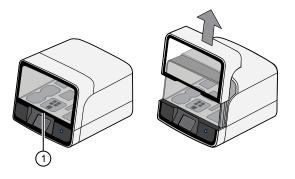
Note: If you cannot sequence a loaded chip immediately, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6–8 hours maximum).

Note: If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge v2 to two new labeled microcentrifuge tubes. See "Supplementary procedures" in the *Ion* $520^{\text{TM}} & \text{Simplementary Procedures}$ is the *Ion* $520^{\text{TM}} & \text{Simplementary Procedures}$ in the *Ion* $520^{\text{TM}} & \text{Simplementary Procedures}$ in the *Ion* $520^{\text{TM}} & \text{Simplementary Procedures}$ is the *Ion* $520^{\text{TM}} & \text{Simplementary Procedures}$ in the *Ion* $520^{\text{TM}} & \text{Simplementary Procedures}$ is t

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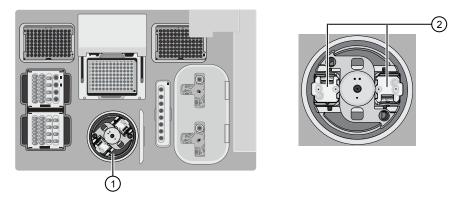
Unload the chips for sequencing

- 1. Open the instrument door:
 - **a.** In the instrument touchscreen, touch (a) (Open Door) then wait for the latch to open.
 - **b.** Lift the instrument door to the top of the travel until the latch mechanism engages.



1 Hold here, then lift

2. Open the lid of the Chip-loading centrifuge, then unload both chip/bucket assemblies from the Ion Chef[™] Instrument.

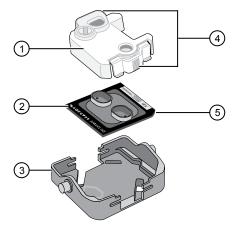


Chip-loading centrifuge
 Remove

To unload each chip:

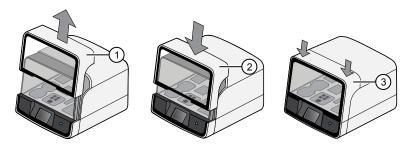
- a. Remove the chip/bucket assembly from the Chip-loading centrifuge.
- **b.** Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.

c. 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.



- 1 Chip Adapter
- Ion chip
- Bucket
- (4) Press and remove adapter
- (5) Remove from bucket carefully
- **3.** 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.



- (1) Lift door first
- 2 Lower
- ③ Press down to lock

4. Load one or both chips into Ion S5[™] Sequencers and promptly start the sequencing runs.

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! If you chose to store a loaded chip, remove the chip from the container and place it on a clean surface in the dark to warm to room temperature at least 20 minutes before running it.



Initialize the sequencer

Initialization takes ~40 minutes.

Ion S5[™] System component positions



- 1 Touchscreen
- Power button
- $\overline{(3)}$ Ion S5TM Sequencing Reagents cartridge
- 4 Chip clamp

- (5) Ion S5[™] Wash Solution bottle. Waste reservoir located behind the Wash Solution bottle (shown on the right).
- (6) Ion S5[™] Cleaning Solution bottle
- Waste reservoir

Note: The system uses RFID technology to verify that the proper reagents are loaded in positions 3, 5, and 6. Reagents that exceed their expiration date or usage count generate an error message prompting the user to replace the reagent before performing the run.

Note: RFID regulatory information can be found on the main screen under **Options → Regulatory info**.



Before you begin

The Ion $S5^{\text{TM}}$ and Ion $S5^{\text{TM}}$ XL Sequencers are equipped to verify the compatibility of each chip and consumable loaded during initialization and sequencing, and that these components do not exceed their expiration date. To avoid exceptions during initialization, inspect this information for each consumable before installing onto the instrument.

• Unbox the Ion S5[™] Sequencing Reagents cartridge 45 minutes before use, then allow it to equilibrate to room temperature.

Do not remove the Ion S5[™] Sequencing Reagents cartridge from its foil packaging until immediately before loading, so that you can return the unused cartridge to storage if your sequencing run is delayed.

- Unbox the Ion S5[™] Wash Solution bottle. Invert the bottle 5 times, then swirl at an angle to mix thoroughly.
- Remove the red cap from the Ion S5[™] Wash Solution and Ion S5[™] Cleaning Solution bottles immediately before installing on the instrument.

When a manual cleaning of the sequencer is required

The Ion S5[™] Sequencer and Ion S5[™] XL Sequencer require that a cleaning be performed before initialization. This 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. However, if the "Enable post-run clean" checkbox (see "Start the sequencing run" on page 46) is unchecked to allow a second run, and a second run is not performed, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed. See "Perform a manual cleaning of the sequencer" on page 56 for more information on how to perform a manual cleaning.

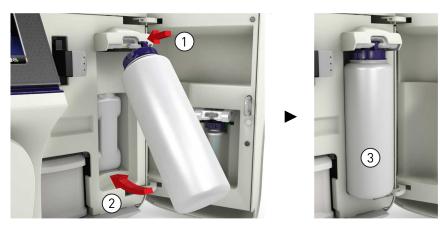
If an Ion S5[™] Sequencer or an Ion S5[™] XL Sequencer is initialized and a sequencing run is not started within 27 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. A purge and clean sequencing run is required before reinitialization. See "Perform a purge and clean run with an initialized, unused Sequencing Reagents cartridge" on page 57 for more information.



Initialize the sequencer

- Ion S5
- 1. In the instrument touchscreen main menu, touch **Initialize**. The door, chip, and Reagent cartridge clamps unlock.

- **2.** When prompted, remove the Ion S5[™] Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.
- **3.** Reinstall the empty waste reservoir.
- **4.** Replace the expended Ion S5[™] Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.
- **5.** Ensure the new Ion S5[™] Wash Solution bottle is thoroughly mixed. Then 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.

Note: The Ion S5[™] Cleaning Solution bottle contains sufficient reagent to complete 4 cleanings.

8. Close the door, then touch Next.

The instrument confirms that the consumables and chip are properly installed and that the Ion S5[™] Cleaning Solution bottle contains sufficient reagent to perform the post-run clean. Follow all on-screen recommendations to ensure proper installation of required consumables.

IMPORTANT! If the allowed number of post-run cleans has been met, the instrument prompts the user to replace the Ion S5[™] Cleaning Solution bottle.

Note: See Appendix A, "Troubleshooting" if a Checking Reagent: Failed warning appears.

9. When initialization is complete (~30–40 minutes), touch Home.

The instrument is now ready for a sequencing run.

Reagent consumables disposal

IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Ion S5[™] reagent consumables.

CO₂ scrubber removal and disposal To properly dispose of the CO_2 scrubber, you must first remove it from the Ion $S5^{TM}$ Sequencing Reagents cartridge.

- Invert the Ion S5[™] Sequencing Reagents cartridge over an appropriate receptacle to drain all residual liquid.
- Wearing gloves, insert the Ion S5[™] Cartridge Tool firmly into the CO₂ scrubber until the flange stops on the top of the cartridge.



Ion S5[™] Cartridge Tool



3. Pull straight up on the tool while holding the nucleotide reagent cartridge down.



4. Remove the scrubber from the cartridge tool, then dispose of the scrubber according to applicable hazardous waste regulations.

The Ion $S5^{TM}$ Wash solution and Ion $S5^{TM}$ Cleaning solution bottles are made of recyclable plastic.

- 1. Open the expended bottle by unscrewing the cap.
- **2.** Remove the cap, sipper, and filter, then pour any residual liquid into an appropriate receptacle.
- **3.** Rinse the empty bottle with water. Pour out the rinse water into the same liquid waste receptacle.
- **4.** Recycle or dispose of the clean bottle, cap, sipper, and filter according to applicable regulations.

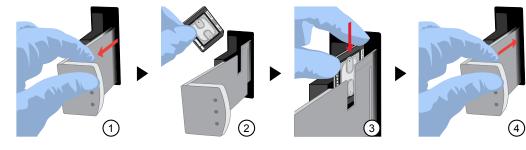
Recycle Ion S5[™] Wash and Cleaning solution bottles



Start the sequencing run

Chip handling guidelines

To place a chip in the chip clamp:



- 1 Slide the chip clamp out.
- (2) Remove the chip currently in the clamp.
- 3 Place the appropriate loaded chip in the chip clamp with the chip notch in the bottom -front corner.

Note: Do not force the chip into the clamp. If the chip does not fit easily in the clamp, confirm that the notch is oriented as shown in the drawing.

(4) Slide the metal tab in fully to engage the clamp, then close the instrument door.



Start the sequencing run

We recommend that you sequence loaded chips on the Ion $S5^{\mathbb{M}}$ or Ion $S5^{\mathbb{M}}$ XL Sequencer 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.

Note: Do *not* press the power button during a run. Interrupting power to the instrument during a run may result in sequencing run failure and loss of sample.

- 1. After completion of initialization, press **Run** in the instrument touchscreen. The door and chip clamp unlock.
- **2.** Remove the used sequencing chip, then secure a chip loaded with template-positive Ion Sphere[™] Particles in the chip clamp.
- **3.** Push the chip clamp all the way in to engage, close the instrument door, then press **Next**.
- 4. Confirm that the correct Planned Run has auto-populated, then press Review.
- **5.** (*Optional*) If this is to be the first of two sequencing runs on this initialization, deselect the **Enable post-run clean** checkbox, then press **Review**.

Note: When starting the second sequencing run on a single initialization, ensure the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.

	Run Selection r10-test	
	Choose a run plan	
Planned Run:	A7RK6 - Oncomine_Plan	~
Chip Barcode:	DACA00615	
	Enable post-run clean	
	Cancel	Review

6. Confirm the remaining pre-populated settings are correct, or make changes using the buttons and dropdown lists if necessary.

Select Run r10-test				
	Confirm the	selected run		
R_2016_06_2		ce_r10-test-67-Oncomine uencing Kit	e_cfDNA	
Run type: TA	G_SEQUENCING	Library name: hg19		
Sample: Chip barcode	e: DACA00615	Project: Post-run clean: true		
Flows: 500		Notes:		
	Data Mgmt	Cancel	Start run	
Edit	Data Mgmt	Cancel	Start run	

7. Confirm that the instrument door is closed, then press **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 may reduce the quality of the measurements.

When the sequencing run is complete, the instrument automatically performs the cleaning procedure unless the **Enable post-run clean** checkbox was deselected. After cleaning, the touchscreen returns to the main menu. Use the Torrent Browser to review the results.



Maintain the sequencer

Required materials	 Lint-free wipes 70% isopropanol (<i>optional</i>)– 10% bleach solution
Clean or decontaminate the sequencer	 In the event of a spill or leak on or inside the instrument, perform the following steps. Note: Dispose of all waste in appropriate liquid or solid waste containers. 1. Remove the Ion S5[™] Wash Solution bottle, then remove and empty the waste reservoir. 2. Remove the Ion S5[™] Sequencing Reagents cartridge.

- **3.** Inspect the waste and nucleotide reagent bays for liquid.
- **4.** Using absorbent paper soak up as much liquid as possible, then wash the affected area with 10% bleach solution.
- 5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.



Clean the Ion Chef[™] System

About the cleaning protocol

The Ion Chef[™] System includes an automated cleaning function that must be performed following every run. The cleaning routine is initiated from the Ion Chef[™] Instrument touchscreen and is designed to minimize potential contamination. During the routine, the instrument irradiates the deck with ultraviolet light for 1 minute after all consumables have been removed from the instrument.

IMPORTANT! Although the Ion Chef[™] Instrument cleaning routine provides some protection against contamination, it is not a substitute for good laboratory technique or precautions. When preparing DNA libraries for use or when preparing the Ion Chef[™] Instrument, make certain to observe sterile laboratory procedures at all times to ensure minimal contamination.

Materials required

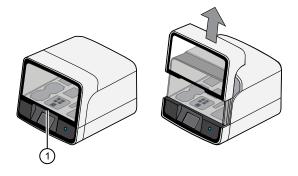
- Gloves, powder-free nitrile
- Isopropanol, 70% solution
- Wipes, lint-free



Clean the Ion Chef[™] instrument

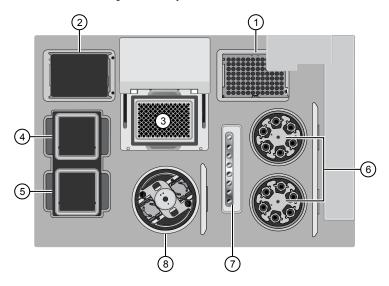
IMPORTANT! Clean the Ion $Chef^{TM}$ Instrument as described below after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.

- 1. Open the instrument door:
 - a. In the instrument touchscreen, touch (a) (Open Door) then wait for the latch to open.
 - **b.** Lift the instrument door to the top of the travel until the latch mechanism engages.



1 Hold here, then lift

2. Remove, then dispose of any used consumables from the instrument.



- (1) Thermal cycler sample block
- (2) Used pipette tip station
- (3) Empty Tip Cartridge v2: move to Used pipette tip station
- (4) Reagents station
- (5) Solutions station
- 6 Chip-loading centrifuge
- (7) Enrichment station

6

(8) Recovery centrifuges

- a. Remove, then discard the PCR plate from the PCR sample block.
- **b.** Remove, then discard the box of used pipette tips from the waste tip position.

IMPORTANT! Handle the disposable reservoir in the waste tip position with care. During the run, liquid waste collects in the reservoir. Dispose of the liquid waste by tipping the reservoir on one corner and pouring the waste into an appropriate waste container:



IMPORTANT! Do not reuse the waste pipette tip rack. Always move the empty Tip Cartridge v2 from the new tip position to the waste tip position.

c. Move the empty Tip Cartridge v2 to the waste tip position.

IMPORTANT! Do not discard the empty Tip Cartridge v2.

d. Remove, then discard the Ion 520[™] & Ion 530[™] Chef Reagents Cartridge.

IMPORTANT! Make sure to transfer the QC samples before you remove and discard the Reagents cartridge.

- e. Remove, then discard the Ion S5[™] Chef Solutions Cartridge.
- f. Close the lid of the Chip-loading centrifuge.
- g. Remove, then discard the Enrichment Cartridge v2.
- h. Remove, then discard the consumables from the Recovery centrifuges, including the:
 - Recovery Station Disposable Lids v2
 - Recovery Tubes v2



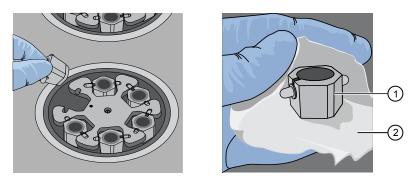
3. Inspect the Recovery centrifuges, then clean the components if excess liquid is present.

Is liquid present?	Action	
No	Go to step 4.	
Yes	Clean the centrifuge bowl and buckets as described below. IMPORTANT! Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do <i>not</i> need to clean the centrifuge after every run.	

To clean the Recovery centrifuge bowl and buckets:

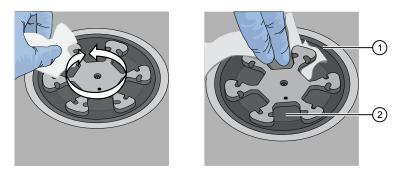
IMPORTANT! Wear powder-free, nitrile gloves when cleaning the Recovery centrifuge.

a. Remove the buckets from the Recovery centrifuges. Clean the inside and outside of each bucket using a lint-free wipe, then place the buckets on a clean, dry surface while you clean the centrifuge.



Bucket
 Lint-free wipe

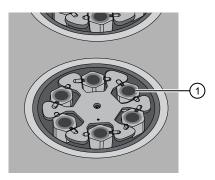
b. Use lint-free wipes to clean the inside rim of the centrifuge, then remove all fluid from the bottom of centrifuge bowl.



- (1) Inside rim of the centrifuge
- Bottom of the centrifuge bowls

Note: When cleaning the centrifuge bowl, fold the wipe in half to provide extra absorbency.

- c. Use lint-free wipes treated with 70% isopropanol to clean the:
 - Inside rim of the centrifuge.
 - Bottom of the centrifuge bowl.
 - Outside and inside of the centrifuge buckets.
- d. Use lint-free wipes to dry the centrifuge and buckets.
- e. Install the centrifuge buckets, then close the centrifuge lid.



(1) Buckets (cleaned and installed)

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

IMPORTANT! Before closing the door, close the lids of the centrifuges.

- 5. From the Ion Chef[™] Instrument touchscreen, start the cleaning:
 - a. On the screen that appears after run completion, touch Next.



Note: You may also clean the instrument at any time starting from the home touchscreen. Touch **Settings**, then touch **Clean Ion Chef**.



b. Confirm that you have removed all consumables from the Ion Chef[™] Instrument, then touch **Next**.



c. With the door closed, touch **Start**. The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef[™] Instrument stops ventilation, then illuminates the ultraviolet (UV) light in the instrument for ~1 minute.





CAUTION! The Ion Chef[™] Instrument emits UV light at 254 nm. Wear appropriate eye wear, protective clothing, and gloves when working near the instrument. Do not look directly at the UV light while it is illuminated during the cleaning routine.



Troubleshooting

Refer to Ion 520TM & Ion 530TM Kit – Chef User Guide

Refer to *Ion* $520^{\text{TM}} & \text{Ion} 530^{\text{TM}} \text{Kit} - Chef User Guide (Pub. No. MAN0010846) for troubleshooting information.$



Supplemental procedures

Perform a manual cleaning of the sequencer

A cleaning protocol is normally performed automatically at the completion of each sequencing run. In the event that a cleaning is necessary, follow the listed steps:

- On the home screen, select Settings ➤ Clean Instrument. The instrument door unlocks allowing access to the consumables.
- **2.** Remove the Ion S5[™] Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.



- **3.** Reinstall the empty waste reservoir and the *used* Ion S5[™] Wash Solution bottle.
- **4.** Ensure the Ion S5[™] Sequencing Reagents cartridge and Ion S5[™] Cleaning Solution bottle are properly installed.

IMPORTANT! Perform the cleaning with a used reagent cartridge and wash solution bottle installed. The cleaning procedure pumps cleaning solution into the wash solution bottle and reagent cartridge making them unsuitable for sequencing.

- **5.** Place a used sequencing chip in the chip clamp, then push the chip clamp in all the way to engage.
- Close the instrument door, then press Next.
 Cleaning takes ~35 minutes to complete. Upon completion the instrument door automatically unlocks and the chip and cartridge clamps disengage.
- **7.** Proceed to "Initialize the sequencer".



Cleaning is normally performed at completion of a sequencing run automatically. If an Ion $S5^{\text{TM}}$ Sequencer or an Ion $S5^{\text{TM}}$ XL Sequencer is initialized and

- a sequencing run is not started within 27 hours after initialization, or
- a sequencing run is not completed due to a power failure or an abort,

a purge and clean sequencing run is required to ensure proper cleaning before reinitialization. Do NOT perform a manual cleaning with an unused, initialized Ion S5[™] Sequencing Reagents cartridge.

Note: If a power failure or abort occurs during the second of two runs started after a single initialization, a manual cleaning (page 56) is sufficient.

To perform a purge and clean run, use the following procedure before reinitialization:

- 1. In the instrument touchscreen main menu, press **Run**. The instrument door and chip clamp unlocks.
- **2.** Ensure that a used sequencing chip is in the chip clamp, then push the chip clamp in all the way to engage.
- 3. Close the instrument door, then press Next.
- **4.** When prompted, select **Planned Run (none)**. Ensure that the **Enable post-run clean** checkbox is selected, then press **Review**.
- In the Select Run screen, press Edit, then in the Detail screen set the number of flows to 500 manually. Ensure that the Post-Run/Clean checkbox is selected, then press Close.
- **6.** Press **Start run**, then press **Accept** to confirm that Post-Run Clean is enabled, and to start the run.

When the purge sequencing run completes, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.



Maintain the Ion Chef[™] System

The Ion $\mathsf{Chef}^{^{\scriptscriptstyle{\mathsf{T}}}}$ System supports the user-performed maintenance procedures found in this section.

Install a firmware update To ensure proper operation of the Ion Chef[™] Instrument, we recommend periodically confirming that your instrument is running the most current firmware. We occasionally release updates to the instrument firmware, which can include important changes to the Ion Chef[™] System operation. To ensure that your instrument is running the most current firmware, check the firmware version as explained below.

Check the Ion Chef[™] Instrument firmware

- 1. On the Ion Chef[™] Instrument touchscreen, touch **Settings**.
- 2. On the Settings screen, touch Check for updates.
- **3.** On the **Software Update** screen, touch **Release** to search the server for updates to the Ion Chef[™] Instrument firmware.

Software Update		
Release	Component	
□ª USB	InstrumentServer-123	
	lonChef_gui-62	
	OS_update-27	
	GM_update-23	
	Cancel Update	

Note: Users are also notified of the availability of firmware updates on the Notifications screen.

4. Select the available component update, touch **Update**, then wait for the instrument to complete the update.

Note: If the Ion Chef[™] Instrument is operating on an isolated network and cannot connect to our website, you must transfer the firmware update manually. To perform a manual update, transfer the firmware files to a USB drive and insert the drive into the USB port at the rear of the instrument. Touch **USB** on the Software Update screen, select from the list of available software components, then touch **Update**.

When finished, the Ion ChefTM Instrument displays the update status.

5. Power off and on the Ion Chef[™] Instrument to complete the update.

IMPORTANT! If you are updating the firmware manually, remove the USB drive before powering on the Ion Chef[™] Instrument.

Change the instrument name

The following procedure describes how to change the name used to identify the Ion $Chef^{TM}$ Instrument both on the network and in the data that it generates.

- 1. In the Ion Chef[™] Instrument home screen, touch **Settings**.
- 2. In the Settings screen, touch Instrument settings.
- **3.** In the Instrument settings screen, touch **Set Instrument Name**. Touch the name field and enter a new instrument name using the keypad that appears, then touch **Save**.

Note: Use only alphanumeric characters. Do not use special characters or spaces in the name.

	Instrument Name	
Instrument name		
	Cancel	

4. In the next screen, touch **Save** again. Power off and on the Ion Chef[™] Instrument to effect the name change.

Download and install BED files

Up-to-date Oncomine[™] cfDNA Assay BED files are available from **thermofisher.com**.

- 1. In the Thermo Fisher Scientific homepage, enter "Oncomine[™] cfDNA Assay" (or the product catalog number) into the search field, then press **Enter**.
- 2. Navigate to the appropriate assay product page, scroll down to Product literature, then click on the desired Target Regions or Hotspots .zip BED file. The file will automatically begin to download.
- **3.** Log in to the Torrent Server where you want to install the Target Regions and Hotspots BED files.
- 4. Click the 🇱 "Gear" tab, then select **References** from the dropdown list.
- 5. Upload the Target Region panel .BED file:

Note: It is not necessary to extract the .zip file prior to uploading the .bed file.

- a. In the left navigation menu, click **Target Regions**, then click the **Add Target Regions** button.
- b. Select hg19 Homo sapiens from the Reference dropdown list.

- **c.** Click **Select File**, then navigate to and select the downloaded Target Regions .zip file:
 - Oncomine_Lung_cfDNA.06012016.Designed.bed
 - Oncomine_Colon_cfDNA.10242016.Designed.bed
 - Oncomine_Breast_cfDNA.08032016.Designed.bed

New Target Regions

Target Regions File :	Select File Please select a BED file to upload.
Reference :	ImmuneResponse_v3.1 - Immune Response (v3.1)
Description :	optional
Notes :	optional
Upload Target Regions Fil	e Cancel

- d. Click Open, then click Upload Target Regions File.
- 6. Upload the Hotspots .BED file:

Note: It is not necessary to extract the .zip file prior to uploading the .bed file.

- **a.** In the left navigation menu, click **Hotspots**, then click the **Add Hotspots** button.
- b. Select hg19 Homo sapiens from the Reference dropdown list.
- **c.** Click **Select File**, then navigate to and select the downloaded Hotspots .zip file:
 - Oncomine_Lung_cfDNA.06012016.Hotspots.bed
 - Oncomine_Colon_cfDNA.10242016.Hotspots.bed
 - Oncomine_Breast_cfDNA.08032016.Hotspots.bed
- d. Click Open, then click Upload Hotspots File.

The Target Regions and Hotspots BED files upload to your Torrent Server and appear in the respective dropdown lists.



Guidelines for Ion OneTouch[™] 2 System users

Proceed to template preparation and sequencing using the following kits appropriate to your instrument setup. To create a specific Run Plan for use in templating and sequencing see "Create a Planned Run" on page 14. Refer to the user guide listed in the following table for more information.

Template System	Sequencer	Kit	User Guide
lon OneTouch [™] 2		lon 520 [™] & lon 530 [™] Kit – 0T2 (Cat. No. A27751)	<i>Ion 520[™] & Ion 530[™] Kit – 0T2 User Guide</i> (Pub. No. MAN0010844)

Safety





WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!** Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

	Symbol	English	Français
	Â	Caution, risk of danger	Attention, risque de danger
	Consult the manual for further safety information.	Consulter le manuel pour d'autres renseignements de sécurité.	
	ŧ	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)

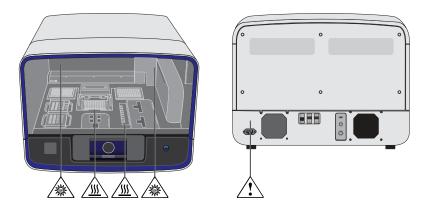
Symbol	English	Français
Symbol	English Do not dispose of this product in unsorted municipal waste ▲ CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible	 Français Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. CAUTION! Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets munici-
	disposal options.	paux pour les dispositions d'élimination et communi- quer avec le service à la cli- entèle pour des renseigne- ments sur les options d'élimi- nation responsable.

Conformity symbols

The following table applies only to the Ion Chef[™] Instrument.

Conformity mark	Description	
	Indicates conformity with safety requirements for Canada and U.S.A.	
CE	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.	
C	Indicates conformity with Australian standards for electromagnetic compatibility.	

Location of safety labels on this instrument



Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical **WARNING!** Ensure appropriate electrical supply. For safe operation of the instrument: Plug the system into a properly grounded receptacle with adequate current capacity. • Ensure the electrical supply is of suitable voltage. · Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument. WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible. Cleaning and **CAUTION!** Cleaning and Decontamination. Use only the cleaning and decontamination decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met: No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment. • The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service). Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment. Ultraviolet (UV) 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 Safety protocol. Safety interlocks are used to ensure that the UV lamp is not powered when

Safety and electromagnetic compatibility (EMC) standards

the door is open.

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:



Safety compliance

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i>
IEC 61010-2-010 EN 61010-2-010	Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials
IEC/EN 61010-2-020	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges</i>
IEC 61010-2-081 EN 61010-2-081	Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
FCC Part 15	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS 2064	<i>Limits and Methods of Measurement of Electromagnetic</i> <i>Disturbance Characteristics of Industrial, Scientific, and</i> <i>Medical (ISM) Radiofrequency Equipment</i>
ICES-001, Issue 3	Industrial, Scientific and Medical (ISM) Radio Frequency Generators

Environmental design

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive" – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
- www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
 World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
 www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Description
<i>Oncomine[™] cfDNA Assays, Part I: Library Preparation User Guide</i> (Pub. No. MAN0014688)	Describes the preparation of Oncomine [™] cfDNA Assay libraries.
Oncomine [™] cfDNA Assays, Part II: Plan a	Describes the automated template
Run, Template Preparation, and	preparation of Oncomine [™] cfDNA Assay
Sequencing User Guide	libraries using the Ion Chef [™] System for
(Pub. No. MAN0015873)	sequencing on the Ion S5 [™] System.
<i>Oncomine[™] cfDNA Assays, Part III: Variant</i>	Describes how to perform variant analysis
Analysis User Guide	on Oncomine [™] cfDNA Assay sequence data,
(Pub. No. MAN0015874)	and how to view, sort, and filter results.

Note: For additional documentation, see "Customer and technical support" on page 69.

Obtain information from the Help system

The Torrent Suite ${}^{{}^{\mathrm{M}}}$ Software has a Help system that describes how to use each feature of the user interface.

In the toolbar of the Torrent Suite[™] Software window, click **Help** → **Software Help**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic

Customer and technical support

Visit thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support

- Product documentation, including:
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)
 - **Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/ terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

