ion torrent

Oncomine™ Myeloid Research Assay

USER GUIDE

Catalog Numbers A36940, A36941

Publication Number MAN0016113

Revision G.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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

Revision history: Pub. No. MAN0016113

Revision	Date	Description
G.0	27 October 2020	Updated to include new library preparation instruction on Ion Chef™ Instruments with Torrent Suite™ Software 5.14 or later.
F.0	29 May 2018	Updated to include: • New Ion Reporter™ Software 5.10 "w3.0" workflows. • Incorporation of FLT3ITD algorithm in Ion Reporter™ Software 5.10 workflows. • Ion GeneStudio™ S5 Series Systems.
E.0	23 February 2018	Correction to the kit contents table, indicating that the materials provided are sufficient to prepare libraries from 24 research samples.
D.0	11 December 2017	*Oncomine™ Myeloid Research Assay User Guide*. Provides instruction for the preparation, templating, sequencing, and data analysis of Oncomine™ Myeloid Research Assay libraries.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Oncomine™ Myeloid Research Assay contains three pools of Ion AmpliSeq™ oligonucleotide primers and associated reagents to generate amplicon libraries for next-generation sequencing (NGS) on an Ion GeneStudio™ S5 Series or Ion PGM™ System. The assay is designed to detect variants across 40 key DNA genes and a broad panel of 29 fusion driver gene targets relevant in major myeloid disorders: acute myeloid leukemia (AML), myeloid dysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML).

Features include:

- Compatible with as little as 10 ng/pool input DNA or RNA per library from blood or bone marrow samples
- Excellent coverage of challenging targets such as CEBPA and FLT3-ITDs
- Validated detection of somatic variants down to 5% allele frequency
- Includes the Ion AmpliSeq™ Sample ID Panel primers to prevent research sample misidentification and provide gender determination.

Designed for research use only (RUO), the Oncomine™ Myeloid Research Assay is a comprehensive, targeted NGS assay designed to assist scientists in the understanding of myeloid cancer. The assay is a complete kit facilitating the amplification of multiple gene targets from blood or bone marrow samples with as little as 10 ng of input DNA or RNA per pool. Leveraging the power of Ion AmpliSeq™ technology, this highly multiplexed NGS assay enables the generation of results from multiple samples (12 samples (DNA and RNA) on a single Ion 530™ Chip, 4 samples on an Ion 318™ Chip) with a single run in days rather than weeks. The assay is aligned with bioinformatic analysis workflows within Torrent Suite™ and Ion Reporter™ Software that utilize optimized variant-calling parameters for SNV, InDel, and FLT3 large internal tandem duplication (ITD) detection.

This guide covers library preparation from DNA and RNA using the following products:

- Oncomine™ Myeloid Research Assay (Cat. No. A36940, manual library preparation)
  - Oncomine™ Myeloid DNA Panel (2 pools)
  - Oncomine™ Myeloid RNA Panel (1 pool)
- Oncomine™ Myeloid Research Assay – Chef-Ready (Cat. No. A36941, automated library preparation)
- Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990)
- Ion Xpress™ Barcode Adapters (various Cat. Nos.)

- IonCode™ Barcode Adapters (various Cat. Nos.)
- Ion Library Equalizer™ Kit (Cat. No. 4482298)

Note: The Oncomine™ Myeloid Research Assay – Chef-Ready library preparation kit (Cat. No. A36941) automates preparation and combining of up to 8 barcoded sample libraries (DNA and RNA) to be sequenced on a single Ion 530™ Chip (see page 9), minimizing hands on time and the per-sample sequencing cost. The kit provides the Oncomine™ Myeloid DNA Panel (2-pools) and Oncomine™ Myeloid RNA Panel (1-pool) at 2X concentration premeasured in barcoded Primer Pool tubes ready to load into an Ion AmpliSeq™ Chef Reagents DL8 cartridge.

Prerequisites

This guide also assumes that you have:

- A general understanding of Ion Torrent™ sequencing chemistry and workflow (See "Related documentation" on page 82 for more information)
- Knowledge of techniques for handling and preparing DNA and RNA libraries

Contents and storage

Oncomine™ Myeloid Research Assay

The Oncomine™ Myeloid Research Assay (Cat. No. A36940) consists of the Oncomine™ Myeloid DNA Panel (2-pool), Oncomine™ Myeloid RNA Panel (single pool), and two Ion AmpliSeq™ Library Kit Plus kits. Sufficient reagents are provided for the rapid preparation of 24 sample libraries (barcoded DNA- and RNA-libraries).

Component	Amount	Storage
Oncomine™ Myeloid Research Assay–DNA (24 reactions)		
5X Oncomine™ Myeloid DNA Panel (pool 1 of 2)	48 µL	–30ºC to –10ºC
5X Oncomine™ Myeloid DNA Panel (pool 2 of 2)	48 µL	
Oncomine™ Myeloid Research Assay–RNA (24 reactions)		
5X Oncomine™ Myeloid RNA Panel (pool 1 of 1)	96 µL	–30ºC to –10ºC
Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990)		
5X Ion AmpliSeq™ HiFi Mix (red cap)	2 × 120 µL each	–30ºC to –10ºC
FuPa Reagent (brown cap)	2 × 48 µL each	
Switch Solution (yellow cap)	2 × 96 µL each	
DNA Ligase (blue cap)	2 × 48 µL each	
25X Library Amp Primers[1] (white cap)	2 × 48 µL each	
1X Library Amp Mix (black cap)	2 × 1.2 mL each	
Low TE (clear cap)	2 × 6 mL each	15°C to 30°C[2]

[1] Not used with the Ion Library Equalizer™ Kit, but can be used for library amplification if quantifying amplified library with the Qubit™ 4 or Qubit™ 3.0 Fluorometer.

[2] Can be stored at –30ºC to –10ºC for convenience.

Oncomine™ Myeloid Research Assay – Chef-Ready

The Oncomine™ Myeloid Research Assay – Chef-Ready Kit (Cat. No. A36941, ordered separately) provides the Oncomine™ Myeloid DNA Panel (2-pools) and the Oncomine™ Myeloid RNA Panel (1-pool) at 2X concentration pre-measured in barcoded Primer Pool tubes ready to load into an Ion AmpliSeq™ Chef Reagents DL8 cartridge. In addition, the kit provides all the reagents and supplies in an Ion AmpliSeq™ Kit for Chef DL8 (Cat. No. A29024) sufficient for preparing 32 libraries. See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for detailed information on preparing Oncomine™ Myeloid Research Assay libraries on the Ion Chef™ System.

Component	Amount	Storage
Oncomine™ Myeloid DNA Research Assay – Chef-Ready (32 reactions)		
2X Oncomine™ Myeloid DNA Panel (pool 1 of 2)	4 × 150 µL	–30°C to –10°C
2X Oncomine™ Myeloid DNA Panel (pool 2 of 2)	4 × 150 µL	
Oncomine™ Myeloid RNA Research Assay – Chef-Ready (32 reactions)		
2X Oncomine™ Myeloid RNA Panel (pool 1 of 1)	8 × 150 µL	–30°C to –10°C
Ion AmpliSeq™ Kit for Chef DL8 (Cat. No. A29024)		
Ion AmpliSeq™ Chef Reagents DL8	2 × 4 cartridges	–30°C to –10°C
Ion AmpliSeq™ Chef Solutions DL8	2 ×4 cartridges	2°C to 8°C[1]
Ion AmpliSeq™ Chef Supplies DL8 (per insert) • Ion AmpliSeq™ Tip Cartridge L8 • PCR Frame Seal • Enrichment Cartridge	2 boxes with 4 inserts	15°C to 30°C
IonCode™ 0101–0132 in 96 Well PCR Plates (dried) Set includes 4 PCR plates: • IonCode™ 0101–0108 in 96 Well PCR Plate (red) • IonCode™ 0109–0116 in 96 Well PCR Plate (yellow) • IonCode™ 0117–0124 in 96 Well PCR Plate (green) • IonCode™ 0125–0132 in 96 Well PCR Plate (blue)	2 sets of 4 plates	15°C to 30°C

[1] Ion AmpliSeq™ Chef Solutions DL8 cartridges are shipped at ambient temperature, but need to be stored at 2°C to 8°C upon arrival.

Materials required but not supplied

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

Ion Library Equalizer™ Kit

The Ion Library Equalizer™ Kit, ordered separately, provides a streamlined method for normalizing library concentration without quantification. The Ion Library Equalizer™ Kit (Cat. No. 4482298) contains reagents for 96 reactions.

Note: Manual library preparation only, the Ion Library Equalizer™ Kit is not required for use with the Oncomine™ Myeloid Research Assay – Chef-Ready kit (Cat. No. A36941).

Component	Amount	Storage
Equalizer™ Primers (pink cap)	200 µL	2°C to 8°C
Equalizer™ Capture (purple cap)	1 mL	
Equalizer™ Elution Buffer (clear cap)	10 mL	
Equalizer™ Beads (orange cap)	300 µL	
Equalizer™ Wash Buffer (clear cap)	35 mL	15–30°C[1]

[1] Can be stored at 2–8°C.

Ion Xpress™ Barcode Adapters Kits

Each kit, ordered separately, provides 16 different barcode adapters sufficient for 640 total reactions.

Note: Not required for use with the Oncomine™ Myeloid Research Assay – Chef-Ready kit (Cat. No. A36941). For use with manual library preparation only.

Component	Quantity	Volume	Storage
Ion Xpress™ P1 Adapter (violet cap)	1 tube	320 µL	–30°C to –10°C
Ion Xpress™ Barcode X (white cap)	16 tubes (1 per barcode)	20 µL each	

The following Ion Xpress™ Barcode Adapters Kits are available:

- Ion Xpress™ Barcode Adapters 1–16 (Cat. No. 4471250)
- Ion Xpress™ Barcode Adapters 17–32 (Cat. No. 4474009)
- Ion Xpress™ Barcode Adapters 33–48 (Cat. No. 4474518)
- Ion Xpress™ Barcode Adapters 49–64 (Cat. No. 4474519)
- Ion Xpress™ Barcode Adapters 65–80 (Cat. No. 4474520)
- Ion Xpress™ Barcode Adapters 81–96 (Cat. No. 4474521)
- Ion Xpress™ Barcode Adapters 1–96 (Cat. No. 4474517, complete set of adapters)

Additional required materials

Note: Ion GeneStudio™ S5 Series System users.

- When preparing template with the Ion Chef™ System, refer to the *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854) for required materials not supplied.

IMPORTANT! The Oncomine™ Myeloid Research Assay is not compatible with the Ion 520™ & Ion 530™ Kit – Chef (Cat. No. A27757) or Ion 540™ Kit – Chef (Cat. No. A27759).

Ion PGM™ System users.

- If preparing template with the Ion OneTouch™ 2 System, refer to the *Ion PGM™ Hi-Q™ View OT2 Kit User Guide* (Pub. No. MAN0014579) and *Ion PGM™ Hi-Q™ View Sequencing Kit User Guide* (Pub. No. MAN0014583) for required materials not supplied.
- If preparing template with the Ion Chef™ System, refer to the *Ion PGM™ Hi-Q™ View Chef Kits User Guide* (Pub. No. MAN0014571) for required materials not supplied.

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

Item	Source
Library preparation	
Agencourt™ AMPure™ XP Kit	Beckman Coulter A63880 or A63881
(RNA only) SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme	11766050
One of the following: • SimpliAmp™ Thermal Cycler • AB™ 2720 Thermal Cycler • Veriti™ 96-Well Thermal Cycler • ProFlex™ 96-well PCR System • GeneAmp™ PCR System 9700[1] or Dual 96-well Thermal Cycler	See web product pages
MicroAmp™ Optical 96-Well Reaction Plate	N8010560 4306737 (with barcode)
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
DynaMag™–96 Side Magnet, or other plate magnet	12331D
Nuclease-free Water	AM9932
Absolute ethanol	**MLS**
Pipettors, 2–200 µL, and low-retention filtered pipette tips	**MLS**

(continued)

Item	Source
Template preparation and sequencing	
with Ion GeneStudio™ S5 Series Systems	
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef	A34019 A34461[2]
Ion 530™ Chip Kit	A27764
with Ion PGM™ System	
Ion PGM™ Hi-Q™ View OT2 Kit	A29900
Ion PGM™ Hi-Q™ View Chef 400 Kit	A30798
Ion PGM™ Hi-Q™ View Sequencing Kit	A30044
Ion 318™ Chip Kit v2 BC	4488150

[1] Supported but no longer available for purchase.

[2] Cat. No. A34461 only provides sufficient reagents for a single sequencing run per initialization when performing 400 bp read sequencing.

Recommended materials

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

Item	Source
Recommended additional equipment	
Real-time PCR instrument (e.g., Applied Biosystems™ 7900HT, 7500, StepOne™, StepOnePlus™, ViiA™ 7 Systems, or QuantStudio™ 12K Flex Real–Time PCR System)	See web product pages
Qubit™ 4 Fluorometer[1]	Q33238
96-well plate centrifuge	**MLS**
Recommended for nucleic acid quantification	
TaqMan™ RNase P Detection Reagents Kit[2]	4316831
Qubit™ dsDNA HS Assay Kit (DNA)	Q32851/Q32854
Qubit™ RNA HS Assay Kit (RNA)	Q32855
Recommended for library quantification	
Ion Library TaqMan™ Quantitation Kit	4468802
Recommended controls	
AcroMetrix™ Oncology Hotspot Control[3]	969056
Seraseq™ Myeloid Mutation DNA Mix[4]	0710-0408
Seraseq™ Myeloid Fusion RNA Mix[5]	0710-0407

[1] Qubit™ 2.0 & Qubit™ 3.0 Fluorometers are supported but no longer available for purchase.
[2] Other qPCR targets such as GUSB can be used for DNA and RNA quantification. Contact your local Field Application Scientist for details.
[3] See page 74 for detailed mutation detection information.
[4] See page 77 for detailed mutation detection information.
[5] See page 78 for detailed mutation detection information.

Sequencer compatibility

Ion GeneStudio™ S5 Series Sequencer or Ion GeneStudio™ S5 Series System refers generically to following systems, unless otherwise specified.

- Ion GeneStudio™ S5 System (Cat. No. A38194)
- Ion GeneStudio™ S5 Plus System (Cat. No. A38195)
- Ion GeneStudio™ S5 Prime System (Cat. No. A38196)

2 Before you begin

Procedural guidelines

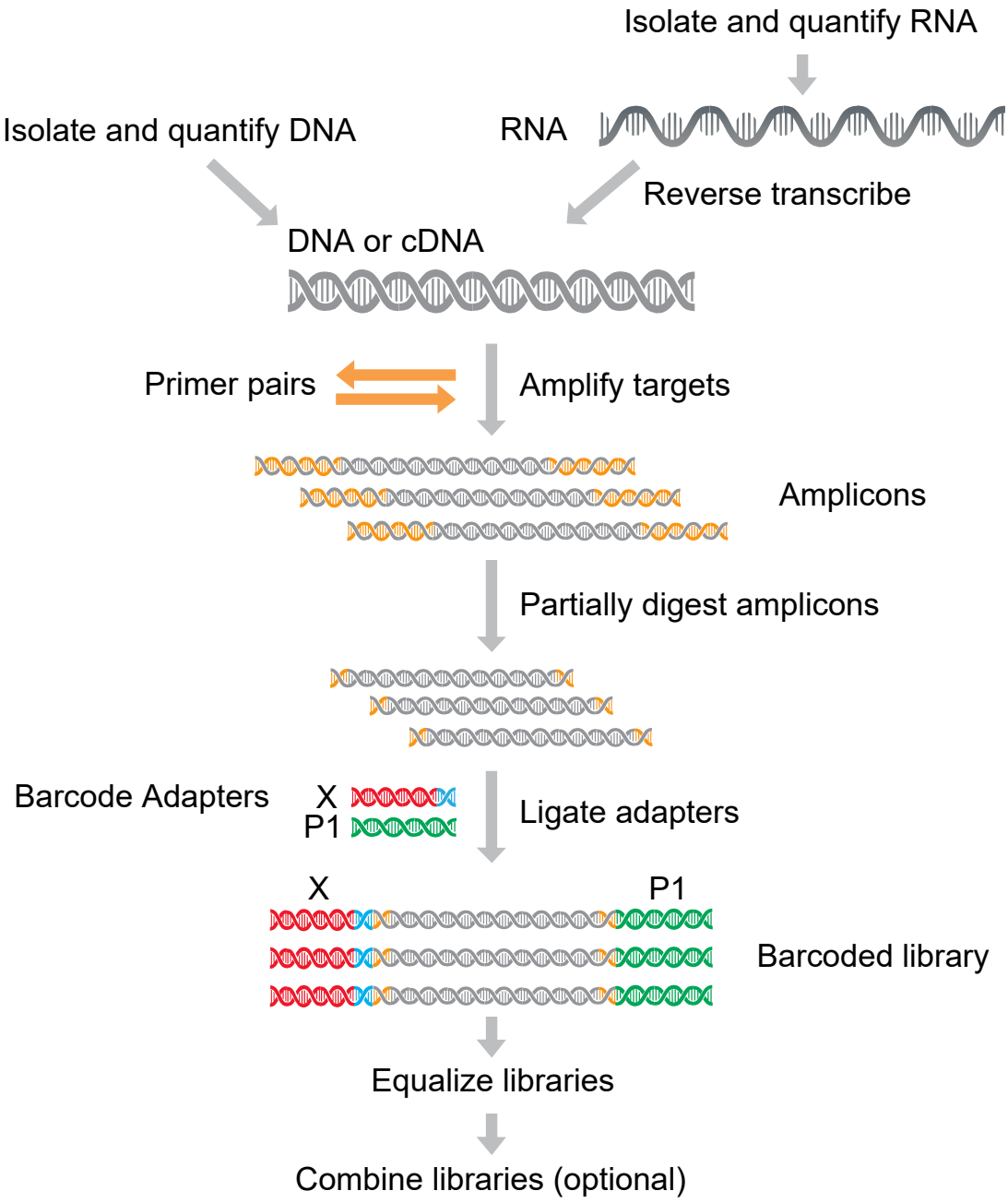
- The Oncomine™ Myeloid Research Assay does not support library preparation from nucleic acids prepared from formalin-fixed paraffin-embedded (FFPE) samples.
- Minimize freeze-thaw cycles of Oncomine™ Myeloid Research Assay panels by aliquoting as needed for your experiments. Panels can be stored at 4°C for one year.
- Use good laboratory practices to minimize cross-contamination of products. If possible, perform PCR setup in an area or room that is free of amplicon contamination. Always change pipette tips between samples.
- Use a calibrated thermal cycler specified in "Additional required materials" on page 11.
- Pipet viscous solutions, such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, Switch Solution, DNA Ligase, and panels, slowly and ensure complete mixing by vortexing or pipetting up and down several times.
- Arrange samples in alternating columns on the plate for easier pipetting with multichannel pipettes during purification with the DynaMag™ Side Magnet.
- Prepare the 70% ethanol the same day you will use it.

Before each use of the kit

- Thaw components that contain enzymes—such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, DNA Ligase, and 1X Library Amp Mix—on ice, and keep on ice during procedure. All other components, including primer pools, can be thawed at room temperature. Gently vortex and centrifuge before use.
- If there is visible precipitate in the Switch Solution after thawing, vortex or pipet up and down at room temperature to resuspend.
- Bring the Agencourt™ AMPure™ XP Reagent to room temperature.

IMPORTANT! Do NOT substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

Workflow


Isolate and quantify RNA
Isolate and quantify DNA
RNA
Reverse transcribe
DNA or cDNA
Primer pairs
Amplify targets
Amplicons
Partially digest amplicons
Barcode Adapters
X
P1
Ligate adapters
X
P1
Barcoded library
Equalize libraries
Combine libraries (optional)

3 Library preparation

Guidelines for RNA isolation, quantification, and input

- Each reverse transcription reaction requires 10 ng (≥2.5 ng/µL) of DNase-treated total RNA. We recommend use of ezDNase™ Enzyme for treatment of total RNA. For more information, see "Digest total RNA with ezDNase™ Enzyme" on page 63.
- We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32855) for quantifying RNA.
- Use your preferred commercially available kit to sequentially isolate high quality gDNA and RNA from research samples for use in library preparation.
- In general, library yield from high quality RNA is greater than from degraded samples. Library yield is not indicative of sequencing performance.
- Increasing the amount of RNA will usually result in higher quality libraries, especially when RNA quality or quantity is unknown.

Guidelines for DNA isolation, quantification, and input

- Each target amplification reaction requires 20 ng of gDNA (≥1.48 ng/µL, 10 ng/pool) from human whole blood or bone marrow.
- Use your preferred commercially available kit to sequentially isolate high quality gDNA and RNA from research samples for use in library preparation.
- We recommend the TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831) for quantifying amplifiable human genomic DNA (refer to the *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan™ RNAse P Detection Reagents Kit* Pub. No. MAN0007732). Other qPCR targets such as GUSB can be used for DNA and RNA quantification. Contact your local Field Application Scientist for details. The Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) can also be used.
- Quantification methods such as densitometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA. Use of these methods can lead to gross overestimation of the sample DNA concentration, under-seeding of the target amplification reaction, and low library yields.
- Increasing the amount of DNA results in higher-quality libraries, especially when DNA quality or quantity is unknown.

Chef Ready: Library preparation

Note: The Oncomine™ Myeloid Research Assay – Chef-Ready does not support library preparation from nucleic acids prepared from formalin-fixed paraffin-embedded (FFPE) samples.

Create a Sample Set manually

Note: Creating a Sample Set is only required in Torrent Suite™ Software 5.14 or later.

Create a Sample Set manually by entering sample information into the Torrent Suite™ Software without the use of an external CSV file. This method of entering sample information is useful for creating small Samples Sets.

To create a Sample Set manually, enter individual samples into the software, then create a new Sample Set and add samples to it. Alternatively, you can add new samples to an existing Sample Set.

For Sample Sets that contain numerous samples, you can import samples using a CSV file. For more information, see “Create a Sample Set by importing samples from a CSV file” on page 67.

1. Sign in to the Torrent Suite™ Software.
2. In the **Plan** tab, click **Samples**, then click **Add or Update Sample Set/Samples**.
3. Click **Enter New Sample**.
   a. Complete the **Add Sample** dialog box. For more information about defining the samples, see the Torrent Suite™ Software Help.

   Note: Sample Name is the only required input.

b. Click **Done**.
   The new sample and sample attributes appear in the **Enter Samples** list.

c. Enter more samples if needed.

4. Add the samples to a sample set. Do one of the following:

   - Click **Add to existing Sample Set**, then select an existing Sample Set to contain the samples.
   - Click **Create Sample Set**, then complete the sample set attributes as indicated in the following table.

Attribute	Action
Sample Set Name (Required)	Enter a name for this Sample Set. Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_).
Group Type	Select DNA and Fusions from the dropdown list.
PCR Plate Serial Number	*Optional*
Description	*Optional*
Library Prep Type	Select AmpliSeq on Chef from the dropdown list.
Library Prep Kit	Select Ion AmpliSeq Kit for Chef DL8 from the dropdown list.
Library Prep Protocol	Select Myeloid from the dropdown list.

5. Click **Save Sample Set**.
   The set name appears in the Sample Sets list.

You can use the Sample Set to create a Planned Run. The information from the Sample Set and individual samples in the Sample Set are prepopulated in the Planned Run workflow bar steps and Planned Run template. For more information, see “Create a Planned Run with Sample Sets” on page 47.

Reverse transcribe RNA for Chef Ready library preparation

If you are starting from RNA, you must first reverse transcribe RNA to cDNA.

IMPORTANT! For ezDNase™ Enzyme total RNA treatment before reverse transcription and cDNA target amplification, see "Digest total RNA with ezDNase™ Enzyme" on page 63.

Prepare the reverse transcription reactions on ice or a cold block pre-chilled to 2–8°C to prevent RNA degradation.

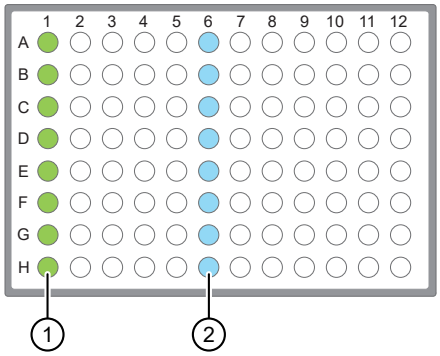
1. Remove and discard the plate seal from an IonCode™ 96-well PCR Plate, then place on ice or in a chilled cold block.

2. For each sample, add the following components into a single well in column 1 of the IonCode™ 96-well plate (provided in the Ion AmpliSeq™ Kit for Chef DL8). Prepare a master mix without sample RNA for multiple reactions.

Component	Volume	
	Total RNA	ezDNase™ Enzyme treated total RNA[1]
SuperScript™ IV VILO™ Master Mix	2 µL	2 µL
Total RNA (10 ng)[2]	≤8 µL	≤5 µL
Nuclease-free Water	to 10 µL	to 10 µL
Total volume per well	**10 µL**	**10 µL**

[1] For more information, see "Digest total RNA with ezDNase™ Enzyme" on page 63.

[2] Do not exceed 5 µL ezDNase™ Enzyme treated total RNA in the reverse transcription reaction. If ezDNase™ Enzyme treated total RNA exceeds 50% of the reverse transcription reaction total volume, reverse transcription may be inhibited. Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).



① Each column 1 well contains a 10 µL reverse transcription reaction, or no-template control reaction.

② Each column 6 well contains a dried-down IonCode™ barcode. The lowest barcode number is in A6, and the highest is in H6. All appear light blue in the actual plates.

3. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

4. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program to synthesize cDNA.

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

STOPPING POINT Samples can be stored at 10°C for up to 16 hours in the thermal cycler. For longer term, store at –20°C.

5. Briefly centrifuge the plate to collect any droplets at the bottom of the wells.

6. Pipet 5 µL of nuclease-free water into each cDNA synthesis reaction in column 1 of the IonCode™ 96-well plate.

7. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

Following completion of cDNA synthesis see "Thaw the reagents and prepare the instrument" in the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for instructions to prepare Oncomine™ Myeloid Research Assay libraries on the Ion Chef™ System.

For information on how to set up the Ion Chef™ Instrument, see “Ion Chef™ Instrument setup information for Chef Ready kit users” on page 22.

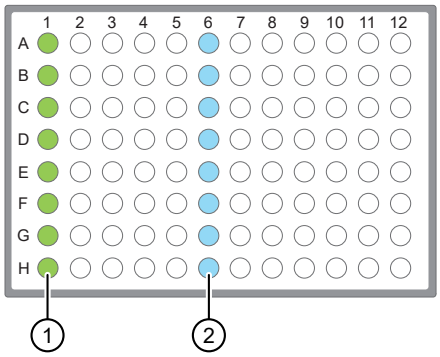
Add DNA to an IonCode™ PCR plate

1. Remove and discard the plate seal from an IonCode™ 96 Well PCR Plate.

2. For each sample, add the following components into a single well in column 1 of the IonCode™ 96-well plate (provided in the Ion AmpliSeq™ Kit for Chef DL8).

Component	Volume
gDNA (10 ng, ≥0.67 ng/µL)[1]	≤15 µL
Nuclease-free Water	to 15 µL
Total volume per well	**15 µL**

[1] Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).



① Each column 1 well contains 15 µL of diluted gDNA sample (0.67 ng/µL, 10 ng total), or Nuclease-free Water as non-template control.

② Each column 6 well contains a dried-down IonCode™ barcode. The lowest barcode number is in A6, and the highest is in H6. All appear light blue in the actual plates.

Note:

- If you are processing fewer than 8 samples, it is preferable to add replicates or positive control samples to the run. Otherwise, pipet 15 µL of Nuclease-free Water as non-template control into column 1 wells that do not contain a DNA sample.
- If processing 5 or fewer samples, we recommend that you quantify the output combined library by qPCR to ensure that an optimal concentration is used in templating reactions.

3. Carefully inspect each well for air bubbles. Remove any air bubbles by gentle pipetting. Alternatively, seal the plate with MicroAmp™ Adhesive Film, then briefly centrifuge the plate in a plate centrifuge.

IMPORTANT! Offset the film to the left so that the adhesive does not cover the barcode label. If the barcode label becomes damaged, you can override the error during Deck Scan on the Ion Chef™ Instrument.

Proceed to "Thaw the reagents and prepare the instrument" in the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for instructions to prepare Oncomine™ Myeloid Research Assay libraries on the Ion Chef™ System.

For information on how to set up the Ion Chef™ Instrument, see “Ion Chef™ Instrument setup information for Chef Ready kit users” on page 22.

Ion Chef™ Instrument setup information for Chef Ready kit users

See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for detailed information on preparing Oncomine™ Myeloid Research Assay libraries on the Ion Chef™ System.

IMPORTANT! When starting the library preparation run on the Ion Chef™ Instrument ensure that the correct Kit Type and Sample set are selected, and that **Myeloid** is displayed in order to properly prepare Oncomine™ Myeloid Assay–Chef Ready libraries. If Myeloid is not displayed the default library preparation script is run which may result in low quality libraries. The Myeloid specific library preparation script is only available in Torrent Suite™ Software 5.14 or later.

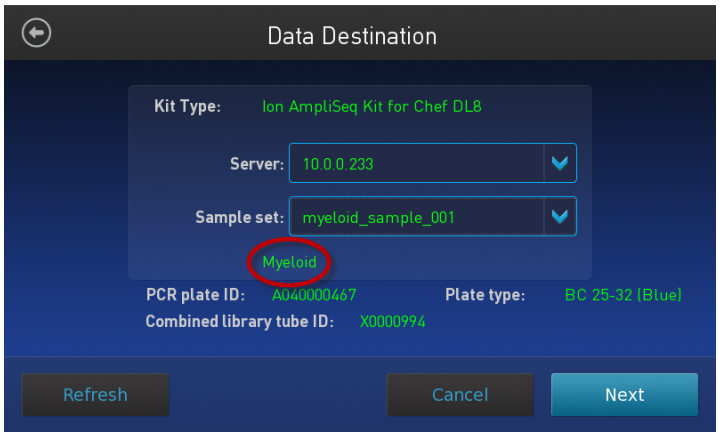


Figure 1 Example of a correct Oncomine™ Myeloid Assay setup

Ensure that Myeloid appears below the Sample set dropdown list.

DNA samples will require between 14–17 cycles of target amplification and RNA samples will require between 25–28 cycles of target amplification. Samples of lower quality may require use of cycling parameters near the high end of the range. During Ion Chef™ Instrument setup, enter the following parameters when prompted.

Stating material	# of primer pools	Target amplification cycles	Anneal & extension time
DNA[1]	2	17	4 minutes
RNA (cDNA)[1]	1	25	4 minutes

[1] If both high quality and low quality nucleic acids are being used in the same reaction, use the low quality parameters.

Manual library preparation

Reverse transcribe RNA for manual library preparation

If you are starting from RNA, you must first reverse transcribe RNA to cDNA.

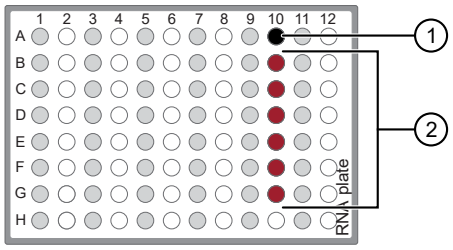
IMPORTANT! For reverse transcription and cDNA target amplification procedures for use with ezDNase™ Enzyme treated total RNA, see "Reverse transcribe ezDNase™ Enzyme treated total RNA for manual library preparation" on page 64.

Prepare the reverse transcription reactions on ice or a cold block pre-chilled to 2–8°C to prevent RNA degradation.

1. For each sample, add the following components into a single well of a 96-well PCR plate. Prepare a master mix without sample RNA for multiple reactions.

Component	Volume
SuperScript™ IV VILO™ Master Mix	1 µL
Total RNA (10 ng)[1]	≤4 µL
Nuclease-free Water	to 5 µL
Total volume per well	**5 µL**

[1] Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).



① No-template control (NTC)
② Samples

2. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

3. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program to synthesize cDNA.

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

STOPPING POINT Samples can be stored at 10°C for up to 16 hours in the thermal cycler. For longer term, store at –20°C.

4. Briefly centrifuge the plate to collect any droplets at the bottom of the wells, then proceed to the next step.

Prepare cDNA target amplification reactions

IMPORTANT! Primer pools and HiFi Mix are viscous. Pipet slowly and mix thoroughly.

1. Place the 96-well plate in a pre-chilled cold block or on ice.
2. Thaw the 5X Ion AmpliSeq™ HiFi Mix on ice, gently vortex to mix, then centrifuge briefly to collect.
3. Remove the seal from the plate, then add the following components to each cDNA synthesis reaction. Prepare a master mix for multiple reactions.

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
5X Oncomine™ Myeloid RNA Panel	4 µL
Nuclease-free Water	7 µL
Total volume per well (includes 5 µL from cDNA synthesis)	**~20 µL**

4. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

Proceed to “Amplify the cDNA targets”.

Amplify the cDNA targets

IMPORTANT! When amplifying multiple samples in a single PCR plate, make sure that the input across all samples is roughly equivalent so that the selected cycle number is optimal for all the samples in the run.

1. Place a MicroAmp™ Compression Pad on the plate, then load the plate into the thermal cycler.
2. Run the following program to amplify the target regions.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
25 Cycles[1]	Denature	98°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold

[1] Adjust cycle number according to the following table.

Input nucleic acid[1]	Recommended cycle number (10 ng)	Cycle number adjustment[2]
		100 ng RNA input
High quality RNA	25	–3
Low quality RNA	28	–3

[1] Cycle numbers can be increased when input material quality or quantity is questionable.

[2] The recommended cycle number is based on 10 ng DNA/RNA input. Adjust the cycle number for lower or higher DNA/RNA input.

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

Proceed to “Transfer the DNA amplicons” on page 28.

Set up DNA target amplification reactions

Prepare DNA target amplification reactions

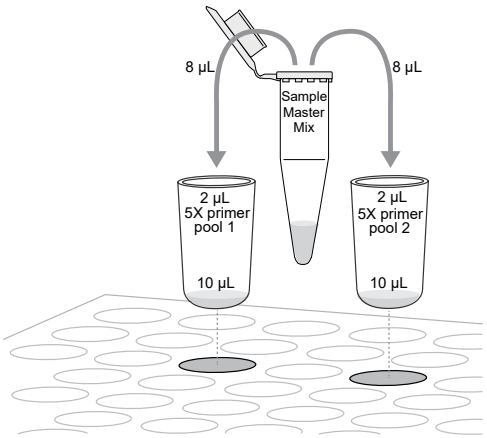
IMPORTANT! Primer pools and 5X Ion AmpliSeq™ HiFi Mix are viscous. Pipet slowly and mix thoroughly.

1. Place a 1.5-mL tube and a 96-well plate in a pre-chilled cold block or on ice.
2. For each sample, prepare a target amplification master mix without primers in the 1.5-mL tube on ice.

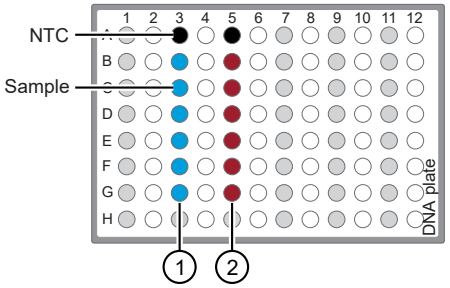
Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4.5 µL
DNA (20 ng)[1]	≤13.5 µL
Nuclease-free Water	to 18 µL

[1] Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).

3. Mix thoroughly by pipetting up and down five times, then transfer 8 µL of each sample-specific master mix to two wells of the 96-well PCR plate.



4. For each sample, add 2 µL of 5X Ion AmpliSeq™ Myeloid DNA Panel pool 1 to the first well, and 2 µL of primer pool 2 to the second well.



① Ion AmpliSeq™ Myeloid DNA Panel pool 1
② Ion AmpliSeq™ Myeloid DNA Panel pool 2

Note: Avoid using columns on the periphery of the plate.

5. Seal the plate with a MicroAmp™ Adhesive Film.

6. Vortex for 5 seconds to mix, then briefly centrifuge to collect the contents. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

Amplify the targets

IMPORTANT! When amplifying multiple samples in a single PCR plate, ensure that equivalent amounts of sample is used in each well so that the selected cycle number is optimal for all the samples in the run.

1. Place a MicroAmp™ Compression Pad on the plate, then load the plate into the thermal cycler.

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

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
13 Cycles[1]	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold

[1] Adjust cycle number according to the following table.

Input nucleic acid[1]	Recommended cycle number (10 ng)	Cycle number adjustment[2]
		100 ng DNA input
High quality gDNA	13–16	–3

[1] When input material quality or quantity is questionable cycle numbers can be adjusted toward the high end of the range.
[2] The recommended cycle number is based on 10 ng DNA input. Adjust the cycle number for lower or higher amounts of sample.

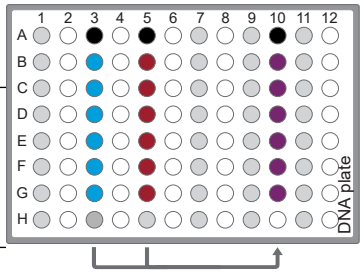
STOPPING POINT Target amplification reactions can be stored at 10°C overnight on the thermal cycler. For longer duration, store at –20°C.

Combine DNA target amplification reactions

1. Remove the plate from the thermal cycler, then centrifuge briefly to collect the contents.

2. Carefully remove the plate seal.

3. For each sample, combine both 10-µL target amplification reactions into a single well.



Note: DNA target amplification reactions can be combined directly onto the RNA/cDNA plate once the RNA target amplification is complete, see step 3 of "Transfer the DNA amplicons."

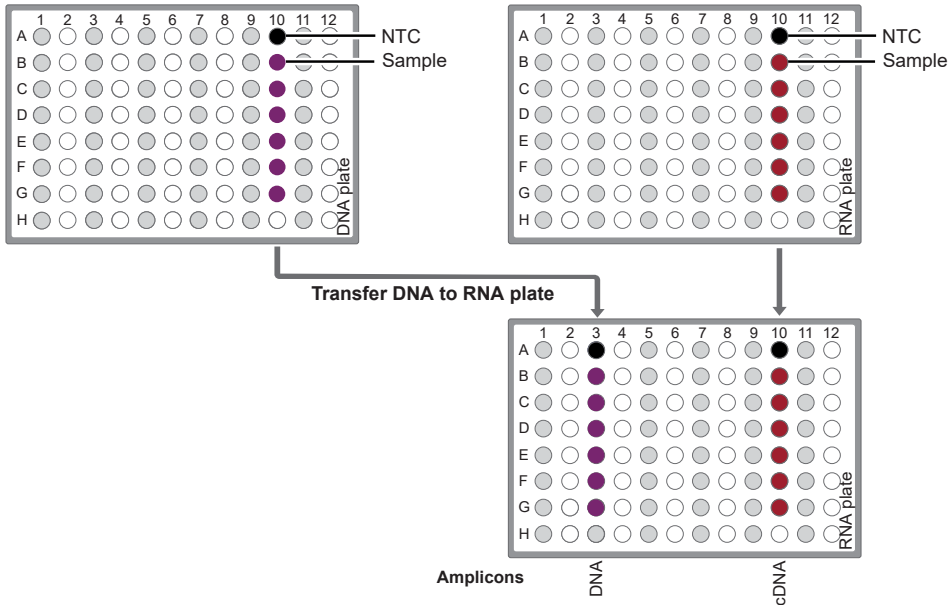
The total volume for each sample should be 20 µL.

Transfer the DNA amplicons

1. Remove the plates from the thermal cycler, then briefly centrifuge them to collect the contents.
2. Carefully remove the adhesive film from both plates.

   IMPORTANT! Be careful when removing the film to minimize contamination.

3. Transfer the amplicons from the DNA plate to the corresponding empty wells of the RNA/cDNA plate. (see below).



Partially digest the amplicons

IMPORTANT! Keep the plate on ice or in a pre-chilled 4°C cold block while preparing the reactions.

1. Thaw the FuPa Reagent (brown cap) on ice, gently vortex to mix, then centrifuge briefly to collect.
2. Add 2 µL of FuPa Reagent to each amplified sample. The total volume is ~22 µL.

   IMPORTANT! FuPa Reagent is viscous. Pipet slowly and mix thoroughly.

3. Seal the plate with a clear adhesive film, vortex thoroughly, then centrifuge briefly to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

4. Place a compression pad on the plate, load in the thermal cycler, then run the following program:

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

STOPPING POINT Store plate at –20°C for longer periods.

Ligate adapters to the amplicons and purify

When sequencing multiple libraries on a single run, you *must* ligate a different barcode to each library. DNA and RNA libraries from the same sample also require different barcodes.

IMPORTANT! When handling barcoded adapters, be careful to avoid cross contamination by changing gloves frequently and opening one tube at a time.

Combine and dilute Ion Xpress™ adapters

For each barcode X selected, prepare a mix of Ion P1 Adapter and Ion Xpress™ Barcode X at a final dilution of 1:4 for each adapter. Store diluted adapters at –20°C.

Substitute 2 µL of this barcode adapter mix for the Ion AmpliSeq™ Adapters in step 3 below. For example, combine the volumes indicated in the following table. Scale volumes as necessary.

Component	Volume
Ion P1 Adapter	2 µL
Ion Xpress™ Barcode X[1]	2 µL
Nuclease-free Water	4 µL
Total	**8 µL**

[1] X = barcode chosen

Perform the ligation reaction

1. If there is visible precipitate in the Switch Solution or the tube cap after thawing, vortex or pipet up and down at room temperature to resuspend before pipeting.

2. Briefly centrifuge the plate to collect the contents.

3. Carefully remove the plate seal, then add the following components in the order listed to each well containing digested amplicons. If preparing multiple non-barcoded libraries, a master mix of Switch Solution and Ion AmpliSeq™ Adapters can be combined before addition.

IMPORTANT! Add the DNA Ligase last. Do not combine DNA Ligase and adapters before adding to digested amplicons.

Order of addition	Component	Volume
1	Switch Solution (yellow cap)	4 µL
2	Diluted Ion Xpress™ barcode adapter mix	2 µL
3	DNA Ligase (blue cap)	2 µL
—	**Total volume** (including ~22 µL of digested amplicon)	**~30 µL**

4. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

5. Place a MicroAmp™ Compression Pad on the plate, load in the thermal cycler, then run the following program:

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

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

Purify the unamplified library

IMPORTANT! Bring the Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the solution slowly.

1. Briefly centrifuge the plate to collect the contents in the bottom of the wells.
2. Carefully remove the plate seal, then add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.

   Note: Visually inspect each well to ensure that the mixture is homogeneous.
3. Incubate the mixture for 5 minutes at room temperature.
4. Place the plate in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears. Carefully remove, then discard the supernatant without disturbing the pellet.
5. Add 150 µL of freshly prepared 70% ethanol, move the plate side-to-side in the two positions of the magnet to wash the beads, then remove and discard the supernatant without disturbing the pellet.

   Note: If your magnet does not have two positions for shifting the beads, remove the plate from the magnet and gently pipet up and down 5 times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.
6. Repeat step 5 for a second wash.
7. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes. Do not overdry.

   IMPORTANT! Residual ethanol drops inhibit library amplification. If needed, centrifuge the plate and remove remaining ethanol before air-drying the beads.

Equalize the library

The Ion Library Equalizer™ Kit (Cat. No. 4482298) provides a method for normalizing library concentration at ~100 pM without the need for special instrumentation for quantification. First amplify the Ion AmpliSeq™ library, then capture the library on Equalizer™ Beads. After elution of the equalized library, proceed directly to combining libraries and/or template preparation.

Note: The Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802) can also be used to normalize library concentration. For more information, see the *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735), or *Ion Library TaqMan™ Quantitation Kit User Guide* (Pub. No. MAN0015802).

Before you begin

Warm all the reagents in the Ion Library Equalizer™ Kit to room temperature. Vortex and centrifuge all reagents before use.

Amplify the library

1. Prepare library amplification mix according to the following table. Prepare a master mix for multiple reactions.

Order of addition	Component	Volume per reaction	Volume for 6 reactions[1]
1	1X Library Amp Mix (black cap)	50 µL	350 µL
2	Equalizer™ Primers (pink cap)	2 µL	14 µL
—	**Total**	**52 µL**	**364 µL**

[1] One additional reaction added as overage to compensate for pipetting error.

Note: Do NOT use the 25X Library Amp Primers (white cap) provided in the Ion AmpliSeq™ Library Kit Plus.

2. Remove the plate from the magnet (at step 7 of "Purify the unamplified library"), then add 52 µL of library amplification mix to each well containing air-dried beads.
3. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge at 100 × *g* for 30 seconds to collect droplets.
4. Place the plate back on the magnet for 2 minutes, then carefully transfer ~50 µL of supernatant from each well to a new plate without disturbing the pellet.

5. Seal the plate with the adhesive film, place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program:

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

Note: During cycling, wash the Equalizer™ Beads for later use (see "Wash the Equalizer™ Beads (if not previously performed)" on page 33).

Wash the Equalizer™ Beads *(if not previously performed)*

1. Bring the Equalizer™ Beads to room temperature, then mix thoroughly.

Note: Beads for multiple reactions can be prepared in bulk, and stored in Equalizer™ Wash Buffer at 4°C for up to 12 months until use. After 12 months, re-wash beads with an equal volume of Equalizer™ Wash Buffer.

2. For each reaction, pipet 3 µL of beads into a clean 1.5-mL tube, then add 6 µL/reaction of Equalizer™ Wash Buffer.
   For example, if you have 4 reactions, add 12 µL of beads and 24 µL of Equalizer™ Wash Buffer.
3. Place the tube in a magnetic rack for 3 minutes or until the solution is clear.
4. Carefully remove the supernatant without disturbing the pellet, then discard.
5. Remove the tube from the magnet, add 6 µL per reaction of Equalizer™ Wash Buffer, then pipet up and down to resuspend.

Add Equalizer™ Capture to the amplified library

1. Carefully remove the seal from the plate, then add exactly 10 µL of Equalizer™ Capture to each library amplification reaction.

Note: The final equalized library concentration is dependent upon accurate pipetting of the Equalizer™ Capture reagent.

2. Seal the plate with a clear adhesive film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
3. Incubate at room temperature for 5 minutes.

Add Equalizer™ Beads and wash

1. Mix the washed Equalizer™ Beads by gentle vortexing or pipetting up and down.
2. Carefully remove the seal from the plate, then **add 6 µL of washed Equalizer™ Beads** to each plate well containing the captured library.
3. Set the pipette volume to 40 µL, then pipet the mixture up and down at least 5 times to mix thoroughly.
4. Incubate at room temperature for 5 minutes.

   Note: Check for droplets on the sides of the plate wells. If droplets are observed, seal the plate, then gently tap the plate on a hard, flat surface, or briefly centrifuge to collect droplets.

5. Place the plate in the magnet, then incubate for 2 minutes or until the solution is clear.
6. If needed, carefully remove the seal from the plate, then remove the supernatant without disturbing the pellet.

   Note: Save the supernatant for repeat analysis if needed.

7. **Add 150 µL of Equalizer™ Wash Buffer** to each reaction.
8. To wash the beads, move the plate side-to-side in the two positions of the magnet.

   Note: If your magnet does not have two positions for shifting the beads. Remove the plate from the magnet, set a pipettor to at least half the total volume, then gently pipet the contents up and down 5 times. Return the plate to the magnet and incubate for 2 minutes or until the solution clears.

9. With the plate still in the magnet, carefully remove, then discard the supernatant without disturbing the pellet.
10. Repeat the bead wash as described in step 7 through step 9.

    Note: Ensure that as much wash buffer as possible is removed without disturbing the pellet.

Elute the Equalized library

1. Remove the plate from the magnet, then add 100 µL of Equalizer™ Elution Buffer to each pellet.
2. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

   Note: Centrifuge with enough force to collect droplets, but not pellet beads. If beads are pelleted, vortex again and centrifuge more gently.

3. Elute the library by incubating in a thermal cycler at 32°C for 5 minutes.

4. Place the plate in the magnet, then incubate at room temperature for 5 minutes or until the solution is clear.
   The supernatant contains the Equalized library at ~100 pM.

Proceed to “Combine libraries”.

STOPPING POINT The Equalized library can be stored with beads for up to 1 month at 4–8°C. For longer term, store at –20°C.

Combine libraries

When comparing genomic DNA- and RNA-libraries that are prepared from the same sample, unequal volumes of libraries can be combined to produce different read depths for the paired DNA and RNA libraries. We recommend combining libraries at 80:20 (DNA:RNA) ratio as described. However, the ratio can be customized to yield the desired read-depths for specific applications. See “Strategies for combining libraries” on page 71 for more information.

Note: Non paired uniquely barcoded DNA and RNA libraries can be run on the same sequencing chip.

1. Combine each equalized uniquely barcoded Oncomine™ Myeloid Research Assay DNA- and RNA-library (~100 pM each) from the same sample at an 80:20 ratio (DNA:RNA—8 µL of DNA library + 2 µL of RNA library).

2. Combine equal volumes of the paired libraries (80:20 DNA:RNA) to be sequenced on the same chip. We recommend sequencing up to:

   - 4 samples (80:20 DNA:RNA) on a single Ion 318™ Chip
   - 12 (11 research samples (80:20 DNA:RNA) + 1 DNA & 1 RNA NTC) manually prepared libraries on a single Ion 530™ Chip
   - 8 (7 research samples (80:20 DNA:RNA) + 1 DNA & 1 RNA NTC) libraries that are prepared on an Ion Chef™ Instrument on a single Ion 530™ Chip

Note: For runs that include a no-template control (NTC), add in the same fractional volumes of equalized NTC library as is added for a sample library.

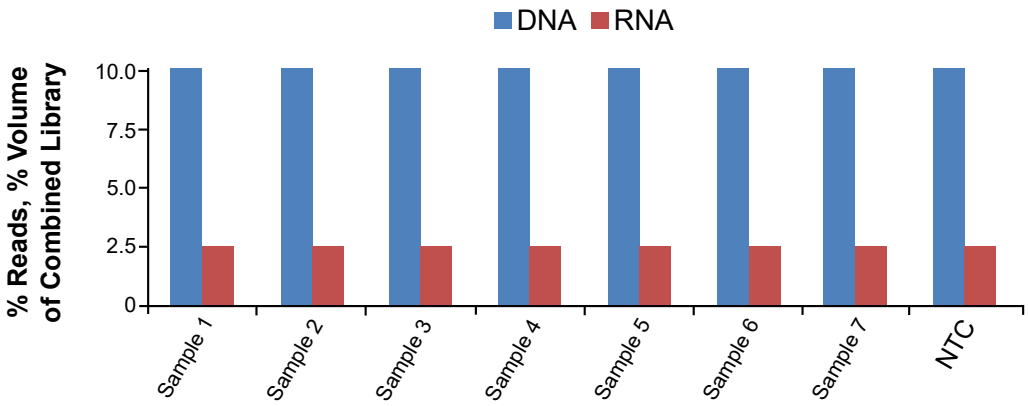


Figure 2 Combined libraries

This figure exemplifies the situation in which libraries (DNA and RNA) from 8 samples (7 research samples (80:20 DNA:RNA) + 1 DNA & 1 RNA NTC) prepared on an Ion Chef™ Instrument. If combining manually prepared libraries combine up to 12 samples (11 research samples (80:20 DNA:RNA) + 1 DNA & 1 RNA NTC).

Table 1 Fractional volumes for combining libraries

Sample	Barcode	Fractional volume (80:20 DNA:RNA)	
		Combining 12 samples	Combining 8 samples
DNA-1	BC_0101	0.067	0.1
RNA-1	BC_0102	0.017	0.025
DNA-2	BC_0103	0.067	0.1
RNA-2	BC_0104	0.017	0.025
DNA-3	BC_0105	0.067	0.1
RNA-3	BC_0106	0.017	0.025
DNA-4	BC_0107	0.067	0.1
RNA-4	BC_0108	0.017	0.025
DNA-5	BC_0109	0.067	0.1
RNA-5	BC_0110	0.017	0.025
DNA-6	BC_0111	0.067	0.1
RNA-6	BC_0112	0.017	0.025
DNA-7	BC_0113	0.067	0.1
RNA-7	BC_0114	0.017	0.025
DNA-8	BC_0115	0.067	—
RNA-8	BC_0116	0.017	—
DNA-9	BC_0117	0.067	—
RNA-9	BC_0118	0.017	—
DNA-10	BC_0119	0.067	—
RNA-10	BC_0120	0.017	—
DNA-11	BC_0121	0.067	—
RNA-11	BC_0122	0.017	—
DNA (NTC)	BC_0123	0.067	0.1
RNA (NTC)	BC_0124	0.017	0.025
Sum	—	**1.0**	**1.0**

4 Guidelines for templating and sequencing

Proceed to template preparation and sequencing using the kits and user guides indicated for your instrument setup.

Ion GeneStudio™ S5 Series Systems

Proceed to template preparation and sequencing using the following kits.

System	Kit	User Guide
Templating		
Ion Chef™ System	Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A34019 or A34461[1])	*Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854)
Sequencing		
Ion GeneStudio™ S5 Series System	Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A34019 or A34461[1])	*Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854)

[1] Cat. No. A34461 only provides sufficient reagents for a single sequencing run per initialization when performing 400 bp read sequencing.

To create a specific Run Plan for use in templating and sequencing see “Create a Planned Run” on page 43. Refer to the appropriate user guide listed in the table for more information.

Dilute the libraries for templating on an Ion 530™ Chip

- Up to 12 (11 research samples (80:20 DNA:RNA) + 1 DNA & 1 RNA NTC) samples can be sequenced on a single Ion 530™ Chip.
- Prepare a fresh dilution of the combined libraries before each Ion Chef™ Instrument run.
- Use the diluted library within 48 hours of dilution.
- Keep diluted libraries on ice until use.
- The starting concentration of combined libraries is ~100 pM for libraries prepared using either manual or automated procedures.

1. Dilute the combined library (see "Combine libraries" on page 36) pool to 25–60 pM final concentration (600–900 × 10^6 molecules/25-µL) for template preparation using the Ion Chef™ Instrument.

   Note: Use polyclonality and low-quality filter results from a sequencing run performed with ISPs templated at the starting concentration, then titrate up or down to achieve optimal concentrations, if needed.

2. In a new 1.5-mL Eppendorf LoBind™ tube, combine:

Component	Volume
Nuclease-free water	21 µL
Combined library pool (100 pM, up to 12 samples 80:20 DNA:RNA)	7 µL
Total volume	28 µL

3. Vortex for 5 seconds, briefly centrifuge to collect the contents, then keep on ice until use.

Use 25 µL of this final combined library dilution (~25 pM) for templating on an Ion Chef™ Instrument.

Ion PGM™ System

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 refer to the appropriate user guide listed in the table for more information.

System	Kit	User Guide
Templating		
Ion OneTouch™ 2 System	Ion PGM™ Hi-Q™ View OT2 Kit (Cat. No. A29900)	*Ion PGM™ Hi-Q™ View OT2 Kit User Guide* (Pub. No. MAN0014579)
Ion Chef™ System	Ion PGM™ Hi-Q™ View Chef 400 Kit (Cat. No. A30798)	*Ion PGM™ Hi-Q™ View Chef Kits User Guide* (Pub. No. MAN0014571)
Sequencing		
Ion PGM™ System	Ion PGM™ Hi-Q™ View Sequencing Kit (Cat. No. A30044)	*Ion PGM™ Hi-Q™ View Sequencing Kit User Guide* (Pub. No. MAN0014583)

IMPORTANT! When diluting the combined libraries and preparing the amplification solution for templating using the Ion OneTouch™ 2 Instrument, follow the instructions provided for Ion AmpliSeq™ DNA Libraries.

Dilute the libraries for templating on an Ion 318™ Chip

IMPORTANT! Keep diluted libraries on ice until use.

Note: Up to 4 samples (80:20 DNA:RNA) can be sequenced on a single Ion 318™ Chip.

- For template preparation using the Ion OneTouch™ 2 System, dilute the combined library pool as follows.
  a. In a new 1.5-mL Eppendorf LoBind™ tube, combine:

Component	Volume
Nuclease-free water	23 µL
Combined library pool (100 pM, up to 4 samples 80:20 DNA:RNA)	2 µL
Total volume	25 µL

  b. Vortex for 5 seconds, then briefly centrifuge to collect the contents.

  c. Proceed immediately to preparing the amplification solution, see *Ion PGM™ Hi-Q™ View OT2 Kit User Guide* (Pub. No. MAN0014579) for more information.

- For template preparation using the Ion Chef™ Instrument, dilute the combined library pool 1:4 (~25 pM final concentration).
  a. In a new 1.5-mL Eppendorf LoBind™ tube, combine:

Component	Volume
Nuclease-free water	21 µL
Combined library pool (100 pM, up to 4 samples 80:20 DNA:RNA)	7 µL
Total volume	28 µL

  b. Vortex for 5 seconds, then briefly centrifuge to collect the contents.

  c. Proceed to "Prepare the libraries and consumables" in the *Ion PGM™ Hi-Q™ View Chef Kits User Guide* (Pub. No. MAN0014571).

Note: Prepare a fresh dilution of the combined libraries before each Ion Chef™ Instrument run. Use the diluted library within 48 hours of dilution.

5 Create a Planned Run

IMPORTANT! This kit is compatible with Torrent Suite™ Software 5.6 and Ion Reporter™ Software 5.10 or later. Before proceeding, check for updates to the Torrent Suite™, Ion Reporter™, and Ion Chef™ System software. Contact your service representative for assistance with upgrading the software.

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, through sequencing, and subsequent data analysis. Each chip prepared requires its own Planned Run.

In Torrent Suite™ Software 5.6 or later, the primary Planned Run template to be used with the Oncomine™ Myeloid Research Assay is the **DNA and Fusions** template.

Research Application	Torrent Suite™ Software template	Description
For use with the Ion PGM™ System		
Oncology-HemeOnc	Oncomine Myeloid Research DNA and Fusions for PGM	DNA and RNA Planned Run template
	Oncomine Myeloid Research Fusions for PGM	RNA-only Planned Run template
	Oncomine Myeloid Research DNA for PGM	DNA-only Planned Run template
For use with Ion GeneStudio™ S5 Series Systems		
Oncology-HemeOnc	Oncomine Myeloid Research DNA and Fusions for S5	DNA and RNA Planned Run template
	Oncomine Myeloid Research Fusions for S5	RNA-only Planned Run template
	Oncomine Myeloid Research DNA for S5	DNA-only Planned Run template

Create a custom Planned Run template

IMPORTANT! Before creating a custom Planned Run template ensure that your Ion Reporter™ account is configured, and that the most current **Reference Library**, **Target Regions**, and **Hotspot Regions** BED files are installed. See Appendix B, "Supplemental information" for more information. Contact your local service representative to obtain the most current BED files.

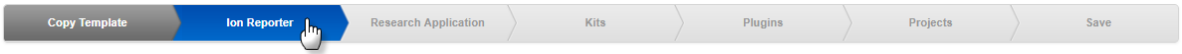
We recommend that you create a customized Planned Run template for reuse when the same conditions will be used for multiple runs. To create a custom Planned Run template, copy an existing system template then edit the settings to meet the requirements for your Planned Run. The following example is for users performing templating with an Ion Chef™ Instrument and sequencing on an Ion GeneStudio™ S5 Series System.

1. Sign in to the Torrent Suite™ Software for the Torrent Server connected to your Ion Chef™ System.
2. Under the **Plan** tab, in the **Templates** screen, click **Oncology - HemeOnc** in the research application list.
3. In the **Oncology - HemeOnc** list, **Oncomine Myeloid Research DNA and Fusions for S5** row, click **(Settings)** ▸ **Copy**.
   The **Copy Template** wizard will open to the **Save** step.
4. Enter or select the required information in each field:

Field[1]	Action…
Template Name	Enter a name for the Planned Run template.
DNA Reference Library	Select **hg19(Human (hg19))**.
DNA Target Regions[2]	Select **Oncomine_Myeloid.20200429.designed.bed**
DNA Hotspot Regions[2]	Select **Oncomine_Myeloid.20191023.hotspots.bed**
Analysis Parameters	Ensure Default (Recommended) is selected.

[1] Fusions Reference Library, and Fusions Target Regions are not required for analysis in Torrent Suite™ software.
[2] Check with your service representative for updates to ensure the most current files are being used. See page 66 for BED file installation instructions.

5. In the **Copy Template** workflow bar, click the **Ion Reporter** step, then select:



- Your Ion Reporter™ account.
- **Oncomine Myeloid Research - 530 - w3.0 - DNA and Fusions - Single Sample** under **Existing Workflow**.
- **DNA and Fusions** under **Sample Grouping**.
- **Automatically upload to Ion Reporter after run completion** under **Ion Reporter Upload Options**.

Note: If the Ion Reporter™ account is not configured, configure it through Ion Reporter Configure settings (see "Configure the IonReporterUploader plugin in Torrent Suite™ Software" on page 70 for more information).

6. Click **Next**.
7. In the **Research Application** step, ensure that **Research Application** is set to **DNA and Fusions**, and that **Target Technique** is set to **AmpliSeq DNA and Fusions**, then click **Next**.
8. In the **Kits** step, select the **Ion Chef Template Kit** radio button, then complete the following fields.

Field	Selection	
	Manual library preparation	Chef-Ready library preparation
Instrument	Select **Ion GeneStudio™ S5 System**.	
Sample Preparation Kit	*(Optional)* Select the sample preparation kit used.	
Library Kit Type	**Ion AmpliSeq™ Library Kit Plus**	**Ion AmpliSeq™ Kit for Chef DL8**
Template Kit	Select **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef**.	
Read length	400	400
Sequencing Kit	Select **Ion S5™ Sequencing Kit**.	
Chip Type	Select **Ion 530™ Chip**.	
Control Sequence	*(Optional)* Select Ion AmpliSeq™ Sample ID Panel.	
Barcode Set	Select **Ion Xpress™**.	Select **IonCode™**.
Flows	850	850

9. Click **Next**.
10. In the **Plugins** step, select the **coverageAnalysis** and **sampleID** plugin, then click **Next**.
11. In the **Projects** step, select the project or projects that will receive data from the runs that use this template, then click **Next**.
12. In the **Save** step, click **Copy Template** to save the new run template.

The customized template is now available in the **Templates** screen within the **Research Application** group from which you copied the system template.

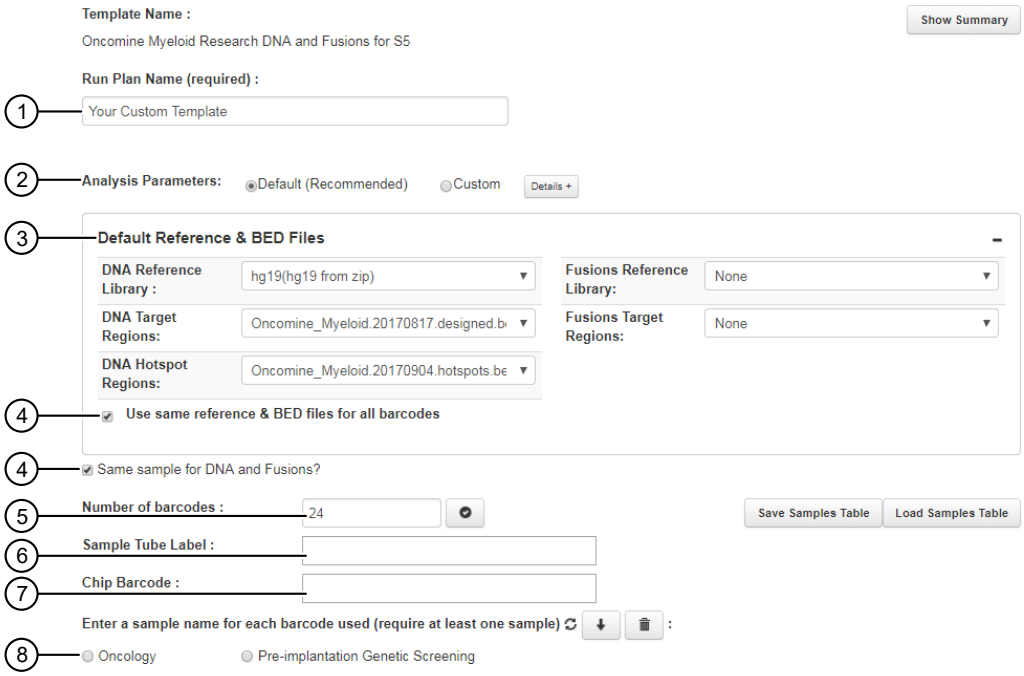
Create a Planned Run

IMPORTANT! Before creating a Planned Run ensure that your Ion Reporter™ account is configured, and that the most current **Reference Library**, **Target Regions**, and **Hotspot Regions** BED files are installed. See Appendix B, "Supplemental information" for more information. Contact your local service representative to obtain the most current BED files.

Note: Users performing templating with the Ion OneTouch™ 2 System should create a Planned Run template for use with their instrument set up. See page 68 for more information.

1. Sign in to the Torrent Suite™ Software on a computer connected to your Ion Chef™ System.
2. Under the **Plan** tab, in the **Templates** screen, click **Oncology - HemeOnc** in the research application list.
3. In the **Oncology - HemeOnc** list, click on your customized Planned Run template name, alternatively click **(Actions)** ▸ **Plan Run**.
   The **Create Plan** wizard will open to the **Plan** step.
4. Enter or select the following information. Row numbers in the table correspond to the callouts in the following figure.

Callout	Field	Action
1	Run Plan Name	Enter a Run Plan name.
2	Analysis Parameters	Ensure the Default (Recommended) radio button is selected.
3	Default Reference & BED Files	• DNA Reference Library: **hg19(hg19 from zip)** • DNA Target Regions: **Oncomine_Myeloid.20200429.designed.bed** • DNA Hotspot Regions: **Oncomine_Myeloid.20191023.hotspots.bed**
4		Ensure both check boxes are selected.
5	Number of barcodes	Enter the number of barcodes that will be used in this run, then click the ✓ button to the right of this field. We recommend: • 8 barcodes (4 DNA and 4 RNA) per Ion 318™ Chip run plans. • 24 barcodes (12 DNA and 12 RNA) for Ion 530™ Chip run plans.
6	Sample Tube Label	Enter or scan the barcode of the Ion Chef™ sample tube that will be used in the run.
7	Chip ID	No entry required.
8	Oncology	Ensure that the radio button is selected. **Note:** Radio button is not visible until an Ion Reporter account has been configured and selected.
	Pre-implantation Genetic Screening	Ensure that the radio button is un-selected. **Note:** Radio button is not visible until an Ion Reporter account has been configured and selected.



5. Enter the sample information. Row numbers in the table correspond to the callouts in the following figure.

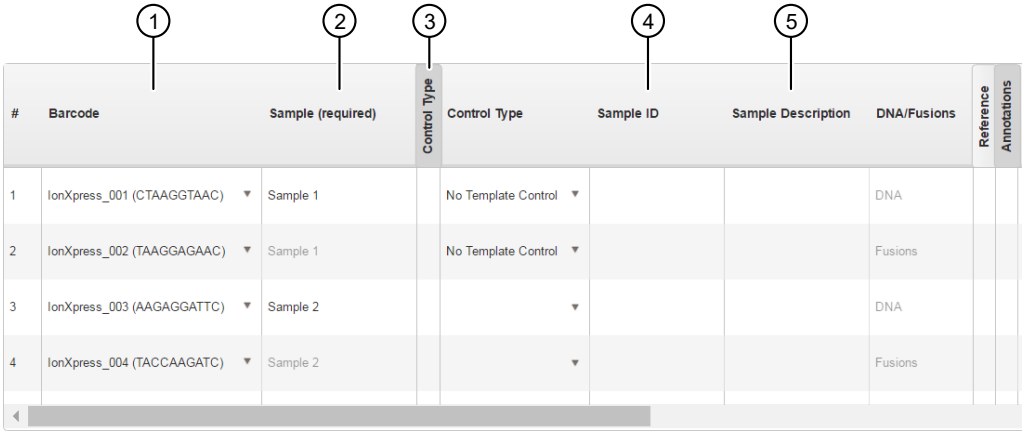
Callout	Field[1]	Action
1	Barcode	For each sample select the **Barcode** that will identify it from the dropdown list.
2	Sample Name	Accept the auto-populated sample names or click in a field, then enter a unique sample name. We recommend sample names be unique even between runs.
3	Control Type (expanded)	Select No Template Control from the dropdown list to designate a sample as a no template control.
4	Sample ID	*(Optional)* Click in the field, then enter a sample ID.
5	Sample Description	*(Optional)* Click in the field, then enter a sample description.
6	Annotations (expanded)	Click to reveal Cancer Type and Cellularity %.
7	Cancer Type	Select from the dropdown list. Click to copy the entry to all the rows.
8	Cellularity %	Enter a value. Click to copy the entry to all the rows.
9	Ion Reporter Workflow	Ensure the appropriate workflow for your system is selected, e.g., **Oncomine Myeloid Research - 530 - w3.0 - DNA and Fusions - Single Sample**.
10	Relation	Ensure the correct value is auto-populated. Select from the dropdown list to change.

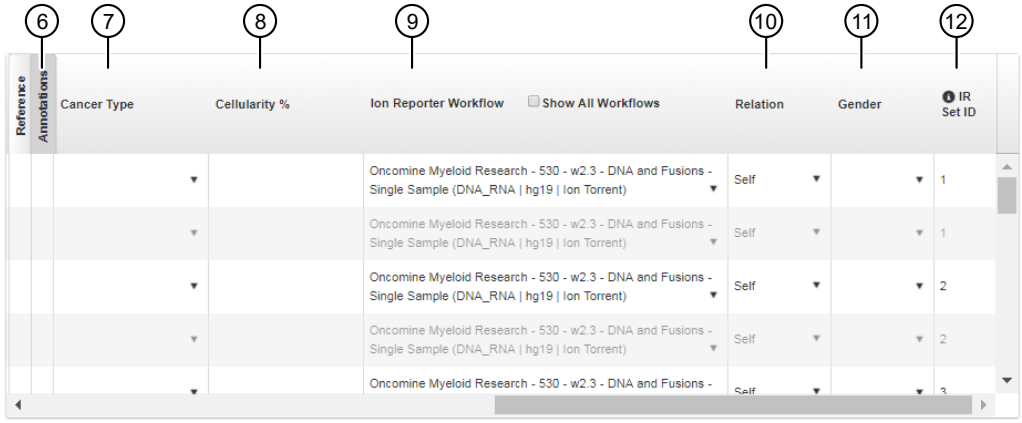
(continued)

Callout	Field[1]	Action
11	Gender	Select from the dropdown list. Click ⬇ to copy the entry to all the rows.
12	IR Set ID[2]	The IR Set ID links individual samples for analysis. Ensure the correct value is auto-populated. Select from the dropdown list to change.

[1] Click vertical column headers (Control Type, Reference, Annotations) to reveal additional columns.

[2] Samples with the same IR Set ID are considered related samples and launched in the same analysis such as a normal sample and its corresponding tumor sample. Do not give unrelated samples the same IR Set ID value (even if that value is zero or blank).





6. Click **Plan Run**.

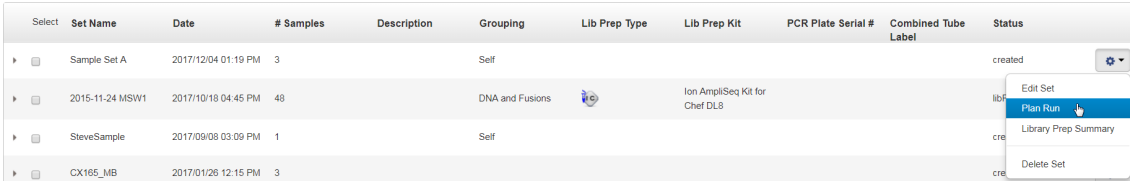
The run is listed in the **Planned Run List** page under the name that you specified and is automatically used by the Ion Chef™ System when the associated Ion Chef™ Library Sample Tube is loaded on the instrument.

Create a Planned Run with Sample Sets

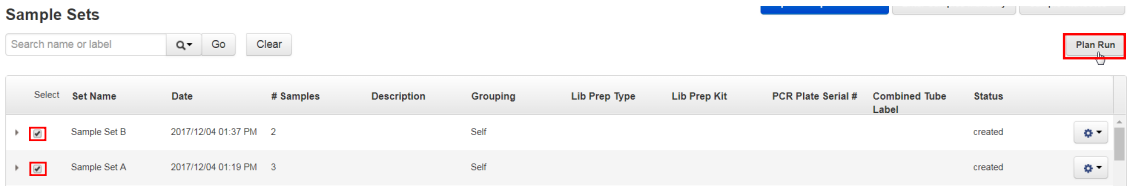
If you set up your samples in Sample Sets before you plan an instrument run, you can add one or more Sample Sets to your Planned Run.

Note that a Sample Set that uses an Ion AmpliSeq™ library preparation kit must also use the corresponding barcode kit that comes with the library preparation kit.

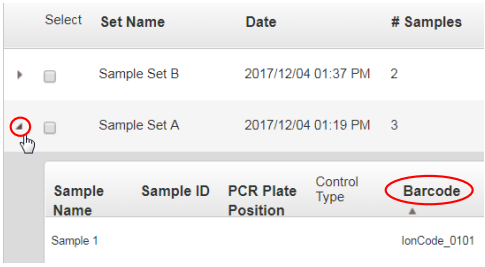
1. In the **Plan** tab, click **Samples**.
2. In the **Sample Sets** screen, select one or more Sample Sets to add to the Planned Run.
   - To plan a run using a single Sample Set, click ⚙ **(Actions)** ▸ **Plan Run** in the row of the Sample Set.



   - To plan a run using multiple Sample Sets, select the checkboxes next to the Sample Sets that you want to add to the Planned Run, then click **Plan Run**.



IMPORTANT! Ensure that all of the selected Sample Sets used in the Planned Run use the same barcode kit. To verify the barcode kit that is used, expand the Sample Set entry to view its details.



The **Select a Run Template to apply to this experiment** dialog box lists Planned Run templates that support your Sample Set.

3. In the **Plan Run from Sample Set** dialog box, select a run template to use for the experiment, then click **Plan Run**.

   If you do not see the template that you are looking for, select **Show All Templates**, then look again for the template.

   The **Create Plan** workflow opens to the **Barcoding** step with the Sample Sets that you selected:

4. In the **Barcoding** step in the workflow bar, enter or select the required information. Depending on your sequencing application, options can vary.

Option	Description
Analysis Parameters	Select **Default** to accept default analysis parameter settings *(recommended)*.
Reference Library	Select the reference library file that is appropriate for your sample. Depending on your application, you may have to select separate DNA, RNA, and fusions reference library files.
Target Regions	Select the target regions BED file appropriate for your sample. Depending on your application, you may have to select separate DNA and fusions target regions files. Ensure that you are using the current BED or VCF files.
Hotspots	Select the hotspots (BED or VCF) file appropriate for your sample. Ensure that you are using the current BED or VCF files.

5. Select the **Use same reference & BED files for all barcodes** checkbox if you are using the same reference, target regions, and hotspots files across all of your barcoded samples in the Planned Run.

   If you are using different reference and/or BED files for one or more of your barcoded samples, deselect the **Use same reference & BED files for all barcodes** checkbox.

6. In **Sample Tube Label**, scan or enter the barcode of the Ion Chef™ sample tubes to be used in the run.

7. In **Chip Barcode**, scan or enter the barcode that is printed on the chip that is used for this run.

8. Complete the samples table.

   - You can save the samples table to a CSV file, fill out all required sample information, and then upload the samples table to populate the **Samples Table** automatically.
     a. Click **Save Samples Table** to save the CSV file to your computer.
     b. Edit the CSV file by entering all required sample information into the appropriate sample information columns, then save the CSV file to your computer.
     c. Click **Load Samples Table**, then select an appropriate CSV file containing sample information specific for this Planned Run.
     d. Click **Load** to populate the **Samples Table** with information from the CSV file.
   - Alternatively, you can manually enter sample information into the samples table. Depending on your sequencing application, options can vary.

Options	Description
Barcode	For barcoded samples, select a barcode.
Sample Name (Required)	Select a sample that is a part of the selected sample set or sets.

(continued)

Options	Description
Control Type	Click the **Control Type** column heading to expand the column, then select the control type from the dropdown list.
Sample ID	*(Optional)* Review sample ID information for each sample.
Sample Description	*(Optional)* Review sample description for each sample.
DNA/Fusions	For DNA and Fusions application, select **DNA** or **Fusions** for each sample.
Reference	If using different reference and BED files for one or more samples, click the **Reference** column heading to expand the sections, then select reference, target regions, and hotspots files for each sample.
Annotations	Click the **Annotations** column heading to expand the annotations specific for your application (for example, cancer type or embryo ID), then complete the required information.
Ion Reporter workflow	Select the Ion Reporter™ Software analysis workflow specific for your run. If you do not see your analysis workflow, select the **Show All Worklows** checkbox in the column heading.
Relation	Select sample relationship group.
Gender	Select **Male**, **Female**, or **Unknown**.
IR Set ID	Set the **IR Set ID** to the same value for related samples. After file transfer, in Ion Reporter™ Software, samples with the same **IR Set ID** are considered related samples and are launched in the same analysis (for example, normal sample and its corresponding tumor sample). Do not give unrelated samples the same **IR Set ID** value even if the value is zero or blank.

9. Click **Next** to continue the steps to create the Planned Run.

   The software takes you to the next step in the workflow bar.

10. Click **Save & Finish**.

The Planned Run is added to the Planned Runs table and can be used in an instrument run.

6 Variant analysis

IMPORTANT! If you are using the Ion Reporter™ Software version 5.10, you must have an off-cycle software package installed by your service representative to perform Oncomine™ Myeloid Research Assay Ion Reporter™ variant analysis. We recommend updating to the latest available version of Ion Reporter™ Software.

Analysis workflows in Ion Reporter™ Software

If the appropriate Ion Reporter™ Software workflow was selected in your Planned Run in the Torrent Suite™ Software, automated analysis has already been performed and you can view the analysis results in the Ion Reporter™ Software. For instructions on manually launching an analysis, see "Manually launch an analysis" on page 51.

Note: Microsoft™ Excel™, or other spreadsheet tool, is required for viewing VCF, CSV, and TSV files.

Available workflows in Ion Reporter™ Software 5.14 include:

Analysis Workflow	Description
For use with the Ion PGM™ System	
Oncomine Myeloid Research - 318 - w3.2 - DNA and Fusions - Single Sample	Detects and annotates somatic variants (SNPs, InDels, FLT3ITD), including those at low frequency, in targeted DNA libraries, as well as gene fusions in targeted RNA libraries, from the Oncomine™ Myeloid Research Assay run on the Ion 318™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 3.2.
Oncomine Myeloid Research - 318 - w3.2 - DNA - Single Sample	Detects and annotates somatic variants (SNPs, InDels, FLT3ITD), including those at low frequency, in targeted DNA libraries, from the Oncomine™ Myeloid Research Assay run on the Ion 318™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 3.2.
Oncomine Myeloid Research - 318 - w3.2 - Fusions - Single Sample	Detects and annotates gene fusions in targeted RNA libraries from the Oncomine™ Myeloid Research Assay run on the Ion 318™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 3.2.
Oncomine Myeloid Research - 318 - w3.2 - Annotate Variants - Single Sample	Annotates VCF files from the Oncomine™ Myeloid Research Assay run on the Ion 318™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 3.2.

(continued)

Analysis Workflow	Description
For use with Ion GeneStudio™ S5 Series Systems	
Oncomine Myeloid Research - 530 - w4.0 - DNA and Fusions - Single Sample	Detects and annotates somatic variants (SNPs, InDels, FLT3ITD), including those at low frequency, in targeted DNA libraries, as well as gene fusions in targeted RNA libraries, from the Oncomine™ Myeloid Research Assay run on the Ion 530™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 4.0.
Oncomine Myeloid Research - 530 - w4.0 - DNA - Single Sample	Detects and annotates somatic variants (SNPs, InDels, FLT3ITD), including those at low frequency, in targeted DNA libraries, from the Oncomine™ Myeloid Research Assay run on the Ion 530™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 4.0.
Oncomine Myeloid Research - 530 - w4.0 - Fusions - Single Sample	Detects and annotates gene fusions in targeted RNA libraries from the Oncomine™ Myeloid Research Assay run on the Ion 530™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 4.0.
Oncomine Myeloid Research - 530 - w4.0 - Annotate Variants - Single Sample	Annotates VCF files from the Oncomine™ Myeloid Research Assay run on the Ion 530™ Chip. Released with: Ion Reporter™ Software 5.14. Workflow Version: 4.0.

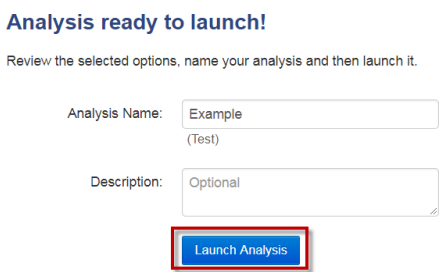
Manually launch an analysis

To launch an Ion Reporter™ Software analysis manually:

1. Sign in to the Ion Reporter™ Software.
2. In the **Workflows** screen, select **DNA and Fusions** from the **Research Application** dropdown list.
3. Type *Myeloid* in the search field, then click **Search** (or press Enter).
4. In the **Workflow Name** column, click the appropriate workflow (e.g., Oncomine Myeloid Research - 530 - w3.0 - DNA and Fusions - Single Sample), then select **Launch Analysis** from the **Actions** dropdown list in the **Details** pane.
5. Search by any unique identifier you used to label your samples during setup, ensure the sample's **Cellularity %** and **Sample Type** are defined.
6. Click the checkbox to select a DNA sample and a Fusions sample.
7. In the **Sample Groups** pane, click **Add Samples** to add to a sample group.
8. Enter a **Group Name**, click **Add to Analysis**, then click **Next**.

9. In the Plugins step, ensure **Oncomine™ Variant Annotator v2.3** is selected, then click **Next**.

10. *(Optional)* Enter an **Analysis Name** and **Description**, then click **Launch Analysis**.



View Oncomine™ Myeloid Research Assay analysis results

Ion Reporter™ Software analyses are performed automatically on uploading of the data files from Torrent Suite™ Software if an Ion Reporter™ account is included in the Planned Run.

The dedicated Myeloid_FLT3_LongITD algorithm in Ion Reporter™ Software Oncomine™ Myeloid Research Assay analysis workflows identify large internal tandem duplications (ITD) in exons 14 and 15 of the FLT3 gene.

The analysis workflow is for use with Oncomine™ Myeloid Research Assay DNA sequencing results.

In-frame insertions in Exons 14 and 15 are detected by both the FLT3 Long ITD algorithm, and the variant calling algorithms in Ion Reporter™ Software. Therefore FLT3-ITD variants can be called by one or both types of variant calling. We recommend obtaining the results from the Myeloid_FLT3_LongITD algorithm for ITDs of 8 bases or larger. For smaller variants the Ion Reporter™ Software variant calling results should be inspected. The Ion Reporter™ Software variant calling module can sometimes call ITDs larger than 8 base pairs, but its sensitivity to variants in that size range is lower than the Myeloid_FLT3_LongITD algorithm.

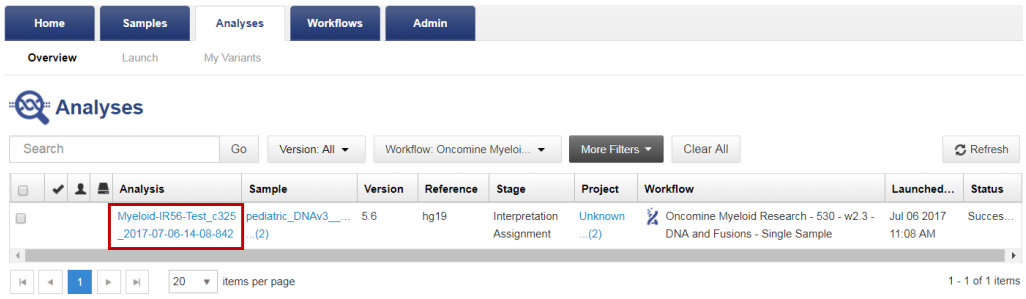
See the following table for guidelines in obtaining FLT3 ITD calls from the Oncomine™ Myeloid Research Assay analysis workflows.

FLT3- ITD variant Length	Called by Myeloid_FLT3_LongITD algorithm	Called by variant calling module in Ion Reporter™ Software	Annotated by Oncomine™ Reporter
≥8 bp	Yes	Yes (with lower sensitivity than the FLT3 algorithm)	Yes
<8 bp	No	Yes	Yes

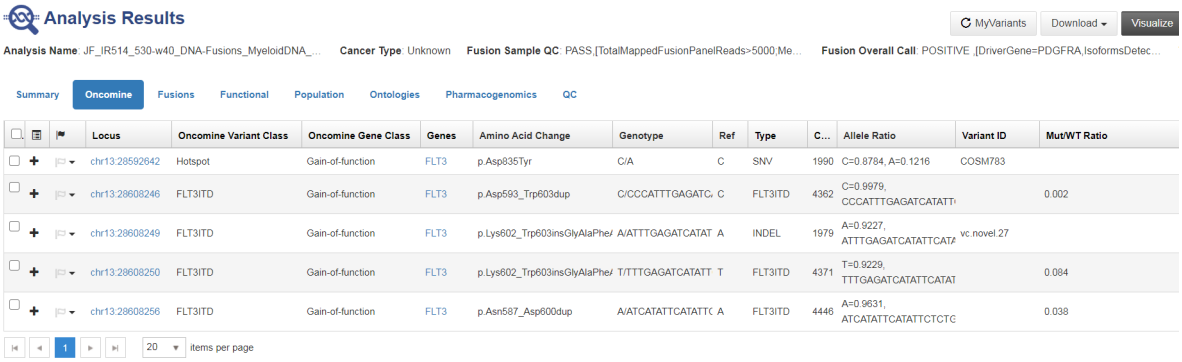
Algorithm description: For standard indel detection, long inserts at the ends of reads can cause a partial alignment, resulting in soft-clipping of the alignment. Since this can soft-clip the FLT3-ITD sequence, and eliminate downstream anchoring sequence, the standard indel parameters might not detect all FLT3-ITD calls. The Myeloid_FLT3_LongITD algorithm analyzes 3' regions of trimmed reads for presence of anchor sequences, and determines the likely position and size of the duplication by looking for copies of sequence within the mapped and trimmed regions.

To view the analysis results:

1. In the Ion Reporter™ Software **Home** tab, click **View analyses** or click the **Analyses** tab. Search, filter, or scroll to find the analysis of interest in the list of **Analyses**, then click the analysis link.



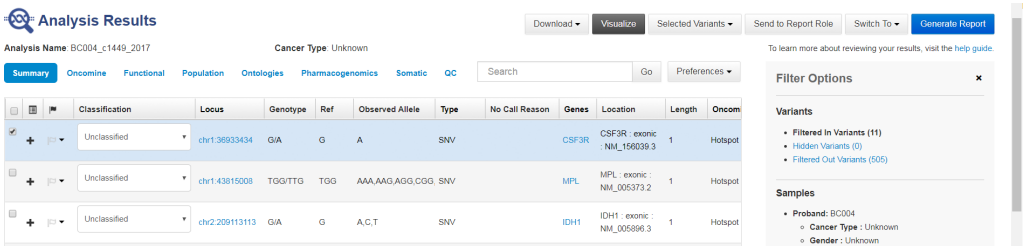
The **Analysis Results** table opens to the list of Oncomine variants.



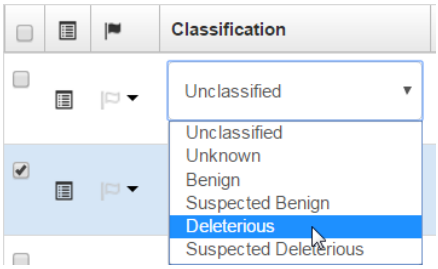
- Variants that are called by the Myeloid_FLT3_LongITD algorithm appear in the default **Oncomine** view, and are identified as **FLT3ITD Oncomine Variant Class** and **Type** in the **Analysis Results** table and as **SVTYPE** in the output VCF file.
- Both the variant calling module in Ion Reporter™ Software and the Myeloid_FLT3_LongITD algorithm can detect the same variant, which appear adjacent in the Ion Reporter™ Software **Analysis Results** as **SNV** or **Indel** and **FLT3ITD** variant types, respectively.
- **Mut/WT** is the ratio of mutant FLT3-ITD variants to the number of the wild type FLT3 read count.

  Note: Values in the **Mut/WT Ratio** column only appear for variants that have FLT3ITD as the **Type**.

2. In the **Analysis Results** table, view a summary of called variants and their genotypic and functional properties. Sort, then select variants of interest by clicking the checkboxes on the left.



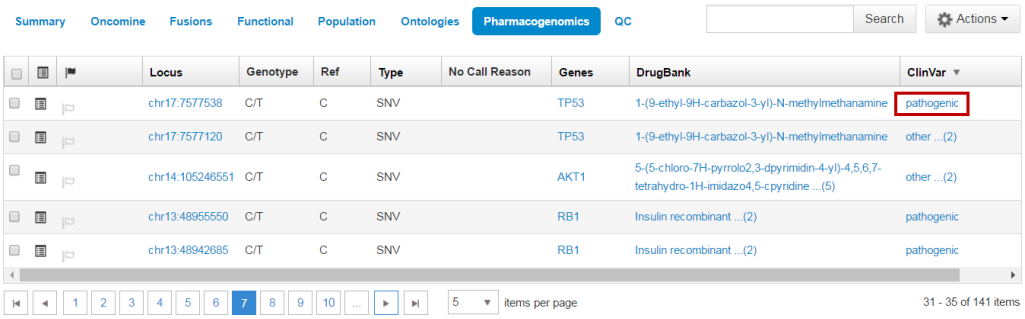
3. *(Optional)* In the **Classification** column, classify variants by selecting a classification from the dropdown list.



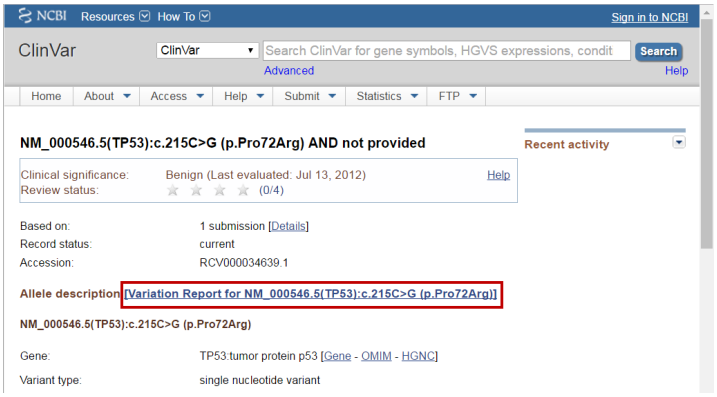
4. Click **Pharmacogenomics**.

5. In the **ClinVar** column, click the ClinVar link to open the NCBI ClinVar variant-specific page where information regarding the ClinVar variant annotation is maintained.

Note: Annotations in the other **Analysis Results** table lists can also be used to classify, sort, and filter variants. See the software help system for more options.



6. In the variant-specific ClinVar website page, click the **Variation Report** link to view additional information about the variant.



Generate a report

Note: Only one Ion Reporter™ Software report can be generated per analysis. You must reanalyze the results to generate a second report.

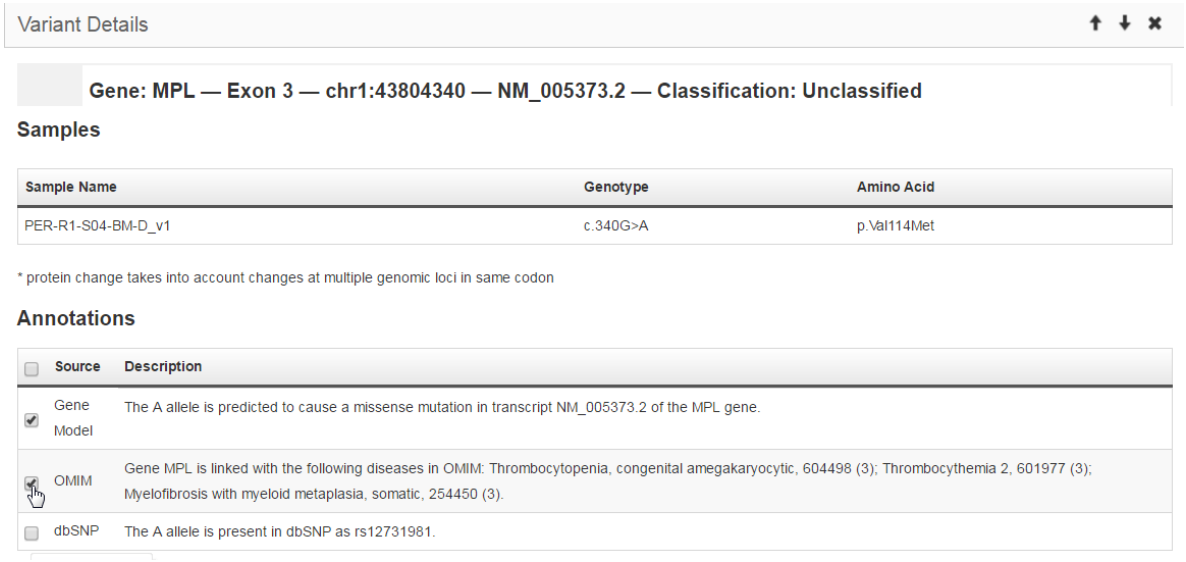
1. Select the variants to be included in the report. In the **Analysis Results** table, click the checkbox to the left of each variant of interest.

2. Click **Generate Report**, a customizable report preview opens.

3. Add, remove or reorder sections of the report, then add or edit text in the available fields if desired.

   Note: You must enter your organization name, the **Organization Name** is a required field.

4. Scroll down to the **Variant Details** section, then select the annotations to be included in the report for each variant.



5. *(Optional)* Click **Save As New Template** to save the current report format as a template for future use.

6. Click **Next**. A preview of your report is displayed. Click **Previous** to edit the report configuration.

7. Click **Lock & Publish** to finalize your report. After generating the report, download it immediately.

To capture all the information from the Ion Reporter™ Software annotation columns, generate a TSV file using the **Download** function. You can then customize the downloaded file to suit your reporting needs.

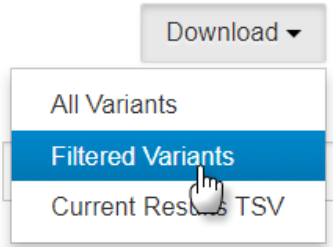
Download the annotated VCF files from Ion Reporter™ Software

VCF (variant call format), or TSV (tab separated value) files of the complete or filtered results can be downloaded from the **Analysis Results** page. You can view the downloaded files individually, or upload a VCF file to a software application that accepts VCF files, such as Ion Torrent™ Oncomine™ Reporter software.

1. In the Ion Reporter™ Software, click the **Analyses** tab, then search, filter, or scroll to find your analysis in the **Analyses** table.

2. In the row of your analysis, click the link in the **Analysis** column.
   The **Analysis Results** table opens to the list of variants.

3. Click **Download** ▸ **Filtered Variants**.



4. In the **Home** tab, click **Notifications** to open the **Notifications** screen.

5. In the **Notifications** table, click **(Download)** in the **Download** column in the row of the appropriate notification to download your analysis results. To download multiple analysis results, select the checkbox in the row of one or more notifications, then click **Download** at the top right above the **Notifications** table.

   The software generates a ZIP file with 4 folders: CNV_VCIB, QC, Variants, and Workflow_Settings for each result. In the Variants folder, you will find the annotated VCF file.

6. Save the filtered Variants ZIP file to your local storage.

A

Tips and troubleshooting

Tips

- Optimize sequencing results by adjusting parameters of ISP template preparation.

Parameter	Range	Effect
Amplification cycles for DNA amplification	13–16 cycles (manual library preparation) 14–17 cycles (Chef library preparation)	Increasing the number of cycles can increase library yield, which can increase total reads per run. However, over amplification during library preparation can increase polyclonality and/or low-quality on-chip reads, which can reduce the total number of useful reads per run.
DNA:RNA pooling ratio	80:20 recommended (modify if needed)	The relative number of reads for either DNA or RNA libraries can be increased or decreased by altering the ratio of DNA to RNA libraries pooled together before templating.
Amount of sample library used in the templating protocol	25–60 pM (Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef templating)	Increasing the amount of sample library used in the templating protocol can increase the number of total reads per chip. However, using an excess of sample library can lead to increased polyclonality.

- Arrange samples in alternating columns on the plate for easier pipetting with multichannel pipettes during purification with the DynaMag™ Side Magnet.
- Plate seals can be firmly applied using the applicator in the MicroAmp™ Optical Adhesive Film Kit. Plate seals can be removed with much less effort when hot. Try removing seals right after taking the plate out of the thermal cycler.
- If library yield is below 50 pM, libraries can still be sequenced by using a proportionally larger volume into a combined library or into template preparation.

- If the unamplified library yield is below 50 pM, libraries can be rescued with library amplification. Combine 25 µL of unamplified library with 72 µL of 1X Library Amp Mix and 4 µL of 25X Library Amp Primers. Perform 5–10 library amplification cycles using the following cycling conditions.

Stage	Temperature	Time
Hold	98°C	2 minutes
5–10 cycles	98°C	15 seconds
	64°C	2 minutes
Hold	10°C	Hold

- When amplifying multiple samples in a single PCR plate, ensure that the input across the samples is roughly equivalent so that the selected cycle number is optimal for all the samples in the run.

Troubleshooting

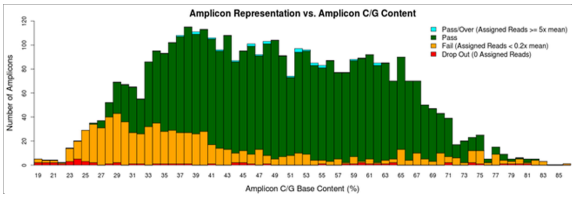
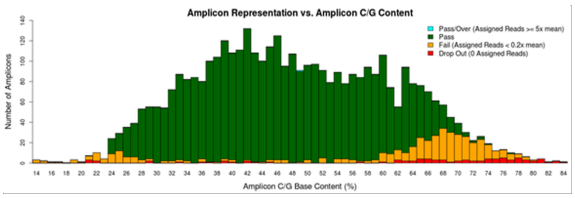
Library yield and quantification

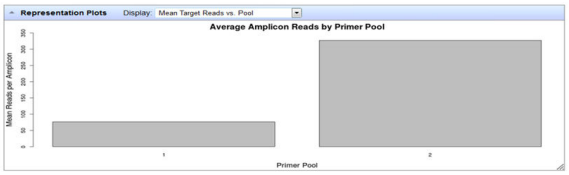
Observation	Possible cause	Recommended action
Library concentration is low—general **Details:** (Library concentration is NOT indicative of quality.)	Sample DNA or RNA was mis-quantified.	Requantify sample DNA using the TaqMan™ RNase P Detection Reagents Kit; quantify RNA with a Qubit™ Fluorometer.
	Residual ethanol in sample DNA or RNA inhibited target amplification.	Incubate uncapped tube in hood for 1 hour.
		Speed-vac tube at room temperature for 5 minutes.
	Residual ethanol from AMPure™ purification inhibited library amplification.	Carefully remove all drops of ethanol before library amplification, then centrifuge plate, if necessary.
	Sample DNA or RNA quality was low.	Add more DNA/RNA or increase target amplification cycles.
	PCR, digestion, or ligation was inefficient.	Ensure proper dispensing and mixing of viscous components at each step.
	AMPure™ XP Beads were over-dried.	Do not dry the AMPure™ XP Beads more than 5 minutes.
	AMPure™ XP Beads inhibited library amplification.	Transfer library off of beads prior to amplification.
	qPCR cycling time is too short.	Use standard qPCR cycling for library designs > 175 bp instead of Fast cycling.
Library concentration with the Ion Library Equalizer™ Kit is less than expected	Equalizer™ Beads were not washed.	Be sure to wash Equalizer™ Beads before use.
	Wrong library amplification primers were used.	Use the Equalizer™ Primers provided in the Ion Library Equalizer™ Kit.
	Residual Equalizer™ Wash Buffer was present after wash.	Carefully remove all of the Equalizer™ Wash Buffer before elution.

Observation	Possible cause	Recommended action
Library concentration is too high	Sample DNA or RNA was mis-quantified.	Requantify sample DNA using the TaqMan™ RNase P Detection Reagents Kit; quantify RNA with a Qubit™ Fluorometer.
	More than 100 ng of sample DNA/RNA was used.	Add less DNA/RNA, or decrease target amplification cycles.

Low amplicon uniformity (DNA only)

Observation	Possible cause	Recommended action
Short amplicons are under-represented	Purification was poor.	Vortex AMPure™ XP Reagent thoroughly before use, and be sure to dispense the full volume.
		100% ethanol is difficult to pipet accurately; it is essential to pre-wet pipette tips.
		In post-ligation library purification, increase AMPure™ XP Reagent volume from 45 µL (1.5X) to 50 µL (1.7X).
Long amplicons are under-represented (short library reads)	Sample DNA or RNA was degraded.	Use an FFPE assay design for degraded or low quality samples.
	PCR was inefficient.	Double the anneal and extend time.
	Too few nucleotide flows were used.	Use an appropriate number of flows to sequence through amplicons. **Note:** Use 550 flows as a default setting when sequencing libraries prepared from most Ion AmpliSeq™ On-Demand panels. In rare instances, amplicons can be longer than 325 bp and can require 650 flows to achieve end-to-end reads, if needed. Determine amplicon length from the panel BED file. Use the templating and sequencing paramaters for 200 bp.

Observation	Possible cause	Recommended action
Long amplicons are under-represented (short library reads) *(continued)*	Sample was over-treated with FuPa Reagent.	Add no more than 2 µL FuPa Reagent per 20 µL target amplification reaction.
		Keep the plate on ice during FuPa Reagent addition, then transfer to a preheated thermal cycler immediately.
	Denaturation temperature was too high.	Use a 97°C enzyme activation/denaturation temperature instead of 99°C in target amplification reactions.
AT-rich amplicons are under-represented  **Example of loss of AT-rich amplicons.** Within the Coverage Analysis Plugin, amplicon representation is plotted by GC content for an Ion AmpliSeq™ Panel. Amplicons with 23-50% GC show an excess failure rate (less than 20% of the mean read depth).	Target amplification was inefficient.	Double the anneal/extend time in the target amplification reaction.
		Decrease the anneal/extend temperature of the target amplification reaction from 60°C to 58°C.
		Decrease the activate the enzyme and denature temperatures of the target amplification reaction from 99°C to 98°C.
	Digested amplicons were denatured.	Do not exceed 60°C during the amplicon digestion step.
	Digestion was inefficient.	Increase amplicon digestion times to 20 minutes for each step.
GC-rich amplicons are under-represented  **Example of loss of GC-rich amplicons.** Within the Coverage Analysis Plugin, amplicon representation is plotted by GC content for an Ion AmpliSeq™ Panel. Amplicons with 60-80% GC show an excess failure rate (less than 20% of the mean read depth).	Denaturation was inadequate during target amplification.	Use a calibrated thermal cycler.
	Target amplification was inefficient.	Increase the anneal/extend temperature of the target amplification reaction from 60°C to 62°C for the first two cycles of the target amplification reaction
	Library amplification was inefficient.	Do not amplify the library (not required for qPCR quantification).
	Sample was over-treated with FuPa Reagent.	Add no more than 2 µL FuPa Reagent per 20 µL target amplification reaction.
		Keep the plate on ice during FuPa Reagent addition, then transfer to thermal cycler immediately.

Observation	Possible cause	Recommended action
Pool representation is not balanced **Details: Example of pool imbalance.** Within the Coverage Analysis Plugin, mean read depth per primer pool is plotted for a 2-pool Ion AmpliSeq™ Panel. In this example, Primer Pool 1 has approximately one quarter the reads of Primer Pool 2. 	Amount of DNA in target amplification reactions varied.	Make a master mix for each sample DNA.
	Pipetting is inaccurate when pools are combined after target amplification.	Centrifuge the plate after target amplification. Ensure that the entire volume of each pool is removed and combined into a single pool.
Uniformity is low (without bias)	Amplification was inadequate.	Double the recommended anneal/extend time for target amplification.

Other

Observation	Possible cause	Recommended action
FLT3 ITD variants cannot be visualized in the IGVS tab	The algorithm uses read information not present in the alignment file displayed by the visualization.	FLT3 ITD variant visualization is not supported in Ion Reporter™ Software 5.10.
Adapter dimers are present during sequencing	Adapter dimers formed during reaction setup or during digestion.	Do not combine Adapters, DNA Ligase, and Switch Solution prior to addition.
		Use a 65°C temperature incubation instead of 60°C during the amplicon digestion step.
	Adapter concentration was too high.	Ensure that barcode adapters are diluted properly.
The number of on-target reads is lower than expected	Unknown.	Increase the number of target amplification cycles by 2.
	Sample ID Panel targets were counted as off-target reads.	Add back the on-target reads from the Sample ID Panel.
Barcode representation is uneven (Equalizer™ kit not used)	Library quantification was inaccurate.	Use the Ion Library TaqMan™ Quantitation Kit for the most specific and accurate library quantification.
	Library combination was inaccurate.	Dilute libraries to 50 pM, then combine equal volumes.
Barcode representation is uneven (Ion Library Equalizer™ Kit used)	Yield of library amplification was inadequate.	When trying the Ion Library Equalizer™ Kit for the first time, quantify with qPCR to ensure libraries are >4 nM. If not the first time, increase input nucleic acid or target amplification cycles.

Observation	Possible cause	Recommended action
Percentage of polyclonal ISPs is high (>40%)	Library input was too high.	Decrease amount of library added to the template preparation reaction by 50%.
	Library was mis-quantified.	Ensure that library was quantified accurately.
	Library was over amplified.	Reduce the number of DNA target amplification cycles.
	Other.	Check the appropriate template preparation user guide for more information.
Low quality ISPs are present at high percentage (>15%)	Library input was too low.	Double the volume of library used in template preparation.
		Use a fresh dilution of library prepared in a low-bind tube.
	Other.	Check the appropriate template preparation user guide for more information.

B

Supplemental information

Digest total RNA with ezDNase™ Enzyme

Note:

- Set thermal cycler to 37°C before you begin.
- Prepare the ezDNase™ Enzyme digestion reactions on ice or a cold block pre-chilled to 2–8°C.

1. Prepare ezDNase™ Master Mix. Add the following components to a 1.5-mL Eppendorf LoBind™ tube.

   IMPORTANT! 10X ezDNase™ Buffer and ezDNase™ Enzyme are viscous. Pipet slowly and mix thoroughly.

Component	Amount
10X ezDNase™ Buffer	(N+1 samples) × 1 µL
ezDNase™ Enzyme	(N+1 samples) × 1 µL

2. Mix by pipetting at least half the total volume up and down at least 5 times.

3. Set up individual sample digestion reactions in a 96-well plate.

   IMPORTANT!

   - For automated library preparation on an Ion Chef™ Instrument, use 1.5X the amount of total RNA required for library preparation. For example, add 15 ng total RNA to the ezDNase™ Enzyme digestion reaction if library preparation requires 10 ng total RNA input.
   - For manual library preparation, use 2X the amount of total RNA required for library preparation. For example, add 20 ng total RNA to the ezDNase™ Enzyme digestion reaction if library preparation requires 10 ng total RNA input.

Component	Amount	
	Ion Chef™ library prep	Manual library prep
Total RNA sample	≤6 µL	≤8 µL
ezDNase™ Master Mix	1.5 µL	2 µL
Nuclease-free Water	to 7.5 µL	to 10 µL
Total volume	7.5 µL	10 µL

4. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

5. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then incubate at 37°C for 2 minutes.

6. Briefly centrifuge the plate to collect any droplets at the bottom of the wells, then keep on ice.

Proceed to the appropriate library preparation-specific reverse transcription procedure topic.

- For automated library preparation on an Ion Chef™ Instrument proceed to "Reverse transcribe RNA for Chef Ready library preparation" on page 19.
- For manual library preparation proceed to "Reverse transcribe ezDNase™ Enzyme treated total RNA for manual library preparation" on page 64.

Reverse transcribe ezDNase™ Enzyme treated total RNA for manual library preparation

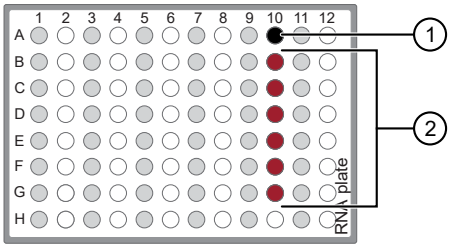
Volumes used in this procedure are for ezDNase™ Enzyme treated total RNA (see, "Digest total RNA with ezDNase™ Enzyme" on page 63). ezDNase™ Enzyme treated total RNA reaction mix must not exceed 50% of the reverse transcription reaction total volume otherwise the reverse transcription can be inhibited.

Note: Prepare the reverse transcription reactions on ice or a cold block pre-chilled to 2–8°C to prevent RNA degradation.

1. For each sample, add the following components into a single well of a 96-well PCR plate. Prepare a master mix without sample RNA for multiple reactions.

Component	Volume
SuperScript™ IV VILO™ Master Mix	2 µL
ezDNase™ Enzyme treated total RNA[1]	5 µL
Nuclease-free Water	3 µL
Total volume per well	**10 µL**

[1] Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).



① No-template control (NTC)
② Samples

2. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

3. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program to synthesize cDNA.

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

STOPPING POINT Samples can be stored at 10°C for up to 16 hours in the thermal cycler. For longer term, store at –20°C.

4. Briefly centrifuge the plate to collect any droplets at the bottom of the wells, carefully remove the seal from the plate, then transfer 5 µL of each cDNA synthesis reaction to a new well.

5. Prepare cDNA target amplification reactions.
   a. Thaw the 5X Ion AmpliSeq™ HiFi Mix on ice, gently vortex to mix, then centrifuge briefly to collect.

   b. Add the following components to each cDNA synthesis reaction. Prepare a master mix for multiple reactions.

Component	Volume
5 µL cDNA synthesis reaction (from step 4)	5 µL
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
5X Oncomine™ Myeloid RNA Panel	4 µL
Nuclease-free Water	7 µL
Total volume per well	**20 µL**

   c. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

Proceed to “Amplify the cDNA targets” on page 25.

Update Oncomine™ Myeloid Research Assay templates in Torrent Suite™ Software

To install or update the Oncomine™ Myeloid Research Assay templates, an off-cycle Torrent Suite™ Software update may be required. Contact your local service representative to schedule a software update.

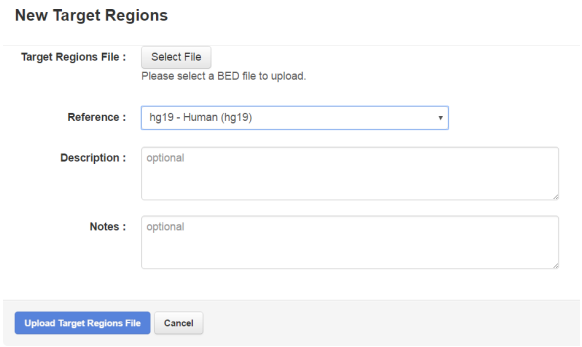
1. Sign in to the Torrent Suite™ Software as an administrator.
2. In the upper right corner, click ⚙ **(Settings)** ▸ **Updates**, then scroll to the **Update Products** section.
3. In the **Name** column find Oncomine™ Myeloid Research Assay, then in that row click **Update**.

The software update begins automatically and displays as **Complete** when finished.

Download and install BED files

Contact your service representative to obtain the latest versions of Oncomine™ Myeloid Research Assay BED files.

1. Extract the ZIP file containing the BED file to a location of your choice.
2. Sign in to the Ion Torrent™ Server on which you want to install the target regions and hotspots BED files.
3. Click the ⚙▾ **(Settings)** tab in the upper right of the screen, then select **References** from the dropdown list.
4. Upload the target regions 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 target regions BED file, which has the following extension: `.designed.bed`



   d. Click **Open**, then click **Upload Target Regions File**.

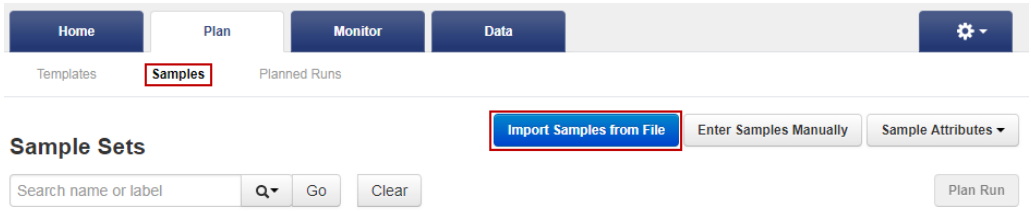
5. Upload the hotspots BED file:
   a. In the left navigation menu, click **Hotspots**, then click the **Add Hotspots** button.
   b. Select **hg19 - Homo sapiens** from the **Reference** dropdown list.
   c. Click **Select File**, then navigate to and select the hotspots file, which has the following extension: `.hotspots.blist.bed`
   d. Click **Open**, then click **Upload Hotspots File**.

The installed BED files appear in the dropdown lists on the Ion Torrent™ Server.

Create a Sample Set by importing samples from a CSV file

If you have many samples, you can import new samples into Torrent Suite™ Software, or update existing samples, using a CSV file that contains sample information. If you do not yet have a samples file, you can create one from an available CSV template that is available in Torrent Suite™ Software. During this process, you can also create a new Sample Set for the new samples.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.



   If you do not yet have a samples file, create a new samples file from an available samples CSV file. For more information, see the Torrent Suite™ Software Help.

2. Upload the samples file, and optionally add a new Sample Set to receive the samples.
   a. In the **Import Samples** section, click **Select file**, then navigate to sample import file, then upload the sample import file.
   b. Select a Sample Set CSV file, then click **Open**.
   c. Select a Sample Set to receive the samples.
      (Optional) To add a new Sample Set to receive the samples, click **Add Sample Set**, then complete the Sample Set information.
      For information about how to define a Sample Set, see the Torrent Suite™ Software Help.

3. Click **Save & Finish**.
   The system loads, parses, and validates the file, then, if no errors are found, saves the samples and Sample Sets.

Create a Planned Run template for use with an Ion OneTouch™ 2 Instrument

We recommend that you create a customized Planned Run template for reuse when the same conditions will be used for multiple runs. To create a custom Planned Run template for use with an Ion OneTouch™ 2 Instrument, copy an existing system template then edit the settings to meet the requirements for your Planned Run.

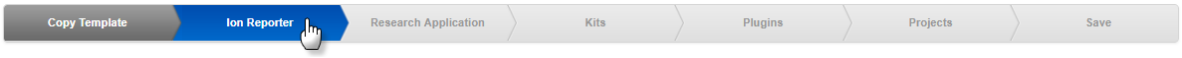
IMPORTANT! Before you create a custom Planned Run template we recommend that you upload the most current **Reference Library**, **Target Regions**, and **Hotspot Regions** BED files on the Torrent Server. See page 66 for more information. Contact your local service representative to obtain the most current BED files.

1. Sign in to the Torrent Browser for the Torrent Server connected to your instrument.
2. Under the **Plan** tab, in the **Templates** screen, click **Oncology - HemeOnc** in the research application list.
3. In the **Oncology - HemeOnc** list, **Oncomine Myeloid Research DNA and Fusions for PGM** row, click **(Settings)** ▸ **Copy**.
   The **Copy Template** wizard will open to the **Save** step.
4. Enter or select the required information in each field:

Field[1]	Action…
Template Name	Enter a name for the Planned Run template.
DNA Reference Library	Select **hg19(Human (hg19))**.
DNA Target Regions[2]	Select the DNA Target Regions BED file **Oncomine_Myeloid.20200429.designed.bed**
DNA Hotspot Regions[2]	Select the DNA Hotspot Regions BED file **Oncomine_Myeloid.20191023.hotspots.bed**
Analysis Parameters	Ensure Default (Recommended) is selected.

[1] Fusions Reference Library, and Fusions Target Regions are not required for analysis in Torrent Suite™ software.
[2] Check with your service representative for updates to ensure the most current files are being used. See page 66 for BED file installation instructions.

5. In the **Copy Template** workflow bar, click the **Ion Reporter** step, then select:



- Your Ion Reporter™ account.
- Select the **Existing Workflow** appropriate to your sequencing instrument.
- **DNA and Fusions** under **Sample Grouping**.
- **Automatically upload to Ion Reporter after run completion** under **Ion Reporter Upload Options**.

Note: If the Ion Reporter™ account is not configured, configure it through Ion Reporter Configure settings (see "Configure the IonReporterUploader plugin in Torrent Suite™ Software" on page 70 for more information).

6. Click **Next**.

7. In the **Research Application** step, ensure that **Research Application** is set to **DNA and Fusions**, and that **Target Technique** is set to **AmpliSeq DNA and Fusions**, then click **Next**.

8. In the **Kits** step, select the **OneTouch Template Kit** radio button, then complete the following fields.

Field	Selection
Instrument	Select the sequencing instrument system being used (for example, Ion PGM™ System).
Sample Preparation Kit	*(Optional)* Select the sample preparation kit used.
Library Kit Type	Select the kit used to prepare the library (for example, Ion AmpliSeq™ Library Kit Plus).
Template Kit	Select Ion 520™ & Ion 530™ Kit – OT2.
Read length	Select 400.
Sequencing Kit	Select the Ion PGM™ Hi-Q™ View Sequencing Kit.
Chip Type	Select Ion 318™ Chip v2.
Control Sequence	*(Optional)* Select the control sequence added to the library preparation. Leave blank if not used.
Barcode Set	Select the barcode set used (for example, Ion Xpress™).
Flows	850

9. Click **Next**.

10. In the **Plugins** step, select the **coverageAnalysis** plugin, then click **Next**.

11. In the **Projects** step, select the project or projects that will receive data from the runs that use this template, then click **Next**.

12. In the **Save** step, click **Copy Template** to save the new run template.

The customized template is now available in the **Templates** screen within the **Research Application** group from which you copied the system template.

Configure the IonReporterUploader plugin in Torrent Suite™ Software

1. Sign in to the Torrent Suite™ Software.
2. Click ⚙ ▾ **(Settings)** ▸ **Ion Reporter Configure**.
3. In the **Ion Reporter Uploader Account Configuration** screen, click + **Add Account** ▸ **Ion Reporter**.
4. In the **Add Ion Reporter account** screen, enter the following information into the fields:

Field	Directions
Server Type	Select:[1]
Display Name	Enter a meaningful name of your choice. This name is used in the run plan template wizard and is seen by other Torrent Suite™ Software users. Use only alphanumeric characters, spaces, and underscores.
Server	Enter:[1]
Port	Enter: 443
Username	Enter your Ion Reporter™ Software username (your email address)
Password	Enter your Ion Reporter™ Software password

[1] Ask your Ion Reporter™ Server administrator for these values.

5. The "Default Account" is the account that is configured by default in run templates and run plans. If this account is the main account to be used for file transfers, enable the **Default Account** checkbox.

Note: You can always change this selection in the Planned Run template workflow bar and in the Upload to IR quick link.

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6. Click **Get Versions**, select **Ion Reporter 5.10 or later**, then click ✓**Add**.

Install Oncomine™ Myeloid Research Assay workflows in Ion Reporter™ Software

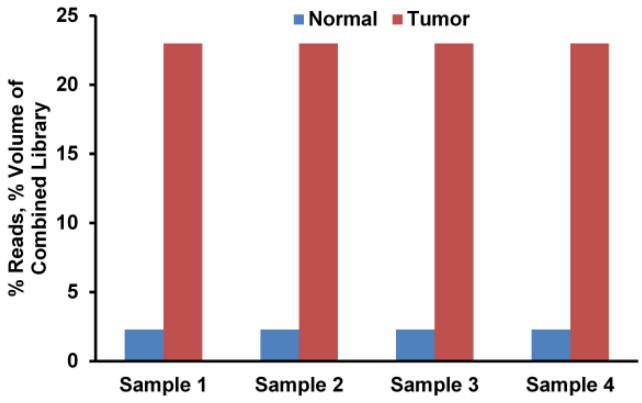
To install or update the Oncomine™ Myeloid Research Assay workflows in Ion Reporter™ Software, contact your service representative to schedule an update.

Strategies for combining libraries

Combine libraries prepared from one panel to vary depth of coverage

When Ion AmpliSeq™ libraries with unique barcodes have been diluted to 100 pM each, unequal volumes of libraries can be combined to produce disproportionate numbers of reads for each barcode.

For example, when comparing tumor and normal sample pairs with the same panel, an average depth of coverage at ~250X may be preferred to type the germline single nucleotide polymorphisms (SNPs) in the normal sample, while an average depth of coverage at ~2,500X may be preferred to type somatic mutations in the tumor sample. In this case, barcoded tumor and normal libraries can be diluted and combined at a 1:10 (normal:tumor) ratio. If the chip capacity is sufficient, multiple tumor/normal sample-pair libraries can be combined in a single chip, as described in the following table:

Sample	Barcode	Fractional volume/ reads	
Sample 1 Normal	BC_0101	0.023	
Sample 1 Tumor	BC_0102	0.23	
Sample 2 Normal	BC_0103	0.023	
Sample 2 Tumor	BC_0104	0.23	
Sample 3 Normal	BC_0105	0.023	
Sample 3 Tumor	BC_0106	0.23	
Sample 4 Normal	BC_0107	0.023	
Sample 4 Tumor	BC_0108	0.23	
Sum	—	**1.0**	

For the example above, if 8 libraries are prepared with the same Ion AmpliSeq™ panel:

1. Dilute all individual libraries to a 100 pM concentration.
2. Add 23 µL of each "tumor" library to a single tube.
3. Add 2.3 µL of each "normal" library into the same tube.
4. Mix the combined libraries and proceed to templating and sequencing.

Ion Chip capacities for combined DNA & RNA Oncomine™ Myeloid Research Assay libraries sequenced at equal depth

The number of combined DNA & RNA libraries that can be accommodated in a single sequencing run depends on the chip, the balance of barcoded library concentration, and the coverage required.

For a given chip, the number of libraries that can be accommodated per sequencing run decreases with the required average read depth. This relationship is shown in the following table. As the number of libraries per chip increases, it becomes more difficult to balance the reads between libraries. We suggest combining fewer libraries initially and determining performance in your laboratory empirically.

	Ion 318™ Chip/ Ion 520™ Chip		Ion 530™ Chip	
Average read depth	**150X**	**1700X**	**150X**	**1700X**
Expected coverage	**95% >30X**	**95% >350X**	**95% >30X**	**95% >350X**
Oncomine™ Myeloid Research Assay library preparation method	**Approximate number of sample libraries[1] per chip**			
Manual library preparation (Cat. No. A36940)	26	4	84	12
Chef Ready library preparation (Cat. No. A36941)[2]	—	—	80	8

[1] Each sample library consists of a combined DNA and RNA library (80:20 DNA:RNA ratio)
[2] Chef Ready library preparation is not recommended for use with Ion 318™ or Ion 520™ sequencing chips.

Using the Ion AmpliSeq™ Sample ID Panel

The Oncomine™ Myeloid Research Assay includes the nine primer pairs that make up the Ion AmpliSeq™ Sample ID Panel. The Sample ID Panel can be used to match a tumor and normal sample. However, copy number variations in the tumor sample may distort the allele balance in the fingerprint.

IMPORTANT! Adding additional Ion AmpliSeq™ Sample ID Panel primer pairs can interfere with target amplification reactions. Do not add additional primers.

If you have not done so already, use the following procedure to configure a Planned Run or Planned Run template in the Torrent Suite™ Software, then view the Sample ID Report following a run.

1. Sign in to the Torrent Suite™ Software.
2. Under the **Plan** tab, in the **Templates** screen, click **Oncology - HemeOnc** in the research application list.
3. In the **Oncology - HemeOnc** list, click your customized Planned Run template name.
4. In the **Kits** page, select **Ion AmpliSeq Sample ID panel** from the **Control Sequence (optional):** dropdown list, then click **Next**.

5. In the **Plugins** page, select the **sampleID plugin** checkbox. Selection of the plugin automatically generates a Sample ID Report after the run.

6. Select the **coverageAnalysis** plugin checkbox, then click **Configure**. In the configuration dialog, select the **Sample Tracking** checkbox. This enables the analysis to produce a statistic for reads mapped to Sample ID targets so that the level of off-target reads is accurately represented in the Coverage Analysis Report.

   Note: If Sample Tracking is not selected, Sample ID reads are counted as off-target reads.

7. Save your new Planned Run template.

After sequencing, select the **Data** tab in the Torrent Suite™ Software, then select **Completed Runs and Results**. Open the report for your run and scroll down to the Plugin Summary section to find the sampleID plugin results.

Sample ID Report

IonXpress_001_R_2012_08_30_15_51_01_user_C02-620--R154979-E292_LT_LN_BT_BN_2-kr

M-TGACASRW

Number of mapped reads	664,406
Number of reads in sample ID regions	24,870
Percent reads in sample ID regions	3.74%
Total base reads in sample ID regions	74,069,093
Percent base reads in sample ID regions	3.55%
Male sample ID region reads	1,798
Female sample ID region reads	1,363

Sample ID Regions		Sample ID SNPs	
Bases in target regions	1,074	Bases in target regions	8
Average base coverage depth	2,449.6	Average base coverage depth	2,620.2
Uniformity of coverage	100.0%	Uniformity of coverage	100.0%
Coverage at 1x	100.0%	Coverage at 1x	100.0%
Coverage at 20x	100.0%	Coverage at 20x	100.0%
Coverage at 100x	100.0%	Coverage at 100x	100.0%

Allele Coverage for Sample Identification SNPs

Chrom	Position	Target ID	TaqMan Assay ID	Call	Ref	AF	Cov	A Reads	C Reads	G Reads	T Reads	Deletions	+Cov	-Cov
chr3	193207380	SNP#1	C_25749280_10	T	T	99.9%	2170	0	3	0	2167	0	1119	1051
chr4	169663615	SNP#2	C_11245682_10	G	T	99.7%	2233	1	0	2219	7	6	961	1266
chr5	178690725	SNP#3	C_3153696_10	A	G	99.1%	2871	2829	25	17	0	0	1407	1464
chr7	137029838	SNP#4	C_3004178_10	C	T	99.8%	2674	0	2666	3	5	0	1244	1430
chr10	17193346	SNP#5	C_2822618	A	A	99.9%	1726	1722	0	2	0	2	895	829
chr12	6945914	SNP#6	C_2184724_1	S	C	66.3%	3369	1	2233	1133	2	0	1952	1417
chr18	9749879	SNP#7	C_1371205_10	R	G	56.6%	2535	1100	0	1433	1	1	1305	1229
chr22	33559508	SNP#8	C_11887110_1	W	T	51.8%	3395	1631	1	5	1756	2	1575	1818

Recommended variants for AcroMetrix™ Oncology Hotspot Control

Note: This table represents representative results using third party commercially available controls. Recommended controls also include variants outside of the Oncomine™ Myeloid Research Assay target space. We only recommend use of these controls but do not guarantee results. Check the user documentation for each specific control for a list of included variants, and consult technical support for performance details.

Gene ID	Coding	Protein	Mutation ID	Target Allele Frequency	Oncomine Myeloid Expected Detection	Oncomine Filtered
TP53	c.818G>A	p.Arg273His	COSM10660	5–15%	Yes	Yes
TP53	c.743G>A	p.Arg248Gln	COSM10662	5–15%	Yes	Yes
TP53	c.916C>T	p.Arg306Ter	COSM10663	5–15%	Yes	Yes
TP53	c.646G>A	p.Val216Met	COSM10667	5–15%	Yes	Yes
TP53	c.469G>T	p.Val157Phe	COSM10670	5–15%	Yes	Yes
TP53	c.892G>T	p.Glu298Ter	COSM10710	5–15%	Yes	Yes
TP53	c.701A>G	p.Tyr234Cys	COSM10725	5–15%	Yes	Yes
TP53	c.659A>G	p.Tyr220Cys	COSM10758	5–15%	Yes	Yes
TP53	c.949C>T	p.Gln317Ter	COSM10786	5–15%	Yes	Yes
TP53	c.404G>A	p.Cys135Tyr	COSM10801	5–15%	Yes	Yes
TP53	c.488A>G	p.Tyr163Cys	COSM10808	5–15%	Yes	Yes
TP53	c.722C>T	p.Ser241Phe	COSM10812	5–15%	Yes	Yes
TP53	c.833C>T	p.Pro278Leu	COSM10863	5–15%	Yes	Yes
TP53	c.1024C>T	p.Arg342Ter	COSM11073	5–15%	Yes	Yes
TP53	c.1015G>T	p.Glu339Ter	COSM11286	5–15%	Yes	Yes
TP53	c.991C>T	p.Gln331Ter	COSM11354	5–15%	Yes	Yes
TP53	c.395A>G	p.Lys132Arg	COSM11582	5–15%	Yes	Yes
TP53	c.166G>T	p.Glu56Ter	COSM12168	5–15%	Yes	Yes
ABL1	c.1052T>C	p.Met351Thr	COSM12578	15–35%	Yes	Yes
ABL1	c.827A>G	p.Asp276Gly	COSM12602	15–35%	Yes	Yes
ABL1	c.1075T>G	p.Phe359Val	COSM12605	15–35%	Yes	Yes

(continued)

Gene ID	Coding	Protein	Mutation ID	Target Allele Frequency	Oncomine Myeloid Expected Detection	Oncomine Filtered
ABL1	c.1064A>G	p.Glu355Gly	COSM12611	15–35%	Yes	Yes
KIT	c.1961T>C	p.Val654Ala	COSM12706	5–15%	Yes	Yes
KIT	c.1727T>C	p.Leu576Pro	COSM1290	5–15%	Yes	Yes
PTPN11	c.226G>A	p.Glu76Lys	COSM13000	5–15%	Yes	Yes
PTPN11	c.181G>T	p.Asp61Tyr	COSM13011	5–15%	Yes	Yes
PTPN11	c.205G>A	p.Glu69Lys	COSM13013	5–15%	Yes	Yes
PTPN11	c.215C>T	p.Ala72Val	COSM13015	5–15%	Yes	Yes
PTPN11	c.1508G>C	p.Gly503Ala	COSM13027	5–15%	Yes	Yes
PTPN11	c.1528C>A	p.Gln510Lys	COSM13031	5–15%	Yes	Yes
KIT	c.1924A>G	p.Lys642Glu	COSM1304	5–15%	Yes	Yes
RB1	c.2053C>T	p.Gln685Ter	COSM13117	5–15%	Yes	Yes
KIT	c.1509_1510insGCCTAT	p.Ser501_Ala502 insAlaTyr	COSM1326	5–15%	Yes	Yes
MPL	c.1544G>T	p.Trp515Leu	COSM18918	5–15%	Yes	Yes
MPL	c.1514G>A	p.Ser505Asn	COSM27286	5–15%	Yes	Yes
FLT3	c.1799_1800insTTT CAGAGAATATGAATATGA	p.Phe594_ Asp600dup	COSM27907	5–15%	Yes	Yes
FLT3	c.1800_1801insTTC AGAGAATATGAATATGAT	p.Phe594_ Asp600dup	COSM27907	5–15%	Yes	Yes
IDH1	c.395G>A	p.Arg132His	COSM28746	5–15%	Yes	Yes
TP53	c.1123C>T	p.Gln375Ter	COSM307348	5–15%	Yes	Yes
IDH2	c.515G>A	p.Arg172Lys	COSM33733	5–15%	Yes	Yes
EZH2	c.1937A>T	p.Tyr646Phe	COSM37028	15–35%	Yes	Yes
IDH2	c.419G>A	p.Arg140Gln	COSM41590	5–15%	Yes	Yes
TP53	c.614A>G	p.Tyr205Cys	COSM43947	5–15%	Yes	Yes
TP53	c.273G>A	p.Trp91Ter	COSM44492	5–15%	Yes	Yes
TP53	c.981T>G	p.Tyr327Ter	COSM44823	5–15%	Yes	Yes
TP53	c.151G>T	p.Glu51Ter	COSM44907	5–15%	Yes	Yes

(continued)

Gene ID	Coding	Protein	Mutation ID	Target Allele Frequency	Oncomine Myeloid Expected Detection	Oncomine Filtered
BRAF	c.1391G>T	p.Gly464Val	COSM450	15–35%	Yes	Yes
BRAF	c.1742A>G	p.Asn581Ser	COSM462	15–35%	Yes	Yes
BRAF	c.1781A>G	p.Asp594Gly	COSM467	15–35%	Yes	Yes
BRAF	c.1790T>G	p.Leu597Arg	COSM471	15–35%	Yes	Yes
BRAF	c.1799T>A	p.Val600Glu	COSM476	15–35%	Yes	Yes
HRAS	c.35G>T	p.Gly12Val	COSM483	5–15%	Yes	Yes
KRAS	c.35G>A	p.Gly12Asp	COSM521	5–15%	Yes	Yes
NRAS	c.35G>A	p.Gly12Asp	COSM564	5–15%	Yes	Yes
HRAS	c.182A>G	p.Gln61Arg	COSM499	5–15%	Yes	Yes
NRAS	c.182A>G	p.Gln61Arg	COSM584	5–15%	Yes	Yes
TP53	c.733G>A	p.Gly245Ser	COSM6932	5–15%	Yes	Yes
FLT3	c.2503G>T	p.Asp835Tyr	COSM783	5–15%	Yes	Yes
RB1	c.2028_2040delAGA ACATATCATC	p.Leu676fs	COSM870	5–15%	Yes	Yes
RB1	c.1072C>T	p.Arg358Ter	COSM879	5–15%	Yes	Yes
RB1	c.1654C>T	p.Arg552Ter	COSM887	5–15%	Yes	Yes
RB1	c.1666C>T	p.Arg556Ter	COSM888	5–15%	Yes	Yes
RB1	c.409G>T	p.Glu137Ter	COSM890	5–15%	Yes	Yes
RB1	c.958C>T	p.Arg320Ter	COSM891	5–15%	Yes	Yes
RB1	c.1735C>T	p.Arg579Ter	COSM892	5–15%	Yes	Yes
RB1	c.1363C>T	p.Arg455Ter	COSM895	5–15%	Yes	Yes
RB1	c.596T>A	p.Leu199Ter	COSM915	5–15%	Yes	Yes
RB1	c.2143A>T	p.Lys715Ter	COSM940	5–15%	Yes	Yes
FLT3	c.1775T>C	p.Val592Ala	COSM19522	5–15%	Yes	Yes
RB1	c.1687T>C	p.Trp563Arg	COSM1367255	5–15%	Yes	Yes

Recommended variants for Seraseq™ Myeloid Mutation DNA Mix control

Note: This table represents representative results using third party commercially available controls. Recommended controls also include variants outside of the Oncomine™ Myeloid Research Assay target space. We only recommend use of these controls but do not guarantee results. Check the user documentation for each specific control for a list of included variants, and consult technical support for performance details.

Gene ID	Coding	Protein	COSMIC ID	Target Allele Frequency	Oncomine Myeloid Expected Detection	Oncomine Myeloid Variant	Oncomine Filtered
ABL1	c.944C>T	p.T315I	12560	10%	Yes	p.Thr315Ile	Yes
ASXL1	c.1900_1922del23	p.E635fs*15	36165	10%	No	—	—
ASXL1	c.1934_1935insG	p.G646fs*12	34210	10%	No	—	—
BRAF	c.1799T>A	p.V600E	476	10%	Yes	p.Val600Glu	Yes
CALR	c.1092_1143del52	p.L367fs*46	1738055	5%	Yes	p.Leu367fs	Yes
CBL	c.1139T>C	p.L380P	34055	10%	Yes	p.Leu380Pro	Yes
CBL	c.1259G>A	p.R420Q	34077	5%	Yes	p.Arg420Gln	Yes
CEBPA	c.68_69insC	p.H24fs*84	18922	15%	No	—	—
CEBPA	c.939_940insAAG	p.K313_V314insK	18099	15%	Yes	p.Lys313dup	Yes
CSF3R	c.1853C>T	p.T618I	1737962	5%	Yes	p.Thr618Ile	Yes
FLT3	Dup chr13:28,608, 250-28,608,277(hg19)	FLT3-ITD	—	10%	Yes	FLT3 Long ITD Plugin	—
FLT3	c.2503G>T	p.D835Y	783	10%	Yes	p.Asp835Tyr	Yes
FLT3	c.1759_1800dup	FLT3-ITD	—	5%	Yes	FLT3 Long ITD Plugin	—
IDH1	c.394C>T	p.R132C	28747	5%	Yes	p.Arg132Cys	Yes
JAK2	c.1624_1629 delAATGAA	p.N542_E543del	24440	10%	Yes	p.Asn542_ Glu543del	No
JAK2	c.1849G>T	p.V617F	12600	5%	Yes	p.Val617Phe	Yes
MPL	c.1544G>T	p.W515L	18918	5%	Yes	p.Trp515Leu	Yes
MYD88	c.794T>C	p.L265P	85940	10%	Yes	p.Leu273Pro	Yes
NPM1	c.863_864insTCTG	p.W288fs*12	17559	5%	Yes	p.Trp288fs	Yes
SF3B1	c.2098A>G	p.K700E	84677	5%	Yes	p.Lys700Glu	Yes
SF3B1	c.1998G>T	p.K666N	131557	5%	Yes	p.Lys666Asn	Yes

(continued)

Gene ID	Coding	Protein	COSMIC ID	Target Allele Frequency	Oncomine Myeloid Expected Detection	Oncomine Myeloid Variant	Oncomine Filtered
SRSF2	c.284_307del24	p.P95_R102del	146289	5%	Yes	p.Pro95_ Arg102del	Yes
U2AF1	c.101C>T	p.S34F	166866	10%	Yes	p.Ser34Phe	Yes

Recommended variants for Seraseq™ Myeloid Mutation RNA Mix control

Note: This table represents representative results using third party commercially available controls. Recommended controls also include variants outside of the Oncomine™ Myeloid Research Assay target space. We only recommend use of these controls but do not guarantee results. Check the user documentation for each specific control for a list of included variants, and consult technical support for performance details.

Fusion Partners	Oncomine™ Myeloid Research Assay Primary Detected Isoform	Oncomine Filtered
MYST3-CREBBP	KAT6A-CREBBP.K17C2	Yes
ETV6-ABL1 (transcript 1)	ETV6-ABL1.E4A2	Yes
ETV6-ABL1 (transcript 2)	ETV6-ABL1.E5A2	Yes
PCM1-JAK2	PCM1-JAK2.P23J12.COSF1001	Yes
FIPIL1-PDGRFA	FIP1L1-PDGFRA.F11P12del45	Yes
TCF3-PBX1	TCF3-PBX1.T16P3.COSF1489	Yes
BCR-ABL1	BCR-ABL1.B14A2.1	Yes
RUNX1-RUNX1T1	RUNX1-RUNX1T1.R3R3	Yes
PML-RARA	PML-RARA.P6del11ins133A3	Yes

C

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, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.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
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide (Pub. No. MAN0016854)	Describes the automated template preparation of Oncomine™ Myeloid Research Assay libraries using the Ion Chef™ System for sequencing on Ion GeneStudio™ S5 Series Systems.
Ion PGM™ Hi-Q™ View Chef Kits User Guide (Pub. No. MAN0014571)	Describes the automated template preparation of Oncomine™ Myeloid Research Assay libraries using the Ion Chef™ System for sequencing on the Ion PGM™ System.
Ion PGM™ Hi-Q™ View OT2 Kit User Guide (Pub. No. MAN0014579)	Describes template preparation of Oncomine™ Myeloid Research Assay libraries on the Ion OneTouch™ 2 System using the Ion PGM™ Hi-Q™ View OT2 Kit for sequencing on the Ion PGM™ System.
Ion PGM™ Hi-Q™ View Sequencing Kit User Guide (Pub. No. MAN0014583)	Describes how to sequence Oncomine™ Myeloid Research Assay libraries on the Ion PGM™ System using the Ion PGM™ Hi-Q™ View Sequencing Kit.
Ion Sphere™ Quality Control Kit User Guide (Pub. No. MAN0017531)	Provides step-by-step instruction for the quantification of template-positive Ion Sphere™ Particles using a Qubit™ Fluorometer.

Note: For additional documentation, see “Customer and technical support” on page 82.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

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- Product documentation
  - 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 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**.