# Oncomine™ Comprehensive Assay Plus
## USER GUIDE

for use with:
Oncomine™ Comprehensive Assay Plus, DNA panel
Oncomine™ Comprehensive Assay Plus, RNA panel

**Catalog Numbers**  A48577, A48578, A49667, and A49671

**Publication Number**  MAN0018490

**Revision**  D.0

**ThermoFisher**
S C I E N T I F I C

Life Technologies Corporation | 7335 Executive Way | Frederick, Maryland 21704 USA

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

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# Product information

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

## Product description

The Oncomine™ Comprehensive Assay Plus is a targeted, next-generation sequencing (NGS) assay that provides a comprehensive genomic profiling solution appropriate for formalin-fixed paraffin-embedded (FFPE) tissues. The assay allows concurrent analysis of DNA and RNA to simultaneously detect multiple biomarkers associated with targeted and immune checkpoint research, including comprehensive targets that are relevant in cancer, in a single workflow.

Features of the Oncomine™ Comprehensive Assay Plus include:

• Enables analysis of variants across 500+ genes

• Detects SNVs, CNVs, INDELs, LOH, TMB, MSI, and gene fusions

• Provides robust performance from as little as 10 ng per pool of nucleic acid isolated from FFPE samples including fine needle biopsies

• Characterized with molecular standards and controls

This guide covers manual library preparation from DNA and RNA using the Oncomine™ Comprehensive Assay Plus, DNA and Oncomine™ Comprehensive Assay Plus, RNA panels, respectively. Each assay panel can be used with barcoded adapters, so that up to 4 paired DNA and RNA samples and DNA and RNA no-template controls (NTCs) can be combined and loaded onto a single Ion 550™ Chip in a single workflow to minimize the per-sample sequencing cost.

The Oncomine™ Comprehensive Assay Plus, DNA panel includes the Ion AmpliSeq™ Sample ID Panel primers to prevent research sample misidentification and provide gender determination.

**Note:** This guide also covers automated library preparation on the Ion Chef™ System using the Oncomine™ Comprehensive Assay Plus – Automated Library Preparation kit (Cat. Nos. A49667 and A49671). The kit provides the Oncomine™ Comprehensive Assay Plus, DNA (2-pools) and Oncomine™ Comprehensive Assay Plus, RNA (2-pools) at 2X concentration pre-measured in barcoded primer pool tubes ready to load into an Ion AmpliSeq™ Chef Reagents DL8 cartridge.

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This guide includes protocols for using the following products:

- Oncomine™ Comprehensive Assay Plus, DNA, Manual Library Preparation (Part No. A45615)
- Oncomine™ Comprehensive Assay Plus, RNA, Manual Library Preparation (Part No. A45616)
- Oncomine™ Comprehensive Assay Plus, DNA, Chef-Ready Library Preparation (Part No. A45617)
- Oncomine™ Comprehensive Assay Plus, RNA, Chef-Ready Library Preparation (Part No. A45618)
- Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990)
- Ion AmpliSeq™ Kit for Chef DL8 (Cat. No. A29024)
- Ion Torrent™ NGS Reverse Transcription Kit (Cat. No. A45003)
- IonCode™ Barcode Adapters 1–384 Kit (Cat. No. A29751)
- Ion Xpress™ Barcode Adapters (various Cat. Nos.)
- Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802)
- Uracil-DNA Glycosylase, heat-labile (Cat. No. 78310100UN)

# Contents and storage

## Oncomine™ Comprehensive Assay Plus – Manual Library Preparation

Oncomine™ Comprehensive Assay Plus (Cat. No. A48577) is designed to prepare barcoded sample libraries from DNA and RNA. The kits consist of the Oncomine™ Comprehensive Assay Plus, DNA, Manual Library Preparation panel (2-pool) (Part No. A45615) and the Oncomine™ Comprehensive Assay Plus, RNA, Manual Library Preparation panel (2-pool) (Part No. A45616), with two Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990). Sufficient reagents are provided to prepare libraries from 24 samples.

| Contents | Amount | Storage |
|---|---|---|
| **Oncomine™ Comprehensive Assay Plus, DNA, Manual Library Preparation** | | |
| 2X DNA OCA Plus, Pool 1 of 2 (blue cap) | 3 × 40 µL | –30ºC to –10ºC |
| 2X DNA OCA Plus, Pool 2 of 2 (blue cap) | 3 × 40 µL | |
| RMC | 24 µL | |
| **Oncomine™ Comprehensive Assay Plus, RNA, Manual Library Preparation** | | |
| 5X RNA OCA Plus, Pool 1 of 2 (red cap) | 3 × 16 µL | –30ºC to –10ºC |
| 5X RNA OCA Plus, Pool 2 of 2 (red cap) | 3 × 16 µL | |
| **Ion AmpliSeq™ Library Kit Plus** | | |
| 5X Ion AmpliSeq™ HiFi Mix (red cap) | 2 x 120 µL | –30ºC to –10ºC |
| FuPa Reagent (brown cap) | 2 x 48 µL | |
| Switch Solution (yellow cap) | 96 µL | |
| DNA Ligase (blue cap) | 2 x 48 µL | |
| 25X Library Amp Primers (pink cap) | 2 x 48 µL | |
| 1X Library Amp Mix (black cap) | 2 x 1.2 mL | |
| Low TE | 2 x 6 mL | 15°C to 30°C[1] |

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

# Oncomine™ Comprehensive Assay Plus, RNA – Manual Library Preparation

Oncomine™ Comprehensive Assay Plus, RNA – Manual Library Preparation (Cat. No. A48578) is designed to prepare barcoded sample libraries from RNA. The kits consist of the Oncomine™ Comprehensive Assay Plus, RNA – Manual Library Preparation panel (2-pool) (Part No. A45616), with one Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990). Sufficient reagents are provided to prepare libraries from 24 samples.

| Contents | Amount | Storage |
|---|---|---|
| **Oncomine™ Comprehensive Assay Plus, RNA – Manual Library Preparation** | | |
| 5X RNA OCA Plus, Pool 1 of 2 (red cap) | 3 × 16 µL | –30ºC to –10ºC |
| 5X RNA OCA Plus, Pool 2 of 2 (red cap) | 3 × 16 µL | |
| **Ion AmpliSeq™ Library Kit Plus** | | |
| 5X Ion AmpliSeq™ HiFi Mix (red cap) | 120 µL | –30ºC to –10ºC |
| FuPa Reagent (brown cap) | 48 µL | |
| Switch Solution (yellow cap) | 96 µL | |
| DNA Ligase (blue cap) | 48 µL | |
| 25X Library Amp Primers (pink cap) | 48 µL | |
| 1X Library Amp Mix (black cap) | 1.2 mL | |
| Low TE | 6 mL | 15°C to 30°C[1] |

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

# Oncomine™ Comprehensive Assay Plus – Automated Library Preparation

The Oncomine™ Comprehensive Assay Plus – Automated Library Preparation (Cat. No. A49667) provides the Oncomine™ Comprehensive Assay Plus, DNA, Chef-Ready panel (2-pool) (Part No. A45617) and Oncomine™ Comprehensive Assay Plus, RNA, Chef-Ready panel (2-pool) (Part No. A45618) 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 samples.

**Note:** For detailed information on preparing Oncomine™ Comprehensive Assay Plus libraries on the Ion Chef™ System, see the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432).

| Component | Amount | Storage |
|---|---|---|
| **Oncomine™ Comprehensive Assay Plus, DNA, Chef Ready** | | |
| 2X DNA OCA Plus (pool 1 of 2) | 4 × 150 µL | –30°C to –10°C |
| 2X DNA OCA Plus (pool 2 of 2) | 4 × 150 µL | |
| RMC | 48 µL | |
| **Oncomine™ Comprehensive Assay Plus, RNA, Chef Ready** | | |
| 2X RNA OCA Plus (pool 1 of 2) | 4 × 150 µL | –30°C to –10°C |
| 2X RNA OCA Plus (pool 2 of 2) | 4 × 150 µL | |
| **Ion AmpliSeq™ Kit for Chef DL8** | | |
| Ion AmpliSeq™ Kit for Chef DL8 (Part No. A29025) | 2 × 4 cartridges | –30°C to –10°C |
| Ion AmpliSeq™ Chef Solutions DL8 (Part No. A29026) | 2 × 4 cartridges | 2°C to 8°C[1] |
| Ion AmpliSeq™ Chef Supplies DL8 (per insert) (Part No. A29027)<br>• Ion AmpliSeq™ Tip Cartridge L8<br>• PCR Frame Seal<br>• Enrichment Cartridge | 2 boxes with 4 inserts | 15°C to 30°C |
| IonCode™ 0101–0132 in 96 Well PCR Plates (dried) (Part No. A29028)<br><br>Set includes 4 PCR plates:<br>• IonCode™ 0101–0108 in 96 Well PCR Plate (red)<br>• IonCode™ 0109–0116 in 96 Well PCR Plate (yellow)<br>• IonCode™ 0117–0124 in 96 Well PCR Plate (green)<br>• 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.

# Oncomine™ Comprehensive Assay Plus, RNA – Automated Library Preparation

The Oncomine™ Comprehensive Assay Plus, RNA – Automated Library Preparation (Cat. No. A49671) provides the Oncomine™ Comprehensive Assay Plus, RNA, Chef-Ready panel (2-pool) (Part No. A45618) 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 samples.

**Note:** For detailed information on preparing Oncomine™ Comprehensive Assay Plus libraries on the Ion Chef™ System, see the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432).

| Component | Amount | Storage |
|---|---|---|
| **Oncomine™ Comprehensive Assay Plus, RNA, Chef-Ready** | | |
| 2X RNA OCA Plus (pool 1 of 2) | 4 × 150 µL | –30°C to –10°C |
| 2X RNA OCA Plus (pool 2 of 2) | 4 × 150 µL | |
| **Ion AmpliSeq™ Kit for Chef DL8** | | |
| Ion AmpliSeq™ Kit for Chef DL8 (Part No. A29025) | 2 × 4 cartridges | –30°C to –10°C |
| Ion AmpliSeq™ Chef Solutions DL8 (Part No. A29026) | 2 × 4 cartridges | 2°C to 8°C[1] |
| Ion AmpliSeq™ Chef Supplies DL8 (per insert) (Part No. A29027)<br>• Ion AmpliSeq™ Tip Cartridge L8<br>• PCR Frame Seal<br>• Enrichment Cartridge | 2 boxes with 4 inserts | 15°C to 30°C |
| IonCode™ 0101–0132 in 96 Well PCR Plates (dried) (Part No. A29028)<br><br>Set includes 4 PCR plates:<br>• IonCode™ 0101–0108 in 96 Well PCR Plate (red)<br>• IonCode™ 0109–0116 in 96 Well PCR Plate (yellow)<br>• IonCode™ 0117–0124 in 96 Well PCR Plate (green)<br>• 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.

# 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 |
|---|---|
| One of the following:<br><br>IonCode™ Barcode Adapters 1–384 Kit<br><br>Ion Xpress™ Barcode Adapters Kit | A29751<br><br>4474517[1] |
| Ion Library TaqMan™ Quantitation Kit and real-time PCR system, see "Recommended materials" on page 14. | 4468802 (A26121) |
| Agencourt™ AMPure™ XP Kit | NC9959336, NC9933872<br>**fisherscientific.com** |
| *(RNA only)* Ion Torrent™ NGS Reverse Transcription Kit | A45003 |
| *(DNA only)* Uracil-DNA Glycosylase, heat-labile | 78310100UN |
| One of the following thermal cyclers, or equivalent:<br>• ProFlex™ 96-well PCR System<br>• Veriti™ 96-Well Thermal Cycler<br>• 2720 Thermal Cycler[2]<br>• GeneAmp™ PCR System 9700 96-Well[2] or GeneAmp™ PCR System 9700 Dual 96-Well[2] | Various |
| MicroAmp™ Optical 96-Well Reaction Plate or<br><br>MicroAmp™ Optical 96-Well Reaction Plate with Barcode | N8010560 or<br><br>4306737 |
| MicroAmp™ Fast Optical 96-Well Reaction Plate | 4346907 |
| MicroAmp™ Clear Adhesive Film | 4306311 |
| MicroAmp™ Optical Film Compression Pad | 4312639 |
| DynaMag™–96 Side Magnet or other plate magnet | 12331D |
| Eppendorf™ DNA LoBind™ Microcentrifuge Tubes, 1.5 mL | 13-698-791<br>**fisherscientific.com** |
| Nuclease-free Water | AM9932 |
| Ethanol, Absolute, Molecular Biology Grade | BP2818500<br>**fisherscientific.com** |
| Pipettors, 2–200 µL, and low-retention filtered pipette tips | MLS |

[1] Various kits are available. For more information, see **thermofisher.com**.

[2] Supported but no longer available for purchase.

# 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** | |
| One of the following Applied Biosystems™ real-time PCR instruments:<br>• 7500 Real-Time PCR System<br>• 7900HT Fast Real-Time PCR System[1]<br>• StepOne™ Real-Time PCR System<br>• StepOnePlus™ Real-Time PCR System<br>• ViiA™ 7 Real-Time PCR System<br>• QuantStudio™ 3 Real-Time PCR System<br>• QuantStudio™ 5 Real-Time PCR System<br>• QuantStudio™ 7 Flex Real-Time PCR System<br>• QuantStudio™ 12K Flex Real–Time PCR System | Various |
| 96-well plate centrifuge | **MLS** |
| Qubit™ 4 Fluorometer[2] | Q33238 |
| **Recommended for nucleic acid isolation** | |
| MagMAX™ FFPE DNA/RNA Ultra Kit | A31881 |
| **Recommended for nucleic acid quantification** | |
| Qubit™ dsDNA HS Assay Kit (DNA) | Q32851, Q32854, |
| Qubit™ RNA HS Assay Kit (RNA) | Q32852, Q32855 |
| TaqMan™ RNase P Detection Reagents Kit | 4316831 |

[1] Supported but no longer available for purchase.
[2] Qubit™ 2.0 Fluorometer or later are supported.

| Recommended controls | Vendor | Part number |
|---|---|---|
| AcroMetrix™ Oncology Hotspot Control | Thermo Fisher Scientific | 969056 |
| MSI FFPE DNA Reference Standard | Horizon | HD830 |
| MSS FFPE DNA Reference Standard | Horizon | HD831 |
| Seraseq™ Tri-Level Tumor Mutation DNA Mix v2 HC | Seracare | 0710-0097 |
| ATCC cell lines with CNV | **www.atcc.org** | ATCC® CRL-2327<br>ATCC® CRL-2336<br>ATCC® CRL-5868D |

*(continued)*

| Recommended controls | Vendor | Part number |
|---|---|---|
| Cell-Ref™ FFPE Human Cancer Cell Slide - HCC - 2998 (5 slides) | AccuRef | ASO-1010 |
| Cell-Ref™ FFPE Human Cancer Cell Slide - T-47D (5 slides) | AccuRef | ASO-1032 |
| Cell-Ref™ FFPE Human Cancer Cell Slide - A549 (5 slides) | AccuRef | ASO-1001 |
| Cell-Ref™ FFPE Human Cancer Cell Slide - SK-MEL-2 (5 slides) | AccuRef | ASO-1028 |
| Cell-Ref™ FFPE Human Cancer Cell Slide - H2228 (5 slides) | AccuRef | ASO-1006 |
| Seraseq™ gDNA TMB Mix Score 7 | Seracare | 0710-1326 |
| Seraseq™ gDNA TMB Mix Score 9 | Seracare | 0710-1325 |
| Seraseq™ gDNA TMB Mix Score 20 | Seracare | 0710-1324 |
| Seraseq™ gDNA TMB Mix Score 26 | Seracare | 0710-1323 |
| Seraseq™ Fusion RNA Mix v4<br><br>18 RNA fusions: RET, ROS1, EGFRvIII, EGFR, ALK, NTRK3, FGFR3, NTRK1, METex14, PPARG1, BRAF, ERG | Seracare | 0710-0497 |
| Seraseq™ FFPE NTRK Fusion RNA Reference Material | Seracare | 0710-1031 |
| Horizon™ ALK RET ROS RNA fusion<br><br>RNA fusions: EML4-ALK, CCDC6-RET, and SLC34A2-ROS1 | Horizon | HD784 |
| CancerSeq™ Plus Paraffin Tissue Curl (5 curls)<br><br>Copy number variation (CNV): CCNE1 ,EGFR, ERBB2, GNAS, KRAS, RB1 (-) | BioChain | T2235152-SC<br>Lot No. B906046 |

# 2 Before you begin

## Procedural guidelines

- Minimize freeze-thaw cycles of Oncomine™ Comprehensive Assay Plus panels and the RMC by aliquoting into low bind tubes as needed for your experiments. Panels can be stored at 4°C for 1 year. Store RMC at –30°C to –10°C.
- 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.
- 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.

## 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.

# Guidelines for RNA isolation, quantification, and input

- We recommend the MagMAX™ FFPE DNA/RNA Ultra Kit (Cat. No. A31881) for isolating RNA.
- We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32855) for quantifying RNA.
- Treat total RNA with DNase before use.
- We recommend using 20 ng of total RNA for reverse transcription. Increasing the amount of total RNA will usually result in higher quality libraries, especially when RNA quality or quantity is unknown. With high-quality, well-quantified samples, as little as 1 ng total RNA can be used.
- In general, library yield from high quality RNA is greater than from degraded samples. Library yield is not indicative of sequencing performance.

# Guidelines for DNA isolation, quantification, and input

- We recommend the MagMAX™ FFPE DNA/RNA Ultra Kit (Cat. No. A31881) for isolating DNA.
- We recommend the TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831) for quantifying amplifiable human genomic DNA (see *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan™ RNAse P Detection Reagents Kit* (Pub. No. MAN0007732) available at **thermofisher.com**).
- The Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) can also be used for quantification, particularly for FFPE DNA, and highly degraded DNA samples.
- Quantification methods such as spectrophotometry (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 concentration of sample DNA, under-seeding of the target amplification reaction, low library yields, and poor chip loading.
- We recommend using 20 ng of DNA for manual library preparation and automated library preparation. Increasing the amount of DNA results in higher-quality libraries, especially when DNA quality or quantity is unknown.

# Library preparation from genomic DNA or RNA

Isolate and quantify DNA

UDG treat FFPE DNA

RMC reagent addition

Isolate and quantify RNA

RNA

Reverse transcribe RNA

DNA or cDNA

Primer pairs          Amplify targets

Amplicons

Partially digest amplicons

Barcode Adapters    X
                    P1          Ligate adapters

X                    P1

Barcoded library

Quantify libraries

Combine libraries (optional)
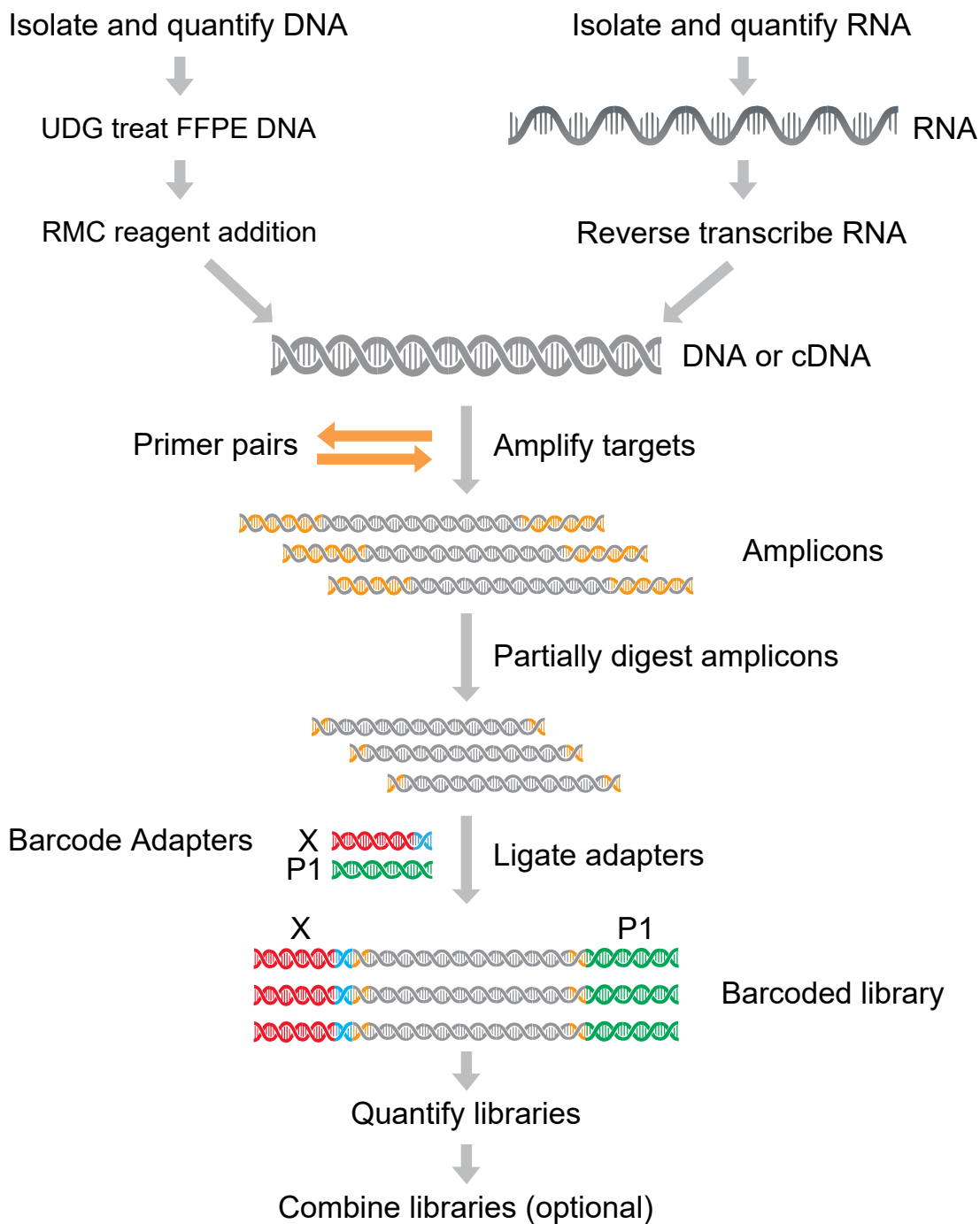
# 3 Automated library preparation on the Ion Chef™ System

This chapter describes library preparation using the following components:

- Oncomine™ Comprehensive Assay Plus, DNA, Chef-Ready Library Preparation (Part No. A45617)
- Oncomine™ Comprehensive Assay Plus, RNA, Chef-Ready Library Preparation (Part No. A45618)
- Ion Torrent™ NGS Reverse Transcription Kit (Cat No. A45003)
- Uracil-DNA Glycosylase, heat-labile (Cat No. 78310100UN)
- Ion AmpliSeq™ Kit for Chef DL8 (Cat No. A29024)

## Create a sample set to prepare 2 pools of 4 libraries each

**IMPORTANT!** The Oncomine™ Comprehensive Assay Plus requires selection of the **2 Library Pools - OCA Plus** Library prep Protocol that limits the number of libraries to 4 per pool in order to generate sufficient read depth when sequencing.

**Note:** In Torrent Suite™ Software 5.16 or later Sample Sets are required for automated library preparation. You can import new samples into Torrent Suite™ Software with the **Import Samples from File** feature, using a CSV template file that is available in Torrent Suite™ Software to simplify the process. 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**. For more information, see the *Torrent Suite™ Software Help*.

3
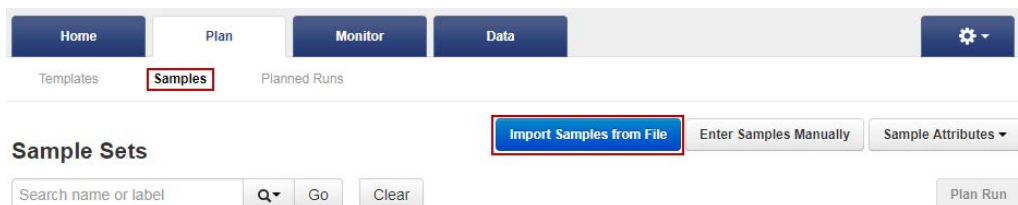
2. In section 1 of the **Import Samples** page, click **Sample File Format** to download a sample CSV template.

**Import Samples**

①: **Sample File :**

[                    ] [Select file]

Select a Sample File (csv) containing the samples to import.

[Sample File Format]

Click Sample File Format button to download the latest Sample File format based upon your latest configuration.

The sample file format CSV contains the version of the CSV file in the top row, and sample attributes in separate columns.

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CSV Version (required) | 2 | | | | | | | | | | | | | | | | | | | | | |
| 2 | Sample Name (required) | Sample ID | Control Type | PCR Plate Position | Barcodekit | Barcode | Gender | Type | Group | Description | Sample Collection Date | Sample Receipt Date | Nucleotide Type | Sample Source | Panel Pool Type | Cancer Type | Cellularity % | Biopsy Days | Cell Number | Couple ID | Embryo ID | Population | Mouse Strains |
| 3 | Sample-1 | | | A | IonCode | IonCode_0101 | Female | Tumor | | | | | DNA | FFPE | Dual Pool | | 50 | | | | | | |
| 4 | Sample-2 | | | B | IonCode | IonCode_0102 | Male | Tumor | | | | | RNA | FFPE | Dual Pool | | 50 | | | | | | |
| 5 | NTC-1 | | No Template Control | C | IonCode | IonCode_0103 | | | | | | | | | | | | | | | | | |

3. Fill out the template CSV file as completely as possible, then save it to the location of your choice.

**Note:**
- Required columns include: Sample name, PCR Plate Position (A–H), Barcode Kit (IonCode Barcodes 1–32), and Barcode.
- Recommended columns include: Sample ID, Gender, Type (sample type, such as self), Group (number that indicates the sample is a single sample, pair or trio), DNA/RNA, Cancer Type, and Cellularity %.

4. When the CSV file is filled out and saved, click **Select File**, navigate to the completed CSV file, then click **Open**.

5. In section 2 of the **Import Samples** page, click **Add Sample Set**, then enter or select the required information in each field.

   a. Enter a Sample Set Name.

   b. Select the Group Type.

   c. Select the Library Prep Type—**AmpliSeq on Chef**.

   d. Select the Library Prep Kit—**Ion AmpliSeq Kit for Chef DL8**.

e. Select the Library Prep Protocol—**2 Library Pools - OCA Plus**.

> **Note:** Completed libraries will be delivered to uncapped library Recovery Tubes in Position C (samples A–D) and Position D (samples E–H) in the Ion AmpliSeq™ Chef Reagents DL8 cartridge. Save the caps. To run 8 RNA samples simultaneously on the same chip do not select a Library Prep Protocol. All 8 sample libraries are combined in a single Recovery Tube v2 in Position D of the Ion AmpliSeq™ Chef Reagents DL8 cartridge.

6. Click **Save & Finish**.

The software automatically imports the samples into the **Sample Sets** table.
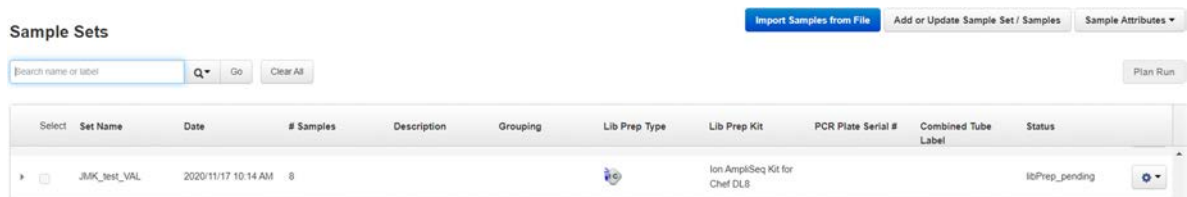


Saved sample sets that enable 2 library pools for OCA Plus can then be selected on the Ion Chef™ Instrument user interface when setting up Ion AmpliSeq™ Kit for Chef DL8 library preparation runs.

# Automated RNA library preparation

## Reverse transcribe RNA for Chef Ready library preparation

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

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). Prepare a master mix without sample RNA for multiple reactions.

| Component | Volume |
|---|---|
| Ion Torrent™ NGS 5X Reaction Buffer | 2 µL |
| Ion Torrent™ NGS 10X RT Enzyme Mix | 1 µL |
| Total RNA (20 ng)[1] | ≤7 µL |
| Nuclease-free Water | to 10 µL |
| **Total volume per well** | **10 µL** |

[1] If preparing an RNA positive control sample along with high quality RNA samples, use 20 ng positive control sample input. If preparing an RNA positive control sample along with FFPE RNA samples, reduce the positive control sample input to 2 ng. Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).
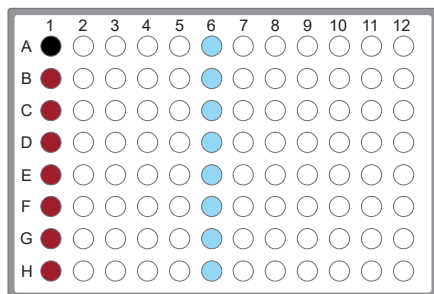
● Column 1 wells contains a 10 µL reverse transcription reaction, or control reaction.

● *(Optional)* Positive control or Non template control (NTC)

○ Each column 6 well contains a dried-down IonCode™ Barcode Adapter. 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 an RNA sample and balance the number of positive samples between rows A–D and E–H.
- We recommend processing at least 6 samples per run. 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.
- If processing RNA samples that are to be combined with a paired DNA library ensure the samples are processed in the correct rows A–D or E–H.

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.

   **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.

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 20 times before sealing the plate.

   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.

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™ Comprehensive Assay Plus libraries on the Ion Chef™ System.

For information on how to set up the Ion Chef™ Instrument, see "Ion Chef™ Instrument setup information for automated RNA library preparation" on page 23.

## Ion Chef™ Instrument setup information for automated RNA library preparation

See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for detailed information on preparing Oncomine™ Comprehensive Assay Plus 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 **2 Library Pools - OCA Plus** is displayed in order to properly prepare Oncomine™ Comprehensive Assay Plus–automated libraries. If 2 Library Pools - OCA Plus is not displayed the default library preparation script is run which results in all 8 libraries combined into a single pool. The 2 Library Pools - OCA Plus library preparation option is only available in Planned Runs created with Torrent Suite™ Software 5.16 or later.



**Figure 1** Example of a correct Oncomine™ Comprehensive Assay Plus setup

Ensure that *2 Library Pools - OCA Plus* appears below the Sample set dropdown list.

During Ion Chef™ Instrument setup, enter the following parameters when prompted.

| Stating material | # of primer pools | Target amplification cycles | Anneal & extension time |
|---|---|---|---|
| High quality RNA[1] | 2 | 23 | 4 minutes |
| FFPE RNA[1] | 2 | 29 | 4 minutes |

[1] Due to the disparity in the required number of target amplification cycles for high quality and FFPE RNA we do NOT recommend running both high quality and FFPE samples on the same plate using the same input amount. If preparing a positive control (high quality) along with FFPE RNA samples, reduce the positive control sample input to 2 ng and use the FFPE cycling parameters.

# Automated DNA library preparation

## RMC in DNA target amplification reactions

Oncomine™ Comprehensive Assay Plus has been developed to support a wide range of biomarkers, including assessment of microsatellite instability (MSI). MSI arises from defects in the mismatch repair (MMR) system and is associated with hypermutability of short DNA sequence repeats (microsatellite locations) throughout the genome.

RMC is composed of in-sample standards that function as internal references in the analysis pipeline to ensure the robustness of MSI assessment in case of variations in sample preparation or run conditions. RMC is added to the DNA target amplification reaction.

## Remove deaminated bases from FFPE DNA

Sample age, storage conditions, and FFPE preservation methods can lead to significant cytosine deamination of the isolated DNA. This deamination can result in an artificially high deamination score when determining the tumor mutational burden result. We have demonstrated that deaminated cytosine (uracil) bases can be enzymatically removed by treatment with Uracil DNA Glycosylase (UDG).

**Note:** We recommend treating all samples including FFPE and high quality (for example, commercial controls or DNA isiolated from cell lines) DNA with UDG to remove deaminated bases before target amplification.

1. Remove and discard the plate seal from an IonCode™ Barcode Adapters 96-well PCR plate.

2. For each FFPE DNA sample, add the following components to a single well in column 1 of the IonCode™ Barcode Adapters 96-well PCR plate.

| Component | Volume |
|---|---|
| 20 ng FFPE DNA[1] | ≤9 µL |
| Uracil-DNA Glycosylase, heat-labile | 1 µL |
| Low TE | to 10 µL |

[1] Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).
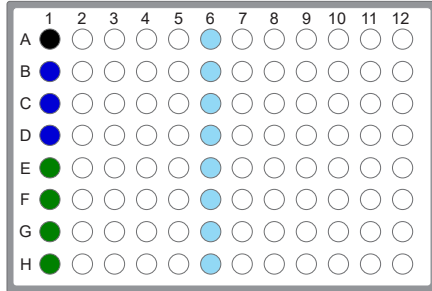
- **●** (blue) Column 1 wells contains 20 ng of FFPE DNA sample in 10 µL, or 10 µL Nuclease-free Water as non-template control. Samples A–D delivered to uncapped library Recovery Tube in Position C in the Ion AmpliSeq™ Chef Reagents DL8 cartridge.

- **●** (green) Column 1 wells contains 20 ng of FFPE DNA sample in 10 µL, or 10 µL Nuclease-free Water as non-template control. Samples E–H delivered to uncapped library Recovery Tube in Position D in the Ion AmpliSeq™ Chef Reagents DL8 cartridge.

- **●** (black) *(Optional)* Non template control (NTC)

- **○** (light blue) Each column 6 well contains a dried-down IonCode™ Barcode Adapter. 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 10 µL of Nuclease-free Water as non-template control into column 1 wells that do not contain a DNA sample and balance the number of positive samples between rows A–D and E–H.
- We recommend processing at least 6 samples per run. We do not recommend processing 1, 2 or 5 samples per run. If you do process 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.
- If processing only 3 or 4 samples, group them together either in rows A–D or E–H.

3. Mix the reaction by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Alternatively, seal the plate, vortex for 5 seconds to mix the reactions, then centrifuge briefly to collect the contents.

   **IMPORTANT!** To prevent evaporation during UDG treatment, use an applicator tool to press the film securely around each reaction well and around the perimeter of the plate. Offset the film to the left so that the adhesive does not cover the barcode label.

4. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

| Temperature | Time |
|---|---|
| 37°C | 2 minutes |
| 50°C | 10 minutes |
| 4°C | Hold (≤1 hour) |

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

STOPPING POINT Reactions can be stored at −20°C long term.

6. Carefully remove the plate seal, then add the following components to each well.

**Note:** If processing multiple samples, prepare a reaction master mix (+ 5–10% overage), then add 5 µL to each well.

| Component | Volume |
|---|---|
| RMC | 1.5 µL |
| Nuclease-free water | 3.5 µL |
| **Total volume per well** (including sample) | **15 µL** |

7. Mix the reaction by pipetting at least half the total volume up and down at least 5 times, then carefully inspect each well for air bubbles. Remove any air bubbles by gentle pipetting. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film, vortex for 5 seconds to mix the reactions, then centrifuge briefly to collect the contents.

**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™ Comprehensive Assay Plus libraries on the Ion Chef™ System.

For information on how to set up the Ion Chef™ Instrument, see "Ion Chef™ Instrument setup information for automated DNA library preparation" .

# Ion Chef™ Instrument setup information for automated DNA library preparation

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

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**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 **2 Library Pools - OCA Plus** is displayed in order to properly prepare Oncomine™ Comprehensive Assay Plus–Chef Ready libraries. If 2 Library Pools - OCA Plus is not displayed the default library preparation script is run which results in all 8 libraries combined into a single pool. This exceeds the capacity of the Ion 550™ Chip. The 2 Library Pools - OCA Plus library preparation option is only available in Planned Runs created with Torrent Suite™ Software 5.16 or later.



**Figure 2** Example of a correct Oncomine™ Comprehensive Assay Plus setup

Ensure that *2 Library Pools - OCA Plus* appears below the Sample set dropdown list.

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During Ion Chef™ Instrument setup, enter the following parameters when prompted.

| Stating material | # of primer pools | Target amplification cycles | Anneal & extension time |
|---|---|---|---|
| High quality DNA[1] | 2 | 13 | 16 minutes |
| FFPE DNA[1] | 2 | 16 | 16 minutes |

[1] Due to the disparity in the required number of target amplification cycles for high quality and FFPE DNA we do NOT recommend running both high quality and FFPE samples on the same plate using the same input amount.

# Manual library preparation

This chapter describes library preparation using the following kits:

• Oncomine™ Comprehensive Assay Plus, DNA, Manual library preparation (Part No. A45615)

• Oncomine™ Comprehensive Assay Plus, RNA, Manual library preparation (Part No. A45616)

• Ion AmpliSeq™ Library Kit Plus (Cat No. 4488990)

• Ion Torrent™ NGS Reverse Transcription Kit (Cat No. A45003)

• Uracil-DNA Glycosylase, heat-labile (Cat No. 78310100UN)

# RNA preparation and cDNA amplification

Use the components of the Oncomine™ Comprehensive Assay Plus, RNA (Cat. No. A45616) for the follow procedures.

## Reverse transcribe RNA for manual library preparation

1. If the RNA was prepared from FFPE tissue and not previously heat-treated, heat at 80°C for 10 minutes, then cool to room temperature.

2. For each sample, add the following components into a single well of a 96-well PCR plate on ice or in a pre-chilled 4°C cold block. Prepare a master mix without sample RNA for multiple reactions.

| Component | Volume |
|---|---|
| Ion Torrent™ NGS 5X Reaction Buffer | 2 μL |
| Ion Torrent™ NGS 10X RT Enzyme Mix | 1 μL |
| Total RNA (20 ng)[1] | ≤7 μL |
| Nuclease-free Water | to 10 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).
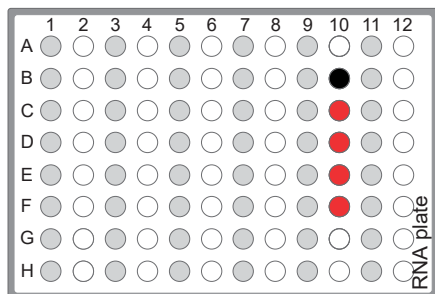
- ● *(Optional)* Non template control (NTC)
- ● RNA sample

3. Seal the plate with MicroAmp™ Clear 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™ Optical Film 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, then proceed to the next step.
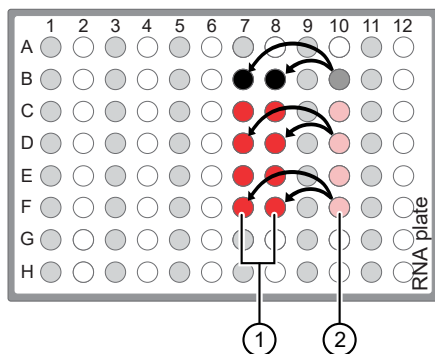
## Prepare cDNA target amplification reactions

**IMPORTANT!** The cDNA synthesis reaction, primer pools, and 5X Ion AmpliSeq™ 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 briefly centrifuge to collect.

3. To each cDNA synthesis reaction add:

| Component | Volume |
|---|---|
| cDNA synthesis reaction | 10 µL |
| 5X Ion AmpliSeq™ HiFi Mix (red cap) | 4.5 µL |
| Nuclease-free Water | 3.5 µL |
| **Final volume** | 18 µL |

4. Mix by pipetting at least half the total volume up and down at least 5 times, then transfer 8 µL to each of two adjacent wells (~2 µL overage remainder).



● cDNA sample                               ● Non template control (NTC)

① 8 µL transferred cDNA target amplification reaction.     ② ~2 µL cDNA target amplification reaction remaining.

5. Add 2 µL of 5X Oncomine™ Comprehensive Assay Plus, RNA primer pool-1 into the first well, then add 2 µL of primer pool-2 into the second well for a total of 10 µL in each well.

6. Seal the plate with a new MicroAmp™ Clear 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, ensure 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™ Optical Film 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 min |
| Cycle; set number according to Table 1 | Denature | 98°C | 15 sec |
| | Anneal and extend | 60°C | 4 min (RNA Panel) |
| Hold | — | 10°C | Hold |

Table 1   Recommended cycle number

| Input nucleic acid[1] | Recommended number of cycles[2] | Cycle number adjustment[3] | |
|---|---|---|---|
| | 10 ng RNA input | 1 ng RNA input | 100 ng RNA input |
| High quality RNA | 22 | +5 | –3 |
| FFPE RNA | 28 | +3 | –3 |

[1] Due to the disparity in the required number of target amplification cycles for high quality and FFPE RNA we do NOT recommend running both high quality and FFPE samples on the same plate using the same input amount. If preparing a positive control (high quality) along with FFPE RNA samples, reduce the positive control sample input to 2 ng and use the FFPE cycling parameters.

[2] Number of cycles can be increased when input material quality or quantity is questionable.

[3] The recommended number of cycles is based on 10 ng RNA input per primer pool. Adjust the cycle number for lower or higher 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.

# Combine cDNA target amplification reactions

**Note:** If preparing both RNA and DNA sample libraries use the same FuPa digestion conditions, samples may be combined onto a single plate for simultaneous processing.

1. Tap the plate gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells.

2. Carefully remove the plate seal.

3. For each sample, combine the 10-µL target amplification reactions. The total volume for each sample should be 20 µL.
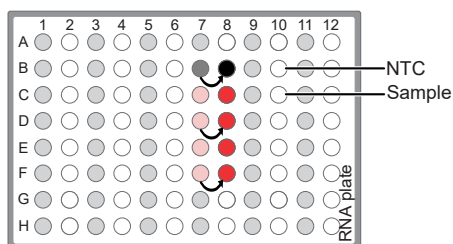
# DNA preparation and amplification

Use the components of the Oncomine™ Comprehensive Assay Plus, DNA, Manual Library Preparation (Cat. No. A47620) for the following procedures.

## RMC in DNA target amplification reactions

Oncomine™ Comprehensive Assay Plus has been developed to support a wide range of biomarkers, including assessment of microsatellite instability (MSI). MSI arises from defects in the mismatch repair (MMR) system and is associated with hypermutability of short DNA sequence repeats (microsatellite locations) throughout the genome.

RMC is composed of in-sample standards that function as internal references in the analysis pipeline to ensure the robustness of MSI assessment in case of variations in sample preparation or run conditions. RMC is added to the DNA target amplification reaction.

## Remove deaminated bases from FFPE DNA

Sample age, storage conditions, and FFPE preservation methods can lead to significant cytosine deamination of the isolated DNA. This deamination can result in an artificially high deamination score when determining the tumor mutational burden result. We have demonstrated that deaminated cytosine (uracil) bases can be enzymatically removed by treatment with Uracil DNA Glycosylase (UDG). For this reason, we recommend treating DNA isolated from FFPE samples with UDG to remove deaminated bases before target amplification.

Note:  We recommend treating all samples including FFPE and high quality (for example, commercial controls or DNA isiolated from cell lines) DNA with UDG to remove deaminated bases before target amplification.

1. For each FFPE DNA sample, add the following components to a single well of a 96-well PCR plate.

| Component | Volume |
|---|---|
| 20 ng FFPE DNA[1] | ≤5.5 µL |
| Uracil-DNA Glycosylase, heat-labile | 1 µL |
| Low TE | to 6.5 µL |

[1]  Do not exceed 40 ng maximum FFPE DNA sample as input. Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).

2. Mix the reaction by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Alternatively, seal the plate, vortex for 5 seconds to mix the reactions, then centrifuge briefly to collect the contents.

Note:  To prevent evaporation during UDG treatment, use an applicator tool to press the film securely around each reaction well and around the perimeter of the plate.

3. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

| Temperature | Time |
|---|---|
| 37°C | 2 minutes |
| 50°C | 10 minutes |
| 4°C | Hold (≤1 hour) |

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

**STOPPING POINT**  Reactions can be stored at −20°C long term.

5. Carefully remove the plate seal, then proceed immediately to "Prepare DNA target amplification reactions" on page 34, adding the target amplification reaction components to the well containing 6.5 µL of UDG treated FFPE DNA.

## 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 96-well plate on ice or in a pre-chilled 4°C cold block.

2. For each sample, prepare a target amplification master mix without primers in a 1.5-mL tube on ice.

| Component | Volume |
|---|---|
| 5X Ion AmpliSeq™ HiFi Mix (red cap) | 5 µL |
| DNA (20 ng) (treated with UDG) | 6.5 µL |
| RMC | 1 µL |
| Nuclease-free Water | to 12.5 µL |

3. Mix thoroughly by pipetting up and down 5 times, then transfer 5 µL of each sample-specific master mix to 2 wells of a 96-well PCR plate on ice or in a pre-chilled 4°C cold block.

   **Note:**  When using multi block thermal cyclers ensure each pair of samples is in the same temperature zone for amplification. For example, use the same VeriFlex block on a Veriti™ Thermal Cycler.

4. Add 5 µL of 2X Oncomine™ Comprehensive Assay Plus, DNA primer pool 1 to the first well, and 5 µL of primer pool 2 to the second well.
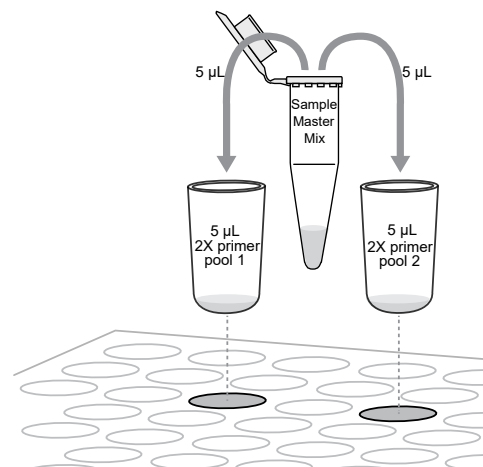
**5.** Seal the plate with MicroAmp™ Clear Adhesive Film.

**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.

Proceed to "Amplify the DNA targets" .

## Amplify the DNA 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™ Optical Film 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 min |
| Cycle; set number according to Table 2 | Denature | 99°C | 15 sec |
| | Anneal and extend | 60°C | 16 min (DNA Panel) |
| Hold | — | 10°C | Hold |

Table 2   Recommended cycle number

| Input nucleic acid[1] | Recommended number of cycles[2] | Cycle number adjustment[3] | |
|---|---|---|---|
| | 10 ng DNA input | 1 ng DNA input | 20 ng DNA input |
| High quality DNA | 12 | +3 | −1 |
| FFPE DNA | 15 | +3 | −1 |

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

[2]   Number of cycles can be increased when input material quality or quantity is questionable.

[3]   The recommended number of cycles is based on 10 ng DNA input per primer pool. Adjust the cycle number for lower or higher DNA input.

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

## Combine the DNA target amplification reactions

**Note:** Perform the following steps on ice or in a pre-chilled 4°C cold block.

1. Remove the DNA 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 DNA target amplification reactions into a single well.

   **IMPORTANT!** Accurate volume transfer in this step is critical. We recommend using a single-channel pipettor. If you are using a multi-channel pipettor, visually check pipette tips to ensure that volumes are equivalent.
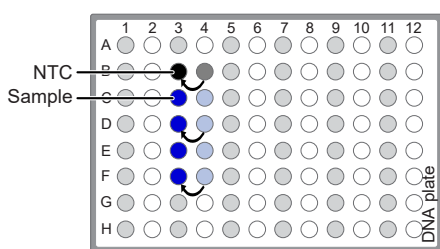


   **Note:** In multi-zone thermal cyclers ensure samples are in the same zone for amplification.

   The total volume for each sample should be ~20 µL.

# Library preparation

## Transfer the DNA amplicons

If preparing both DNA and cDNA libraries with the same FuPa digestion conditions, library preparations can be transferred to a single plate for simultaneous processing. For more information, see "Partially digest the DNA and cDNA amplicons" on page 37.

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

2. Carefully remove the adhesive film from the plate.

   **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.
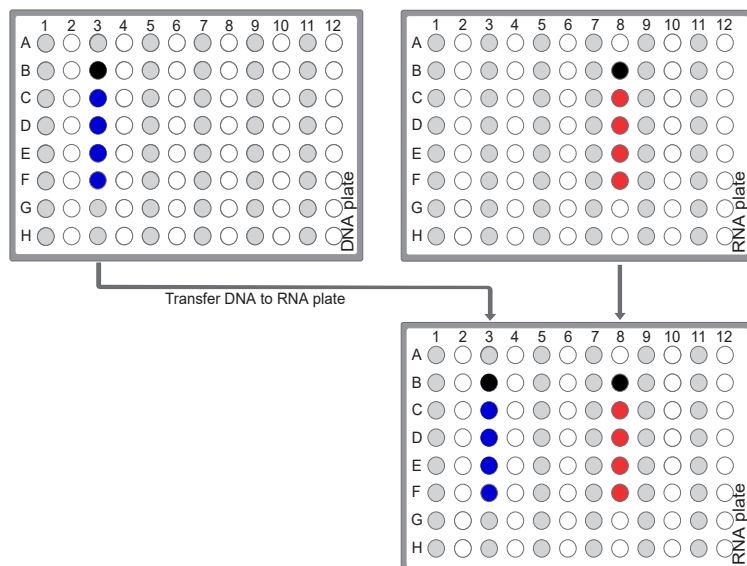


- 🔵 Sample DNA target amplification reactions
- 🔴 Sample cDNA(RNA) target amplification reactions
- ⚫ No template control (NTC) target amplification reaction

## Partially digest the DNA and cDNA amplicons

**IMPORTANT!** Keep each 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 DNA or cDNA sample. The total volume per well is ~22 μL.

3. Seal each DNA or cDNA 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 | 20 min (cDNA/DNA) |
| 55°C | 20 min (cDNA/DNA) |
| 60°C | 20 min (cDNA/DNA) |
| 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.

IonCode™ Barcode Adapters are provided at the appropriate concentration and include forward and reverse adapters in a single well. No further handling is necessary.

Ion Xpress™ Barcode Adapters require handling and dilution as described in "Ion Xpress™ Barcode Adapters only: Combine and dilute adapters".

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

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## Ion Xpress™ Barcode Adapters only: Combine and dilute 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. Scale volumes as necessary. Use 2 µL of this barcode adapter mix in step 3 in "Perform the ligation reaction".

For example, combine the volumes indicated in the following table.

| 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

---

**Note:** Store diluted adapters at –20°C.

---

## 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 pipetting.

2. If you have not already done so, briefly centrifuge the plate to collect the contents, then carefully remove the plate seal.

3. 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 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 | Adapters (IonCode™ Barcode Adapters or diluted Ion Xpress™ barcode adapter mix (for barcoded libraries)) | 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™ Clear 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 for up to 24 hours 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.

## Elute and dilute the library

1. Remove the plate with purified libraries from the plate magnet, then add 50 µL of Low TE to the pellet to disperse the beads.

2. Seal the plate with MicroAmp™ Clear 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. Incubate at room temperature for at least 2 minutes.

4. Place the plate on the magnet for at least 2 minutes.

   STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C. We recommend transferring the supernatant to a 1.5-mL Eppendorf LoBind™ tube for long-term storage.

5. Prepare a 100-fold dilution for quantification. Remove 2 µL of supernatant, containing the library, then combine with 198 µL of Nuclease-free Water.

Proceed immediately to "Quantify the library by qPCR and calculate the dilution factor" .

# Quantify the library by qPCR and calculate the dilution factor

Determine the concentration of each library by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802) using the following steps. Analyze each sample, standard, and negative control in duplicate 20-µL reactions.

---

**IMPORTANT!** The following steps differ from those in the *Ion AmpliSeq™ Library Kit Plus User Guide*. Follow these steps for quantifying your Oncomine™ Comprehensive Assay Plus libraries.

---

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Ion Control Library (~68 pM, from the 2) at 6.8 pM, 0.68 pM, and 0.068 pM. Mark these tubes as standards, then use these concentrations in the qPCR instrument software.

2. Use the volumes per reaction in the following table to prepare a PCR Reaction Mix for all reactions. We recommend duplicate reactions for each sample library, standard, and NTC. Include a 5–10% overage to accommodate pipetting errors.

---

**Note:** Volumes are provided per reaction. Double the volume for duplicate reactions.

---

| Component | Volume per reaction | |
|---|---|---|
| | 96-well plate | 384-well plate |
| 2X Ion Library qPCR Master Mix | 10 µL | 5 µL |
| Ion Library TaqMan™ Quantitation Assay, 20X | 1 µL | 0.5 µL |
| **Total** | **11 µL** | **5.5 µL** |

3. In a MicroAmp™ Optical Reaction Plate , set up duplicate PCR reactions for each sample, standard, and NTC. To each well, add the following components:

| Component | Volume per reaction | |
|---|---|---|
| | 96-well plate | 384-well plate |
| PCR Reaction Mix (from step 2) | 11 µL | 5.5 µL |
| 1:100 dilution of the sample[1,2] | 9 µL | 4.5 µL |

[1] Substitute E. coli DH10B standards prepared in step 1 for standards. Substitute nuclease-free water for NTC.

[2] High quality samples may require further dilution (5–10 fold) to give results within the range of the prepared standards. We recommend starting with 1000X dilution for RNA library quantification.

4. Seal the plate with a MicroAmp™ Optical Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets.

5. Program your real-time instrument as follows:

---

**Note:** The fast cycling program was developed using the StepOnePlus™ Real-Time PCR System in Fast mode.

---

   a. Enter the concentrations of the control library standards.

   **b.** Select ROX™ Reference Dye as the passive reference dye.

   **c.** Select a reaction volume of 20 µL (96-well plate) or 10 µL (384-well plate).

   **d.** Select FAM™ dye/MGB as the TaqMan™ probe reporter/quencher.

| Reaction plate format | Run mode | Stage | Temperature | Time |
|---|---|---|---|---|
| 96-well Standard OR 384-well Standard | Standard | Hold (UDG incubation) | 50°C | 2 min |
| | | Hold (polymerase activation) | 95°C | 2 min |
| | | Cycle (40 cycles) | 95°C | 15 sec |
| | | | 60°C | 1 min |
| 48- / 96-well Fast OR 384-well Standard | Fast | Hold (UDG incubation) | 50°C | 2 min |
| | | Hold (polymerase activation) | 95°C | 20 sec |
| | | Cycle (40 cycles) | 95°C | 1 sec |
| | | | 60°C | 20 sec |

**6.** Following qPCR, calculate the average concentration of the undiluted library by multiplying the determined concentration × 100.

**7.** Based on the calculated library concentration, determine the dilution that results in a concentration of ~50 pM for template preparation on the Ion Chef™ System.
For example:

- The undiluted library concentration is 300 pM.
- The dilution factor is 300 pM/50 pM = 6.
- Therefore, 10 µL of library that is mixed with 50 µL of Low TE (1:6 dilution) yields approximately 50 pM.

---

**Note:**

- Good results have been observed with libraries ≤50 pM. Proceed to the next step without further dilution.
- Libraries that yield significantly less than 50 pM can be rescued with library amplification. See "Tips" .

---

**8.** Proceed to "Combine libraries" then template preparation, or store libraries as described below.

---

STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C. We recommend transferring the supernatant to an RNase-free microcentrifuge tube for long-term storage. Alternatively, seal the plate with a new MicroAmp™ Clear Adhesive Film for long-term storage.

---

# 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.

- Combine DNA and RNA library pools from the same samples prepared on an Ion Chef™ Instrument.

  **Note:** Upon completion of the Ion Chef™ Instrument run libraries have already been adjusted to a final concentration of ~100 pM each and pooled on the Ion Chef™ Instrument. Libraries from samples A–D are in the Recovery Tube in Position C and samples E–H in Position D of the Ion AmpliSeq™ Chef Reagents DL8 cartridge.

  a. Combine the DNA library pool with the RNA library pool for a given set of 4 samples at a 90:10 ratio (DNA:RNA—18 µL of DNA library pool + 2 µL of RNA library pool).

  **Note:** This ratio differs from previous Oncomine™ Comprehensive Assay protocols.

  **IMPORTANT!**
  - The combined DNA and RNA library pools must be from matched Recovery Tubes. For example, combine Position C libraries, do NOT combine sample DNA libraries from Position C (samples A–D) with sample RNA libraries from Position D (samples E–F) or vice versa.
  - Each combined library must have a unique barcode.

  b. Dilute the combined (90:10 DNA:RNA) pooled libraries 2-fold (~50 pM final concentration).

  **Note:** We recommend sequencing up to 4 research samples (one library pool, either samples A–D or E–H) on a single Ion 550™ Chip.
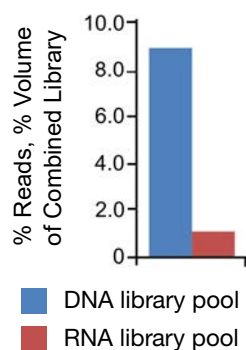


| Library pool | Sample | Barcode | Fractional volume (90:10 DNA:RNA) | |
|---|---|---|---|---|
| DNA–C | DNA-1 | BC_0101 | 0.225 | 0.9 |
| | DNA-2 | BC_0102 | 0.225 | |
| | DNA-3 | BC_0103 | 0.225 | |
| | DNA-4 | BC_0104 | 0.225 | |
| RNA–C | RNA-1 | BC_0109 | 0.025 | 0.1 |
| | RNA-2 | BC_0110 | 0.025 | |

*(continued)*

| Library pool | Sample | Barcode | Fractional volume (90:10 DNA:RNA) | |
|---|---|---|---|---|
| RNA–C | RNA-3 | BC_0111 | 0.025 | 0.1 |
| | RNA-4 | BC_0112 | 0.025 | |
| **Sum** | — | — | **1.0** | **1.0** |

- Combine manually prepared DNA and RNA libraries.

  a. Combine each uniquely barcoded DNA and RNA library (~50 pM each) from the same sample at an 90:10 ratio (DNA:RNA—18 µL of DNA library + 2 µL of RNA library).

  **Note:** This ratio differs from previous Oncomine™ Comprehensive Assay protocols.

  b. Combine equal volumes of the paired libraries (90:10 DNA:RNA) to be sequenced on the same chip.

  **Note:**
  - We recommend sequencing up to 5 samples (4 research samples plus 1 no-template control) on a single Ion 550™ Chip.
  - For runs that include a no-template control (NTC), add in the same fractional volumes of undiluted DNA and RNA NTC libraries as is added for equivalent sample libraries.
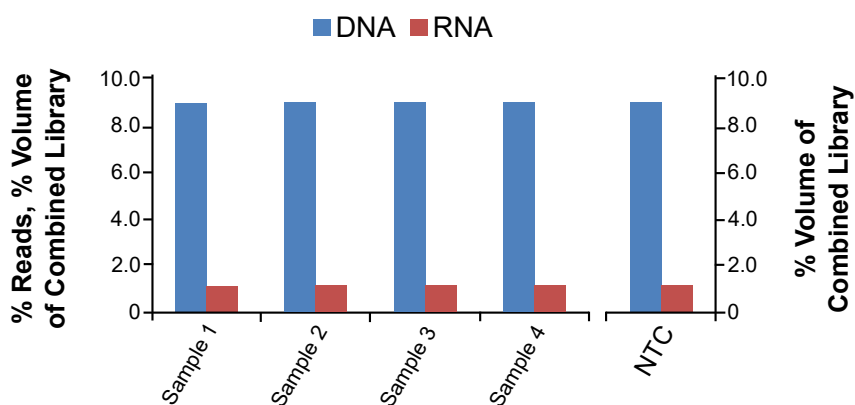


| Sample | Barcode | Fractional volume (90:10 DNA:RNA) |
|---|---|---|
| DNA-1 | BC_0101 | 0.18 |
| RNA-1 | BC_0102 | 0.02 |
| DNA-2 | BC_0103 | 0.18 |
| RNA-2 | BC_0104 | 0.02 |
| DNA-3 | BC_0105 | 0.18 |
| RNA-3 | BC_0106 | 0.02 |
| DNA-4 | BC_0107 | 0.18 |
| RNA-4 | BC_0108 | 0.02 |

*(continued)*

| Sample | Barcode | Fractional volume (90:10 DNA:RNA) |
|---|---|---|
| DNA-5 (NTC) | BC_0109 | 0.18 |
| RNA-5 (NTC) | BC_0110 | 0.02 |
| **Sum** | — | **1.0** |

- Combine only manually prepared RNA libraries to be sequenced on the same chip.

  **Note:** We recommend sequencing up to 32 sample RNA libraries on a single Ion 550™ Chip.

  a. Combine 2 µL of each uniquely barcoded RNA library (~50 pM each), then mix thoroughly.

- Combine only RNA library pools prepared on an Ion Chef™ Instrument to be sequenced on the same chip.

  **Note:**
  - When combining only RNA libraries, library pools in the Recovery Tube in Position C (samples A–D) can be combined with library pools in the Recovery Tube in Position D (samples E–F).
  - We recommend sequencing up to 32 sample RNA libraries on a single Ion 550™ Chip or Ion 540™ Chip.
  - We recommend sequencing up to 8 sample RNA libraries on a single Ion 530™ Chip.

  a. Combine 5 µL from each of 4 pooled RNA libraries (~100 pM each).

  b. Mix thoroughly, then dilute the combined library pools 2-fold (~50 pM final concentration).

- Combine only manually prepared DNA libraries to be sequenced on the same chip.

  **Note:** We recommend sequencing up to 4 sample DNA libraries on a single Ion 550™ Chip.

  a. Combine 10 µL of each uniquely barcoded DNA library (~50 pM each).

- Combine only DNA library pools prepared on an Ion Chef™ Instrument to be sequenced on the same chip.

  **Note:** We recommend sequencing up to 4 sample DNA libraries on a single Ion 550™ Chip. Upon completion of the Ion Chef™ Instrument run each Recovery Tube contains 4 pooled libraries adjusted to a final concentration of ~100 pM.

  a. Dilute 15 µL of the pooled DNA libraries 2-fold (~50 pM final concentration), then mix thoroughly.

# Guidelines for templating and sequencing

Proceed to template preparation and sequencing using the following kits.

| Chip | Template System | Sequencer | Kit | User Guide |
|------|-----------------|-----------|-----|------------|
| Ion 550™ Chip | Ion Chef™ System | Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer | Ion 550™ Kit – Chef (Cat. No. A34541) | Ion 550™ Kit – Chef User Guide (Pub. No. MAN0017275) |
| | | | Ion 550™ Single Chip Supplemental Kit (Cat. No. A36953) | |

To create a specific Run Plan for use in templating and sequencing, see Chapter 5, "Create a Planned Run". Refer to the appropriate user guide listed in the table for more information.

# Create a Planned Run

---

**IMPORTANT!**  This kit is compatible with Torrent Suite™ Software 5.16 or later and Ion Reporter™ Software 5.18 or later. Before proceeding, we recommend that you update to the latest available versions of Torrent Suite™ Software, Ion Reporter™ Software, and Ion Chef™ System software. Contact your service representative for help with upgrading the software.

---

**Note:**  The IonReporterUploader 5.18 plugin must be installed in Torrent Suite™ Software. If your Ion Reporter™ account is not configured, configure it through the software settings (⚙ ▾) see "Configure the IonReporterUploader plugin in Torrent Suite™ Software" on page 101).

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## About Planned Runs

Planned Runs are digital instructions that are created in Torrent Suite™ Software for controlling the template preparation and sequencing instruments. Planned Runs contain settings such as number of flows, kit types, barcodes, sample information, and reference files (if any). Planned Runs are also used to track samples, chips, and reagents throughout the workflow, from template preparation on the Ion Chef™ Instrument through sequencing on an Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer and subsequent data analysis. Each chip that is prepared in an Ion Chef™ run requires its own Planned Run.

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**IMPORTANT!**  For more information on creating a Planned Run in Torrent Suite™ Software, including a complete description of each field in the **Create Plan** workflow bar, see the *Torrent Suite™ Software Help*, available by clicking the **Help** button in the software.

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In Torrent Suite™ Software 5.16 or later, use the **Oncomine™ Comprehensive Plus – DNA and Fusions** template as the primary Planned Run template for the Oncomine™ Comprehensive Assay Plus.

| Application category in Torrent Suite™ Software | Template name | Description |
|---|---|---|
| Oncology – Solid Tumor | Oncomine™ Comprehensive Plus – DNA and Fusions | DNA and RNA Planned Run template |
| | Oncomine™ Comprehensive Plus – DNA | DNA-only Planned Run template |
| | Oncomine™ Comprehensive Plus – Fusions[1] | RNA-only Planned Run template |

[1] Available in Torrent Suite™ Software 5.16 or later.

# Update Oncomine™ Comprehensive Assay Plus templates in Torrent Suite™ Software

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™ Comprehensive Assay Plus, then in that row click **Update**.

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

# Create a custom Planned Run template

**IMPORTANT!** Before creating a Planned Run, you may need to enable the Oncomine™ Comprehensive Plus templates and upload the **Reference Library**, **Target Regions**, and **Hotspots** BED files on the Ion Torrent™ Server. For more information, see Appendix B, "Supplemental information". Contact your local service representative to obtain the most current BED files.

We recommend setting up a customized Planned Run template for reuse when the same conditions will be used for multiple runs. For more information on how to create a new Planned Run, see the *Torrent Suite™ Software Help*.

**Note:** Copy number variation (CNV), microsatelite instability (MSI), and tumor mutational burden (TMB) functionalities may require copy/editing of the Ion Reporter™ Software analysis workflow with user defined optimizations before creating a Planned Run. For more information on creating a CNV baseline (see Appendix C, "CNV baseline creation"), setting MSI thresholds (see "MSI parameters" on page 89), and setting the TMB classification thresholds (see "Set TMB Classification parameters" on page 102) or refer to the Ion Reporter™ Software Help.

1. Sign in to the Torrent Suite™ Software.

2. In the **Plan** tab, in the **Templates** screen, click **Oncology – Solid Tumor** in the left navigation menu.

3. In the list of templates, find **Oncomine™ Comprehensive Plus – DNA and Fusions**, then click ⚙ **(Actions) ▸ Copy**.
   The **Copy Template** workflow opens to the **Save** step.

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

| Entry or selection [1] | Action |
|---|---|
| Template Name | Enter a name for the Planned Run template. |
| DNA Reference Library | Select **hg19 (Human (hg19))**. |
| DNA Target Regions[2] | Select **OCAPlus.20210430.designed.bed**. |

[1] Fusions Reference Library and Fusions Target Regions are not necessary for analysis in Torrent Suite™ Software.
[2] Check with your service representative for updates to ensure that the most current files are used.

5. Click the **Ion Reporter** step, then select your Ion Reporter™ Software account (version 5.18 or later).

   **Note:** If the account is not configured, configure it through **Ion Reporter Configure** settings (see "Configure the IonReporterUploader plugin in Torrent Suite™ Software" on page 101). For instructions on how to install the IonReporterUploader plugin, see the *Torrent Suite™ Software Help*.

6. In the **Existing Workflow** dropdown list, select the appropriate Ion Reporter™ analysis workflow (page 57) for your Planned Run (for example, **Oncomine™ Comprehensive Plus - w2.2 - DNA and Fusions - Single Sample**), then click **Next**.

7. In the **Research Application** step, verify that the appropriate **Research Application** and **Target Technique** are pre-selected, then click **Next**.

8. In the **Kits** step, verify that **Ion Chef** is selected for the **Template Kit**, then complete the following fields as described:

| Field | Selection | |
|---|---|---|
| | Manual library preparation | Automated library preparation |
| Instrument | Ion GeneStudio™ S5 System | |
| Library Kit Type | Ion AmpliSeq™ Library Kit Plus | Ion AmpliSeq™ Kit for Chef DL8 |
| Template Kit | Ion 550™ Kit – Chef | |
| Sequencing Kit | Ion S5™ Sequencing Kit | |
| Chip Type | Ion 550™ Chip | |
| Barcode Set | IonXpress or IonCode | IonCode |
| Flows | 500 | |

9. Select or edit the optional information fields appropriately for your run, then click **Next**.

10. In the **Plugins** step, ensure that the **sampleID** and **coverageAnalysis** plugins are selected.

11. Configure the **coverageAnalysis** plugin as follows:

    a. Click **configure** next to **coverageAnalysis**.

    b. Ensure that the **Sample Tracking** checkbox is selected, then click **Save Changes**.

    c. Click **Next**.

12. In the **Projects** step, select the project appropriate to your run, then click **Next**.

13. In the **Save** step, click **Copy Template** to save the new Planned Run template.

The customized template is now available in the **Templates** screen, under the **Oncology – Solid Tumor** application.

# Create a Planned Run for manual library preparation

1. Sign in to the Torrent Suite™ Software.

2. In the **Plan** tab, in the **Templates** screen, click **Oncology – Solid Tumor** in the left navigation menu.

3. In the list, click on your customized Planned Run template name, or click ⚙ **(Actions)** ▸ **Plan Run**. The **Create Plan** workflow opens to the **Plan** step.

4. Enter a name for the plan in the **Run Plan Name** field.

5. Make or confirm the following selections:

| Field | Action |
|---|---|
| Analysis Parameters | Ensure that **Default (Recommended)** is selected. |
| Use same reference & BED files for all barcodes | Ensure that this checkbox is selected. |
| Same sample for DNA and Fusions? | Ensure that this checkbox is selected. |
| Number of barcodes | The standard number of barcodes for an Oncomine™ Comprehensive Assay Plus DNA and Fusions run is 8 without no-template controls (NTC) and 10 with NTCs. Enter the number of barcodes, then click ✓ button to the right of this field. |
| Sample Tube Label | Enter or scan the barcode of the Ion Chef™ Library Sample Tube that will be used in the run. |
| Chip Barcode | No entry required. |
| Oncology | Ensure that this is selected. |
| Pre-implantation Genetic Screening | Ensure that this is deselected. |

6. Enter the sample and barcode information:

| Field[1] | Action |
|---|---|
| Barcode | Use the default barcode selections, or select the barcode for each sample from the dropdown list. |
| Sample Name | Each sample must have a unique name. Type in the field to overwrite the default name. We recommend that you use unique names even between runs. |
| Control Type (expanded) | Select **No Template Control** from the dropdown list to designate a sample as a no template control. |
| Sample ID | *(Optional)* Click in the field, then enter a sample ID. |
| Sample Description | *(Optional)* Click in the field, then enter a sample description. |
| Annotations (expanded) | Click to reveal **Cancer Type** and **Cellularity %**. |

*(continued)*

| Field[1] | Action |
|---|---|
| Cancer Type | Select from the dropdown list. Click ⊙ to copy the entry to all the rows. |
| Cellularity % | Each DNA sample *must* have a cellularity percentage. This field is optional for RNA samples. Click ⊙ to copy the same entry to all rows. |
| Ion Reporter Workflow | Ensure the correct workflow is selected.<br><br>**Note:** If the workflows are not displayed in the dropdown list, select the **Show All Workflows** checkbox to view all workflows on the selected Ion Reporter™ Server. |
| Relation | Ensure the correct value is auto-populated. Select from the dropdown list to change. |
| Gender | Select from the dropdown list. Click ⊙ to copy the entry to all the rows.<br><br>**Note:** This is a required field to perform CNV analysis. By default Ion Reporter™ Software treats samples as female. |
| IR Set ID[2] | The **IR Set ID** links individual samples for analysis. Ensure the correct value is autopopulated. Select from the dropdown list to change.<br><br>**IMPORTANT!** Ensure that a unique value is entered for each sample. |

[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 the DNA barcode and Fusions barcode of the same sample. Do not give unrelated samples the same IR Set ID value (even if that value is zero or blank).

7. Click **Plan Run**.

The run is listed in the **Planned Runs** screen 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 for automated library preparation using sample sets

Automated Oncomine™ Comprehensive Assay Plus library preparation on the Ion Chef™ System requires use of sample sets for creating your planned run. For more information on creating a sample set, see "Create a sample set to prepare 2 pools of 4 libraries each" on page 19.

If combining more than one sample set, each sample set must correspond to Oncomine™ Comprehensive Assay Plus automated library preparations and use the same barcode kit to be included in a single Planned Run.

1. In the **Plan** tab, in the **Samples** screen, find the Sample Sets that you want to add to the Planned Run.

2. Select one or more Sample Set (for example a DNA Sample Set and an RNA Sample Set) to add to the Planned Run.

   - To plan a run using one 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 you want to add to the Planned Run, then click **Plan Run**.



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



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

3. Select a Run Template to use for the experiment.
Select the samples to Create Plan Run using: All (A–H: Pool 1, 2), Pool 1 (A–D), or Pool 2 (E–H) then click **Plan Run**.

**Note:** 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 bar opens to the **Barcoding** step with the Sample Sets that you selected:

4. In the Barcoding step in the workflow bar, make or confirm the following selections.

| Field | Action |
|---|---|
| Analysis Parameters | Ensure that **Default (Recommended)** is selected. |
| Use same reference & BED files for all barcodes | Ensure that this checkbox is selected. |
| Same sample for DNA and Fusions? | Ensure that this checkbox is selected. |
| Number of barcodes | The standard number of barcodes for an Oncomine™ Comprehensive Assay Plus DNA and Fusions run is 8 without no-template controls (NTC) and 10 with NTCs. Enter the number of barcodes, then click ✓ button to the right of this field. |
| Sample Tube Label | Enter or scan the barcode of each Ion Chef™ Library Sample Tube that will be used in the run. |
| Chip Barcode | No entry required. |
| Oncology | Ensure that this is selected. |
| Pre-implantation Genetic Screening | Ensure that this is deselected. |

5. Make sure your sample set information has been correctly imported. Edit entries in each field as needed in the **Samples Table**, then click **Next**.

| Field[1] | Description |
|---|---|
| Barcode | Use the imported barcode selections, or select the barcode for each sample from the dropdown list. |
| Sample Name (required) | Each sample must have a unique name. Type in the field to overwrite the default name. We recommend that you use unique names even between runs. |
| Control Type (expanded) | Click on the **Control Type** column header to expand the **Control Type** column. Select **No Template Control** from the dropdown list to designate a sample as a no template control. |
| Sample ID | *(Optional)* Click in the field, then enter a sample ID. |

*(continued)*

| Field[1] | Description |
|---|---|
| Sample Description | *(Optional)* Click in the field, then enter a sample description. |
| DNA/Fusions | For DNA and Fusions application, select **DNA** or **Fusions** from the dropdown menu for each samples. |
| Annotations (expanded) | Click to reveal **Cancer Type** and **Cellularity %**. |
| Cancer Type | Select from the dropdown list. Click ⊙ to copy the entry to all the rows. |
| Cellularity % | Each DNA sample *must* have a cellularity percentage. This field is optional for RNA samples. Click ⊙ to copy the same entry to all rows. |
| Ion Reporter Workflow | Ensure the correct workflow is selected.<br>**Note:** If the workflows are not displayed in the dropdown list, select the **Show All Workflows** checkbox to view all workflows on the selected Ion Reporter™ Server. |
| Relation | Select sample relationship group. |
| Gender | Select "Male", "Female", or "Unknown" from the dropdow list. Click ⊙ to copy the entry to all the rows.<br>**Note:** Gender must be defined to perform CNV analysis. By default "Unknown" is treated as female. |
| IR Set ID[2] | The **IR Set ID** links individual samples for analysis. Ensure the correct value is autopopulated. Set the IR Set ID to the same value for related samples.<br>**IMPORTANT!** Ensure that a unique value is entered for each sample. Do not give unrelated samples the same IR Set ID value even if the value is zero or blank. |

[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 the DNA barcode and Fusions barcode of the same sample. Do not give unrelated samples the same IR Set ID value (even if that value is zero or blank).

**Note:** You can save the samples table to a CSV file as a template for future use. Click **Save Samples Table** above the upper right corner of the **Samples Table** to save the CSV file to your computer.

6. Review the **Plugins** and **Projects** tabs, make selections appropriate to your run, then click **Next**.

7. In the **Save & Finish** step in the workflow bar, enter a name for the Planned Run in the provided field.

8. Click **Save & Finish**.

The run is listed in the **Planned Runs** screen 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.

# 6

# Variant analysis

# Analysis workflows in Ion Reporter™ Software

If you selected the appropriate Ion Reporter™ Software analysis workflow when setting up your Planned Run in Torrent Suite™ Software, automated analysis has already been performed and you can view the Oncomine™ analysis results in Ion Reporter™ Software. For instructions to manually launch an analysis, see "Manually launch a DNA analysis" <span>on page 59</span> and "Manually launch a Fusions analysis" <span>on page 59</span>.

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

Ion Reporter™ Software 5.18 or later includes the following analysis workflows:

| Workflow name[1] | Description |
|---|---|
| Oncomine™ Comprehensive Plus - w2.2 - DNA and Fusions - Single Sample | Detects and annotates low frequency somatic variants (SNPs, InDels, CNVs) from targeted Ion AmpliSeq™ DNA manual or Ion Chef™ automated libraries, computes automatic tumor cellularity, Loss-of-Heterozygosity, TMB and microsatellite instability (MSI), as well as gene fusions from targeted Ion AmpliSeq™ RNA manual or Ion Chef™ automated libraries, from Oncomine™ Comprehensive Assay Plus run on the Ion 550™ Chip. TMB uses the TMB (Non-Germline Mutations) filter chain with TMB algorithm v4.0. MSI status is computed using a baseline with MSI algorithm v2.0. Released with: Ion Reporter™ Software 5.18. Workflow Version: 2.2. |
| Oncomine™ Comprehensive Plus - w2.2 - DNA - Single Sample | Detects and annotates low frequency somatic variants (SNPs, InDels, CNVs), computes automatic tumor cellularity, Loss-of-Heterozygosity, TMB and microsatellite instability (MSI) from targeted Ion AmpliSeq™ DNA manual or Ion Chef™ automated libraries from Oncomine™ Comprehensive Assay Plus run on the Ion 550™ Chip. TMB uses the TMB (Non-Germline Mutations) filter chain with TMB algorithm v4.0. MSI status is computed using a baseline with MSI algorithm v2.0. Released with: Ion Reporter™ Software 5.18. Workflow Version: 2.2. |
| Oncomine™ Comprehensive Plus - w2.2 - Fusions - Single Sample | Detects and annotates gene fusions from targeted Ion AmpliSeq™ RNA manual or Ion Chef™ automated libraries from Oncomine™ Comprehensive Assay Plus run on the Ion 550™ Chip. Released with: Ion Reporter™ Software 5.18. Workflow Version: 2.2. |
| Oncomine™ Comprehensive Plus - w2.2 - Annotate Variants - Single Sample | Annotates VCF files from the Oncomine™ Comprehensive Assay Plus. Released with: Ion Reporter™ Software 5.18. Workflow Version: 2.2. |

[1] Workflow names can vary depending on the version of Ion Reporter™ Software.

# Manually launch a DNA and Fusions analysis

1. Sign in to Ion Reporter™ Software.

2. In the **Workflows** tab, in the **Overview** screen, select **DNA and Fusions** from the **Research Application** dropdown list.

3. *(Optional)* In **Search**, enter a term, then click **Go** (or press **Enter**).

4. In the **Workflow Name** column, select the checkbox next to the appropriate workflow (for example, Oncomine™ Comprehensive Plus - w2.2 - DNA and Fusions - Single Sample), then click ⚙ **Actions ▸ Launch Analysis** in the **Details** pane.

5. Select a DNA sample.

   a. In the **Samples** step, search by any unique identifier you used to label your samples during setup, then ensure the sample **Cellularity %** , **Sample Type** and **Gender** are defined.

   ---
   **Note:** We recommend that you define the sample Gender to perform accurate CNV analysis. By default this is treated as female. In the rare instance that the **Cellularity %** can not be auto-calculated the software will use the user entered value. By default the software uses the auto-calculated **Cellularity %** value.

   ---

   b. Click the checkbox to select a DNA sample, then click **Add samples**.

6. Select an RNA sample.

   a. In the **Samples** step, search by any unique identifier you used to label your samples during setup, then ensure the sample **Sample Type** is defined.

   b. Click the checkbox to select an RNA sample, then click **Add samples**.

7. Enter a **Group Name**, then click **Add to Analysis**.

8. In the **Plugins** step, ensure that the **Oncomine™ Variant Annotator 3.2** plugin is selected, then click **Next**.

9. *(Optional)* Enter an **Analysis Name** and **Description**.

10. Click **Launch Analysis**.

    If you selected multiple samples, a separate analysis is launched for each sample for single-sample analysis workflows.

**Analysis ready to launch!**

Review the selected options, name your analysis and then launch it.

Analysis Name: Example
(Test)

Description: Optional

Launch Analysis

# Manually launch a DNA analysis

1. Sign in to Ion Reporter™ Software.

2. In the **Workflows** tab, in the **Overview** screen, select **DNA** from the **Research Application** dropdown list.

3. *(Optional)* In **Search**, enter a term, then click **Go** (or press **Enter**).

4. In the **Workflow Name** column, select the checkbox next to the appropriate workflow (for example, Oncomine™ Comprehensive Plus - w2.2 - DNA - Single Sample ), then click ⚙ **Actions ▸ Launch Analysis** in the **Details** pane.

5. In the **Samples** step, search by any unique identifier you used to label your samples during setup, then ensure the sample **Cellularity %** , **Sample Type** and **Gender** are defined.

   ---
   Note: We recommend that you define the sample Gender to perform accurate CNV analysis. By default this is treated as female. In the rare instance that the **Cellularity %** can not be auto-calculated the software will use the user entered value. By default the software uses the auto-calculated **Cellularity %** value.
   ---

6. Click the checkbox to select a DNA sample, then click **Next**.

7. In the **Plugins** step, ensure that the **Oncomine™ Variant Annotator 3.2** plugin is selected, then click **Next**.

8. *(Optional)* Enter an **Analysis Name** and **Description**.

9. Click **Launch Analysis**.

**Analysis ready to launch!**

Review the selected options, name your analysis and then launch it.

Analysis Name: Example
(Test)

Description: Optional

Launch Analysis

# Manually launch a Fusions analysis

1. Sign in to Ion Reporter™ Software.

2. In the **Workflows** tab, in the **Overview** screen, select **Fusions** from the **Research Application** dropdown list.

3. *(Optional)* In **Search**, enter a term, then click **Go** (or press **Enter**).

4. In the **Workflow Name** column, select the checkbox next to the appropriate workflow (for example, Oncomine™ Comprehensive Plus - w2.2 - Fusions - Single Sample ), then click ⚙ **Actions ▸ Launch Analysis** in the **Details** pane.

5. In the **Samples** step, search by any unique identifier you used to label your samples during setup, then ensure that the **Sample Type** is defined.

6.  Click the checkbox to select a fusions sample, then click **Next**.

7.  In the **Plugins** step, ensure that the **Oncomine™ Variant Annotator 3.2** plugin is selected, then click **Next**.

8.  *(Optional)* Enter an **Analysis Name** and **Description**.

9.  Click **Launch Analysis**.

**Analysis ready to launch!**

Review the selected options, name your analysis and then launch it.

Analysis Name:  Example
                (Test)

Description:    Optional

[Launch Analysis]

# View results

Ion Reporter™ Software analyses are performed automatically on uploading of the data files from the Torrent Suite™ Software.

1.  Sign in to the Ion Reporter™ Software.

2.  Click the **Analyses** tab.
    The **Overview** screen displays a list of analyses in the **Analyses** table.

3.  *(Optional)* Filter the **Analyses** table.

    •   In the **Overview** screen, click **More Filters ▸ Research Application**. In the **Research Application** dropdown list, select the Oncomine™-specific analyses (**DNA**, **Fusions**, **DNA and Fusions**, or **Annotate Variants**).

    •   Enter a search term in the search field, then click **Go** (or press **Enter**).

    To further refine the list of analyses, apply additional filters from the **More Filters** dropdown list or click the column headers. The **Analyses** table automatically updates based on the filter selections and search term.

4.  Click on a row (but not on the sample data set hyperlink) to open the **Details** pane on the right side of the screen for that analysis.

    In the **Details** pane, you can view **Workflow Details** and access the **Actions** dropdown list.

    **Details**          Edit    ⚙ Actions ▾

                                 Audit Log
    👤 (0)                        Delete
    H2228_b_v1_c17(              Download Filtered Variants
    Version:                     Download Mapped BAM

5.  Click a sample result hyperlink in the **Analysis** column to open the **Analysis Results** page.

The **Analysis Results** page opens to the **Oncomine** tab, and displays only variants relevant to cancer. Metrics listed in the header (red box) are described in the table below.



| Item | Description |
|---|---|
| MAPD (Median Absolute Pairwise Difference) | The MAPD metric is a measure of read coverage noise detected across all amplicons in a panel. Higher MAPD typically translates to lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. MAPD score is viewable in downloadable VCF file or review of the Analysis Results of a single sample extended analysis. To make a CNV call the following criteria must be met:<br><br>• MAPD <0.5<br><br>• CNV Ratio for a copy number gain must be >2<br><br>• P-value <$10^{-5}$<br><br>• CNV Ratio for a copy number loss must be <0.85 |
| Sample ID | If the sample data is transferred from Torrent Suite™ Software and the SampleID plugin was run, a Sample ID is displayed at the top of the Analysis Results screen, and on the Generate Report page in the Sample Information section. |
| Tumor Cellularity Percentage | The percent of tumor cells in the sample. Allowed values: Manually input Tumor Cellularity input per user or Auto-calculated tumor cellularity.[1] |
| Tumor Mutational Burden (Mutations/Mb) | The value of the result for the tumor mutational burden calculation. |
| TMB Classification (based on specified parameters) | • High—The Tumor Mutational Burden (Mutations/Mb) is greater than the value entered in the **TMB-High Threshold** parameter.<br><br>• Low—The Tumor Mutational Burden (Mutations/Mb) is less than the value entered in the **TMB-Low Threshold** parameter.<br><br>• Intermediate—The Tumor Mutational Burden (Mutations/Mb) is equal to or greater than the value entered in the **TMB-Low Threshold** parameter AND less than or equal to the value entered in the **TMB-High Threshold** parmeter.<br><br>• Undefined—TMB classification thresholds settings were not included in the analysis workflow or were set incorrectly.<br><br>**Note:** Threshold values are not provided in the factory shipped analysis workflow. The analysis workflow always reports the TMB classification status as Undefined unless a user sets the thresholds in the parameter settings. For more information on setting TMB Classification thresholds, see "Set TMB Classification parameters" on page 102. |

*(continued)*

| Item | Description |
|------|-------------|
| MSI Status | • MSI-High—MSI score is greater than the threshold value set in the MSI-High Threshold parameter.<br>• MSS—MSI score is less than the threshold value set in the MSS Threshold parameter.<br>• No Call—MSI score is equal to or greater than the value entered in the **MSS Threshold** parameter AND less than or equal to the value entered in the **MSI-ThresholdHigh** parmeter.<br>• QCFail—Indicates determination of MSI status was not reliable due to the MSI baseline. For more information, see "MSI QC Failure" on page 99 or for more assistance contact support (see "Customer and technical support" on page 124). |
| MSI Score | A sample-level MSI score that is calculated with individual MSI marker scores. The overall score is used to determine the MSI status of the sample. |
| BRCA CNV QC | A quality control score that is based on the MAPD threshold. BRCA CNV QC is PASSED or FAILED. If failed, a reason is provided in a message. |
| LOH Percentage | Genomic loss-of-heterozygosity (sample level LOH percentage (%LOH)) is the percentage of genomic segments with (LOH) detected. Calculated as the sum of the sizes of the genomic segments with LOH detected / total size of genomic segments assessed for LOH. |

[1] If tumor cellularity percentage is determined to be not reliable by auto-calculation, then "Manual" is displayed.

6. In the **Analysis Results** table, sort or filter the data using the Oncomine™-specific annotations. See the Ion Reporter™ Software help menu for more options.

   a. In the **Filter Options** pane, select the desired **Filter Chain**.

   ---

   **Note:**
   • The default **Filter Chain** is **Oncomine™ Extended**, which limits the results that are displayed to variants relevant to cancer research only. Each variant that is called must meet all the conditions of the filter chain to be filtered-in. For more information on filter chains, see the *Ion Reporter™ Software Help*.
   • Select **No Filter** to view all the variant calls attempted by the variant caller.
   • Saving the analysis using a filter chain other than **Oncomine™ Extended** changes the variant calls that are saved in the VCF file and can affect downstream workflows.

   ---

   b. In the **Oncomine** tab, click the column headers to sort the list of variants by **Oncomine Variant Class** or **Oncomine Gene Class**.

c. In the **Ontologies** tab, click the column headers to sort the list by variant **Type** or **Genes**.



d. Click **Pharmacogenomics** to view the **ClinVar** column. Click the link in the **ClinVar** column for a selected variant to open an NCBI ClinVar website where information about the ClinVar variant annotation is available.



After you review, filter, and sort your Analysis Result, you can create a report (see "Generate a final report" on page 91), or download the analysis files (see "Download Ion Reporter™ annotation VCF or TSV files" on page 92). You can view the extracted files individually, or upload a VCF file to a software that requires VCF files, such as Ion Torrent™ Oncomine™ Reporter Software.

## Fusion results

The **Fusions** results table lists the calls and other information for the fusions, including intragenic MET exon skipping variants, analyzed in each sample in a run.

1. Click the **Analyses** tab.
   The **Overview** screen displays a list of analyses in the **Analyses** table.

2. Click a sample result hyperlink in the **Analysis** column to open the **Analysis Results** summary screen.

3. Click the **Fusions** tab to view the **Fusions** results table.
   If using a Fusions analysis workflow, only the **Fusions** results table is displayed in the **Analysis Results** summary screen. Metrics listed in the header (red box) are described in the table below.

| Column | Description |
|---|---|
| **Classification** | A user-defined classification selected from the list. |
| **Locus** | The chromosome positions in the reference genome that define the fusion junction. |
| **Type** | Assay type (for example, Fusion, RNA exon variant (exon skipping), RNAExon Tile, Proc Control). |
| **Filter** | Indicates the presence or absence of a fusion or RNA exon variant. When the default filter chain is applied, only the fusion/RNA exon variant calls that are designated with **PASS** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** filter chain or download the **Variants (.vcf)** file. <br><br> • **PASS** – indicates a high confidence call that passes all filter thresholds at a given variant position. <br><br> • **FAIL** – indicates the absence of a fusion due to the variant call failing internal quality control. <br><br> • **NO CALL** – while some evidence for the presence of a fusion exists, the call does not pass one or more filters that are required for a high confidence fusion call. |
| **No Call Reason** | The reason for reporting a fusion as **NOCALL** in **Filter** column. |
| **Genes (Exon)** | The name of fusion target and representative acceptor and donor exons. |
| **Read Counts** | The frequency that the fusion was detected in the sample. |
| **Oncomine Variant Class** | Variants that are known Oncomine™ annotated hotspots. |
| **Oncomine Gene Class** | Gain-of-function, or Loss-of-function |
| **Detection** | Returns: **Present**, **Absent**, **Present-Non-Targeted** or **NoCall** as suppported by the read counts dependent upon the thresholds set for detection. |
| **Ratio to Wild Type** | The ratio of a given variant within all wild type variants of that gene. |
| **Norm Count Within Gene** | The ratio of a given variant within all variants of that gene. |

*(continued)*

| Column | Description |
|---|---|
| Imbalance P-value | The P-value calculated for a given variant Imbalance score. |
| Read Counts Per Million | The ratio of target fusion read counts within the total mapped reads X $10^6$ |

After you review, filter, and sort your Analysis Result, you can create a report (see "Generate a final report" ), or download the analysis files (see "Download Ion Reporter™ annotation VCF or TSV files" ).

## View RNA Exon Tile Fusion Imbalance

The **RNA Exon Tile Fusion Imbalance** data view provides a visual representation of the RNA fusion imbalance analyses.

1. In the menu bar, click **Analyses ▸ Overview**.

2. In the **Analysis** column, click the hyperlinked name of the analysis of interest.

3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results.

4. In the top right corner of the screen, click **Visualize**, then select the **RNA Exon Tile Fusion Imbalance** tab to view the plots.

### Representative RNA Exon Tile Fusion Imbalance plots
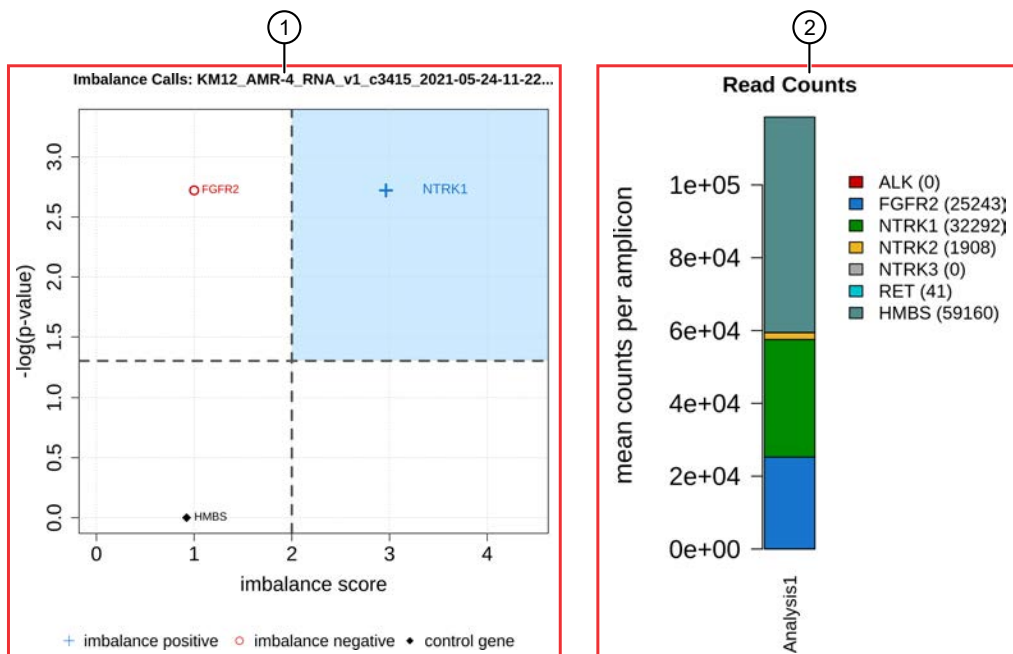


① The **Fusion Imbalance Call: Imbalance Score & P-value** plot shows the imbalance scores and p-values for all the genes in the selected sample. The dashed gray lines mark the threshold for an imbalance call, which is applied to all genes across all samples. Points that fall within the blue shaded area of the plot represent fusion-positive genes (✚). All other points that are outside of the blue shaded area represent fusion-negative genes (◯). Control genes are marked with ◆.

② The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip for the selected sample. For each gene, the read counts are normalized to the number of amplicons.
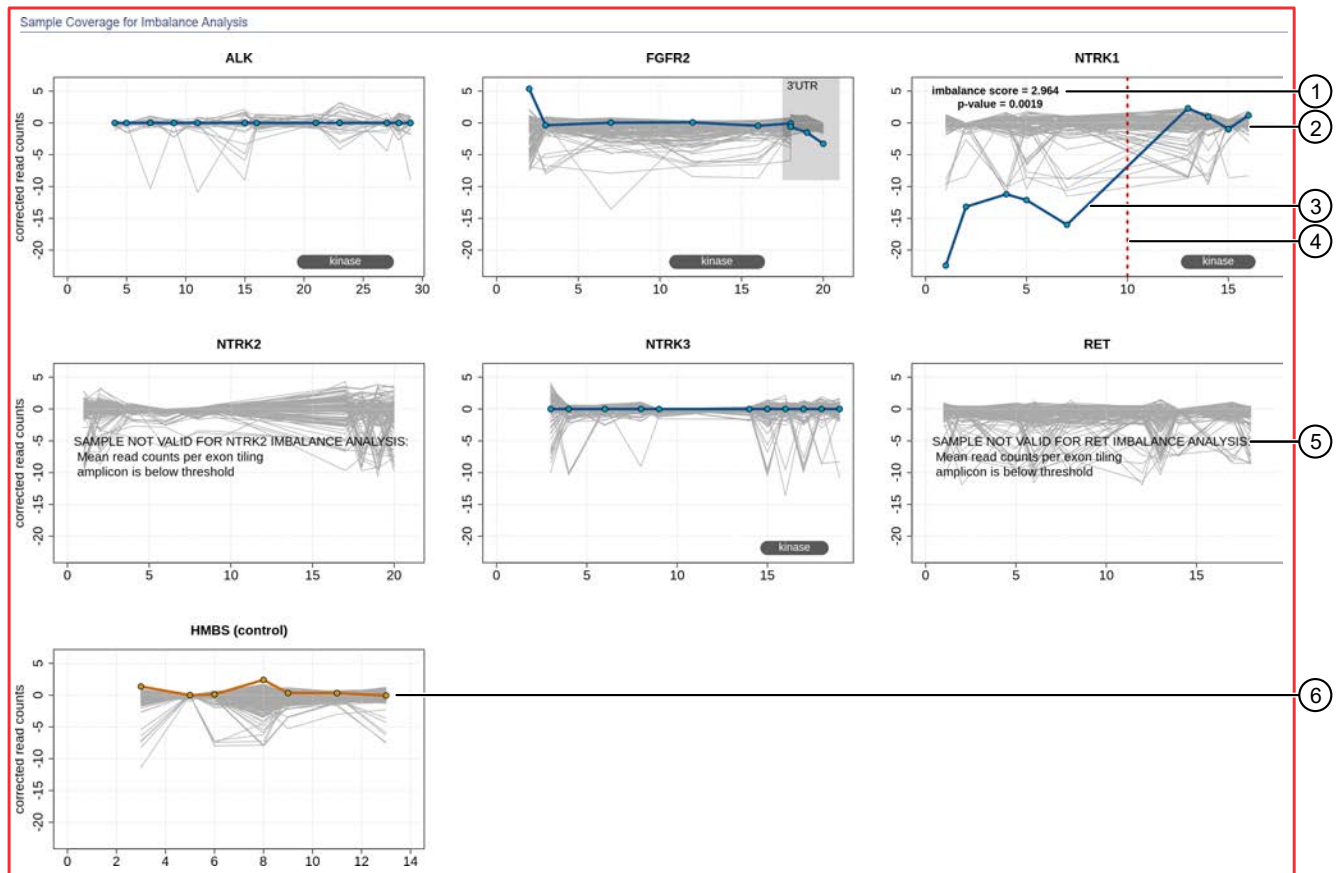
① The **Sample Coverage for Imbalance Analysis** plots show the expression profile for each exon-exon tiling amplicon for each gene. The Y-axis represents the normalized coverage after baseline correction. The X-axis represents individual exon-exon junctions, which are listed from 5′ to 3′. The **imbalance score** and **p-value** are listed in the panel of each gene that was called positive for fusion.

② Baseline (a cluster of gray lines), generated from a fusion-negative sample.

③ Test sample corrected read coverage (blue line), normalized to the baseline. Each point on the line represents a unique exon-exon junction that was covered by the assay and normalized to the baseline.

④ Predicted range for the fusion break point for a fusion-positive gene (dashed red line).

⑤ If the collected data are insufficient to determine an imbalance score, the **SAMPLE NOT VALID FOR <gene> IMBALANCE ANALYSIS** message appears in the panel for that gene.

⑥ Sample coverage profile for the control gene (orange line).

To return to the table view of fusions, click the browser **Back** arrow.

## Non-targeted fusion detection

In the non-targeted fusion detection method, the panel primers are used to detect fusions between novel combinations of known driver and partner genes. The sequencing reads are mapped to a broader reference, such as the whole-exome. Mapping the reads to a broader reference allows for the detection of multiple configurations of driver and partner genes as well as detection of novel breakpoints between the known partner and driver genes.

Low confidence non-targeted fusions are identified in VCF output files with key value pairs as follows.

Table 3   Key value pairs for non-targeted fusions

| Type of non-targeted fusion identified | Definition | Key value pair |
|---|---|---|
| Out-of-frame sequence | The expected non-targeted sequence from the amplicon reference file is used to check whether the sequence is in frame. If the non-targeted sequence is out of frame (compared to the original partner or driver genes), the key-value pair `NT_FUSION_IN_FRAME=FALSE` is included in the VCF file. | `NT_FUSION_IN_FRAME`[1] <br><br>Values<br><br>• `FALSE`<br>• `TRUE` |
| Partner-partner fusion | Non-targeted fusions are identified if one of the genes in an isoform is not a driver gene. The input reference BED file includes annotations for driver genes, such as, in the case of EML4-ALK.E6A19.COSF1296.2, the DRIVER_GENE=ALK. By default, the software uses a 3p-partner of a targeted fusion isoform as the driver gene if that targeted isoform BED entry does not include the `DRIVER_GENE` parameter, as is the case with earlier panel files and also a few targets in later panel files. In this case, the software will check if at least one of the two genes involved in non-targeted fusion is a 3p gene. | `NT_FUSION_DRIVER_INVOLVED`[2]. <br><br>Values<br><br>• `FALSE`<br>• `TRUE` |
| Secondary isoform fusion | In some samples, a non-targeted fusion isoform might be detected in a sample while additional targeted isoforms with the same gene pair are also detected (that is, isoforms that are defined in the panel). In this case, the non-targeted fusion isoform should be identified as `NT_FUSION_SECONDARY=TRUE`, if its read count coverage is below *<x%* of the reads of any of the other isoforms with the same gene pair. By default, the threshold for secondary isoforms is *<5%* of another targeted fusion isoform. | `NT_FUSION_SECONDARY`[3] <br><br>Values<br><br>• `FALSE`<br>• `TRUE` |

[1]   If the key value pair is not included then `NT_FUSION_IN_FRAME` is assumed to be `True`, indicating that an out-of-frame sequence is not found.

[2]   If the key value pair is not included then `NT_FUSION_DRIVER_INVOLVED` is assumed to be `True`, indicating that a partner-partner fusion is not found.

[3]   If the key value pair is not included then `NT_FUSION_SECONDARY` is assumed to be `False`, indicating that a secondary isoform fusion is not found.

Additional attributes that indicate whether the non-targeted fusion is out of frame, involves partner-partner fusion, or is a secondary isoform, are added to the VCF files. This information is used by the Oncomine™ Variant Annotator plugin to annotate variants in Ion Reporter™ Software.

## View RNA exon variants

The RNA Exon Variant data view displays a bar graph summary of intragenic exon rearrangements or fusions. The displayed RNA exon variants are defined in the BED file that is associated with an assay. The RNA Exon Variant data view is available for all RNA and Fusion assays.

1. In the menu bar, click **Analyses ▸ Overview**.

2. In the **Analysis** column, click the hyperlinked name of the analysis of interest.

3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results.

4. In the top right corner of the screen, click **Visualization ▸ RNA Exon Variant** to view the **RNA Exon Variant** plot.

To return to the table view of fusions, click the browser **Back** arrow.

### Representative RNA Exon Variant plots
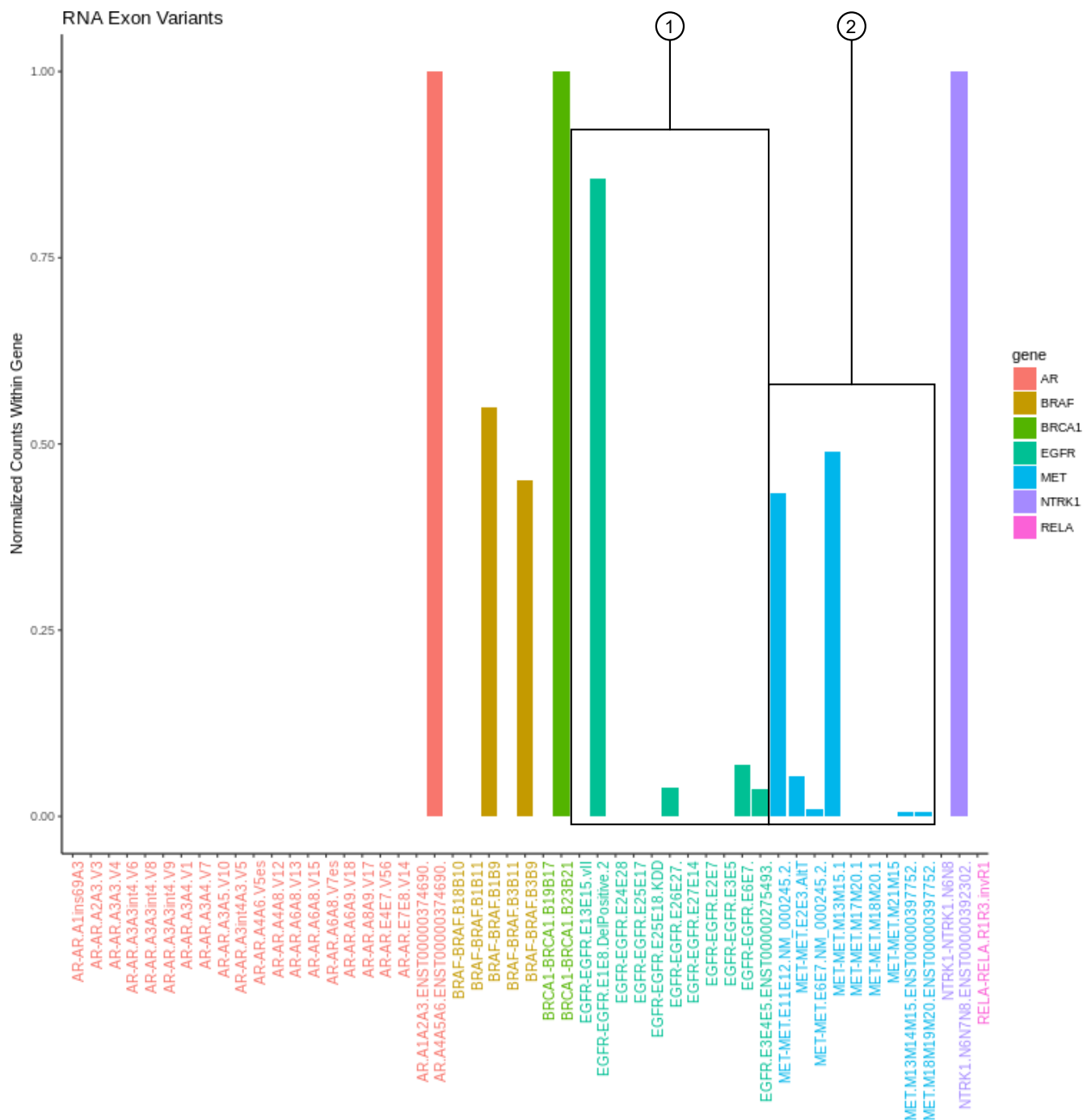
RNA Exon Variants



The X-axis represents specific exon variants, where each variant is labeled with a gene ID followed by a sequence of adjacent exons. The Y-axis measures the read counts for each variant, normalized to the wild type.

① Example analysis where RNA exon 2–7 deletion occurred in the EFGR gene. The deletion of exons 2–7 resulted in an increase of normalized read counts for the EFGR variant that contains the intragenic fusion of exon 1 and exon 8 (EGFR-EGFR.E1E8.DelPositive.2), and a decrease of normalized read counts for the wild type EFGR (EFGR.E6E7).

② Example analysis where exon-skipping (i.e., exon deletion) of exon 14 in the MET gene was detected. Normalized read counts for the variant representative of the exon-skip event (MET-MET.M13M15.1) are higher relative to the MET wild-type assays (for example, MET.M13M14M15.ENST00000397752.WT and MET.M17M18M19M20.ENST00000397752.WT).

# Visualize tumor mutational burden analysis results

To visualize tumor mutational burden analysis results in Ion Reporter™ Software, the analysis workflow must be any DNA-single sample, or DNA and Fusions-single sample analysis workflow that has tumor mutational burden enabled.

1. Do one of the following to open tumor mutational burden analysis results:

| Option | Description |
|---|---|
| Visualize analysis results from an individual sample or from multiple samples simultaneously from the **Analyses** table. | In the **Analyses** table, select an individual sample result row, or select the checkbox next to each sample result that you want to visualize simultaneously, then click **Visualize**. Alternatively, click **Actions ▸ Visualize**. |
| Visualize analysis results individually from the **Analysis Results** screen. | In the **Analyses** table, click a sample result hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**. |

The **Tumor Mutational Burden (Mutations/Mb)** is displayed at the top of each section of sample results.



2. Click **Download Report** to download the **Tumor Mutational Burden** visualization report in PDF format, which includes the results with graphs and metrics.

3. Scroll down to view graphical representations of the analysis results and QC metrics, and download additional files.

   **Note:** Multiple sample results are listed sequentially.

4. In the lower right corner of the screen:

   • Click **Download Variant Details TSV** to download a tab-separated list of detected variants that contributed to the tumor mutational burden count. Open the file with compatible software, such as Microsoft™ Excel™.

   • Click **Download the TMB statistic.txt file** to download the tumor mutational burden output file. The file is named `<analysis name>_statistic.txt`. For example, `NCI-1395_c3495_2020-03-03-10-58-54-007_statistic.txt`.

# QC metrics for tumor mutational burden

**QC metrics** for tumor mutational burden are listed at the top of **Analysis Visualization** in the **TMB** tab when you visualize analysis results with Ion Reporter™ Software.

| QC metric | Definition |
|---|---|
| **Average Coverage** | The following formula is used to calculate average coverage: $$\frac{\text{Total base coverage across the genomic positions defined by the assay BED file}}{\text{Number of bases in the DNA BED file}}$$ If the average coverage is below 150, the analysis completes successfully but Tumor Mutational Burden is reported as $-1$. **Note:**  This setting cannot be changed in Ion Reporter™ Software. |
| **Number of bases used in calculating TMB** | The number of bases used as a denominator for the tumor mutational burden calculation. Only bases with sufficient base coverage are used in the calculation, as defined in the workflow parameters. In the parameters, you can also select only the genomic regions covered by the panel to be used instead of all exonic regions. See the *Ion Reporter™ Software Help* for more information. |
| **Number of variant calls** | The number of somatic variants that are identified in the sample. This value is reported in the `statistic.txt` file as **Total Somatic Filtered Variants Count (numerator for TMB calculation)** and **Variant Count**. For more information, see *Ion Reporter™ Software Help*. |
| **Deamination score** | Deamination score was previously reported as the estimated SNP proportion consistent with deamination (mainly FFPE). The deamination score can be used to determine the quality of the FFPE sample. For more information on how to minimize the impact of high deamination on a tumor mutational burden score, see *Ion Reporter™ Software Help*. A deamination QC status of *PASS/FAIL* is also reported. The threshold for this QC can be adjusted in the analysis workflow parameter settings. For more information, see *Ion Reporter™ Software Help*. |

# Sample results

Tumor mutation burden results are represented graphically in the **TMB** tab when you visualize analysis results with Ion Reporter™ Software.

### Germline and Somatic Variants

This histogram shows the frequency distribution of allele ratio for total called germline and somatic variants. Listed below the figure is the combined total of called germline and somatic variants. The value is reported in the `statistic.txt` file as **Variant count (Germline + somatic)**. For more information, see *Ion Reporter™ Software Help*.
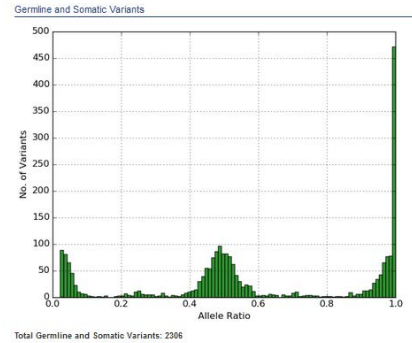


### Only Somatic Variants

This histogram shows the frequency distribution of allele ratio for only somatic mutations as determined by the selected TMB filter chain. Listed below the figure are:

- The number of **Total Somatic Variants** , reported in the `statistic.txt` file as **Total Somatic Filtered Variants Count (numerator for TMB calculation)**.

- Of the **Total Somatic Variants** , the number of SNVs that are determined to be nonsynonymous (detrimental) and synonymous (non-detrimental) as annotated by Ion Reporter™ Software. Values are reported by different Ion Reporter™ annotation types in the `statistic.txt` file.

- The number of detected somatic variants found in the COSMIC database. The value is reported in the `statistic.txt` file as **COSMIC Annotated Somatic Variants**.

For more information, see step 4 of "Visualize tumor mutational burden analysis results" on page 71 .

## Substitution Type and Context of Somatic Mutations



| | | |
|---|---|---|
| ■ C>A | | ■ T>A |
| ■ C>G | | ■ T>C |
| ■ C>T | | ■ T>G |

Somatic mutations can be divided into 6 base substitution classes (that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type. After incorporating information on the bases immediately 5′ and 3′ to each mutated base, 96 possible mutation types are in this classification. These 96 mutation types are represented on the x-axis, and variant frequency for mutation type on the y-axis. Bars for each substitution class are grouped and displayed with different color. A summary TXT file of these results is also available, see "Download Ion Reporter™ annotation VCF or TSV files" on page 92.

## Substitution Type of Somatic Mutations

A pie chart dividing somatic mutations into 6 base substitution classes
(that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type.



| | | |
|---|---|---|
| ■ C>T | | ■ T>G |
| ■ C>G | | ■ T>C |
| ■ C>A | | ■ T>A |

## Signature Pattern of Somatic Mutations

A pie chart dividing somatic mutations in groups consistent with specific mechanisms.



- 🟥 C>T at [AG]CG
- 🟦 C>T at [CT]CG
- 🟩 C>T at NCC,CC[ACT],TC[ACT]
- 🟪 C>T at other sites
- 🟧 C>A
- 🟨 T>A and T>C
- 🟫 Rest
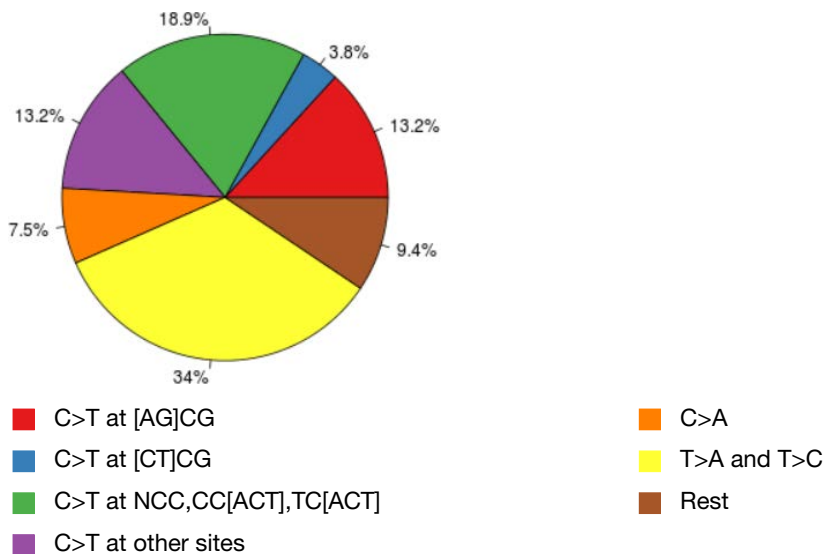
In the pie chart, a small fraction of multiple signature types can be observed in the sample. However, significant dominance of a single signature pattern often correlates to the respective tumor type. For example, 56.7% of the variants detected (sum of blue, green, and yellow) are an observed UV damage signature in this sample.

- High C>T at CpC, CpC, TpC, T>A, and T>C is consistent with UV damage. (Blue + Green + Yellow)
  For more information, see **https://www.nature.com/articles/nature22071**

- High C>T at CpG is consistent with spontaneous deamination of 5-methylcytosine. (Red + Blue).
  For more information, see **https://www.nature.com/articles/nature12477**

- High C>A is consistent with smoking damage.(Orange)
  For more information, see **https://science.sciencemag.org/content/354/6312/618.full**

- High C>T (site independent) is consistent with FFPE processing. (Green + Purple)
  For more information, see **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4032349/**

**Note:** Underlined bases represent the reference base being substituted (for example, CpG in the first bullet is same as to [ACGT]CG).

# View Oncomine™ BRCA analysis results

The provided Ion Reporter™ Software 5.18 or later Oncomine™ Comprehensive Assay Plus analysis workflows now include end-to-end coverage of both BRCA1 and BRCA2 genes with optimized variant calling for SNV, long deletions, exon deletion and duplication, and whole somatic gene deletion variants in somatic and germline samples with high sensitivity.

1. In the **Analyses** tab, click **Overview**.

2. Search, sort, or filter the **Analyses** table to find the sample result of interest.

3. In the **Analysis** column, click the hyperlink for the sample result of interest to open the **Analysis Results** screen for your BRCA analysis.

4. The following data for the BRCA results is available in the **Analysis Results** screen:

   - A summary of called variants and their genotypic and functional properties. Sort and select variants of interest. Variants that are listed include SNVs, INDELs, germline long exon deletions, duplications, and germline and somatic whole gene deletions and duplications.

   - Metrics and information at the top of the screen.

| Item | Description |
|---|---|
| Analysis Name | The name of the analysis. |
| Cancer Type | The type of cancer, as defined by the sample attribute in Torrent Suite™ Software, if sample data is transferred by Torrent Suite™ Software. |
| MAPD | The MAPD (Median of the Absolute values of all Pairwise Differences) metric is an estimate of coverage variability between adjacent amplicons. The default threshold is 0.65. As a result, sample results with a MAPD above this value should be viewed with lower confidence. |
| BRCA CNV QC | A quality control score that is based on the MAPD threshold. **BRCA CNV QC** is PASSED or FAILED. If failed, a reason is provided in a message. |
| Sample ID | **Note:** If the sample data is transferred from Torrent Suite™ Software and the SampleID plugin was run, a Sample ID is displayed at the top of the **Analysis Results** screen, and on the **Generate Report** page in the **Sample Information** section. |

   - Click **Summary** to view a summary of the called variants. Select a classification from the dropdown list to assign a classification to a variant. The following variant types are available for a BRCA analysis.

| Type | CNV Subtype | Description |
|---|---|---|
| CNV | BigDel | Deletion of at least one exon |
| | BigDup | Duplication of at least one exon |
| | GeneCNV | Whole BRCA1/BRCA2 gene deletion or duplication |
| | NOCALL | Read count differs from baseline by non-integer amount; evidence for a BigDel or BigDup call is weak |
| | REF | Read count matches reference baseline |

*(continued)*

| Type | CNV Subtype | Description |
|------|-------------|-------------|
| LongDel | — | A specific 40 bp deletion in BRCA1 (c.1176_1214del) |
| SNV | — | Single nucleotide substitution |
| MNP | — | Multiple nucleotide polymorphism at adjacent nucleotide positions |
| INDEL | — | Single or multiple nucleotide insertion or deletion |

- Click **Functional** to view other functional annotations and use the annotations to classify, sort, and filter variants.

- Click **Pharmacogenomics** to view the **ClinVar** column. Click the link in the **ClinVar** column for a selected variant to open an NCBI ClinVar website where information about the ClinVar variant annotation is available.



## Visualize the BRCA report

Oncomine™ Comprehensive Assay Plus, DNA Ion Reporter™ Software analysis workflows enable the detection and visualization of whole exon and multiple exon deletions in BRCA1 and BRCA2 genes in somatic and germline samples with high sensitivity.

1. In the **Analysis Results** screen, click **Visualize**.

   When you open a visualization for the analysis results, you can view a **BRCA Report** that shows a boxplot of read counts of each BRCA1 and BRCA2 exon that are normalized to the the CNV baseline for the panel. The **Post-Corrected** view is shown by default. Click, **Pre-Corrected** to view the uncorrected raw data.

## BRCA Report
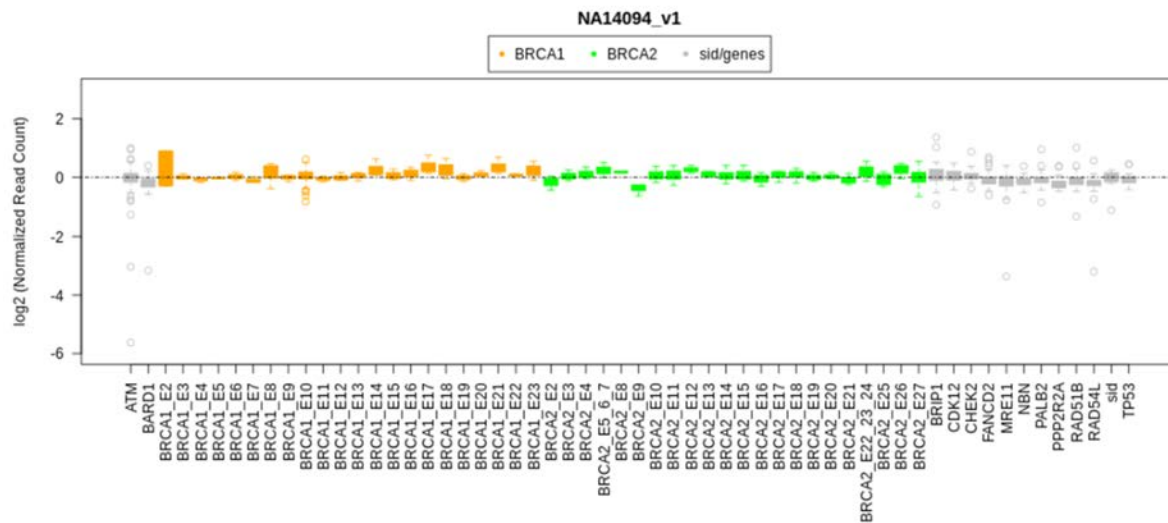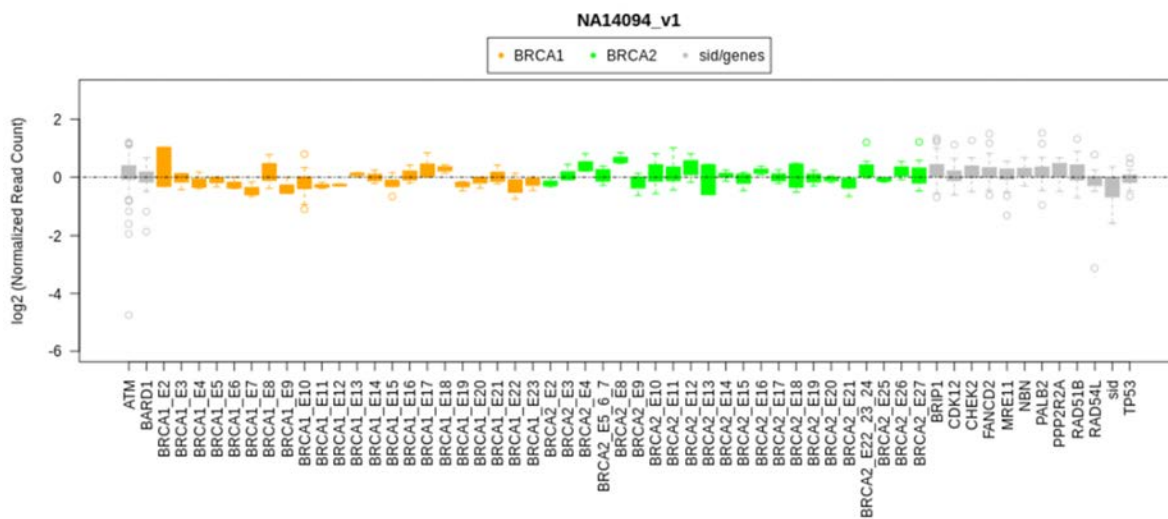




**Figure 3   Normal sample, no BRCA whole-gene or exon deletion**

Example post-correction boxplot (Figure 3 **a**) of a tissue normal sample without whole-gene or exon deletions in either the BRCA1 or BRCA2 genes. The data have less variability relative to the pre-corrected view (Figure 3 **b**).

In Ion Reporter™ Software 5.16 or later, the **Post-Corrected** view normalizes the abundance of the amplicons in BRCA1 and BRCA2, bringing the median coverage of amplicons in BRCA1 and BRCA2 to comparable values. This correction is used to detect exon deletions in the BRCA1 or BRCA2 genes (Figure 4). As a result the **Post-Corrected** view does not show any whole-gene deletions or duplications in BRCA1 or BRCA2 (for example, somatic CNVs). This behavior is different than in the earlier version of Ion Reporter™ Software (5.14), where whole-gene deletions

were visible in the **Post-Corrected** view. To view whole-gene deletions or duplications, review the **Pre-Corrected** view (Figure 5) or the **IRGV & Generate Report** tab.
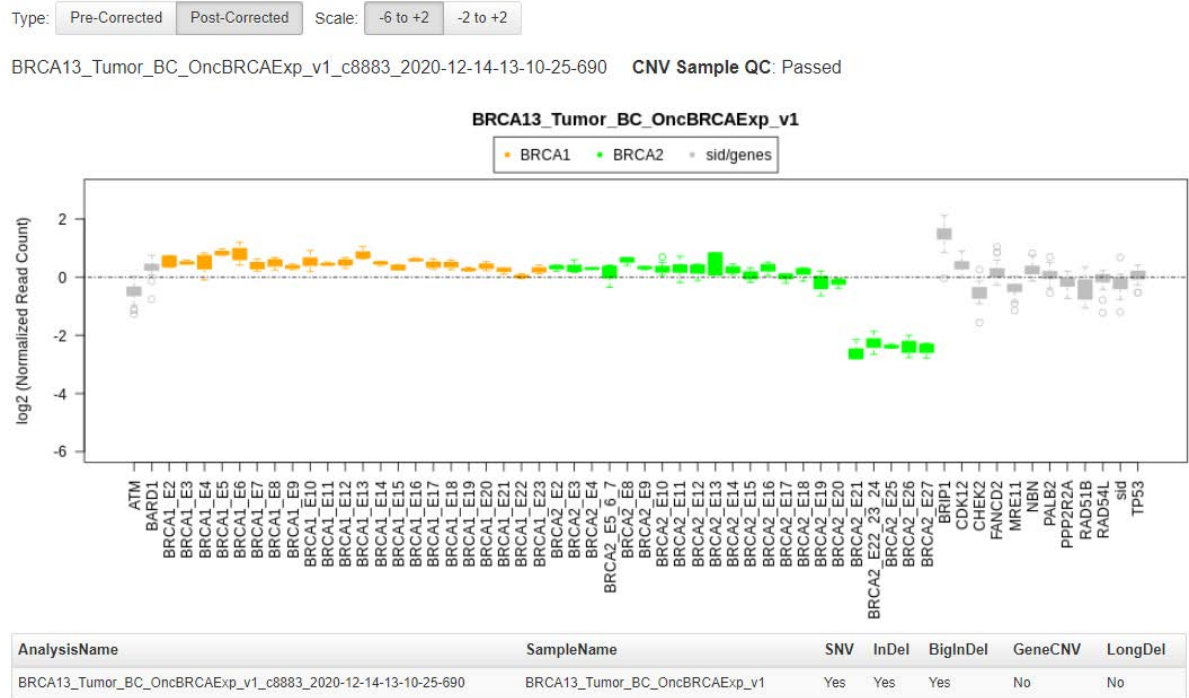


**Figure 4   Tumor sample with BRCA exon deletion**

Example Post-Corrected boxplot of a tumor sample with an exon deletion in the BRCA2 gene.
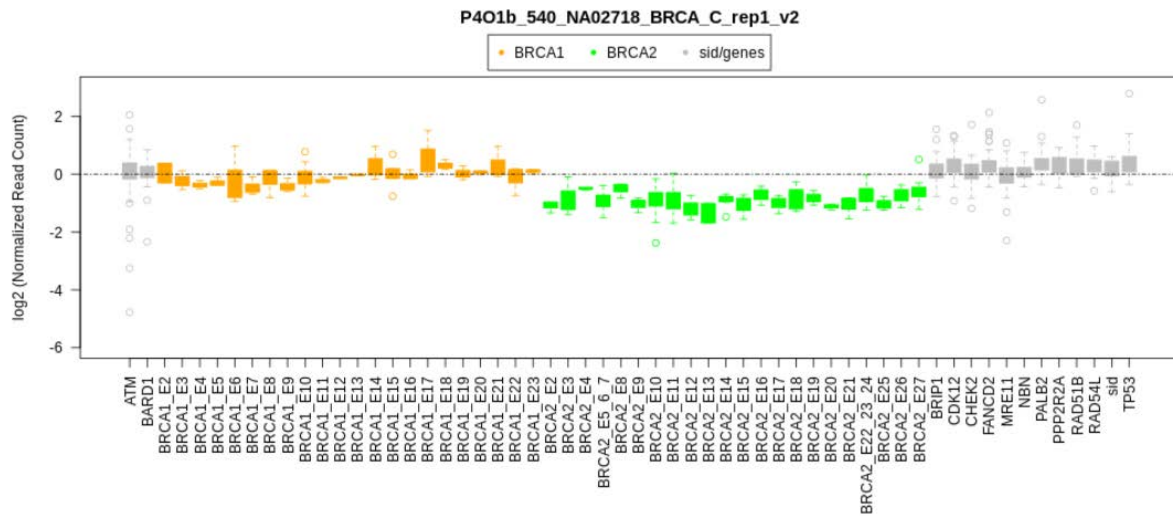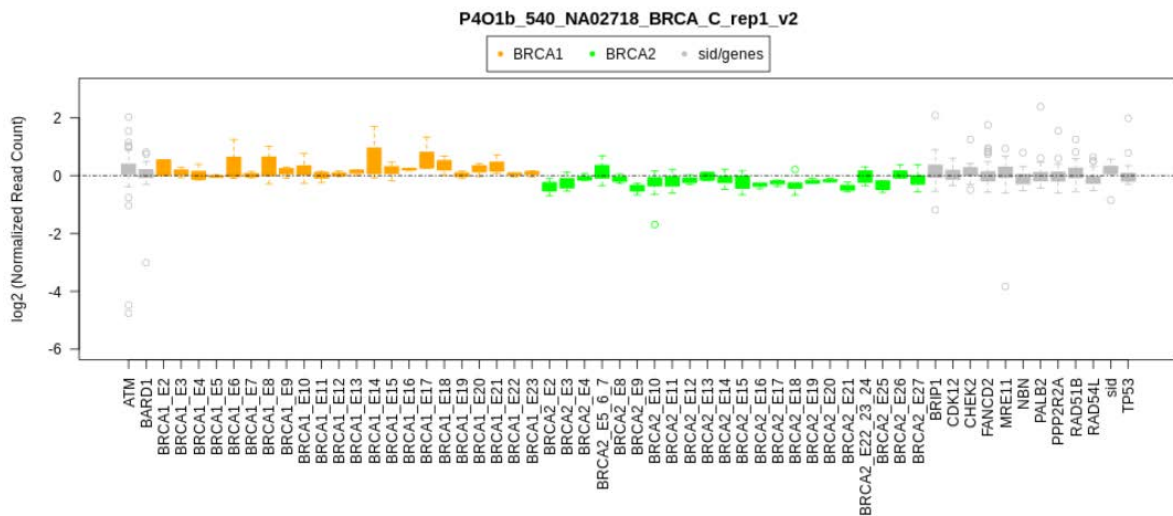
**Figure 5   Tumor sample with BRCA2 whole-gene deletion**

Example Pre-Corrected boxplot (Figure 5 **a**) of a tumor sample with a whole-gene deletion in the BRCA2 gene. The whole-gene deletion is not visible in the Post-Corrected view (Figure 5 **b**).

---

**Note:**  If either of the BRCA1 or BRCA2 genes is amplified, the other BRCA gene could be incorrectly described as being deleted. To identify whether a BRCA gene has been amplified or the other BRCA gene deleted, review the Pre-Corrected boxplot to compare the abundance of the amplicons in both the BRCA1 and BRCA2 genes compared to all the other genes.

---

2. Click the **IRGV & Generate Report** tab to view exon deletions or duplications on chromosomes 13 and 17.



① Click to open the **Sample Analysis Summary** in a separate browser window

② Zoom in and zoom out for the selected chromosome

③ Click to select and view a chromosome

④ Slide the **MAPD filter** to adjust the threshold value. This filter applies to the entire sample. If the reported MAPD value is higher than the selected **MAPD filter** value, no CNVs are shown in the BRCA Report.

⑤ Scroll down in this section to see more of the alignments or hotspots.

⑥ Oncomine BRCA Expanded designed BED file alignment. This **IRGV** track shows the alignment of the amplicons to the reference sequence.

3. Click **IRGV Export & Preferences** to open the visualization in IGV, export the whole genome view or the IRGV tracks to a PNG file, or show IRGV preferences.

# Genomic segmentation analysis results

The Ion Reporter™ Software Oncomine™ Comprehensive Assay Plus, DNA analysis workflow utilizes heterozygous population SNPs covered by the assay to determine the ploidy levels of genomic segments. The genome is divided into contiguous segments of similar ploidy levels using a circular binary segmentation (CBS) algorithm. Log odd ratios for variant allele frequency of observed population SNPs (by TVC) and copy-number (CN) ratios (by CNV pipeline) for each segment are calculated. Log odd ratio and CN ratios are then used to infer:

- Tumor cellularity percentage (percentage of tumor cells in the sample)
- Loss-of-heterozygosity (LOH) for each genomic segment. Segment level LOH events are intersected with targeted gene boundaries to determine LOH events in selected genes. Segment level LOH events are also aggregated to determine genomic (%LOH).

## Visualization of genomic segmentation analysis, Allele Specific Copy Number plots

1. There are two ways to visualize analysis results in Ion Reporter™ Software:

| Option | Description |
|---|---|
| Visualize analysis results from one or more analyses simultaneously from the **Analyses** table. | In the **Analyses** table, select a row for an analysis or select the checkbox next to one or more analyses that you want to visualize simultaneously, then click **Visualize**. Alternatively, click **Actions ▸ Visualize**. |
| Visualize analysis results individually from the **Analysis Results** screen. | In the **Analyses** table, click an analysis hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**. |

**Note:** The **Analysis Visualization** screen opens to the **TMB** tab by default.

2. Click the **Allele Specific Copy Number** tab.

   A series of plots showing copy number variation across the entire genome is displayed. Click **Export Image** to save a PNG image of all three plots to a location of your choice. If visualizing multiple analyses simultaneously, individual sample results are listed sequentially.

Variants Table    Variant Impact    CNV Heat Map    IRGV & Generate Report    BRCA    TMB    MSI    **Allele Specific Copy Number**    HRR

BRCA_SN17-16850 _10ng_15cyc_rep2_v1_c39881_2021-03-31-09-44-32-609
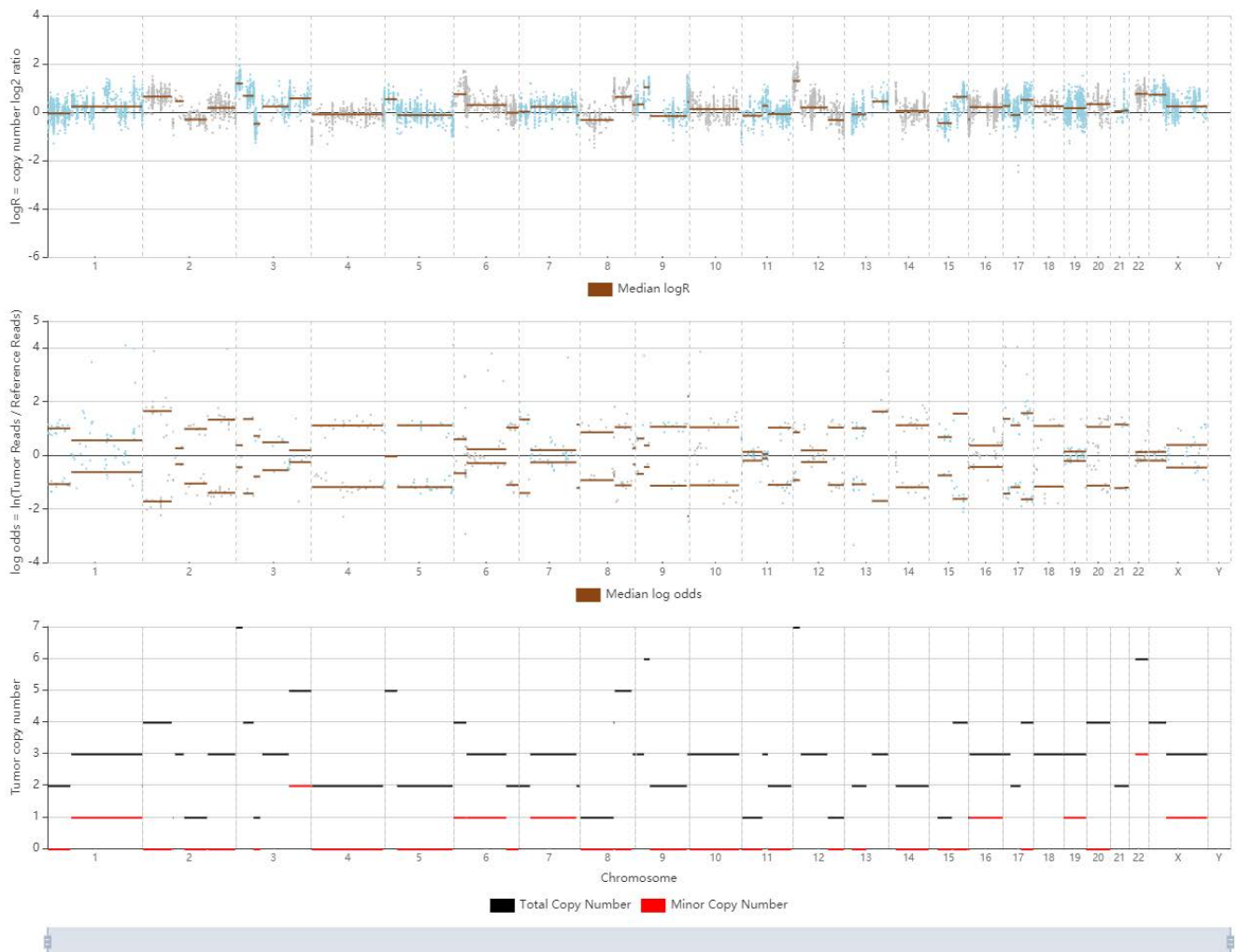
This visualization shows identified genomic segments (shown as horizontal lines) using log2 ratios from the Copy Number Variation (CNV) analysis and log odds from the Torrent Variant Caller (TVC) analysis. Segments are identified using the heterozygous population SNPs (shown as dots in the upper two panels) targeted by assay amplicons.

| Plot scale (x-axis) | Description |
|---|---|
| logR = copy number log2 ratio | Log2 ratios (top panel) of the copy-number estimates relative to the baseline copy-number as calculated by the CNV algorithm for each amplicon in the assay. Ratio representative Genome segmentation overlay (horizontal brown bars) shows segments with similar log2 ratios clustered together. |
| log odds = ln(Tumor Reads / Reference Reads) | Log odds (middle panel) for each heterozygous SNP in the assay. Log odds is calculated as the natural logarithm of the ratio of the sequencing reads with variant allele and reference allele that are obtained using Torrent Variant Caller (TVC). Genome segmentation overlay (horizontal brown bars) shows segments with similar log odds clustered together. Since the variant allele could be major or minor for any SNP, the corresponding log odds could be positive or negative and are therfore displayed as segments that are mirror images around 0. |
| Tumor Copy Number | Bottom panel shows the total (black horizontal line) and minor (red horizontal line) copy-number estimates for each of the identified genomic segments (top two panels). Genomic segments where total CN ≥1 and minor CN = 0 are segments with LOH. There may exist genomic segments for which minor copy-number estimates can not be determined. |

# Visualize HRR analysis results

You can visualize data that is relevant to Homologous Recombination Repair (HRR) in Ion Reporter™ Software, with the Oncomine™ Comprehensive Assay Plus panel.

HRR is a pathway to repair double-strand breaks which are generated as by-products of cellular metabolism, DNA replication errors, or exogenous factors. The biomarkers that are associated with HRR can indicate alterations in genes and genomes. An HRR visualization shows details about the genomic loss-of-heterozygosity in the sample (%LOH). Additionally, information about Telomeric Allelic Imbalance (%TAI) and Large-Scale State Transitions (%LST) biomarkers is available in the VCF file that you can download when you open HRR analysis results.

1.  Do one of the following to open HRR analysis results:

    | Option | Description |
    |---|---|
    | Visualize analysis results from an individual sample or from multiple samples simultaneously from the **Analyses** table. | In the **Analyses** table, select an individual sample result row, or select the checkbox next to each sample result that you want to visualize simultaneously, then click **Visualize**. Alternatively, click ⚙ **Actions** ▸ **Visualize**. |
    | Visualize analysis results individually from the **Analysis Results** screen. | In the **Analyses** table, click a sample result hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**. |

    The **Analysis Visualization** opens to the **TMB** tab by default.

2.  Select the **HRR** tab to review a summary of the analysis results about the genes and genomic alterations in the HRR pathway. For example, you can view information about genomic LOH (sample %LOH), CNVs, SNVs, and INDELs. You can also view any exon-level deletions and duplications that are associated with BRCA1 and BRCA2 genes.

3. Click **Download ▸ Current Results TSV** to download a tab-separated list of detected variants in the analysis results. Open the file with compatible software, such as Microsoft™ Excel™.

   For more information on the download files, see "Download Ion Reporter™ annotation VCF or TSV files" <span>on page 92</span>.

4. Click **Download ▸ Filtered Variants** or **Download ▸ All Variants** to download a ZIP folder of files that list the variants in the analysis results.

# Mutation Signature

## Mutational Signature profiles

Cancer genomes are subject to diverse mutational processes that generate recognizable mutational signatures. Some processes are driven by defects in specific DNA repair pathways (for example, HRR, MMR) whereas others are characteristic of environmental mutagens (for example, ultra-violet light, tobacco smoke). These processes generate unique combinations of mutation types, a "Mutational Signature".

Somatic SNVs in the sample are filtered to remove germline mutations and are used to construct a normalised single base substitution (SBS) matrix, or mutational signature. The normalised sample mutational signature is then compared with 54 signatures from the COSMIC Mutational Signatures v3.1 and the cosine similarity is calculated. COSMIC signatures with cosine similarity score ≥0.7 to the sample signature are then analyzed with the deconstructSigs algorithm to identify which COSMIC Mutational Signatures are the best match.

## View the Mutational Signature Prediction report

To view the mutational signature prediction report (`signature_identification_<analysis_name>_report.pdf`) you must first download the ZIP folder. For more information, see "Download Ion Reporter™ annotation VCF or TSV files" <span>on page 92</span>. The folder contains the following files.

- PDF Report
- Profile of Mutational signature of Sample (Only available if a mutational signature match is found.)
- Profile of enriched COSMIC signature (One or More, only available if a mutational signature match is found.)
- deconstructSigs Weights Pie Chart (Only available if a mutational signature match is found.)
- Trinucleotide distribution of Variants

1. Extract the downloaded ZIP folder to your hard drive.

2. Open the **signature_prediction** folder, then double-click on the PDF Report file.

# Mutational Signature Prediction report

| File | Description |
|------|-------------|
| PDF Report | FileName format is "signature_identification_<analysis_name>_report.pdf". The PDF report consists of 4 sections:<br><br>1. Header<br> • Report Name - "Mutational Signature Prediction Report"<br> • Sample Name<br> • Analysis Name (If a sample is re-analysed, the Analysis name will change)<br>2. Summary<br> • Table<br>  a. Name of enriched signature, from COSMIC<br>  b. Cosine similarity between sample mutational profile and COSMIC Signature<br>  c. Signature Description or Aetiology<br> • COSMIC Signature Weights—pie chart denoting weights from deconstructSigs. This reflects the strength of the evidence for the match between the data and the Cosmic signature.<br>3. Mutation Profiles<br>4. Signature Specific Gene Mutations |
| Profile of Mutational signature of Sample | File name format is "<analysis_name>_normalized_sample_profile.png". The image is present in the PDF report under "Mutation Profile" section. |
| Profile of COSMIC signature | File name format is "<analysis_name>_<cosmic_signature>.png". The image is present in the PDF report under "Mutation Profile" section. One image is generated per enriched COSMIC signature. The output will not be generated if any enriched signature is not found. |
| deconstructSigs Weights Pie Chart | File name format is "<analysis_name>_signatures_pie.png". The image is present in the PDF report under "Summary" section. The output will not be generated if any enriched signature is not found. |
| Trinucleotide distribution of Variants | File name format is "<analysis_name>_contextFile.txt". It contains freqeuencies of mutations within each of the 96 trinucleotides. |

# Mutation Signature Prediction report example

① **Mutation Signature Identification Report**

Sample Name:
LN_AT0035_MSI_10_Tumor_FFPE_DNA_20ng
Analysis Name:
LN_AT0035_MSI_10_Tumor_FFPE_DNA_20ng_20210525102124898

② **Summary**

| Enriched Signature | Cosine Similarity | Signature Description |
|---|---|---|
| SBS14 | 0.835 | Concurrent polymerase epsilon mutation and defective DNA mismatch repair (with microsatellite instability, MSI). |
| SBS44 | 0.719 | Associated with DNA mismatch repair(with microsatellite instability, MSI). |

COSMIC Signature Contribution

SBS44 (57.7%)
SBS14 (42.3%)

③ **Mutation Profiles**



④ **Mutation Signature Identification Report**

Sample Name:
LN_AT0035_MSI_10_Tumor_FFPE_DNA_20ng
Analysis Name:
LN_AT0035_MSI_10_Tumor_FFPE_DNA_20ng_20210525102124898

**Signature specific gene mutations**

**SBS14**

| Locus | Type | Gene | Frequency | Protein |
|---|---|---|---|---|
| chr2:47690230 | SNV | MSH2 | 38.74 | p.Glu483Ter |
| chr2:47705502 | SNV | MSH2 | 36.74 | p.Glu768Ter |
| chr2:190728929 | SNV | PMS1 | 32.37 | p.Glu773Ter |
| chr3:37055998 | SNV | MLH1 | 36.64 | p.Tyr251Ter |
| chr12:133210839 | SNV | POLE | 39.19 | p.Asn1979Lys |
| chr12:133226285 | SNV | POLE | 60.84 | p.Pro1258His |
| chr14:75500154 | SNV | MLH3 | 34.97 | p.His1228Pro |
| chr14:75515362 | SNV | MLH3 | 41.86 | p.Trp333Arg |

**SBS44**

| Locus | Type | Gene | Frequency | Protein |
|---|---|---|---|---|
| chr2:47690230 | SNV | MSH2 | 38.74 | p.Glu483Ter |
| chr2:47705502 | SNV | MSH2 | 36.74 | p.Glu768Ter |
| chr2:190728929 | SNV | PMS1 | 32.37 | p.Glu773Ter |
| chr3:37055998 | SNV | MLH1 | 36.64 | p.Tyr251Ter |
| chr14:75500154 | SNV | MLH3 | 34.97 | p.His1228Pro |
| chr14:75515362 | SNV | MLH3 | 41.86 | p.Trp333Arg |

① Header
② Summary
③ Mutation Profiles
④ Signature Specific Gene Mutations

# MSI analysis results

You can determine whether microsatallite instability (MSI) is present using Oncomine™ Comprehensive Assay Plus - w2.2 - DNA (or DNA and Fusions) Ion Reporter™ Software analysis workflows. In these analysis workflows parameters are applied that detect MSI markers, which can identify a form of genomic instability in the replication of repetitive DNA.

**Note:** MSI parameters in the software are not applied when you use the Oncomine™ Comprehensive Plus - w2.2 - Fusions - Single Sample workflow as MSI is a DNA variant.

## View MSI parameters

The parameters for MSI analysis are located in the Oncomine™ Comprehensive Plus - w2.2 - DNA - Single Sample workflow in Ion Reporter™ Software.

1. Under the **Workflows** tab, in the **Overview** screen, search for the workflow, then select it in the list.

2. In the **Details** pane to the right of the list, scroll down, then click **View** next to **Parameters**.



3. In the **Parameters** dialog, select **MSI** from the left navigation bar.

## MSI parameters

You can adjust MSI parameters to optimize your analysis results when you create or edit analysis workflows in Ion Reporter™ Software. MSI parameters are available only for some Oncomine™ analysis workflows. For a complete description of all MSI parameters see the *Ion Reporter™ Software Help*.

| Parameter name | Description |
|---|---|
| **Main tab** | |
| Enable MSI Detection | When set to true, this parameter enables detection of MSI markers for the workflow. These markers can identify a form of genomic instability in the replication of repetitive DNA. MSI often occurs in tumor cells. It leads to the appearance of multiple alleles at microsatellite loci, which can be easily identified. |
| | This parameter is set to **True** by default in the Oncomine™ Comprehensive Assay Plus analysis workflows. If you do not want to include MSI results in an analysis, you can set to the parameter to **False**. Do not change this setting in other Oncomine™ analysis workflows. |
| MSI-High Threshold | The MSI score above which a sample is considered MSI-High. This score is reported in analysis results. [1,2] |
| MSS Threshold | The MSI score below which a sample will report MSS in analysis results.[1,2] |

[1] If the MSI Score falls between the **MSI-High Threshold** and the **MSS Threshold**, a No Call is reported in the analysis results.

[2] The MSI-High and MSS thresholds are optimized based on the MSI baseline of the workflow. Do not change parameters from the default settings unless you understand how the change can affect your analysis.

## Visualize MSI analysis results

1.  There are two ways to visualize MSI analysis results in Ion Reporter™ Software:

    | Option | Description |
    |---|---|
    | Visualize MSI results from one or more analyses simultaneously from the **Analyses** table. | In the **Analyses** table, select a row for an analysis or select the checkbox next to one or more analyses that you want to visualize simultaneously, then click **Visualize**. Alternatively, click **Actions ▸ Visualize**. |
    | Visualize analysis results individually from the **Analysis Results** screen. | In the **Analyses** table, click an analysis hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**. |

    The **Analysis Visualization** screen opens to the **TMB** tab.

2.  Click the **MSI** tab.
    A table with **MSI Status**, **MSI Score**, **MSI Coverage**, and **MSI QC** is shown.

| Item | Description |
|---|---|
| **MSI Status** | A sample is assigned an MSI status that is based on the MSI Score. The MSI status can be one of the following:<br><br>• MSI-High—MSI score is greater than the threshold value set in the MSI-High Threshold parameter.<br><br>• MSS—MSI score is less than the threshold value set in the MSS Threshold parameter.<br><br>• No Call—MSI score is equal to or greater than the value entered in the **MSS Threshold** parameter AND less than or equal to the value entered in the **MSI-ThresholdHigh** parmeter.<br><br>• QCFail—Indicates determination of MSI status was not reliable due to the MSI baseline. |
| **MSI Score** | A sample-level MSI score that is calculated with individual MSI marker scores. The overall score is used to determine the MSI status of the sample. |
| **MSI QC** | Alert messages for quality control of the MSI analysis results. For more information, see "Troubleshooting" on page 97. |
| **MSI Coverage** | A combined sample-level coverage that is calculated with the individual MSI marker-level coverage. |

3. Click **Download Results** to download a report of the MSI results. The report is downloaded to the folder that is used for downloads, depending on the browser settings.

## View downloaded MSI results files

The ZIP file of downloaded MSI results contains three files.

| Filename | File type | Description |
|---|---|---|
| Details.tsv | Tab separated values (TSV) | File contains detailed information on each MSI marker in the assay. Details include Ion Reporter™ Software sample and analysis name, marker level coverage, MSI score, and MSI algorithm version. |
| Summary.tsv | Tab separated values (TSV) | File contains sample level summary of MSI results. This includes sample and analysis name in Ion Reporter™ Software, total coverage across all MSI markers, MSI score, MSI status, and MSI algorithm version. |
| MSIQC.json | JavaScript Object Notation (JSON) | File contains "flags" and warning messages associated with MSI status used by support personnel. See "Customer and technical support" on page 124. |

Extract the `MSIResults_<date>.zip` folder, then open the enclosed folder.

# Generate a final report

After you have reviewed, filtered, and sorted your analysis results, you can download a final report. The procedure described here includes creating and formatting a final report template.

1. In the Analysis Results screen for your sample, click **Generate Report**.
   The **Generate Report** workflow bar opens to the **Configuration** step. The sections of the final report can be rearranged, deleted, or edited.

2. Hover the cursor over the various sections and icons to view instructional text to help you format your final report output.

3. Enter information in editable fields (for example, edit the report name or enter background information).

4. *(Optional)* Click **Save As New Template** to save your reconfigured final report template for future use with other sample results.

5. Click **Next**, a live preview of your final report is displayed.

6. Click **Lock & Publish** to generate the final report.

7. Click **Download**.

# Download Ion Reporter™ annotation VCF or TSV files

Variant call format (VCF), and tab separated values (TSV) files of the complete or filtered results can be downloaded from the **Analysis Results** screen.

1. Click **Download**, then select **All Variants**, **Filtered Variants**, **Current Results TSV**, or **Selected Variants**.



- All variants—A VCF file that contains all variants that are included in the analysis.
- Filtered variants—A VCF file that contains the variants which were filtered IN for the analysis.
- Selected variants—A VCF file that contains your selected variants from the analysis. Each variant to be included in the selected variants VCF file must be selected in the Analysis Results before the files are downloaded.
- Current Results TSV—A tab separated values (TSV) file of the analysis results of the current analysis.

2. Click **Home ▸ Notifications** to open the **Notifications** screen, then click ⬇(Download) next to the file name to download your results.

   Alternatively, select one or more rows, then click **Download**.

   The software generates a compressed directory named <analysis name>_All.zip that is downloaded. Extract the contents of the ZIP file to access the following 9 folders: CNV_VCIB, CnvActor, HRR_Results, QC, Variants, RESULTS, MSI, signature_prediction, and Workflow_Settings. Within the Variants folder, you'll find the Oncomine™ annotated VCF file.

| Folder | Contents of folder |
|---|---|
| CNV_VCIB | <ul><li>`amplicon_data.txt` Open the file with a text editor application.</li><li>`cn_results.png`, an image file that represents the CNV amplicons in the X scale and log2 ratio in Y scale.</li></ul>  |

*(continued)*

| Folder | Contents of folder |
|---|---|
| QC | Contains a PDF of the QC report, and a folder containing coverage statistics files. |
| Variants | Contains VCF and TSV files for the CNV variants. You can open these files in Microsoft™ Excel™.<br><br>• Intermediate and Oncomine™-annotated .VCF files<br><br>• TSV files that contain Oncomine™-filtered and all somatic variants |
| Results | If the Tumor Mutational Burden parameter is enabled a Results folder is generated. The Results folder contains:<br><br>• A PDF report of the tumor mutational burden results<br><br>• TSV files that contain post-filter and somatic variants<br><br>• Image files that graphically represent the results<br><br>• TXT files that contain summaries of the TMB output<br><br>• TXT files of the base changes |
| MSI | Contains summary and detailed marker-level files with data about microsatellite markers. These markers can identify a form of genomic instability in the replication of repetitive DNA.<br><br>• `Summary.tsv`—Provides a summary of results for microsatellite markers, as shown in the **Analysis Results** table.<br><br>• `Details.tsv`—Provides marker-level information, such as individual marker MSI scores, and read coverage for individual microsatellite markers.<br><br>• `MSIQC.json`—Provides the MSI status and MSI score results for the sample. If present, the file also contains "flags" and warning messages associated with MSI status used by support personnel. |
| Workflow_Settings | Contains folders with the following files:<br><br>• A text file that describes settings used for the analysis. Open the file with a text editor.<br><br>• Configuration files used by the Ion Reporter™ Software in the analysis workflow settings. |
| CnvActor | `Gene_deletions.xls`, a file that contains information about whole gene deletions and amplicon coverage that are found in BRCA analyses. Values in the third column of the file indicate the following information for the BRCA1 and BRCA2 genes in the sample. |
| HRR_Results | Contains a .TSV file that contains a summary of the HRR results. |
| signature_prediction | The signature_prediction folder contains:<br><br>• A PDF report of the mutational signature prediction results<br><br>• Image files that graphically represent the results (Only available if a mutational signature match is found.)<br><br>• A TXT file of the trinucleotide distribution of Variants |

You can view the extracted files individually, or upload the downloaded ZIP file to an ancillary software application such as the Ion Torrent™ Oncomine™ Reporter for further analysis.

## Requirements for variant annotation in Ion Reporter™ Software

The following table summarizes the requirements that must be met for the Oncomine™ Variant Annotator to annotate variants in Ion Reporter™ Software for the Oncomine™ Comprehensive Assay Plus.

For each variant type in this table, an annotation is generated only if all conditions in the corresponding **Annotation Criteria** column are satisfied.

**Note:** You can find all relevant annotation criteria in VCF files.

| Variant type | Oncomine™ Gene Class | Oncomine™ Variant Class | Annotation criteria |
|---|---|---|---|
| Copy number amplification | Gain-of-Function | Amplification | • SVTYPE = "CNV"<br>• FILTER = "GAIN"<br>• Occurs in a designated copy-gain gene |
| Copy number deletion | Loss-of-Function | Deletion | • SVTYPE = "CNV"<br>• FILTER = "LOSS"<br>• Occurs in a designated copy-loss gene |
| Copy number exon deletion | Loss-of-Function | ExonDeletion | • SVTYPE = "CNV"<br>• FILTER = "LOSS"<br>• SUBTYPE = "BigDel" |
| Loss of Heterozygosity | Loss-of-Function | LOH | • SVTYPE = "LOH"<br>• LOH = "1"<br>• FILTER = "GAIN" or "LOSS"or "PASS" |
| Long deletion | Loss-of-Function | LongDel | • Positive mutation call<br>• SVTYPE = "LongDel" |
| Gene fusion | Gain-of-Function | Fusion | • SVTYPE = "Fusion"<br>• FILTER = "PASS"<br>• Is a targeted fusion isoform |
| Non-targeted fusion | Gain-of-Function | NonTargeted Fusion | • SVTYPE = "Fusion"<br>• FILTER = "PASS"<br>• Non-Targeted field is present<br>• NT_FUSION_IN_FRAME is not FALSE<br>• NT_FUSION_DRIVER_INVOLVED is not FALSE<br>• NT_FUSION_SECONDARY is not TRUE |

*(continued)*

| Variant type | Oncomine™ Gene Class | Oncomine™ Variant Class | Annotation criteria |
|---|---|---|---|
| RNA exon variant | Gain-of-Function | RNAExonVariant | • SVTYPE = "RNAExonVariant" or "Fusion"<br>• FILTER = "PASS"<br>• Is a targeted RNA exon variant |
| Expression imbalance | Gain-of-Function | Expression Imbalance | • SVTYPE = "RNAExonTiles"<br>• FILTER = "PASS"<br>• Record meets Targeted Isoforms Detected Requirement |
| Loss-of-function truncating de novo mutation | Loss-of-Function | Truncating | • Positive mutation call<br>• Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense<br>• Occurs in a loss-of-function gene |
| Missense hotspot mutation | Gain-of-Function / Loss-of-Function | Hotspot | • Positive mutation call<br>• Functional impact is missense<br>• Transcript and codon position occur in predefined missense hotspot list |
| In-frame hotspot mutation | Gain-of-Function / Loss-of-Function | Hotspot | • Positive mutation call<br>• Function, transcript, and coding syntax occur in predefined in-frame hotspot list |
| Splice site hotspot mutation | Gain-of-Function / Loss-of-Function | Hotspot | • Positive mutation call<br>• Transcript, location, and exon occur in predefined splice site hotspot list |
| Intronic hotspot mutation | Gain-of-Function / Loss-of-Function | Hotspot | • Positive mutation call<br>• Transcript, location, and coding syntax occur in predefined intronic hotspot list |
| Promoter hotspot mutation | Gain-of-Function / Loss-of-Function | Hotspot | • Positive mutation call<br>• Transcript, location, and coding syntax occur in predefined promoter hotspot list |
| Gain-of-function truncating hotspot mutation | Gain-of-Function | Hotspot | • Positive mutation call<br>• Function, transcript, and coding syntax occur in predefined truncating hotspot list<br>• Occurs in a gain-of-function gene |

*(continued)*

| Variant type | Oncomine™ Gene Class | Oncomine™ Variant Class | Annotation criteria |
|---|---|---|---|
| Loss-of-function truncating hotspot mutation | Loss-of-Function | Truncating | • Positive mutation call<br>• Function, transcript, and coding syntax occur in predefined truncating hotspot list<br>• Occurs in a loss-of-function gene |
| MNV hotspot mutation | Gain-of-Function / Loss-of-Function | Hotspot | • Positive mutation call<br>• Transcript and coding syntax occur in MNV hotspot list |
| EGFR exon 19 deletion | Gain-of-Function | EGFRExon19 Deletion | • Positive mutation call<br>• Functional impact is nonframeshift deletion, or nonframeshift block substitution<br>• Deletion impacts codons 744-761 of EGFR |
| EGFR exon 20 insertion | Gain-of-Function | EGFRExon20 Insertion | • Positive mutation call<br>• Functional impact is nonframeshift insertion, or nonframeshift block substitution<br>• Insertion impacts codons 762-775 of EGFR or variant is COSM26720 |
| ERBB2 exon 20 insertion | Gain-of-Function | ERBB2Exon20 Insertion | • Positive mutation call<br>• Functional impact is nonframeshift insertion, or nonframeshift block substitution<br>• Insertion impacts codons 770-783 of ERBB2 |
| MET exon 14 skipping | Gain-of-Function | METExon14 Skipping | • Positive mutation call<br>• Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET Exon 14 Skipping confirmed list |

# A

# Tips and troubleshooting

## Tips

- Arrange samples in alternating columns on the plate for easier pipetting with multichannel pipettes during purification with the DynaMag™–96 Side Magnet.
- Plate seals can be firmly applied using the applicator in the MicroAmp™ Adhesive Film Applicator. Plate seals can be removed with less effort when hot. Try removing seals right after taking the plate out of the thermal cycler.
- Use IonCode™ Barcode Adapters to avoid handling and diluting adapters. Alternatively, combine and dilute Ion Xpress™ Barcode Adapters in large batches, then carefully aliquot into 96-well plates.
- If library yield is below 50 pM, libraries can still be sequenced by adding a proportionally larger volume to a combined library or template preparation.
- 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<br><br>**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.<br><br>Requantify sample RNA with a Qubit™ Fluorometer. |
| | Sample DNA or RNA quality was low. | Add more DNA or RNA or increase target amplification cycles. |
| | PCR, digestion, or ligation was inefficient. | Ensure proper dispensing and mixing of viscous components at each step. |
| | Residual ethanol in the sample DNA or RNA inhibited target amplification. | Incubate uncapped tube in hood for 1 hour. |
| | | Speed-vac tube at room temperature for 5 minutes. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Library concentration is low– general<br><br>**Details:** (Library concentration is NOT indicative of quality.)<br>*(continued)* | Residual ethanol from AMPure™ purification inhibited library amplification. | Carefully remove all drops of ethanol before library amplification, then centrifuge plate, if needed. |
| | AMPure™ XP beads were over-dried. | Do not dry the AMPure™ XP beads more than 5 minutes. |
| | FFPE RNA was not heat treated before reverse transcription. | Heat FFPE RNA at 80°C for 10 minutes, then cool to room temperature before reverse transcribing. |
| 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 |
|---|---|---|
| Pool representation is not balanced<br><br>**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.<br><br> | 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. |
| 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). |

## Other

| Observation | Possible cause | Recommended action |
|---|---|---|
| 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. |
| 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. |
| | 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. |
| MSI QC Failure<br><br>**Details:** Error message: "Not enough markers with coverage X" | MSI marker(s) had insufficient coverage to reliably calculate MSI. This is common in NTC samples. | If the sample is not an NTC, review the previous "Troubleshooting" topics to ensure sufficient coverage. For further assistance, contact support (see "Customer and technical support" on page 124). |
| MSI QC Failure<br><br>**Details:** Error messge: "RMC Calibration Failure" | Insufficient RMC read coverage for calibration. RMC reagent may have been omitted during library preparation. | Remake library including RMC in the target amplification reaction, then sequence the new library. |

# B Supplemental information

## Update Oncomine™ Comprehensive Assay Plus templates in Torrent Suite™ Software

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™ Comprehensive Assay Plus, then in that row click **Update**.

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

## Install Oncomine™ Comprehensive Assay Plus analysis workflows in Ion Reporter™ Software

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

## Download and install BED files

Contact your service representative to obtain the latest versions of Oncomine™ Comprehensive Assay Plus 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.bed
```

    d. Click **Open**, then click **Upload Hotspots File**.

The installed BED files appear in the dropdown lists in the Ion Reporter™ Software.

# 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.

6. Click **Get Versions**, select **Ion Reporter 5.18 or later**, then click ✓**Add**.

# Set TMB Classification parameters

Before the Ion Reporter™ Software analysis workflow can determine the TMB classification the threshold parameters must be set in the analysis workflow. Default threshold values (0) result in a value of **Undefined**.

1. Under the **Workflows** tab, in the **Overview** screen, search for the workflow, then select it in the list.

2. In the **Details** pane to the right of the list, click ✿ ▸ **Copy** (to create a new analysis workflow) or **Edit** to modify the existing analysis workflow.

3. Advance to the **Parameters** step in the workflow bar.

4. Click the **Tumor Mutation Burden** tab.
   The **Tumor Muational Burden Filter Chain** and the **Tumor Mutational Burden Calculation Version** are displayed.

Main | Tumor Mutational Burden

**Tumor Mutational Burden Filter Chain**

Analysis filter chain applied post variant calling to filter in variants to be included for TMB calculation

ion  TMB (Non-germline Mutatio...  ▼

**Tumor Mutational Burden Calculation Version**

Analysis version for generating tumor mutational burden results

V4.0

5. Scroll to **TMB-Low Threshold**, then enter threshold values for the following parameters.

| Item | Description |
|------|-------------|
| TMB-Low Threshold | Tumor Mutational Burden (mut/mb) threshold below which a sample is defined TMB-Low. |
| TMB-High Threshold | Tumor Mutational Burden (mut/mb) threshold above which a sample is defined TMB-High. |

6. Click **Next** to advance to the **Confirm** step, then click **Save Workflow**.

# C

# CNV baseline creation

In Ion AmpliSeq™ assays, Copy Number estimates are made by counting reads for each amplicon, making adjustments to account for certain types of variability, comparing those read counts to expected counts for those amplicons in a "normal" sample, and then making further adjustment.

Known sources of variability include pool imbalance (when the assay has more than one pool of amplicons), total number of reads and per amplicon attributes of GC proportion, and length of the amplicon insert. In practice, we observe other variability that does not associate with known attributes yet is systematic. The method that we use trains on many diverse samples, captures systematic effects, and encodes these into a file (the "baseline").

When augmenting a baseline, new samples are run, the size of each systematic effect encoded in the baseline is estimated, and a correction is applied to remove the effect. These added samples need not be normal, and should be diverse so as to capture likely systematic variation.

The following instructions walk you through using the new Variability Correction Information Baseline (VCIB) CNV baseline, creating a new VCIB CNV baseline, or augmenting an existing VCIB CNV baseline for Oncomine™ Comprehensive Assay panels.

## Use VCIB CNV baseline

If you want to use the VCIB CNV baseline included in Ion Reporter™ Software 5.18, simply select it when creating your workflow.

Note:  The VCIB CNV baseline is currently noncompatible with the Ion GRCh38 human reference.

# Create a CNV baseline

Ion Reporter™ Software provides a wizard to guide you through Copy Number Variation (CNV) Baseline creation.

1.  In the **Workflows** tab, in the **Presets** screen, click **Create Preset**, then select **Copy Number Baseline** from the dropdown list.

    | Create Preset ▾ |
    | --- |
    | Annotation Set |
    | Filter Chain |
    | Copy Number Baseline |
    | Final Report Template |

2.  Click **AmpliSeq**, select **Oncomine™ Comprehensive Plus DNA Regions v1.3** as your Target Regions file, then click **Next**.

3.  Select at least 48 samples, flag at least 6 of the selected samples as "Normal" by selecting the checkbox in the **Normal** column, then click **Next**.
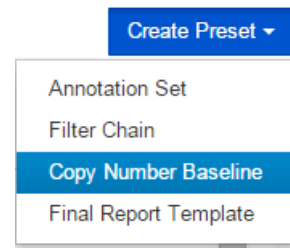
    Note: Male or Female gender must be specified for Normal samples, but samples that are not flagged as normal can be male, female, or unknown. You can use the Summary panel to see your totals.



4.  Enter a name for your baseline, then click **Create Baseline**.

    Note: Log files for both successful and failed analyses include the **BaselineCreation.log** file, which has the BAM files named that were rejected due to similarity to other files in the baseline, as well as the **map.TmapMergeActor-00.err** file that has the BAM files named that were rejected due to QC failure.

To add this new baseline to your workflow, proceed to "Create an Ion Reporter analysis workflow" on page 108.

# Augment (add Samples to) an existing VCIB CNV baseline

Ion Reporter™ Software provides a wizard to guide you through Copy Number Variation (CNV) baseline creation. This example describes how to add additional samples to an existing CNV baseline.

1. Under the **Workflows** tab, in the **Presets** screen, click **Create Preset**, then select **Copy Number Baseline** from the dropdown list.
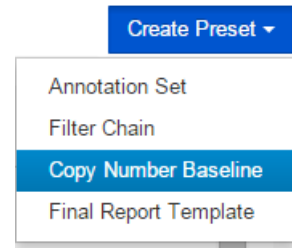
2. Click **AmpliSeq**, select **Oncomine™ Comprehensive Plus DNA Regions v1.3** as your **Targets Region** file, then click **Next**.

3. Select the **Start with an existing CNV Baseline** checkbox, then select a baseline from the dropdown list.
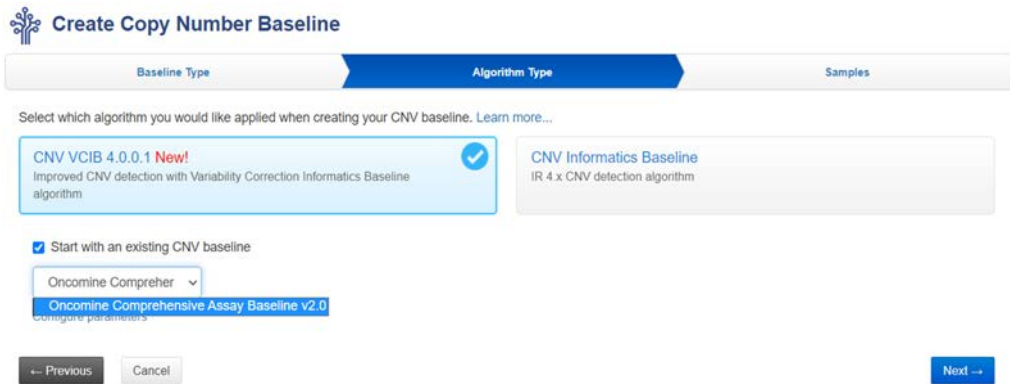


   **Note:** By default, the software prompts you to add another 48 samples. However, you can set the number to 1 or more. Add non-Normal samples. Marking samples as "Normal" in the augmentation workflow has no effect, only the original Normals in the first baseline creation are treated as Normals in the augmented baseline.

4. Click the **Configure Parameters** link.

5. In the **Configure Parameters** dialog, click **Cnv Baseline Creation**, then **Advanced**. Set the **Minimum number of samples required to add to an existing baseline** to the number you are adding, click **Done**, then click **Next**.



6. Select additional samples, then click **Next**.



7. Enter a name for your baseline, then click **Create Baseline** to save.

# Create an Ion Reporter analysis workflow

Ion Reporter™ Software provides a wizard to guide you through creating a workflow. However, it can be easier to copy an existing Oncomine™ workflow and edit it by adding your newly created baseline.

1. In the **Workflows** tab, in the **Overview** screen, select an appropriate Oncomine™ Comprehensive workflow.

2. In the **Details** pane, click ⚙ **Actions** ▸ **Copy**.
   The workflow wizard opens to the **Research Application** step in the **Create** screen.

3. Click **Next** to advance to the **Reference** step.

4. Confirm that **hg19** is the selected **Reference**, then select a **Target Regions**, **Hotspot Regions**, and **Fusions** BED file from the respective dropdown lists. Click **Next**.

5. Select an **Annotation Set** from the dropdown list, then click **Next**.

6. Select a **Filter Chain** from the dropdown list, then click **Next**.

7. In the **Copy Number** step, select the baseline that you want to use from the **Baseline** dropdown list, then click **Next**.

| Application | Reference | Annotation | Filters | **Copy Number** | Plugins | Final Report |
|---|---|---|---|---|---|---|

Baselines provide a reference point against which CNVs can be detected. This is required if you wish to detect CNVs in a single sample analysis. If not provided no CNVs will be called. Learn more…

**Baseline**

FC20_CNVBase_Oncomi...  ▾

**Settings**

Name:  FC20_CNVBase_Oncomine_Panel_v1_2_Regions_Comprehensive_100Sample_10Normals_0901_np

Can't find what you're looking for? Configure in Workflow Presets.

8. In the **Plugins** step, ensure that all **In-Analysis** plugins are deselected, then click **Next**.

9. Select a **Final Report Template** from the dropdown list, then click **Next**.

10. In the **Parameters** step, review the default settings, then click **Next**.

    **Note:**  Although **Read Mapping** parameters are exposed in workflow creation, it is not necessary to change any settings.

11. In the **Confirm** step, enter a **Workflow Name** and **Description**, then click **Save Workflow**.

# Launch an analysis

1. In the **Home** tab, in the **Dashboard** screen, click **Launch Analysis**.

2. In the **Launch Analysis** wizard, in the **Workflow** step, select your custom workflow or one of the pre-installed Oncomine™ workflows, then click **Next**.

3. Select the samples to include in the analysis.

    a. Use the **Samples** dropdown list to filter the available samples.

    b. Click within a sample row to select each sample to include in a sample group, then click **Add Samples** in the **Sample Groups** pane.

    c. Enter a **Group Name**, then click **Add to Analysis**.
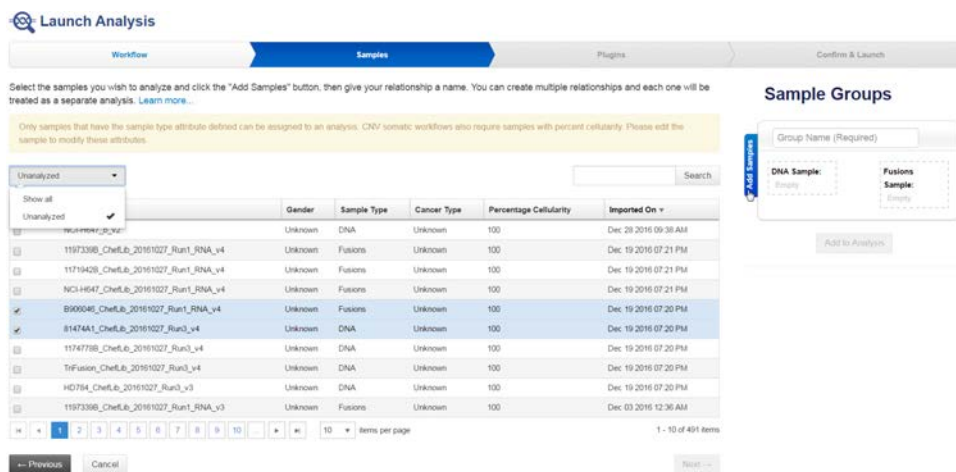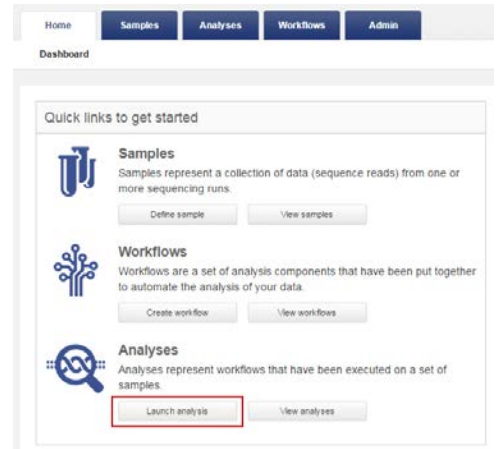
**Note:** The **Percentage Cellularity** sample attribute is required for DNA samples.

4. Repeat substep 3b and substep 3c to add additional **Sample Groups**.

5. Click **Next** 2 times to move to the **Confirm & Launch** step.

6. In the **Confirm & Launch** step, enter an **Analysis Name** and **Description** for the analysis, then click **Launch Analysis**.
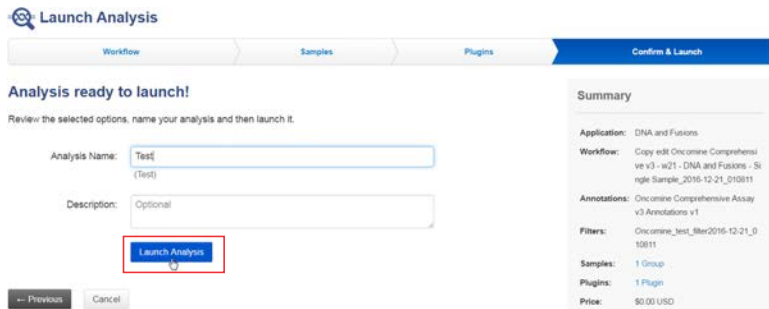


7. Review your results in the **Analyses** tab. Confirm the CNV workflow and baseline used in the **Details** pane.

8. *(Optional)* Download the analysis results, then review the results visually with the cn_results.png.
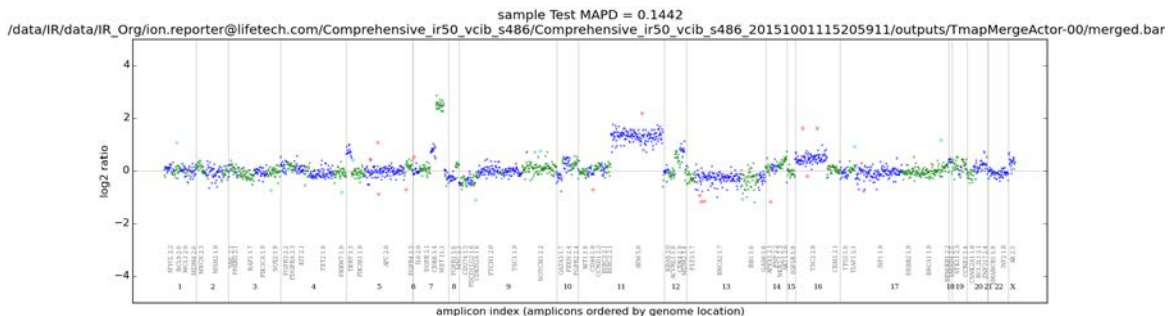


Interpretation example: This plot shows log2 ratios across the genome and highlights panel CNV IDs. The alternating blue and green color is used to distinguish between adjacent CNV IDs. The outliers data are the small pink circles. The numbers on the X axis are the chromosomes. Above this are the CNV ID names and the mean CN call for each CNV ID. You can see copy number gains on chromosome 7 and chromosome 11. The MAPD number at the top of the plot is a QC metric measuring the noisiness of the sample. A low MAPD is good. MAPD >0.5 is considered to fail QC. Below the MAPD is the BAM file name. Log2 ratios of 0 are equivalent to a copy number call of 2 (normal). If the sample was a male, you would expect to see a copy number of 1 on chromosome X.

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# CNV somatic confidence filter

## CNV somatic confidence range

The somatic CNV algorithms in Ion Reporter™ Software deliver not only a ploidy estimate call, but also a 90% confidence interval. The 5% lower confidence bound value is the ploidy estimate where there is 95% confidence that the true ploidy is above that value. The 95% upper confidence bound is the ploidy estimate where it is 95% certain that the true ploidy is below that value. For calling focal amplification, the lower bound is important and not the upper bound.

Note:  The Oncomine™ Variant Annotator plugin annotates somatic CNVs in the Oncomine™ Comprehensive Assay Plus results in the following categories:

- Copy-number gain genes whose 5% confidence bound is greater than or equal to the expected normal ploidy plus 2.
- Copy-loss number genes whose 5% confidence bound is less than or equal to the expected normal ploidy minus 2.

## How to change the CI threshold default value

Note:  The default CI threshold value of 0.0 will find all copy-gain genes whose 5% CI value is of ploidy 2 or greater and all copy-loss genes whose 95% CI value is of ploidy less than 2.

To change the confidence interval threshold default value, you must create a custom somatic CNV filter, then reanalyze the sample.

1. Sign in to the Ion Reporter™ Software.

2. In the **Workflows** tab, in the **Presets** screen, click **Create Preset ▸ Filter Chain** .
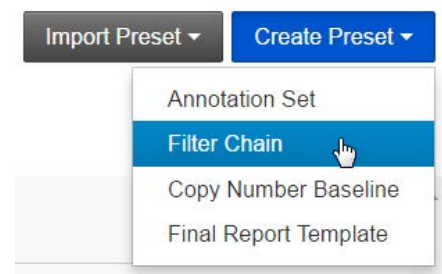
3. Enter a **Name** *(required)*, and **Description** *(optional)*, for the new Filter Chain.



4. Ensure the **Reference** hg19 radio button is selected.

5. Select **CNV Somatic Confidence - CNVs Only** from the **Choose Filter** dropdown list.

6. Ensure **Enabled** is selected for both **Minimum Ploidy Gain (5% CI) over expected** and **Minimum Ploidy Loss (95% CI) under expected**.

---

Note: Deselect **Minimum Ploidy Loss (95% CI) under expected** to filter only for copy-gain genes.

---

7. Enter your desired threshold values in the respective **GREATER_THAN** fields, then click **Set**.

---

Note: The new value should be the ploidy of gain you want to threshold on when looking at the respective confidence interval value.

---

For example, setting the **Minimum Ploidy Gain (5% CI) over expected** threshold value to 1.0 will cause the filter to look for all copy-gain genes whose 5% CI value is of ploidy 3 or greater.

8. Click **Save**.

---

Note: See the *Ion Reporter™ Software Help* for more information on creating complex filter chains.

---

In the image in step 3, the **Minimum Ploidy Gain (5% CI) over expected greater_than** was set to 2.0 (over expected normal), so copy-gain genes with ploidy >4 will be filtered in. So if looking for copy-gain genes whose 5% CI value is anything over the expected normal of autosomes (2), leave the value set

at 0.0. The **Minimum Ploidy Loss (95% CI) under expected to be greater_than** was set to 0.0 (under the expected normal), so only genes with a ploidy <2 will be filtered in as copy-loss. For example, the following example CNV call confidence interval data would result in the CNV data to be filtered in or out of the results:

- A gene with suspected gain with 5% CI = 4.1% and 95% CI = 10.3 will be filtered in (5% CI >4).

- A gene with suspected loss with 5% CI = 0% and 95% CI = 1.0 will be filtered in (95% CI <2).

- A gene with suspected gain with 5% CI = 2.2% and 95% CI = 3.6 will be filtered out (5% CI 2.2 <4% and 95% CI 3.6>2).

- A gene with 5% CI = 0.8% and 95% CI = 2.1 will be filtered out (0.8 is less than 4 and 2.1 is greater than 2) (5% CI 0.8<4% and 95% CI 2.1>2).

# Subset filter creation

If you do not want to generate information on all the Oncomine™ variants, you can create subset filters to look at only certain genes and variants. This appendix provides examples for creating subset filters.

## Create a gene-level filter

If you just want to filter for a set of genes in your Oncomine™ analyses, applying the Gene Symbol filter is the easiest approach.

1. In the **Analyses** tab, in the **Overview** screen, click on the desired **Analysis** hyperlink in the **Analyses** table.

2. In the **Filter Options** pane, click 📄 **(New)**.

3. In the **Create Filter Chain** dialog, enter a **Name** and **Description** for your gene-level filter.

4. Click **Choose Filter**, then select **Gene Symbol** from the dropdown list.

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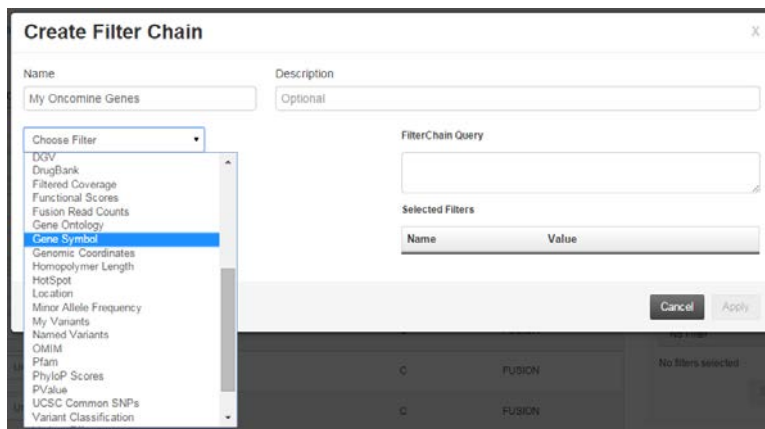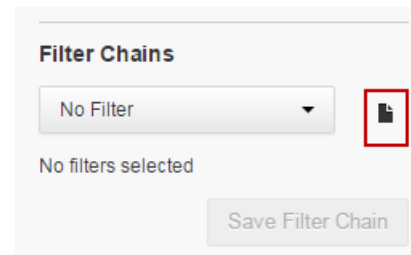
5. In the **Search** field, enter a gene symbol of interest (for example, EGFR), then click **Go** or press Enter.



6. Select each search result to be included, then click **Set**. Repeat step 5 and step 6 to add additional **Gene Symbols**.

7. In the **Choose Filter** dropdown list, select **Oncomine™**.

8. In the **Filter value** dropdown list, select **In**, then click **Set**.



9. Click **Apply**.

10. In the **Filter Options** pane, click **Save Filter Chain**.

   Your new filter is now available for use.

Next, copy a relevant workflow, then select this new filter chain as the default.

# Create a variant-level filter

If you only want to review a subset of variants from the <assay name>_variantDB.vcf file, you must first create a new workflow in the Ion Reporter™ Software. To create a new workflow you must first prepare a custom variantDB file and new annotation set, then copy and edit an existing workflow to use the newly created custom variantDB file and annotation set.

## Create a new variantDB from the provided file

1. In the **Workflows** tab, in the **Presets** screen, click **Create Preset ▸ Annotation Set**.
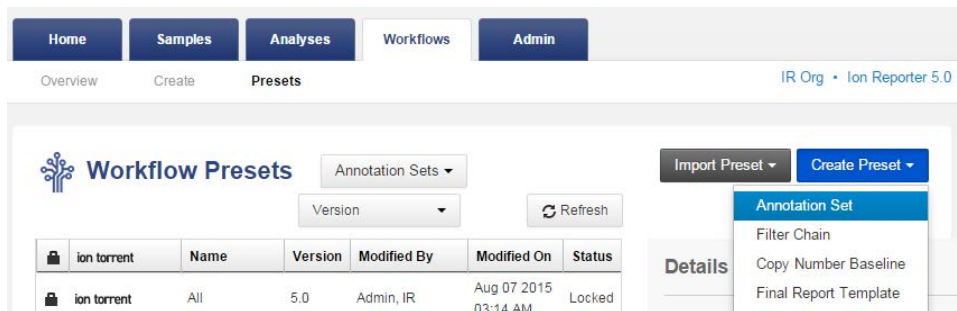
2. In the **Create Annotation Set** dialog, enter a **Name**, and **Description** for your **Annotation Set**.

3. Click **Choose Type**, then select **VariantDB (Custom)** from the dropdown list.

4. In the **Create New** tab, enter a **Name**, and **Version** for the new variantDB.

5. Click **Select File**.

6. Navigate to the <assay name>_variantDB.vcf file, click **Open**, then click **Upload**.

7. Click **Save**.
   Your new variantDB is now available in the **Workflow Presets** table in the **Presets** screen.

## Create a new annotation set from the new variantDB and existing Oncomine™ annotation sources

1. In the **Workflows** tab, in the **Presets** screen, click **Create Preset ▸ Annotation Set**.

2. In the **Create Annotation Set** dialog, enter a **Name** and **Description** for your new **Annotation Set**.

3. Click **Choose Type**, then select **VariantDB (Custom)** from the dropdown list.

4. In the **Choose Existing** tab, scroll down, select the variantDB file that you previously created, then click **Use**.
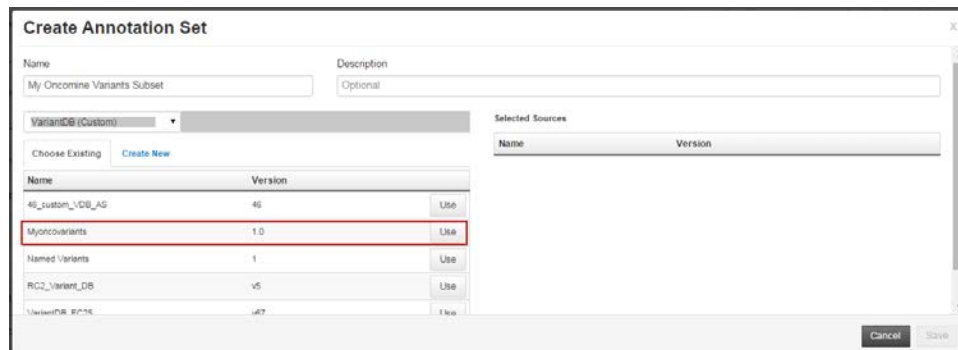


5. In the **Choose Type** dropdown list, select **5000Exomes**, then click **Use**. Repeat for the following sources:

   - ClinVar
   - dbSNP
   - DGV
   - DrugBank
   - Gene Ontology
   - Pfam

   - PhyloP Scores
   - RefGene Functional Canonical Transcripts Scores
   - RefSeq GeneModel
   - Oncomine™ Canonical Transcripts

   Click **Use** after selecting each.

   ---

   Note:  Some annotation sets are under existing selections in the dropdown list:
   - **RefGene Functional Canonical Transcripts Scores** is under **SIFT/PolyPhen**
   - **RefSeq GeneModel** is under **Gene Model**
   - **Oncomine™ Canonical Transcripts** is under **Transcript Set (Custom)**

   ---

6. Click **Save**.

Your new **Annotation Set** is now available in the **Workflow Presets** table in the **Presets** screen.

## Create a new filter chain using the new variantDB

1. In the **Workflows** tab, in the **Presets** screen, click
**Create Preset ▸ Filter Chain**.

2. In the **Create Filter Chain** dialog, enter a **Name** and
**Description** for your new **Filter Chain**.

3. Click **Choose Filter**, then select the variantDB you
created in the dropdown list.

4. Select the variants that you want to filter in, then click
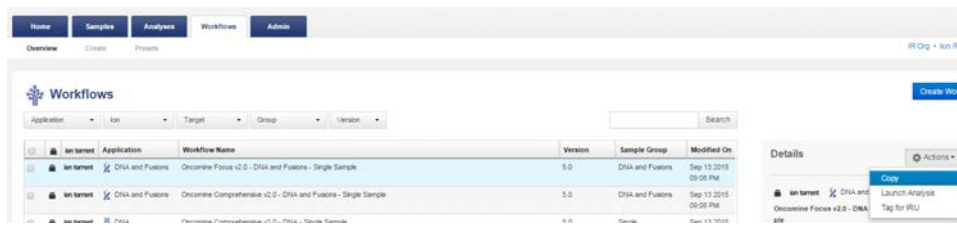**Set**.

5. Click **Save**.

You can now apply your new variant subset filter to analyses.
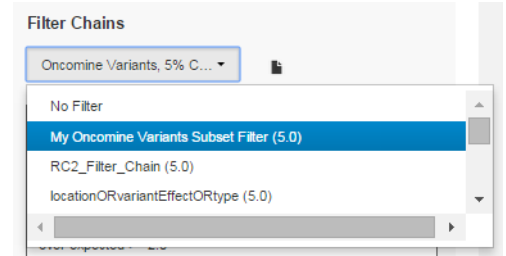
## Create a copied workflow with the new annotation set and filter chain

Now you need to create a new workflow to use the annotation set and filter chain that you created.
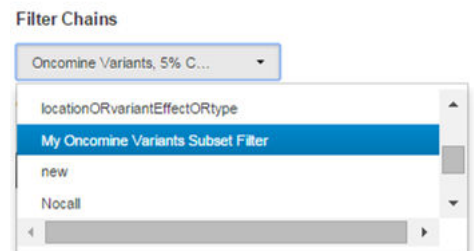
1. In the **Workflows** tab, in the **Overview** screen, select the workflow to be copied, then click **Actions ▸ Copy** in the **Details** pane.

2. In the **Annotation** step, add the new annotation set.
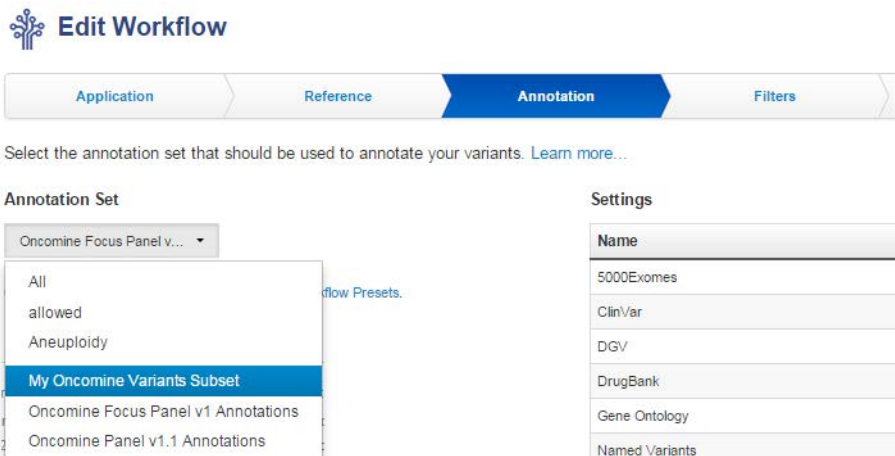
3. In the **Filters** step, add the new filter chain.

4. In the Confirm step, name the workflow.

5. Click **Save Workflow**.
   Your new workflow is now available for use in the **Workflows** tab.

# Use the new workflow

Your variants subset workflow is now ready for use.

Select your new workflow, then click **Actions ▸ Launch Analysis**.

# F 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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

# Documentation and Support

## Related documentation

| Document | Description |
|---|---|
| *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) | Describes the automated preparation and templating of Oncomine™ Comprehensive Assay Plus libraries using the Ion Chef™ System. |
| *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003) | Comprehensive instruction for the preparation of Ion AmpliSeq™ libraries and provides detailed instruction and troubleshooting for use of the Ion Library Equalizer™ Kit. |
| *Ion 550™ Kit – Chef User Guide* (Pub. No. MAN0017275) | Describes the automated template preparation of Oncomine™ Comprehensive Assay Plus libraries using the Ion Chef™ System for sequencing on the Ion S5™ System. |
| *Ion Library TaqMan™ Quantitation Kit User Guide* (Pub. No. MAN0015802) | Provides detailed instruction and troubleshooting for use of the Ion Library TaqMan™ Quantitation Kit. |
| *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan™ RNAse P Detection Reagents Kit* (Pub. No. MAN0007732) | Provides detailed instruction for sample quantification using the TaqMan™ RNAse P Detection Reagents Kit. |
| *Ion Reporter™ Software 5.18 User Guide* (Pub. No. MAN0024777) | Comprehensive instruction for use of Ion Reporter™ Software. |

**Note:** For additional documentation, see "Customer and technical support" .

## Customer and technical support

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

- Worldwide contact telephone numbers
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  - Product FAQs
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  - Training for many applications and instruments

- Order and web support
- 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**.

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