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Oncomine™ Comprehensive Assay v3

USER GUIDE

Catalog Number A35805, A35806, A36111

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Revision C.0

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ThermoFisher SCIENTIFIC

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Revision history: Pub. No. MAN0015885

Revision	Date	Description
C.0	18 April 2019	• Moved "Transfer the DNA amplicons" after "Partially digest the amplicons" in "Manual library preparation". • Updated product information in "Required materials not supplied" and "Recommended materials".
B.0	26 February 2019	• Updated the catalog numbers for the Oncomine™ Comprehensive Assay v3. • Updated the information in "Contents and storage" to reflect the new catalog numbers, including the amount of each component provided. • Updated the list of recommended nucleic acid isolation kits in "Recommended materials". • Added information for the 96-reaction kit size. • Added new procedural guidelines. • Updated the Chef-Ready protocol and re-organized Chapter 3, "Library preparation" to accommodate both, Chef-Ready and Manual library preparation procedures. • Added information on updating Alignment Analysis Parameter when creating a custom Planned Run template in Torrent Suite™ Software (see page 31). • Updated Chapter 4, "Create a Planned Run" to include the latest BED files and screen shots. • Updated workflow names in "Analysis workflows in Ion Reporter™ Software". • Updated the procedure in "View results". • Added new topic: "Generate an Analysis Results Report". • Updated "Download Ion Reporter™ annotation VCF or TSV files" to include ZIP file folder descriptions. • Updated Variant Type and Annotation Criteria information in "Oncomine™ Comprehensive Assay v3 with Ion Reporter™ Software 5.4 or later". • Removed reference to the discontinued Ion 520™ & Ion 530™ Kit – Chef and the corresponding user guide. • Updated "Related documentation".
A.0	5 April 2017	Oncomine™ Comprehensive Assay v3 Library Preparation User Guide, provides instruction for library preparation, templating, sequencing, and results analysis of Oncomine™ Comprehensive Assay v3 libraries.

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1 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 v3 contains targeted, multi-biomarker panels that enable simultaneous detection of hundreds of variants across 161 genes relevant to solid tumors. This assay allows concurrent analysis of DNA and RNA to simultaneously detect multiple types of variants, including hotspots, single nucleotide variants (SNVs), insertions and deletions (INDELs), copy number variants (CNVs), and gene fusions, in a single workflow.

This guide covers library preparation from DNA or RNA using the Ion AmpliSeq™ Library Kit Plus and the DNA Oncomine™ Comprehensive Panel v3M or RNA Oncomine™ Comprehensive Panel v3M. The assay can be used with barcoded adapters so that up to seven paired DNA and RNA samples plus DNA and RNA no template controls (NTCs) can be combined and loaded onto a single Ion Chip™ in a single workflow to minimize the per-sample sequencing cost. The DNA Oncomine™ Comprehensive 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 v3C kit (Cat. No. A35806, see page 8). The kit provides DNA Oncomine™ Comprehensive Panel v3C (2-pools) and RNA Oncomine™ Comprehensive Panel v3C (2-pools) at 2X concentration pre-measured in barcoded primer pool tubes ready to load into an Ion AmpliSeq™ Chef Reagents DL8 cartridge.

This guide covers the following products:

- Oncomine™ Comprehensive Assay v3M (Cat. Nos. A35805, A36111)
- Oncomine™ Comprehensive Assay v3C (Cat. No. A35806)
- Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990)
- Ion Xpress™ Barcode Adapters (various Cat. Nos.)
- IonCode™ Barcode Adapters 1–384 Kit (Cat. No. A29751)
- Ion Library Equalizer™ Kit (Cat. No. 4482298)

Contents and storage

Oncomine™ Comprehensive Assay v3M

The 24-reaction Oncomine™ Comprehensive Assay v3M (Cat. No. A35805) consists of the DNA Oncomine™ Comprehensive Panel v3M (2-pool) (Part No. A33636), RNA Oncomine™ Comprehensive Panel v3M (2-pool) (Part No. A33637), and two Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990) for the rapid preparation of barcoded sample libraries from DNA and RNA.

The 96-reaction Oncomine™ Comprehensive Assay v3M (Cat. No. A36111) consists of the DNA Oncomine™ Comprehensive Panel v3M (2-pool) (Part No. A35403), RNA Oncomine™ Comprehensive Panel v3M (2-pool) (Part No. A35408), and two Ion AmpliSeq™ Library Kit Plus (Cat. No. A35907) for the rapid preparation of barcoded sample libraries from DNA and RNA.

Contents	Amount		Storage
	24 reactions	96 reactions	
DNA Oncomine™ Comprehensive Panel v3M			
2X DNA Oncomine™ Comprehensive Panel v3M (blue cap) (pool 1 of 2)	3 × 40 µL	480 µL	–30ºC to –10ºC
2X DNA Oncomine™ Comprehensive Panel v3M (blue cap) (pool 2 of 2)	3 × 40 µL	480 µL	
RNA Oncomine™ Comprehensive Panel v3M			
5X RNA Oncomine™ Comprehensive Panel v3M (red cap) (pool 1 of 2)	3 × 16 µL	192 µL	–30ºC to –10ºC
5X RNA Oncomine™ Comprehensive Panel v3M (red cap) (pool 2 of 2)	3 × 16 µL	192 µL	
Ion AmpliSeq™ Library Kit Plus			
5X Ion AmpliSeq™ HiFi Mix (red cap)	120 µL	480 µL	–30ºC to –10ºC
FuPa Reagent (brown cap)	48 µL	192 µL	
Switch Solution (yellow cap)	96 µL	384 µL	
DNA Ligase (blue cap)	48 µL	192 µL	
25X Library Amp Primers (pink cap)	48 µL	192 µL	
1X Library Amp Mix (black cap)	1.2 mL	4 × 1.2 mL	
Low TE	6 mL	2 × 6 mL	15°C to 30°C[1]

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

Oncomine™ Comprehensive Assay v3C

The Oncomine™ Comprehensive Assay v3C (Cat. No. A35806) provides the DNA Oncomine™ Comprehensive Panel v3C (Part No. A33638) and RNA Oncomine™ Comprehensive Panel v3C (Part No. A33639) 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 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
DNA Oncomine™ Comprehensive Panel v3C (32 reactions)		
2X DNA Oncomine™ Comprehensive Panel v3C (pool 1 of 2)	4 × 150 µL	–30°C to –10°C
2X DNA Oncomine™ Comprehensive Panel v3C (pool 2 of 2)	4 × 150 µL	
RNA Oncomine™ Comprehensive Panel v3C (32 reactions)		
2X RNA Oncomine™ Comprehensive Panel v3C (pool 1 of 2)	4 × 150 µL	–30°C to –10°C
2X RNA Oncomine™ Comprehensive Panel v3C (pool 2 of 2)	4 × 150 µL	
Ion AmpliSeq™ Kit for Chef DL8 (Cat. No. A29024)		
Ion AmpliSeq™ Chef Reagents 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) • Ion AmpliSeq™ Tip Cartridge L8 • PCR Frame Seal • Enrichment Cartridge	2 boxes with 4 inserts	15°C to 30°C
IonCode™ 0101–0132 in 96 Well PCR Plates (dried) (Part No. A29028) Set includes 4 PCR plates: • IonCode™ 0101–0108 in 96 Well PCR Plate (red) • IonCode™ 0109–0116 in 96 Well PCR Plate (yellow) • IonCode™ 0117–0124 in 96 Well PCR Plate (green) • IonCode™ 0125–0132 in 96 Well PCR Plate (blue)	2 sets of 4 plates	15°C to 30°C

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

Required materials not supplied

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

Item	Source
IonCode™ Barcode Adapters 1–384 Kit or Ion Xpress™ Barcode Adapters Kit	A29751 or 4474517[1]
Ion Library Equalizer™ Kit[2]	4482298
Agencourt™ AMPure™ XP Kit	Beckman Coulter A63880 or A63881
(RNA only) SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme	11766050
One of the following thermal cyclers: • ProFlex™ 96-well PCR System • SimpliAmp™ Thermal Cycler • Veriti™ 96-Well Thermal Cycler • 2720 Thermal Cycler[3] • GeneAmp™ PCR System 9700 96-Well[3] or GeneAmp™ PCR System 9700 Dual 96-Well[3]	Various
MicroAmp™ Optical 96-Well Reaction Plate or MicroAmp™ Optical 96-Well Reaction Plate with Barcode	N8010560 or 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 **fisherscientific.com**
Nuclease-free Water	AM9932
Ethanol, Absolute, Molecular Biology Grade	BP2818500 **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] Not required for use with Chef Ready kits.
[3] Supported but no longer available for purchase.

Recommended materials

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

Item	Source
Recommended additional equipment	
One of the following Applied Biosystems™ real-time PCR instruments: • 7500 Real-Time PCR System • 7900HT Fast Real-Time PCR System[1] • StepOne™ Real-Time PCR System • StepOnePlus™ Real-Time PCR System • ViiA™ 7 Real-Time PCR System • QuantStudio™ 3 Real-Time PCR System • QuantStudio™ 5 Real-Time PCR System • QuantStudio™ 7 Flex Real-Time PCR System • QuantStudio™ 12K Flex Real-Time PCR System	Various
96-well plate centrifuge	MLS
Qubit™ 4 Fluorometer[2]	Q33226
Recommended for nucleic acid isolation	
RecoverAll™ Multi-Sample RNA/DNA Workflow	A26069
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
Ion AmpliSeq™ Direct FFPE DNA Kit	A31133, A31136
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
Recommended for library quantification	
Ion Library TaqMan® Quantitation Kit	4468802
Recommended controls	
Horizon Quantitative Multiplex Reference Standard	HD200
Horizon ALK-RET-ROS1 Fusion RNA Reference Standard	HD784
AcroMetrix™ Oncology Hotspot Control	969056

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

2 Before you begin

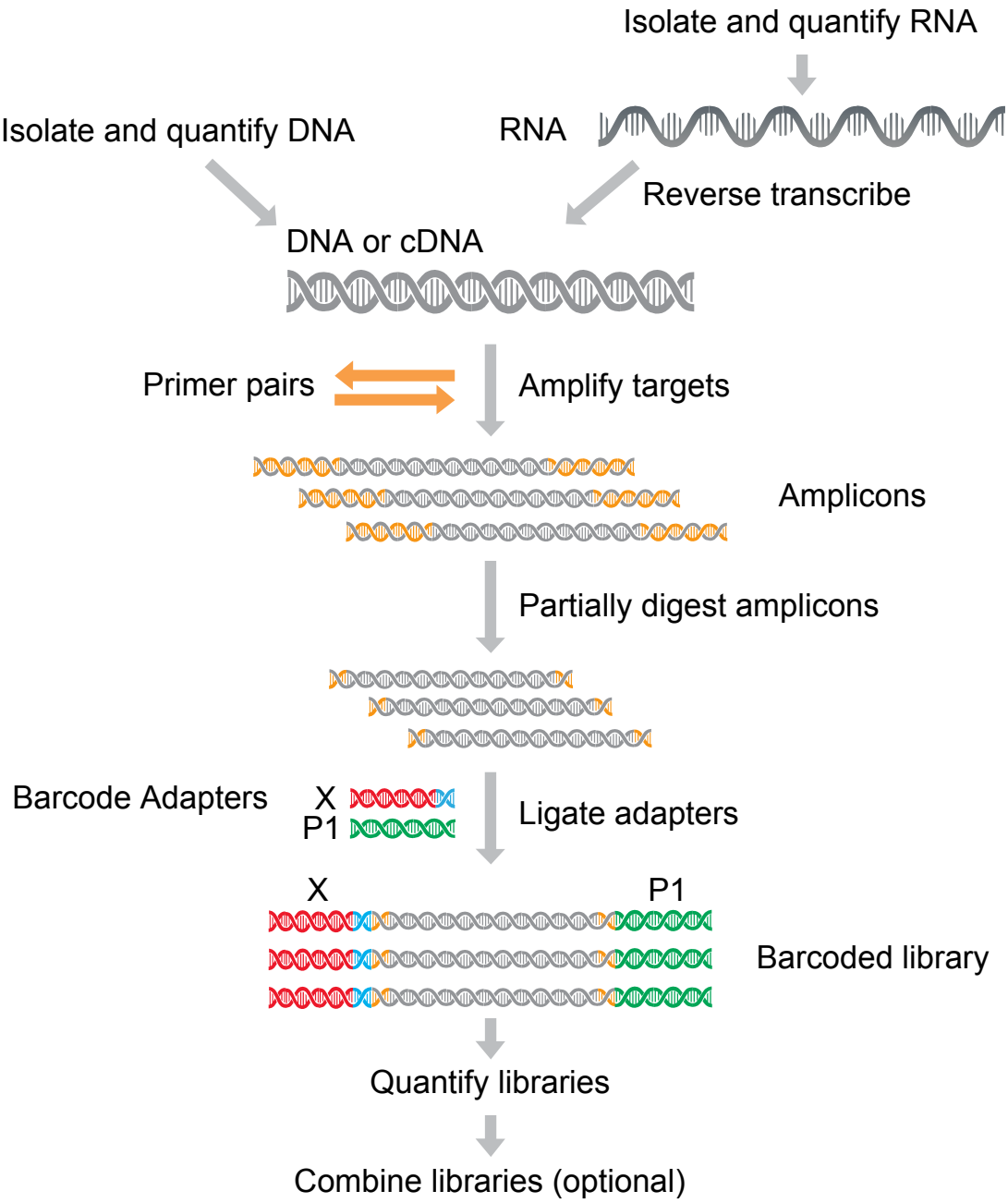
Procedural guidelines

- Minimize freeze-thaw cycles of Oncomine™ Comprehensive Assay panels by aliquoting as needed for your experiments. Panels can be stored at 4°C for one year.
- Use good laboratory practices to minimize cross-contamination of products. If possible, perform PCR setup in an area or room that is free of amplicon contamination. Always change pipette tips between samples.
- Use a calibrated thermal cycler specified in "Required materials not supplied".
- 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.

Library preparation from genomic DNA or RNA



3 Library preparation

IMPORTANT! If you are using the Oncomine™ Comprehensive Assay v3C (Cat. No. A35806) with RNA Oncomine™ Comprehensive Panel v3C (Part No. A33639), we recommend that you perform the reverse transcription as described in "Chef Ready: Library preparation" on page 14. Following completion of cDNA synthesis, see the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for instructions to prepare Oncomine™ Comprehensive Assay RNA libraries on the Ion Chef™ System.

Guidelines for RNA isolation, quantification, and input

- We recommend the RecoverAll™ Multi-Sample RNA/DNA Workflow (Cat. No. A26069) for isolating total RNA.
- We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32855) for quantifying RNA.
- Reverse transcription of each sample requires 20 ng of DNase-treated total RNA (≥2.5 ng/µL).
- In general, library yield from high quality RNA is greater than from degraded samples. Library yield is not indicative of sequencing performance.
- Increasing the amount of RNA will usually result in higher quality libraries, especially when RNA quality or quantity is unknown. We recommend using 1 ng total RNA only with high-quality, well-quantified samples.

Guidelines for DNA isolation, quantification, and input

- We recommend the RecoverAll™ Multi-Sample RNA/DNA Workflow (Cat. No. A26069) for isolating gDNA.
- 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.
- The Ion AmpliSeq™ Direct FFPE DNA Kit bypasses nucleic acid isolation when preparing libraries from FFPE sections on slides. Refer to the *Ion AmpliSeq™ Direct FFPE DNA Kit User Guide* (Pub. No. MAN0014881) for using this kit to prepare gDNA from FFPE tissue.
- The Direct FFPE DNA preparation can be stored for up to 6 months at –20°C before library preparation.
- For each target amplification reaction, use 10 ng (≥1.82 ng/µL) of mammalian gDNA from normal or FFPE tissue.
- Increasing the amount of DNA results in higher-quality libraries, especially when DNA quality or quantity is unknown.

Chef Ready: Library preparation

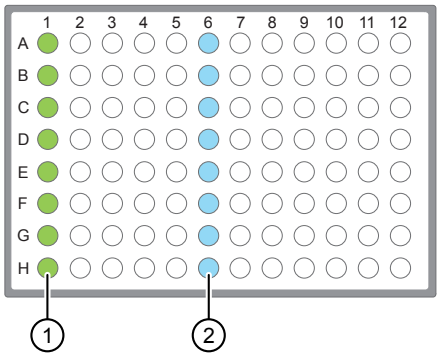
Reverse transcribe RNA for Chef Ready library preparation

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
SuperScript™ IV VILO™ Master Mix	2 µL
Total RNA (10 ng)[1]	≤8 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).



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

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

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

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

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

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

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

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

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

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

For information on how to set up the Ion Chef™ Instrument, see “Ion Chef™ Instrument setup information for Chef Ready kit users“ on page 17.

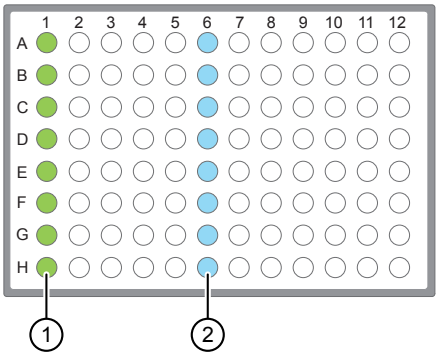
Add DNA to an IonCode™ PCR plate

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

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

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

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



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

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

Note:

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

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

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

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

For information on how to set up the Ion Chef™ Instrument, see “Ion Chef™ Instrument setup information for Chef Ready kit users“ on page 17.

Ion Chef™ Instrument setup information for Chef Ready kit users

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	15	8 minutes
FFPE DNA[1]	2	18	8 minutes
High quality RNA[1]	2	28	4 minutes
FFPE RNA[1]	2	31	4 minutes

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

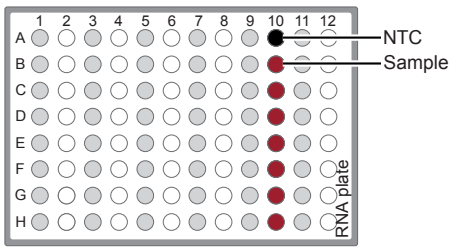
Manual library preparation

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
SuperScript™ IV VILO™ Master Mix	2 µL
Total RNA (20 ng)[1]	≤8 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).



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

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

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

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

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

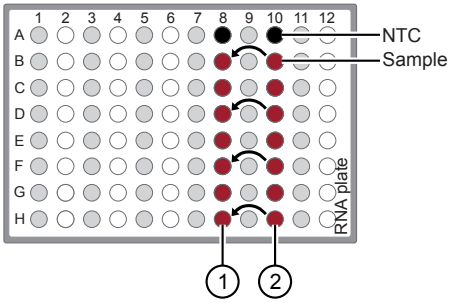
Prepare cDNA target amplification reactions

IMPORTANT! The cDNA synthesis reaction, primer pools, and HiFi Mix are viscous. Pipet slowly and mix thoroughly.

1. Place the 96-well plate in a pre-chilled cold block or on ice.
2. Thaw the 5X Ion AmpliSeq™ HiFi Mix on ice, gently vortex to mix, then briefly centrifuge to collect.
3. To each cDNA synthesis reaction add:

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
Nuclease-free Water	2 µL
Final volume (includes 10 µL cDNA synthesis reaction)	16 µL

4. Mix by pipetting at least half the total volume up and down at least 5 times, then transfer 8 µL (half the total volume) to an adjacent well.



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

5. Add 2 µL of 5X RNA Oncomine™ Comprehensive Panel primer pool 1 into the first well, then add 2 µL of primer pool 2 into the other well.
   Each well should have a final volume of 10 µL.

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

Proceed to "Amplify the cDNA targets" on page 19 .

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™ 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	99°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	27	+3	–3
FFPE RNA	30	+3	–3

[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 RNA input. 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.

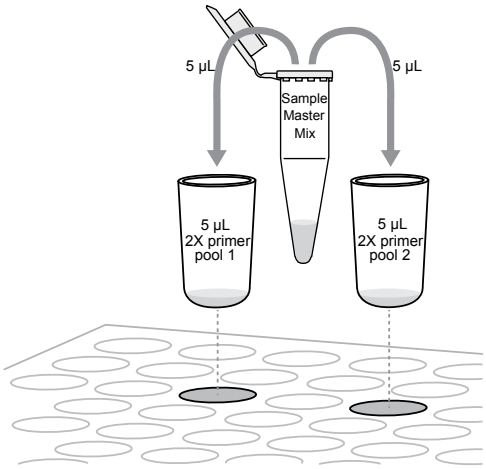
Prepare DNA target amplification reactions

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)	≤7.5 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.



4. Add 5 µL of 2X DNA Oncomine™ Comprehensive Panel primer pool 1 into the first well, and 5 µL of primer pool 2 to the second well.
5. Seal the plate with a MicroAmp™ Adhesive Film.
6. Vortex for 5 seconds to mix, then briefly centrifuge to collect the contents. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

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™ 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	8 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	100 ng DNA input
High quality DNA	14	+3	–3
FFPE DNA	17	+3	–3

[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. Adjust the cycle number for lower or higher DNA input.

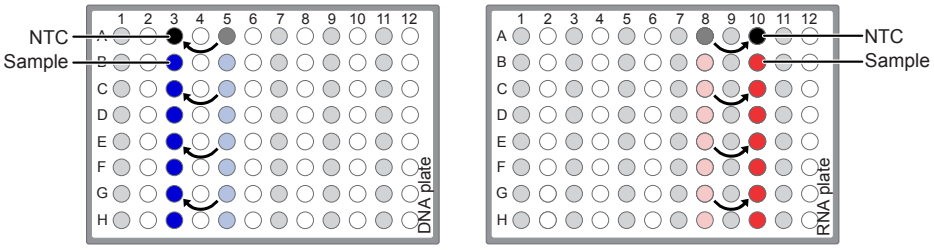
STOPPING POINT Target amplification reactions may be stored at 10°C overnight on the thermal cycler. For longer periods, store at –20°C.

Combine target amplification reactions

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

1. Remove the plate from the thermal cycler, then centrifuge briefly to collect the contents.
2. Carefully remove the plate seal.
3. For each sample, combine both 10-µL target amplification reactions into a single well.

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.



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

Partially digest the amplicons

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

1. Thaw the FuPa Reagent (brown cap) on ice, gently vortex to mix, then centrifuge briefly to collect.
2. Add 2 µL of FuPa Reagent to each amplified sample. The total volume is ~22 µL.
3. Seal the plate with a clear adhesive film, vortex thoroughly, then centrifuge briefly to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

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

Temperature	Time
50°C	10 min (RNA) 20 min (DNA)
55°C	10 min (RNA) 20 min (DNA)
60°C	20 min (DNA/RNA)
10°C	Hold (for up to 1 hour)

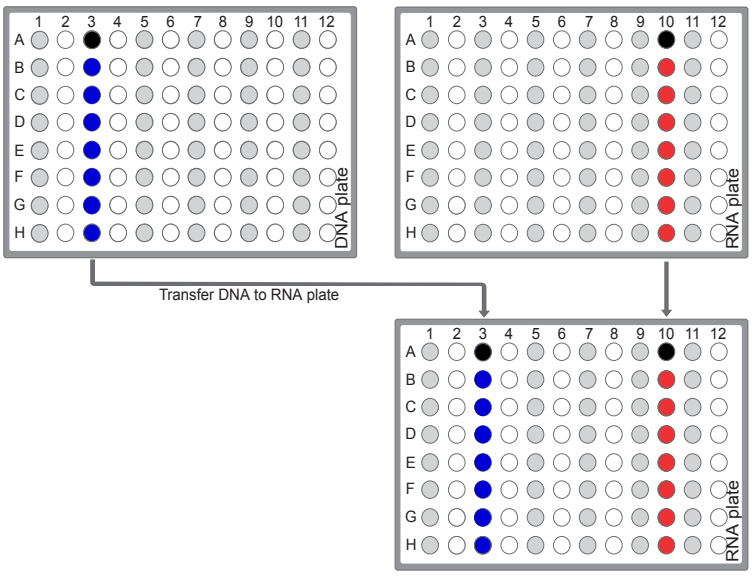
STOPPING POINT Store plate at –20°C for longer periods.

Transfer the DNA amplicons

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

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".

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

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.

Proceed immediately to "Equalize the library" on page 25.

Equalize the library

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

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

Before you begin

- Thaw the 1X Library Amp Mix (black cap) on ice. Keep on ice until use.
- Warm all the reagents in the Ion Library Equalizer™ Kit to room temperature. Vortex briefly, then centrifuge to collect the contents before use.

Amplify the library

1. Remove the plate with purified libraries from the plate magnet, then **add 50 µL of 1X Library Amp Mix** (black cap) and **2 µL of Equalizer™ Primers** (pink cap) to each bead pellet. The 1X Library Amp Mix and primers can be combined before addition.

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

3. Place the plate back on the magnet for at least 2 minutes, then carefully transfer ~50 µL of supernatant from each well to a new well or a new plate without disturbing the pellet.

4. Seal the plate with a new clear adhesive film, place a compression pad on the plate, then load in the thermal cycler. Run the following program. During cycling, wash the Equalizer™ Beads, if they have not been previously washed.

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

5. Briefly centrifuge the plate to collect the contents in the bottom of the wells.

   Note: The concentration of the amplified library can be confirmed by removing 2 µL of the reaction and evaluating with the Ion Library TaqMan® Quantitation Kit. The Ion Library Equalizer™ Kit should only be used when library concentrations are routinely >4,000 pM after library amplification.

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

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

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

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

Add Equalizer™ Capture to the amplified library

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

   Note: The final equalized library concentration is dependent upon accurate pipetting of the Equalizer™ Capture reagent.
2. Seal the plate with a clear adhesive film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
3. Incubate at room temperature for 5 minutes.

Add Equalizer™ Beads and wash

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

   Note: Check for droplets on the sides of the plate wells. If droplets are observed, seal the plate, then gently tap the plate on a hard, flat surface, or briefly centrifuge to collect droplets.
5. Place the plate in the magnet, then incubate for 2 minutes or until the solution is clear.
6. If needed, carefully remove the seal from the plate, then remove the supernatant without disturbing the pellet.

   Note: Save the supernatant for repeat analysis if needed.
7. **Add 150 µL of Equalizer™ Wash Buffer** to each reaction.
8. To wash the beads, move the plate side-to-side in the two positions of the magnet.

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

9. With the plate still in the magnet, carefully remove, then discard the supernatant without disturbing the pellet.

10. Repeat the bead wash as described in step 7 through step 9.

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

Elute the Equalized library

1. Remove the plate from the magnet, then add 100 µL of Equalizer™ Elution Buffer to each pellet.

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

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

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

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

Proceed to "Combine libraries".

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

Combine libraries

When comparing genomic DNA and RNA libraries that are prepared from the same sample, unequal volumes of libraries can be combined to produce different read depths for the paired DNA and RNA libraries.

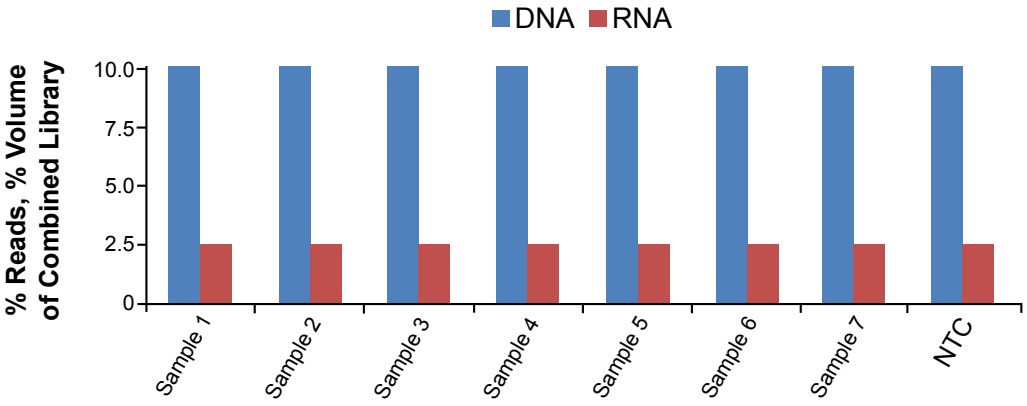
1. Combine each equalized uniquely barcoded Oncomine™ Comprehensive Assay DNA and RNA library (~100 pM each) from the same sample at an 80:20 ratio (DNA:RNA—8 µL of DNA library + 2 µL of RNA library).

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

   Note:
   - We recommend sequencing up to 8 (7 research samples + 1 DNA & RNA NTC) samples on a single Ion 540™ Chip.
   - For runs that include a no-template control (NTC), add in the same fractional volumes of equalized DNA and RNA NTC libraries as is added for equivalent DNA and RNA sample libraries.

3. Dilute the combined library pool 1:2 (14 µL of combined library pool + 14 µL of nuclease-free water, ~50 pM final concentration).

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



Sample	Barcode	Fractional volume (80:20 DNA:RNA)
DNA-1	BC_0101	0.1
RNA-1	BC_0102	0.025
DNA-2	BC_0103	0.1
RNA-2	BC_0104	0.025
DNA-3	BC_0105	0.1
RNA-3	BC_0106	0.025
DNA-4	BC_0107	0.1
RNA-4	BC_0108	0.025
DNA-5	BC_0109	0.1
RNA-5	BC_0110	0.025
DNA-6	BC_0111	0.1
RNA-6	BC_0112	0.025
DNA-7	BC_0113	0.1
RNA-7	BC_0114	0.025
DNA-8 (NTC)	BC_0115	0.1
RNA-8 (NTC)	BC_0116	0.025
Sum	—	**1.0**

Guidelines for templating and sequencing

Proceed to template preparation and sequencing using the following kits.

Chip	Template System	Sequencer	Kit	User Guide
Ion 540™ Chip	Ion Chef™ System	Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer	Ion 540™ Kit – Chef (Cat. Nos. A27759, A30011)	*Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)

Note: In this guide, Ion GeneStudio™ S5 Series Sequencer or Ion GeneStudio™ S5 Series System refers generically to the following systems, unless otherwise specified:

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

To create a specific Run Plan for use in templating and sequencing, see Chapter 4, "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 5.2 or later and Ion Reporter™ Software 5.2 or later. Before proceeding, we recommend that you update to the latest available versions of Torrent Suite™, Ion Reporter™, and Ion Chef™ System software. Contact your service representative for assistance with upgrading the software.

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™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer and subsequent data analysis. Each chip that is prepared in an Ion Chef™ run requires its own Planned Run.

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.

In Torrent Suite™ Software 5.2 or later, use the **Oncomine™ Comprehensive v3 DNA and Fusions** template as the primary Planned Run template for the Oncomine™ Comprehensive Assay v3 .

Application	Torrent Suite™ Software template	Description
DNA and Fusions	Oncomine™ Comprehensive v3 DNA and Fusions	DNA and RNA Planned Run template
	Oncomine™ Comprehensive v3 Fusions	RNA-only Planned Run template
AmpliSeq DNA	Oncomine™ Comprehensive v3 DNA	DNA-only Planned Run template

Create a custom Planned Run template

IMPORTANT! Before creating a Planned Run, you may need to enable the Oncomine™ Comprehensive Assay v3 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 about creating Planned Runs manually or from the generic application template, see the *Torrent Suite™ Software Help*.

1. Sign in to the Torrent Suite™ Software.
2. In the **Plan** tab, in the **Templates** screen, click **DNA and Fusions** in the left navigation menu.
3. In the **DNA and Fusions** list, find **Oncomine™ Comprehensive v3 DNA and Fusions**, then click ⚙ ▸ **Copy**.
   The **Copy Template** workflow opens to the **Save** step.
4. Enter or select the required information in each field:

Field[1]	Action
Template Name	Enter a name for the Planned Run template.
DNA Reference Library	Select **hg19(Human (hg19))**.
DNA Target Regions[2]	Select **OCAv3.20180426.designed.bed**
DNA Hotspot Regions[2]	Select **OCAv3.20170621.hotspots.blist.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 the most current files are being used. For BED file installation instructions, see "Download and install BED files" on page 48.

5. Update the Alignment Analysis Parameter.
   a. In the **Analysis Parameters**, select the **Custom** radio button.
   b. Scroll down to the **Alignment** pane, then replace the existing text string with "`tmap mapall ... -J 25 --end-repair 15 --context --bed-file --max-one-large-indel-rescue 60 --max-amplicon-overrun-large-indel-rescue 10 stage1 map4`".
6. Click the **Ion Reporter** step, then select your Ion Reporter™ account (version 5.2 or later).

   Note: If the Ion Reporter™ account is not configured, configure it through Ion Reporter Configure settings (see "Configure the IonReporter Uploader plugin in the Torrent Suite™ Software" on page 49).

7. In the **Existing Workflow** dropdown list, select the appropriate Ion Reporter™ workflow for your Planned Run (for example, **Oncomine™ Comprehensive v3 - w3.2 - DNA and Fusions - Single Sample**), then click **Next**.

   Note: If you are using the Ion Reporter™ Software version 5.2, you must have an off-cycle software package installed by your service representative for the Oncomine™ Comprehensive Assay Ion Reporter™ workflows to appear in the **Existing Workflow** dropdown list.

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

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

Field	Selection
Instrument	Ion GeneStudio™ S5 System
Library Kit Type	Ion AmpliSeq Library Kit Plus
Template Kit	Ion 540™ Kit – Chef
Sequencing Kit	Ion S5™ Sequencing Kit
Base Calibration Mode	Default Calibration
Chip Type	Ion 540™ Chip
Barcode Set	Ion Xpress™
Flows	400

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

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

12. 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 **DNA and Fusions** application.

Create a Planned Run

1. Sign in to the Torrent Suite™ Software.

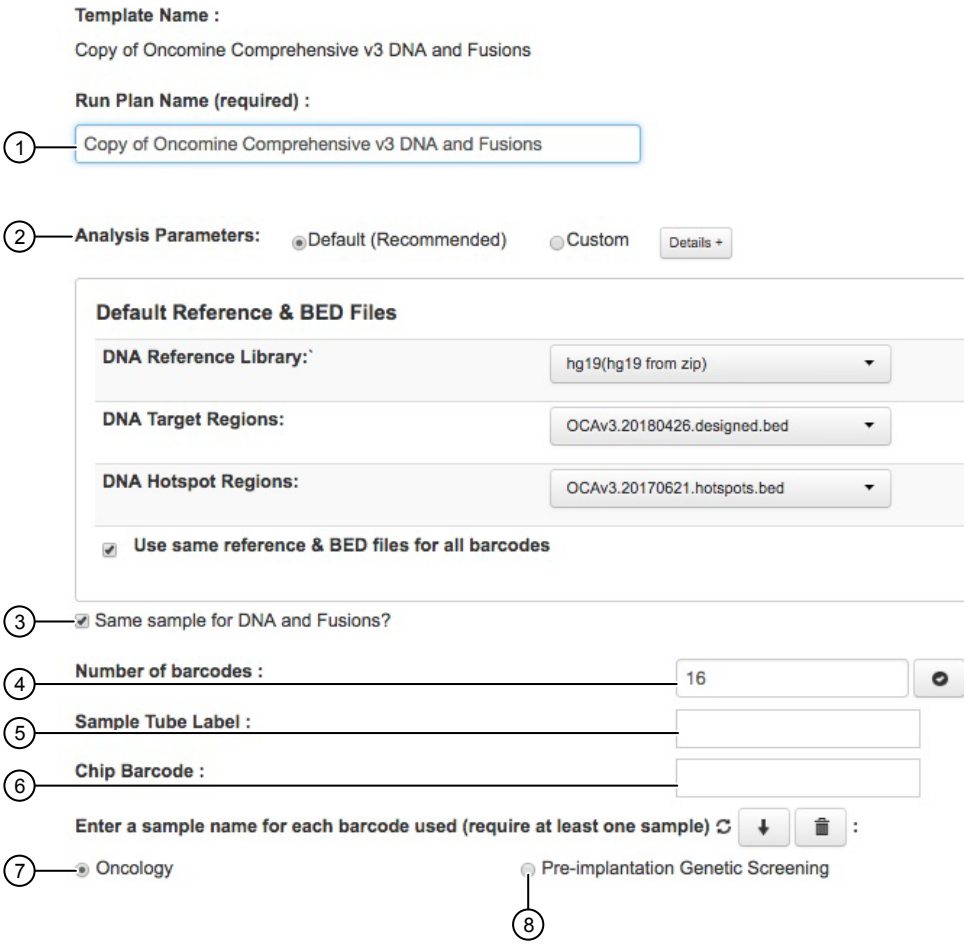
2. In the **Plan tab**, in the **Templates**, click **DNA and Fusions** in the left navigation menu.

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

4. Enter or select the following information.

   Note: Row numbers in the table correspond to the callouts in the figure.

Callout	Field	Action
1	Run Plan Name	Enter a Planned Run name.
2	Analysis Parameters	Ensure the **Default (Recommended)** radio button is selected.
3	Use same reference & BED files for all barcodes	Ensure that the checkbox is selected.
4	Number of barcodes	Enter the number of barcodes that will be used in this run, then click the ✔ button to the right of this field. The default value is 16 barcodes.
5	Sample Tube Label	Enter or scan the barcode of the Ion Chef™ Library Sample Tube that will be used in the run.
6	Chip ID	No entry required.
7	Oncology	Ensure that the radio button is selected.
8	Pre-implantation Genetic Screening	Ensure that the radio button is deselected.



5. Enter sample information.

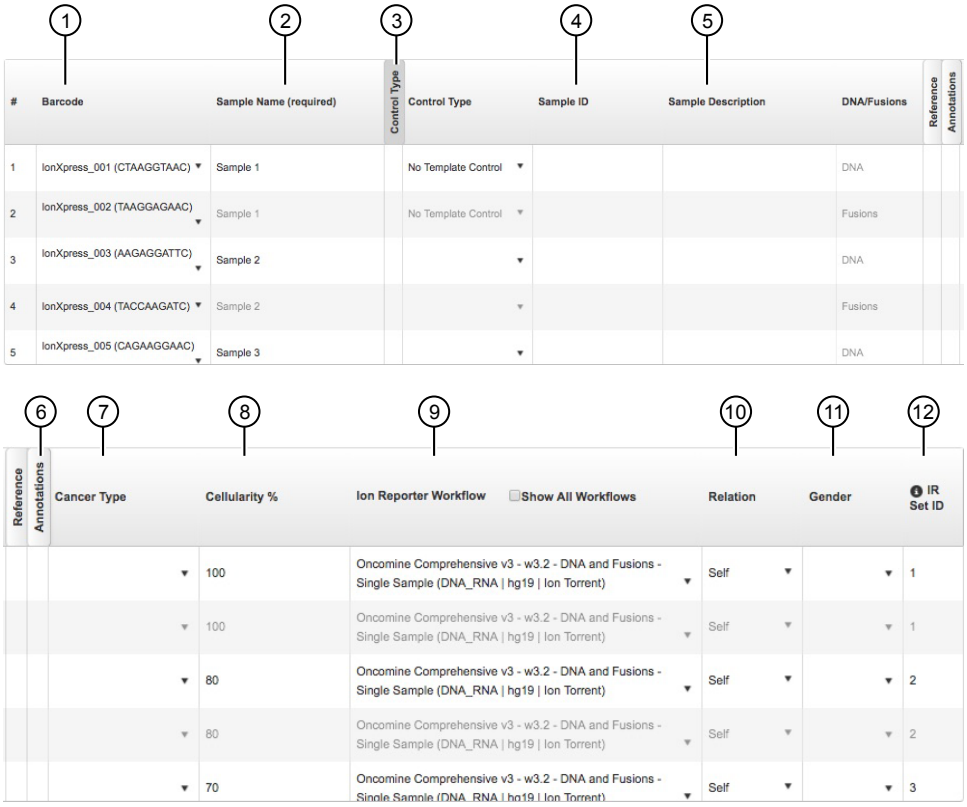
   Note: Row numbers in the table correspond to the callouts in the figure.

Callout	Field[1]	Action
1	Barcode	For each sample select the **Barcode** that will identify it from the dropdown list.
2	Sample Name	Accept the auto-populated sample names or click in a field, then enter a unique sample name. We recommend that the sample names (either auto-populated or user defined) that you pick are unique even between runs.
3	Control Type (expanded)	Select **No Template Control** from the dropdown list to designate a sample as a no template control.
4	Sample ID	*(Optional)* Click in the field, then enter a sample ID.
5	Sample Description	*(Optional)* Click in the field, then enter a sample description.
6	Annotations (expanded)	Click to reveal **Cancer Type** and **Cellularity %**.

Callout	Field[1]	Action
7	Cancer Type	Select from the dropdown list. Click to copy the entry to all the rows.
8	Cellularity %	Enter a value. Click to copy the entry to all the rows.
9	Ion Reporter Workflow	Ensure the correct workflow is selected.
10	Relation	Ensure the correct value is auto-populated. Select from the dropdown list to change.
11	Gender	Select from the dropdown list. Click to copy the entry to all the rows.
12	IR Set ID[2]	The IR Set ID links individual samples for analysis. Ensure the correct value is auto-populated. Select from the dropdown list to change.

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

[2] Samples with the same IR Set ID are considered related samples and launched in the same analysis such as 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).



6. 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.

5 Variant analysis

IMPORTANT! If you are using the Ion Reporter™ Software version 5.2, you must have an off-cycle software package installed by your service representative to perform Oncomine™ Comprehensive Assay Ion Reporter™ variant analysis. We recommend updating to the latest available version of Ion Reporter™ Software.

Analysis workflows in Ion Reporter™ Software

If you selected the appropriate Ion Reporter™ 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 on manually launching an analysis, see "Manually launch an analysis" on page 37.

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

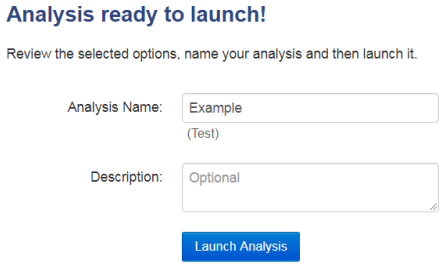
Ion Reporter™ Software 5.2 or later includes the following analysis workflows:

Workflow name[1]	Description
Oncomine™ Comprehensive v3 - w3.2 - DNA and Fusions - Single Sample	Detects and annotates somatic variants (SNPs, INDELs, CNVs), including those at low frequency, in targeted DNA libraries, as well as gene fusions in targeted RNA libraries, from the Oncomine™ Comprehensive Assay v3 run on the Ion 540™ Chip.
Oncomine™ Comprehensive v3 - w3.2 - DNA - Single Sample	Detects and annotates somatic variants (SNPs, INDELs, CNVs), including those at low frequency, in targeted DNA libraries from the Oncomine™ Comprehensive Assay v3 run on the Ion 540™ Chip.
Oncomine™ Comprehensive v3 - w3.2 - Fusions - Single Sample	Detects and annotates gene fusions in targeted RNA libraries from the Oncomine™ Comprehensive Assay v3 run on the Ion 540™ Chip.
Oncomine™ Comprehensive v3 - w3.2 - Annotate Variants - Single Sample	Annotates VCF files from the Oncomine™ Comprehensive Assay v3.

[1] The workflows listed in this table are available in Ion Reporter™ Software 5.10. Workflow names can vary depending on the version of the Ion Reporter™ Software used.

Manually launch an analysis

1. Sign in to the Ion Reporter™ Software.
2. In the **Workflows** tab, in the **Overview** screen, select **DNA and Fusions** from the **Research Application** dropdown list.
3. Type *Comprehensive* in the search field, then click **Go** (or press Enter).
4. In the **Workflow Name** column, click the appropriate workflow (for example, Oncomine™ Comprehensive v3 - w3.2 - DNA and 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 the sample **Cellularity %** and **Sample Type** are defined.
6. Click the checkbox to select a DNA sample and a Fusions sample.
7. In the **Sample Groups** pane, click **Add Samples** to add the selected samples to a sample group.
8. Enter a **Group Name**, click **Add to Analysis**, then click **Next**.
9. In the **Plugins** step, ensure that the **Oncomine™ Variant Annotator v2.1 or later** plugin is selected, then click **Next**.
10. *(Optional)* Enter an **Analysis Name** and **Description**.
11. Click **Launch Analysis**.



View results

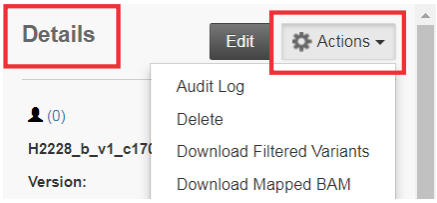
Ion Reporter™ Software analyses are performed automatically on uploading of the data files from the Torrent Suite™ Software.

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 ***Comprehensive*** in the search field, then click **Go** (or press **Enter**).

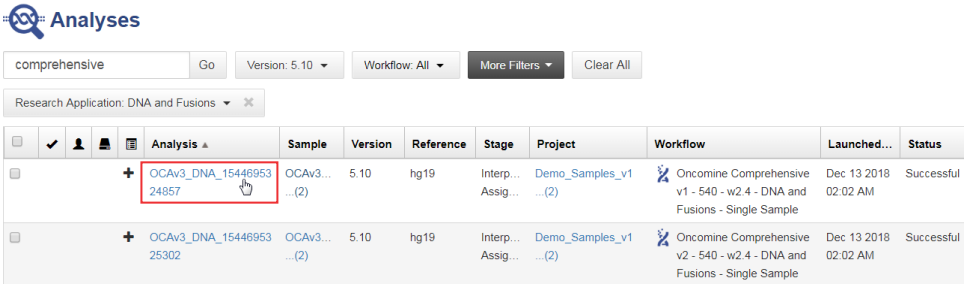
You can further refine the list of analyses by applying additional filters that are available in the **More Filters** dropdown list, or clicking column headers. The **Analyses** table automatically filters the content appropriate to your filter selection and search term.

4. Click within a row (but not on the sample data set hyperlink) to view the **Details** of the analysis.

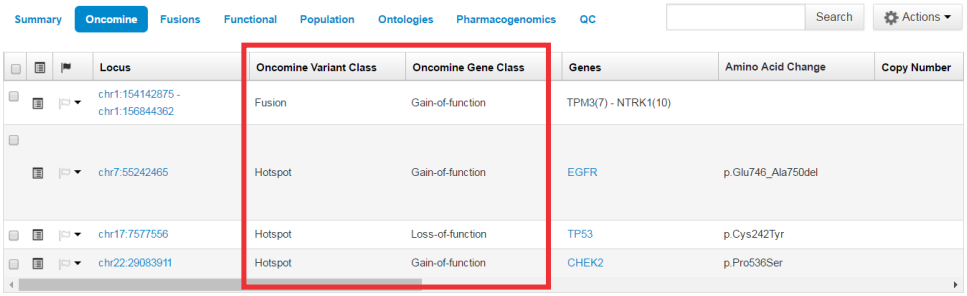
   In the **Details** pane, you can view **Workflow Details** and access the **Actions** dropdown list.



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 displaying only Oncomine™ annotated variants relevant to cancer.



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.

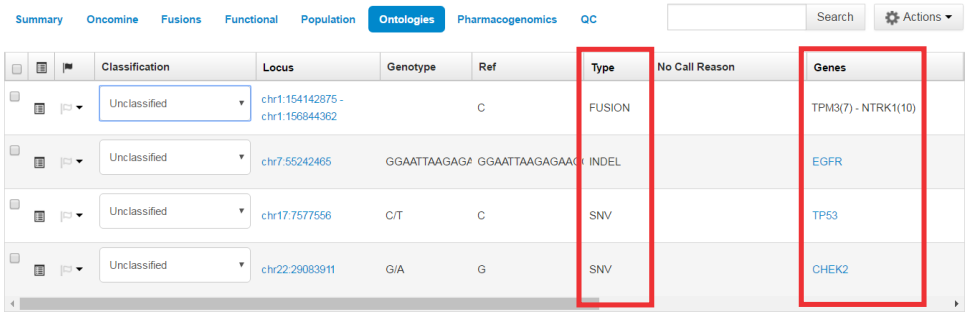
   a. In the **Filter Options** pane, select the desired **Filter Chain**.

   Note:

   - The default **Filter Chain** is **Oncomine™ Variants (5.2 or later)**, which limits the results that are displayed to variants relevant to cancer 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 5.10 User Guide* (Pub. No. MAN0017605).
   - Select **No Filter** to view all the variant calls attempted by the variant caller.
   - Saving the analysis using a filter chain other than **Oncomine™ Variants (5.2 or later)** changes the variant calls that are saved in the VCF file and can affect downstream workflows, such as with Oncomine™ Reporter Software.

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**.



After you review, filter, and sort your Analysis Result, you can create a report (see "Generate an Analysis Results Report" on page 39), or download files for use by the Oncomine™ Reporter Software (see "Download Ion Reporter™ annotation VCF or TSV files" on page 40).

Generate an Analysis Results Report

After you have reviewed, filtered, and sorted your Analysis Result, you can download an Analysis Report. The procedure described here includes creating and formatting a 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 report can be rearranged, deleted, or edited.

2. Hover over the various sections and icons to view instructional text to help you format your 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 report template for future use with other sample results.

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

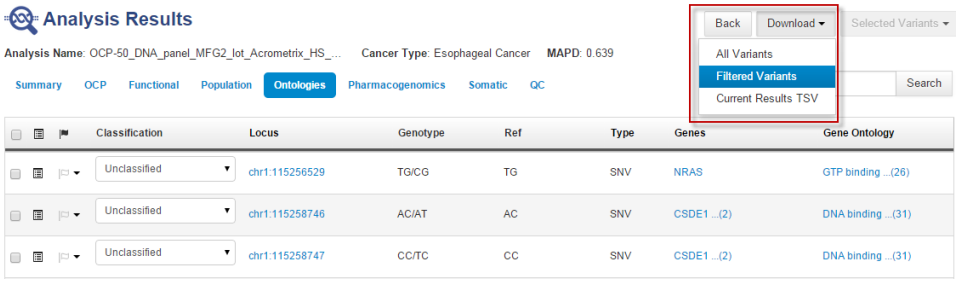
6. Click **Lock & Publish** to generate the final Analysis Report.

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.

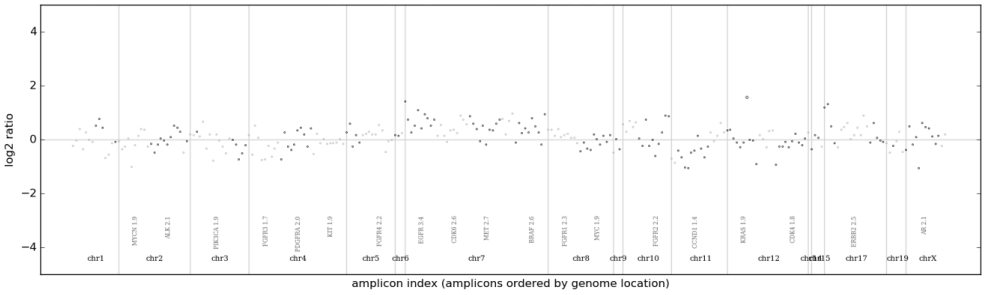
1. Click **Download**, then select **All Variants, Filtered Variants** or **Current Results TSV**.



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

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

   The software generates a ZIP file with 4 folders: CNV_VCIB, QC, Variants, and Workflow_Settings. Within the Variants folder, you'll find the Oncomine™ annotated VCF file.

Folder	Contents of folder
CNV_VCIB	Contains an image file (`cn_results.png`) of the copy number determination for all amplicons. 
QC	Contains a PDF of the QC report, and a folder containing coverage statistics files.
Variants	Contains a folder with: • Intermediate and Oncomine™ annotated .VCF files, which are used by Oncomine™ Reporter Software. For more information, see the *Oncomine™ Reporter User Guide* (Pub. No. MAN0018068). • TSV files that contain Oncomine™-filtered and all somatic variants.
Workflow_Settings	Contains folders with: • A text file that describes settings used for the analysis. Open the file with a text editor. • Configuration files used by the Ion Reporter™ Software in the workflow settings.

Oncomine™ Comprehensive Assay v3 with Ion Reporter™ Software 5.4 or later

The following table summarizes the requirements that must be met in order for the Oncomine™ Variant Annotator v2.2 or later plugin to annotate variants in Ion Reporter™ Software 5.4 or later for the Oncomine™ Comprehensive Assay v3.

For each variant type in this table, Oncomine™ Variant Annotator plugin annotates a variant 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	Positive amplification call (SVTYPE = "CNV" and FILTER = "GAIN") in one of the 43 Oncomine™ Comprehensive Assay v3 copy-gain genes
Gene Fusion	Gain-of-Function	Fusion	Positive fusion call (SVTYPE = "Fusion" and FILTER = "PASS") in one of the 760 Oncomine™ Comprehensive Assay v3 fusion variants
RNA Exon Variant	Gain-of-Function	RNAExonVariant	Positive RNA exon variant call (SVTYPE = "RNAExonVariant" or "Fusion" and FILTER = "PASS") in one of the Oncomine™ Comprehensive Assay v3 RNA exon variants
Loss of Function Mutation	Loss-of-Function	Deleterious	• Positive mutation call • Variant's functional impact is frameshift block substitution, frameshift insertion, frameshift deletion, or nonsense • Variant occurs in a loss-of-function gene
Gain of Function Missense Hotspot Mutation	Gain-of-Function	Hotspot	• Positive mutation call • Variant's functional impact is missense • Variant occurs in a gain-of-function gene • Variant's transcript and codon position occur in predefined missense hotspot list
Loss of Function Missense Hotspot Mutation	Loss-of-Function	Hotspot	• Positive mutation call • Variant's functional impact is missense • Variant occurs in a loss-of-function gene • Variant's transcript and codon position occur in predefined missense hotspot list
Gain of Function In Frame Hotspot Mutation	Gain-of-Function	Hotspot	• Positive mutation call • Variant occurs in a gain-of-function gene • Variant's function, transcript, and coding syntax occur in predefined in-frame hotspot list
Loss of Function In Frame Hotspot Mutation	Loss-of-Function	Hotspot	• Positive mutation call • Variant occurs in a loss-of-function gene • Variant's function, transcript, and coding syntax occur in predefined in-frame hotspot list

Variant Type	Oncomine™ Gene Class	Oncomine™ Variant Class	Annotation Criteria
Gain of Function Splice Site Hotspot Mutation	Gain-of-Function	Hotspot	• Positive mutation call • Variant occurs in a gain-of-function gene • Variant's transcript, location, and exon occur in predefined splice site hotspot list
Loss of Function Splice Site Hotspot Mutation	Loss-of-Function	Hotspot	• Positive mutation call • Variant occurs in a loss-of-function gene • Variant's transcript, location, and exon occur in predefined splice site hotspot list
Gain of Function Promoter Hotspot Mutations	Gain-of-Function	Hotspot	• Positive mutation call • Variant occurs in a gain-of-function gene • Variant's transcript, location, and coding syntax occur in predefined promoter hotspot list

Tips and troubleshooting

Tips

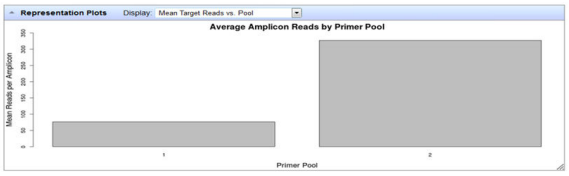
- Arrange samples in alternating columns on the plate for easier pipetting with multichannel pipettes during purification with the DynaMag™–96 Side Magnet.
- Plate seals can be firmly applied using the applicator in the MicroAmp™ Adhesive Film Applicator. Plate seals can be removed with less effort when 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.
- If the unamplified library yield is below 100 pM, libraries can be rescued with library amplification. Combine 25 µL of unamplified library with 71 µL of 1X Library Amp Mix and 4 µL of 25X Library Amp Primers. Perform 5–10 library amplification cycles (for cycling conditions, see step 4 of "Amplify the library").
- 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 (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. 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.
	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 with the Ion Library Equalizer™ Kit is less than expected	Equalizer™ Beads were not washed.	Be sure to wash Equalizer™ Beads before use.
	Wrong library amplification primers were used.	Use the Equalizer™ Primers provided in the Ion Library Equalizer™ Kit.
	Residual Equalizer™ Wash Buffer was present after wash.	Carefully remove all of the Equalizer™ Wash Buffer before elution.
Library concentration is too high	Sample DNA or RNA was mis-quantified.	Requantify sample DNA using the TaqMan® RNase P Detection Reagents Kit; quantify RNA with a Qubit™ Fluorometer.
	More than 100 ng of sample DNA/RNA was used.	Add less DNA/RNA, or decrease target amplification cycles.

Low amplicon uniformity (DNA only)

Observation	Possible cause	Recommended action
Short amplicons are under-represented	Purification was poor.	Vortex AMPure™ XP Reagent thoroughly before use, and be sure to dispense the full volume.
		100% ethanol is difficult to pipet accurately; it is essential to pre-wet pipette tips.
		In post-ligation library purification, increase AMPure™ XP Reagent volume from 45 µL (1.5X) to 50 µL (1.7X).
Pool representation is not balanced  **Example of pool imbalance.** Within the Coverage Analysis Plugin, mean read depth per primer pool is plotted for a 2-pool Ion AmpliSeq™ Panel. In this example, Primer Pool 1 has approximately one quarter the reads of Primer Pool 2.	Amount of DNA in target amplification reactions varied.	Make a master mix for each sample DNA.
	Pipetting is inaccurate when pools are combined after target amplification.	Centrifuge the plate after target amplification. Ensure that the entire volume of each pool is removed and combined into a single pool.

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.
Barcode representation is uneven (Ion Library Equalizer™ Kit used)	Yield of library amplification was inadequate.	When trying the Ion Library Equalizer™ Kit for the first time, quantify with qPCR to ensure libraries are >4 nM. If not the first time, increase input nucleic acid or target amplification cycles.
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.

Observation	Possible cause	Recommended action
Low quality ISPs are present at high percentage (>15%)	Library input was too low.	Double the volume of library used in template preparation.
		Use a fresh dilution of library prepared in a low-bind tube.
	Other.	Check the appropriate template preparation user guide for more information.

B Supplemental information

Update Torrent Suite™ Oncomine™ Comprehensive Assay templates

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

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

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

Install Oncomine™ Comprehensive Assay Ion Reporter™ workflows

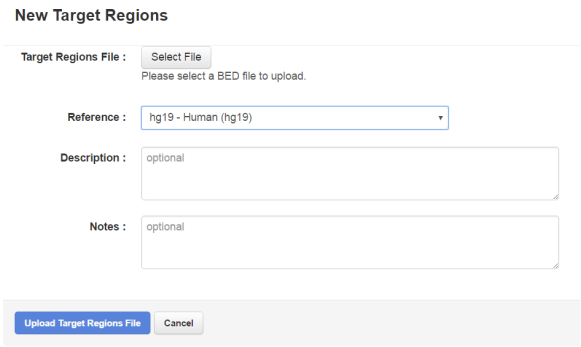
To install or update the Oncomine™ Comprehensive Assay Ion Reporter™ workflows, contact your service representative to schedule an update.

Note: For more information refer to the *Torrent Suite™ Software 5.6 Release Notes* (Pub. No. MAN0017340).

Download and install BED files

Contact your service representative to obtain the latest versions of Oncomine™ Comprehensive Assay v3 BED files.

1. Extract the BED file containing ZIP file to a location of your choice.
2. Sign in to the Ion Torrent™ Server where 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 panel 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 file: **OCAv3.20180426.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: **OCAv3.20170621.hotspots.blist.bed**
   d. Click **Open**, then click **Upload Hotspots File**.

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

Configure the IonReporter Uploader plugin in the Torrent Suite™ Software

1. Sign in to the Torrent Suite™ Software.
2. Click the ⚙ ▾ **(Settings)** tab (upper right), then select **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 local Ion Reporter™ Server System 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 and in the Upload to IR quick link.

6. Click **Get Versions**, select **Ion Reporter 5.2 or later**, then click ✓**Add**.

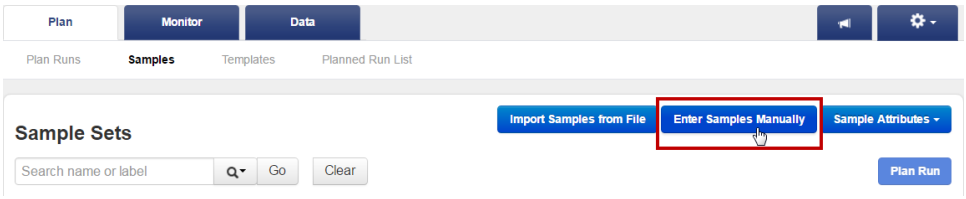
C

Sample uploading tips in Torrent Suite™ Software

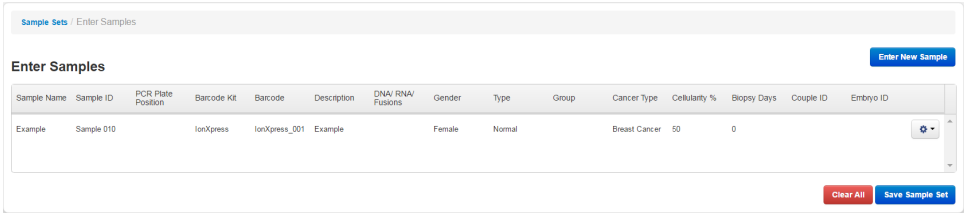
The following topics describe how to use sample sets and create Planned Runs and templates in Torrent Suite™ Software to support Oncomine™ Comprehensive Assay v3 sequencing runs. Setting up mixed samples (i.e., samples for which paired DNA and RNA libraries are not being run) on a chip is also described.

Create sample sets manually

1. In the **Plan** tab, in the **Samples** screen, click **Enter Samples Manually**.



2. Click **Enter New Sample**, then define samples in the **Add Sample** dialog.
3. Click **Done**.



   Your sample appears in the **Enter Samples** table.

4. Repeat step 2 and step 3 to enter additional samples.

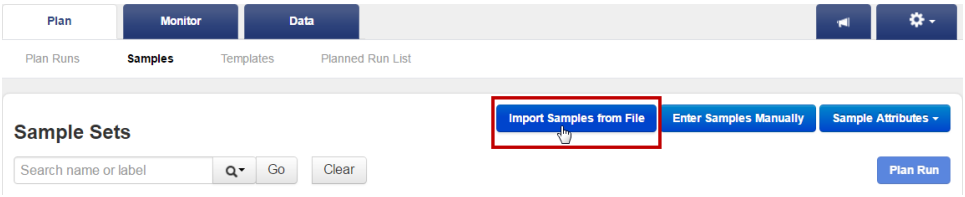
5. If you have sample pairs, set the **Relationship Group** numbers to reflect pairs (for example, DNA and RNA from the same sample would have the same Relationship Group number).

6. Click **Save Sample Set**, then name the **Sample Set** or add the samples to an existing **Sample Set**.

Import samples to create a sample set

If you are importing many samples, you can use the **Import Samples from File** feature.

1. In the **Plan** tab, in the **Samples** screen, click **Import Samples from File**, then click **Sample File Format** to download a template CSV file.



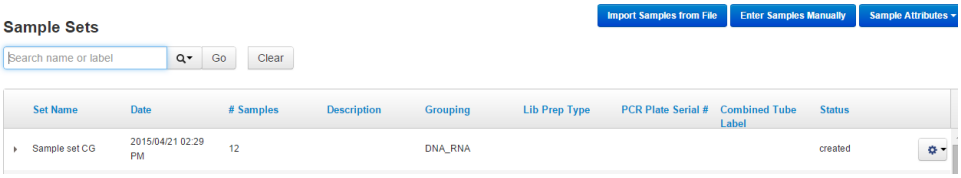
2. Fill out the template CSV file as completely as possible, then save it to the location of your choice.

   Note: Recommended columns include: Sample name, 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, Cellularity %, Barcode Kit, and Barcode.

	A	B	C	D	E	F	G	H	I	J	K
1	Sample Name (required)	Sample ID	Barcodekit	Barcode	Gender	Type	Group	Description	DNA/RNA /Fusions	Cancer Type	Cellularity %
2	CG00001	x101	IonXpress	IonXpress_001	Female	Self	1		DNA	Bladder Cancer	71
3	CG00001	x101	IonXpress	IonXpress_002	Female	Self	1		RNA	Bladder Cancer	71
4	CG00002	x102	IonXpress	IonXpress_003	Male	Self	2		DNA	Colorectal Cancer	55
5	CG00002	x102	IonXpress	IonXpress_004	Male	Self	2		RNA	Colorectal Cancer	55
6	CG00003	x103	IonXpress	IonXpress_005	Female	Self	3		DNA	Colorectal Cancer	62
7	CG00003	x103	IonXpress	IonXpress_006	Female	Self	3		RNA	Colorectal Cancer	62
8	CG00004	x104	IonXpress	IonXpress_007	Female	Self	4		DNA	Glioblastoma	74
9	CG00004	x104	IonXpress	IonXpress_008	Female	Self	4		RNA	Glioblastoma	74
10	CG00005	x105	IonXpress	IonXpress_009	Female	Self	5		DNA	Glioblastoma	51
11	CG00005	x105	IonXpress	IonXpress_010	Female	Self	5		RNA	Glioblastoma	51
12	CG00006	x106	IonXpress	IonXpress_011	Female	Self	6		DNA	Glioblastoma	77
13	CG00006	x106	IonXpress	IonXpress_012	Female	Self	6		RNA	Glioblastoma	77

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

4. Click **Add Sample Set**, enter or select the required information in each field, then click **Save & Finish**.
   The software automatically imports the samples into the **Sample Sets** table.



Create a Planned Run with Sample Sets

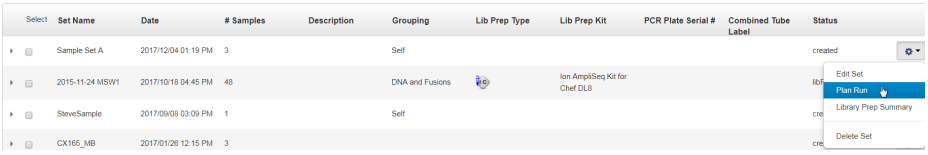
If you set up your samples before you plan an instrument run, you can add one or multiple Sample Sets to your Planned Run.

Sample Sets must correspond to Oncomine™ Comprehensive Assay v3 library preparations and use the same barcode kit to be included in a single Planned Run.

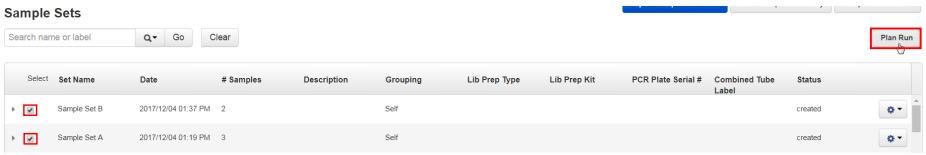
1. In the **Plan** tab, in the **Samples** screen, find the Sample Sets that you want to add to the Planned Run.

2. Select one or more Sample Sets to add to the Planned Run.

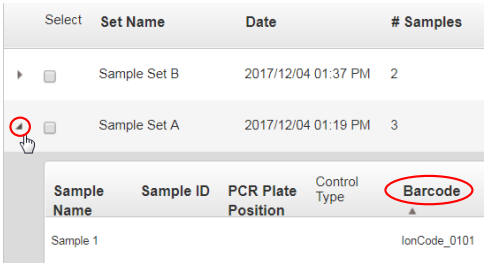
   - To plan a run using one Sample Set, click ⚙ **(Actions)** ▸ **Plan Run** in the row of the Sample Set.



   - 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**.

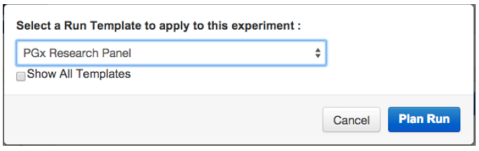


IMPORTANT! Ensure that all Sample Sets used in the Planned Run use the same barcode kit. To verify the barcode kit used, expand the Sample Set entry to view its details.



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, 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, enter or select the required information in the following fields.

Field[1]	Description
Analysis Parameters	Select the **Default** radio button to accept default analysis parameter settings *(recommended)*. Advanced users can customize analysis parameters by selecting the **Custom** radio button, then editing the appropriate analysis fields.
Reference Library	Select the reference library file appropriate for your sample. Depending on your application, you may have to select separate DNA, RNA, and Fusions reference library files.
Target Regions[2]	Select the Target Regions BED file appropriate for your sample. Depending on your application, you may have to select separate DNA and Fusions Target Regions file.
Hotspot Regions[2]	Select the Hotspot Regions (BED or VCF) file appropriate for your sample.

[1] Depending on your sequencing application, fields can vary.
[2] Ensure that you are using the current BED or VCF files

5. Select the **Use same reference & BED files for all barcodes** checkbox if you are using the same reference, Target Regions, and Hotspot Regions files across all of your barcoded samples in the Planned Run. If you are using different reference and/or BED files for one or more of your barcoded samples, deselect the **Use same reference & BED files for all barcodes** checkbox.

6. In the **Sample Tube Label** field, enter or scan the barcode of each Ion Chef™ Library Sample Tube that will be used in the run.

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

8. Fill out or select the following fields in the **Samples Table**.

   - You can save the samples table to a CSV file, fill out all required sample information, then upload the samples table to automatically populate the **Samples Table**.
     a. Click **Save Samples Table** above the upper right corner of the **Samples Table** to save the CSV file to your computer.
     b. Edit the CSV file by entering all required sample information into the appropriate sample information columns, then save the CSV file to your computer.
     c. Click **Load Samples Table**, then select an appropriate CSV file containing sample information specific for this Planned Run.
     d. Click **Load** to populate the **Samples Table** in Torrent Suite™ Software with sample information supplied by the CSV file.
   - Alternatively, you can manually enter sample information into the **Samples Table** using the Torrent Suite™ Software.

Field[1]	Description
Barcode	For barcoded samples, select a barcode from the dropdown menu.
Sample (required)	Enter a unique sample name for each sample. Do not duplicate samples names.
Control Type	Click on the **Control Type** column header to expand the **Control Type** column, then select the control type from the dropdown menu.
Sample ID	*(Optional)* Enter sample ID for each sample.
Description	*(Optional)* Enter sample description for each sample.
DNA/Fusions	For DNA and Fusions application, select **DNA** or **Fusions** from the dropdown menu for each samples.
Reference	If using different reference and BED files for one or more samples, click the **Reference** column header to expand the **Reference** sections and select Reference, Target Regions, and Hotspot Regions files from the dropdown menu for each sample.
Annotations	Click the **Annotations** column header to expand the annotation fields specific for your application (for example, cancer type or Embryo ID) and complete the required field information.
Ion reporter workflow	Select the Ion Reporter™ workflow specific for your run from the dropdown menu. If you do not see your workflow, select the **Show All Worklows** checkbox in the column header.
Relation	Select sample relationship group.

Field[1]	Description
Gender	Select "Male", "Female", or "Unknown" from the dropdown menu.
IR Set ID	Set the IR Set ID to the same value for related samples. After file transfer, in Ion Reporter™ Software, samples with the same Set ID are considered related samples and are launched in the same analysis (for example, normal sample and its corresponding tumor sample). Do not give unrelated samples the same Set ID value even if the value is zero or blank.

[1] Depending on your sequencing application, fields can vary.

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

10. Click **Save & Finish**.

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

Create a Planned Run with a mixed Sample Set

The following example is a mixed sample set consisting of 4 sample pairs, 2 DNA-only samples and 2 RNA-only samples.

1. In the **Plan** tab, in the **Samples** screen, click **Import Samples from File**, then click **Sample File Format** to download a template CSV file.

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

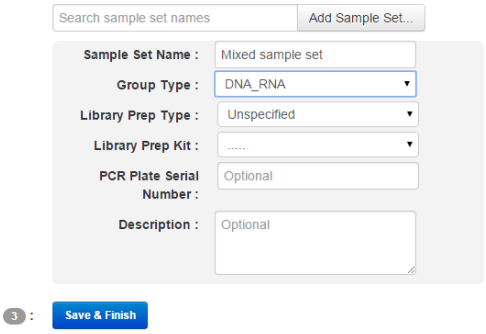
   IMPORTANT! Ensure that you identify sample groups with **Group** numbers (i.e., paired samples use the same **Group** number, whereas unpaired samples receive different **Group** numbers as in the following example).

	A	B	C	D	E	F	G	H	I	J	K
1	Sample Name (required)	Sample ID	Barcodekit	Barcode	Gender	Type	Group	Description	DNA/RNA /Fusions	Cancer Type	Cellularity %
2	CG00001	x101	IonXpress	IonXpress_001	Female	Self	1		DNA	Bladder Cancer	71
3	CG00001	x101	IonXpress	IonXpress_002	Female	Self	1		RNA	Bladder Cancer	71
4	CG00002	x102	IonXpress	IonXpress_003	Male	Self	2		DNA	Colorectal Cancer	55
5	CG00002	x102	IonXpress	IonXpress_004	Male	Self	2		RNA	Colorectal Cancer	55
6	CG00003	x103	IonXpress	IonXpress_005	Female	Self	3		DNA	Colorectal Cancer	62
7	CG00003	x103	IonXpress	IonXpress_006	Female	Self	3		RNA	Colorectal Cancer	62
8	CG00004	x104	IonXpress	IonXpress_007	Female	Self	4		DNA	Glioblastoma	74
9	CG00004	x104	IonXpress	IonXpress_008	Female	Self	4		RNA	Glioblastoma	74
10	CG00005	x105	IonXpress	IonXpress_009	Female	Self	5		DNA	Glioblastoma	51
11	CG00006	x106	IonXpress	IonXpress_010	Female	Self	6		RNA	Glioblastoma	66
12	CG00007	x107	IonXpress	IonXpress_011	Female	Self	7		DNA	Glioblastoma	77
13	CG00008	x108	IonXpress	IonXpress_012	Female	Self	8		RNA	Glioblastoma	59

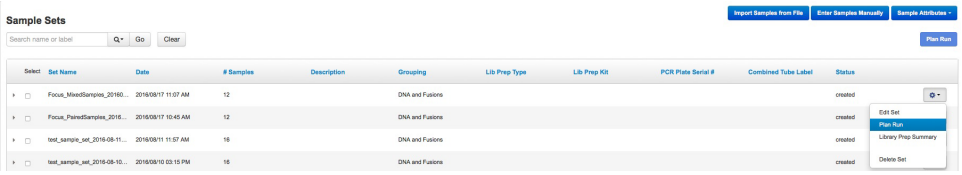
3. After you complete and save the CSV file, click **Select File**, navigate to the completed CSV file, then click **Open**.

4. Click **Add Sample Set**, enter or select the required information in each field, then click **Save & Finish**.

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



5. In the sample set row, click ⚙ **(Actions)**, then select **Plan Run** in the dropdown list.

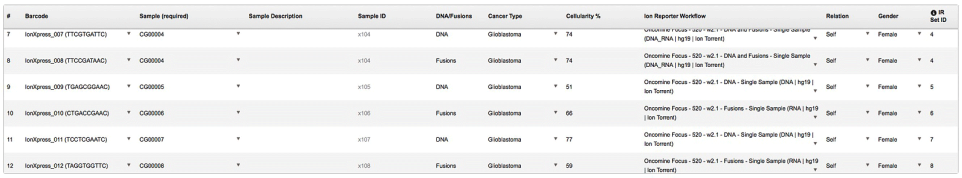


6. Select a **Run Template** to apply to this experiment, then click **Plan Run**.

7. Review the samples section at the bottom of the page.

8. Select appropriate Ion Reporter™ workflows for the non-paired samples.

Torrent Suite™ Software template	Description
Oncomine™ Comprehensive v3 DNA and Fusions	Paired DNA and RNA samples.
Oncomine™ Comprehensive v3 DNA	DNA-only samples.
Oncomine™ Comprehensive v3 Fusions	RNA-only samples.



9. Click **Next** to advance to the **Projects** tab.

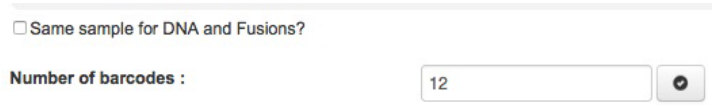
10. *(Optional)* Select a Project, then click **Next**.

11. Enter a plan name, then click **Save & Finish**.

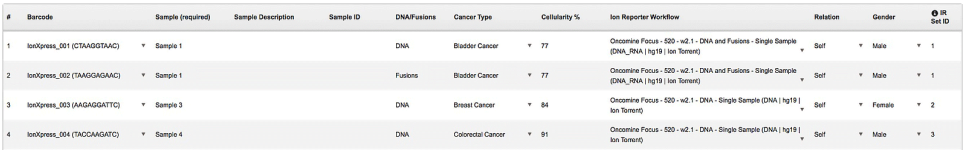
The Planned Run for mixed samples is available under the **Plan** tab, in the **Templates** screen.

Create a Planned Run for mixed samples with a template

1. Select the Oncomine™ Comprehensive v3 DNA and Fusions template or a copied version of this template.
2. Deselect the **Same sample for DNA and Fusions?** option.



3. Enter the number of barcodes that will be used in this run in the **Number of barcodes** field, then click the check mark button to the right of this field.
   The **Samples table** is populated with barcode information for each sample.
4. For each sample:
   - Rename the sample (Sample Names must be unique to each sample).
   - Change the **DNA/Fusions** selection.
   - Select the **Cancer Type**.
   - Enter the **Cellularity %**.
   - Select the appropriate **Ion Reporter™ Workflow**.
   - Select the **Relation**.
   - Select the **Gender**.
   - Enter IR Set IDs.



#	Barcode	Sample (required)	Sample Description	Sample ID	DNA/Fusions	Cancer Type	Cellularity %	Ion Reporter Workflow	Relation	Gender	IR Set ID
1	IonXpress_001 (CTAAGGTAAC)	Sample 1			DNA	Bladder Cancer	77	Oncomine Focus - 520 - w2.1 - DNA and Fusions - Single Sample (DNA_RNA | hg19 | Ion Torrent)	Self	Male	1
2	IonXpress_002 (TAAGGAGAAC)	Sample 1			Fusions	Bladder Cancer	77	Oncomine Focus - 520 - w2.1 - DNA and Fusions - Single Sample (DNA_RNA | hg19 | Ion Torrent)	Self	Male	1
3	IonXpress_003 (AAGAGGATTC)	Sample 3			DNA	Breast Cancer	84	Oncomine Focus - 520 - w2.1 - DNA - Single Sample (DNA | hg19 | Ion Torrent)	Self	Female	2
4	IonXpress_004 (TACCAAGATC)	Sample 4			DNA	Colorectal Cancer	91	Oncomine Focus - 520 - w2.1 - DNA - Single Sample (DNA | hg19 | Ion Torrent)	Self	Male	3

5. Click **Plan Run**.

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

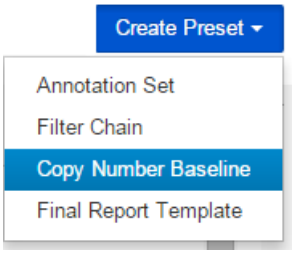
If you want to use the VCIB CNV baseline included in Ion Reporter™ Software 5.2, simply select it when creating your workflow.

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.

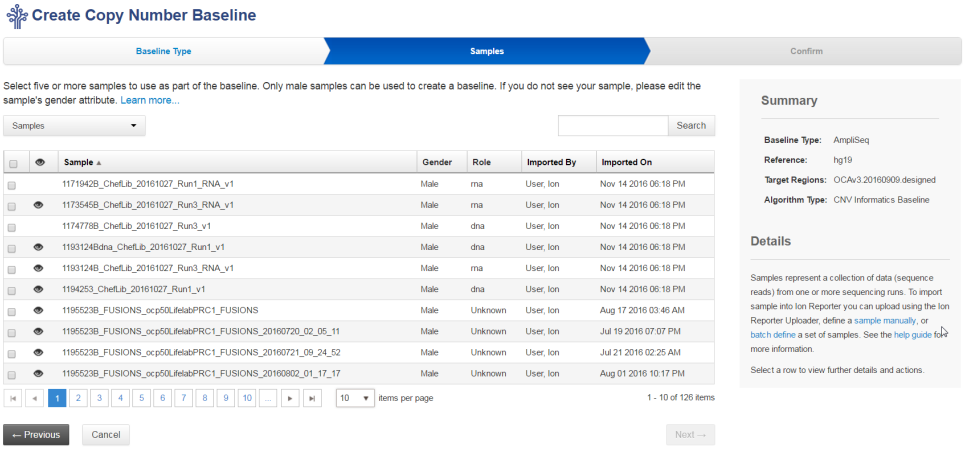
1. In the **Workflows** tab, in the **Presets** screen, click **Create Preset**, then select **Copy Number Baseline** from the dropdown list.



2. Click **AmpliSeq**, select **Oncomine™ Comprehensive DNA v3 Regions v1.0** 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**.

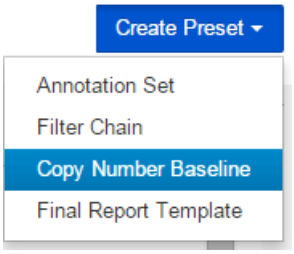
   Note: Log files for both successful and failed analyses include the **BaselineCreation.log** file, which has the BAM files named that were rejected due to similarity to other files in the baseline, as well as the **map.TmapMergeActor-00.err** file that has the BAM files named that were rejected due to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

To add this new baseline to your workflow, proceed to “Create an Ion Reporter analysis workflow“ on page 62.

Augment (add Samples to) an existing VCIB CNV baseline

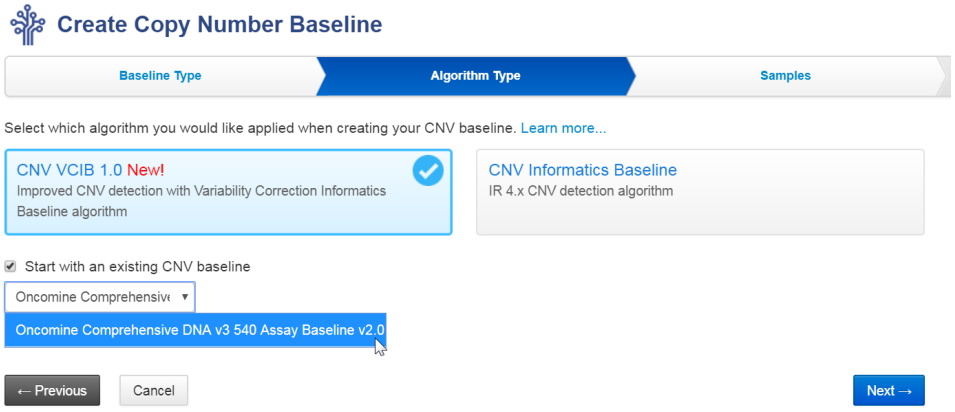
Ion Reporter™ Software provides a wizard to guide you through Copy Number Variation (CNV) baseline creation. This example describes how to add additional samples to an existing CNV baseline.

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 DNA v3 Regions v1.0** as your **Targets Region** file, then click **Next**.

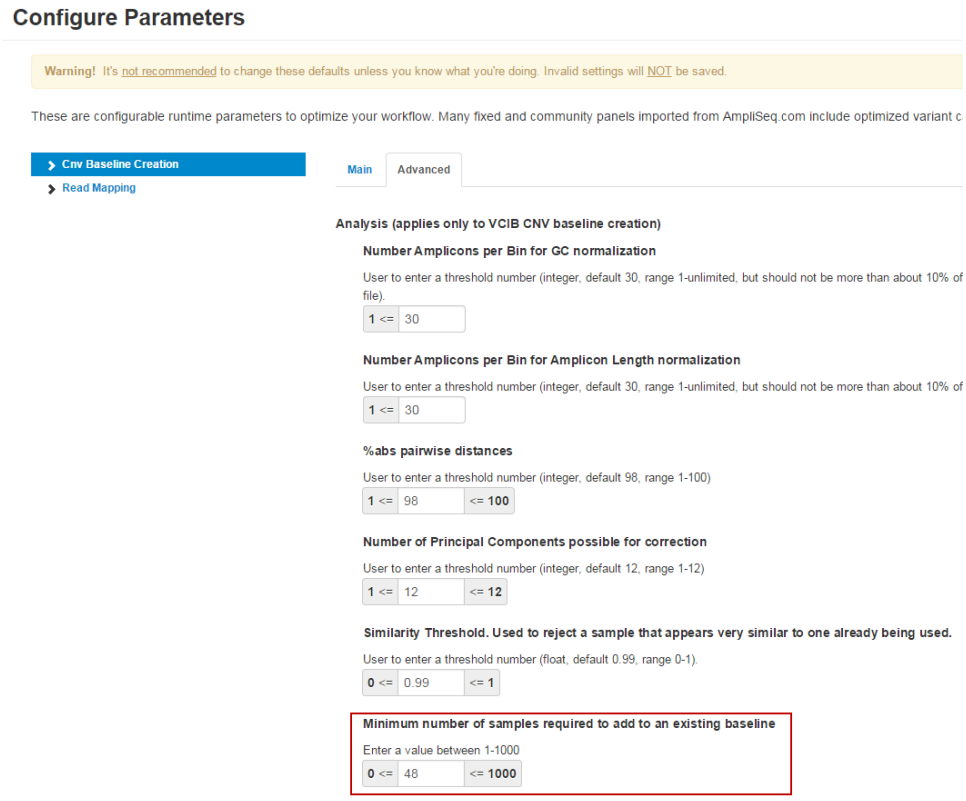
3. Select the **Start with an existing CNV Baseline** checkbox, then select a baseline from the dropdown list.



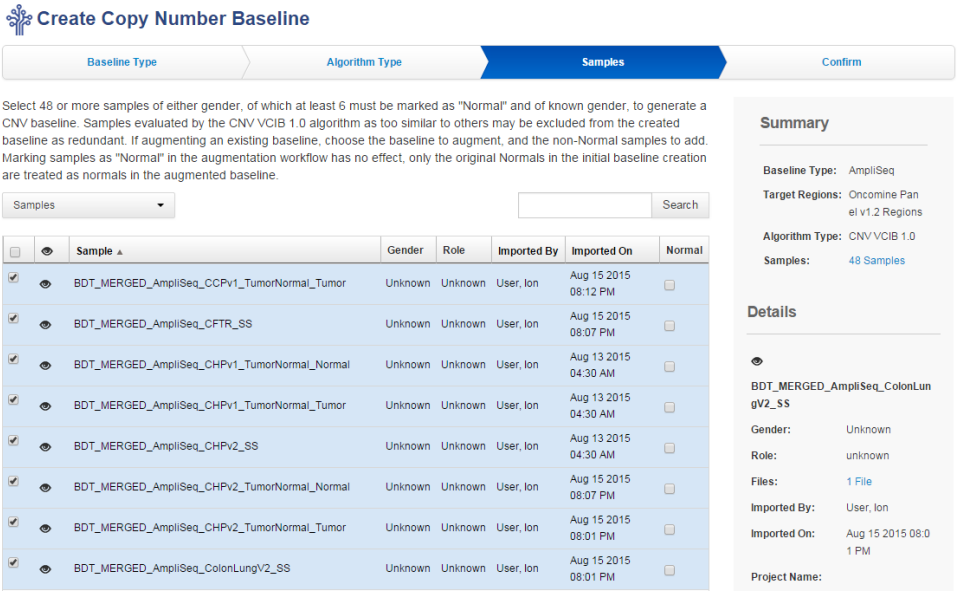
Note: By default, the software prompts you to add another 48 samples. However, you can set the number to 1 or more. Add non-Normal samples. Marking samples as "Normal" in the augmentation workflow has no effect, only the original Normals in the first baseline creation are treated as Normals in the augmented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

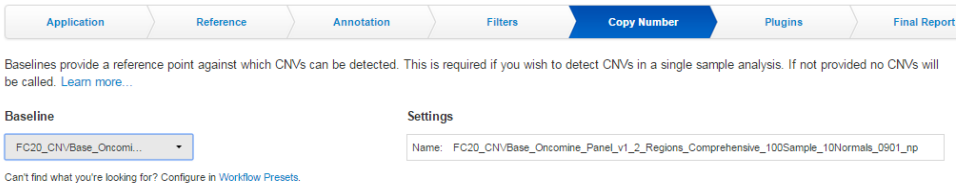


7. Enter a name for your baseline, then click **Create Baseline** to save.

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**.

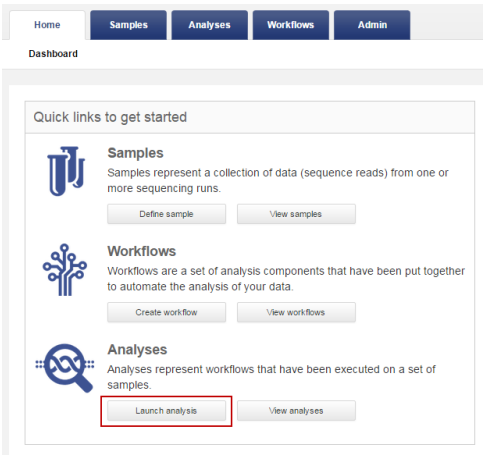


8. In the **Plugins** step, ensure that all **In-Analysis** plugins are deselected, then click **Next**.
9. Select a **Final Report Template** from the dropdown list, then click **Next**.
10. In the **Parameters** step, review the default settings, then click **Next**.

    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**.

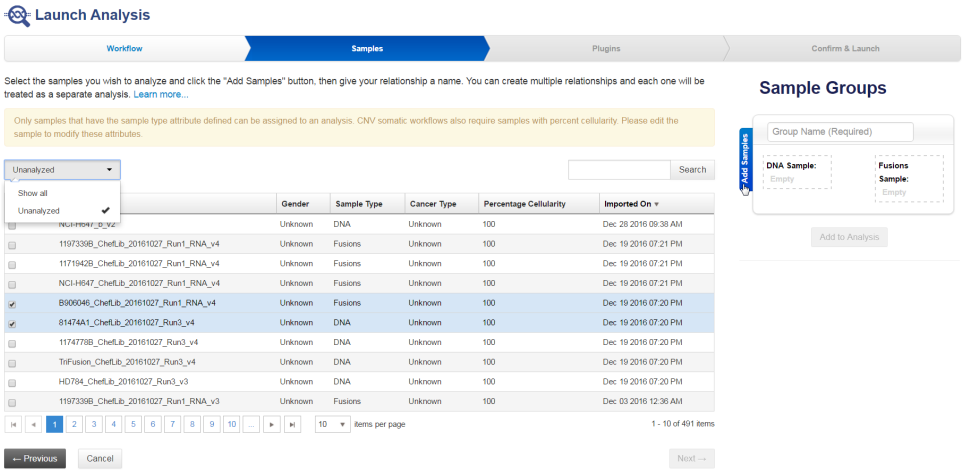
Launch an analysis

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**.

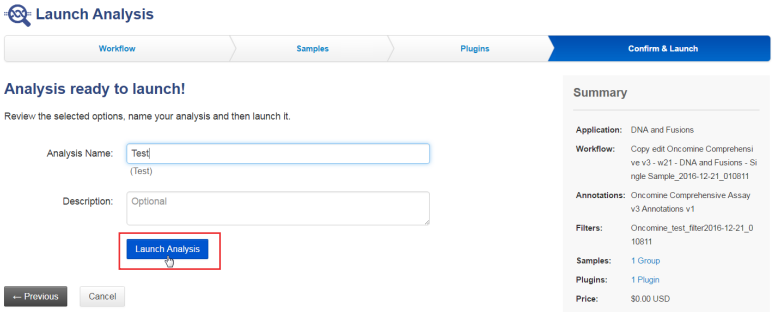


Note: The **Percentage Cellularity** sample attribute is required for DNA samples.

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

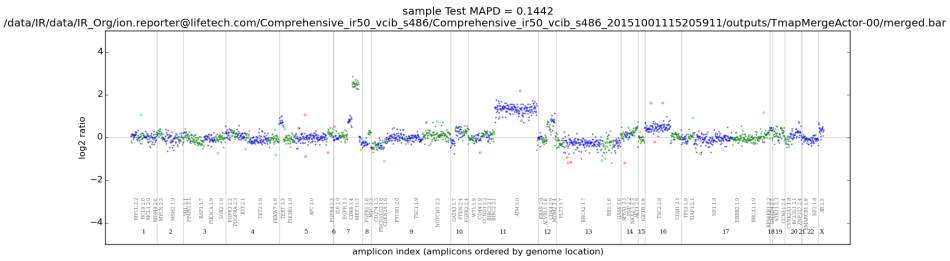
5. Click **Next** 2 times to move to the **Confirm & Launch** step.

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.

CNV somatic confidence filter

Set 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.

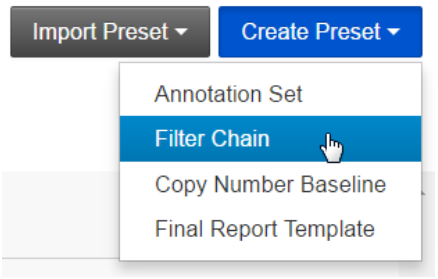
Note: The Oncomine™ Variant Annotator plugin annotates somatic CNVs on all chromosomes for the known copy-gain genes in the Oncomine™ Comprehensive Assay v3 results whose 5% confidence bound is greater than or equal to the expected normal ploidy plus 2.

How to change the confidence interval threshold default value

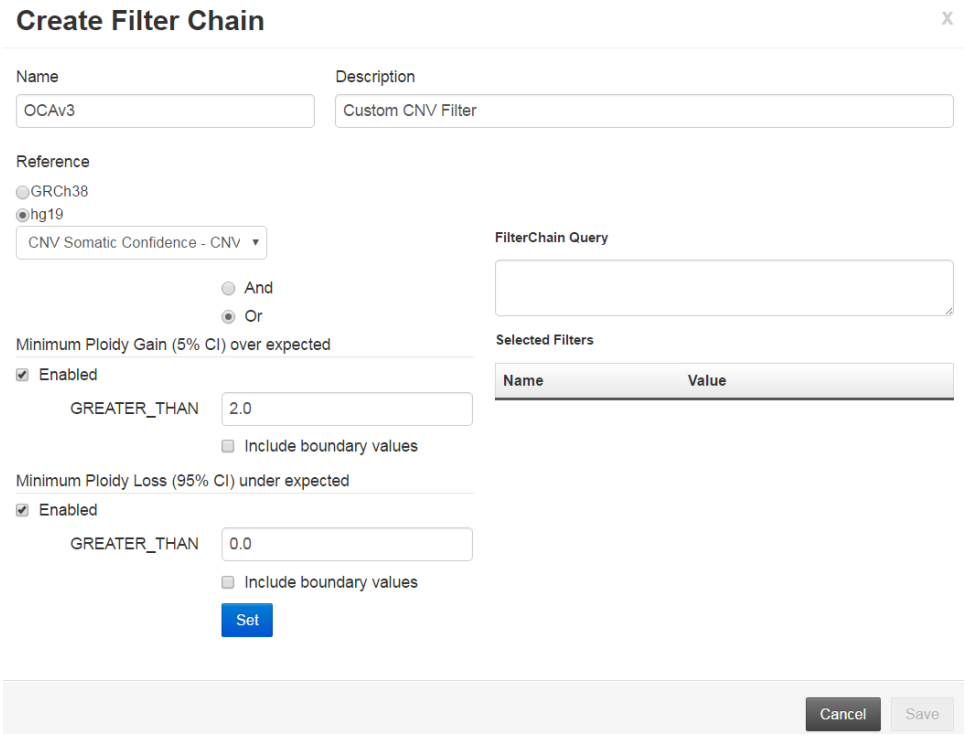
Note: The default threshold values of 0.0 will find all copy-gain genes whose 5% CI value is of ploidy 2 or greater and all copy-loss genes whose 95% CI value is of ploidy less than 2.

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).

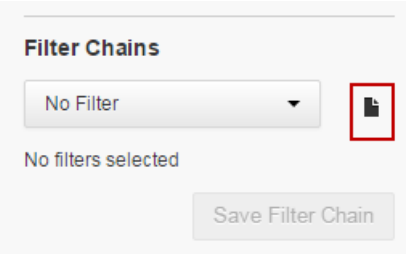
Subset filter creation

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.

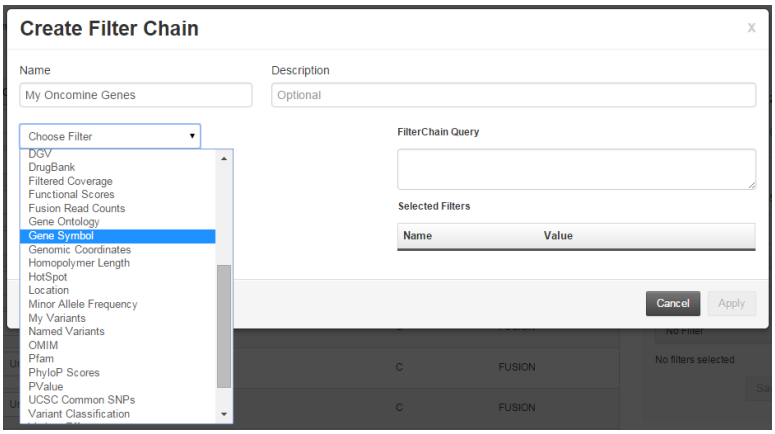
Create a gene-level filter

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

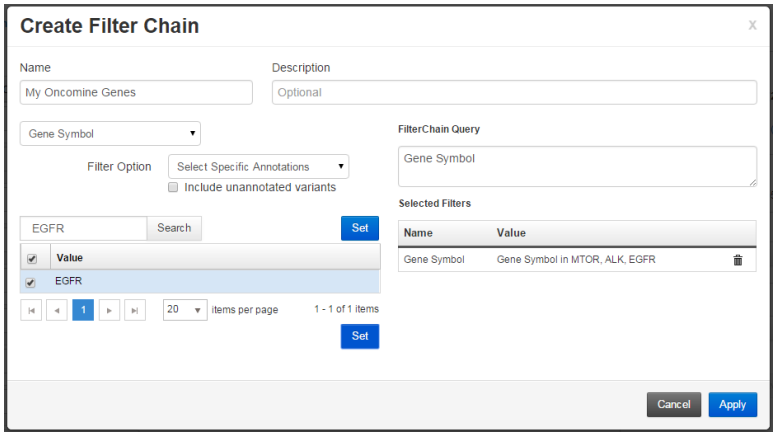
1. In the **Analyses** tab, in the **Overview** screen, click on the desired **Analysis** hyperlink in the **Analyses** table.
2. In the **Filter Options** pane, click **(New)**.



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.



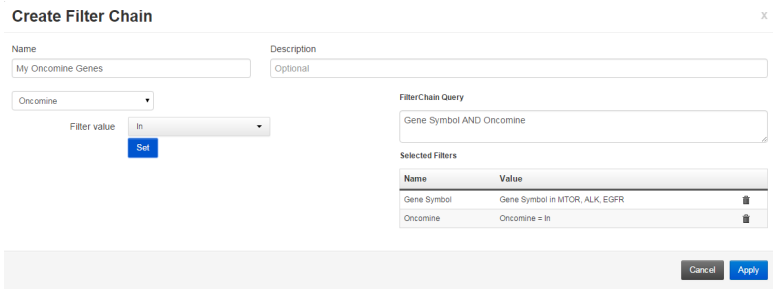
5. In the **Search** field, enter a gene symbol of interest (for example, EGFR), then click **Go** or press Enter.



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™**.

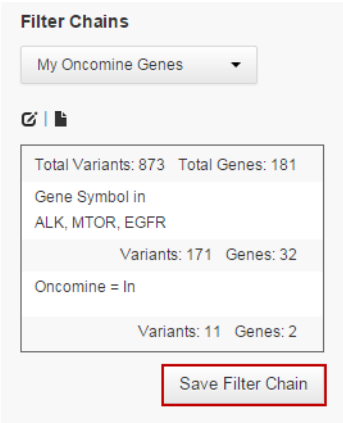
8. In the **Filter value** dropdown list, select **In**, then click **Set**.



9. Click **Apply**.

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

    Your new filter is now available for use.



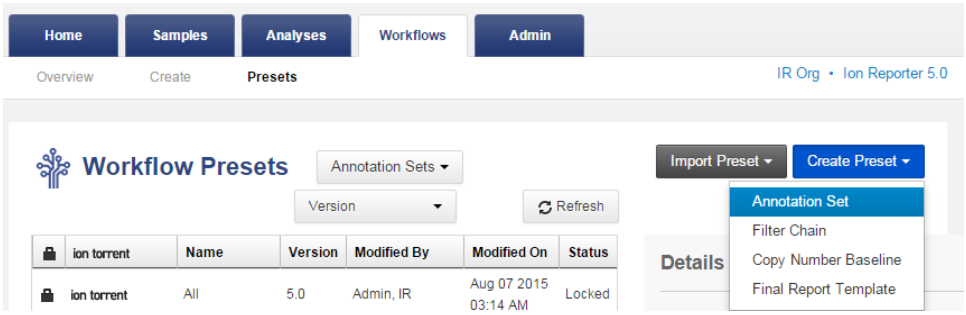
Next, copy a relevant workflow, then select this new filter chain as the default.

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.

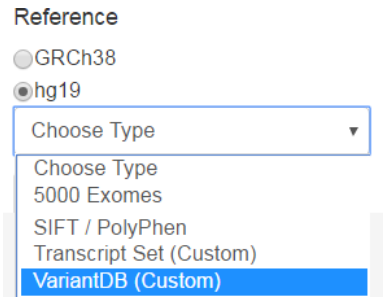
Create a new variantDB from the provided file

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.

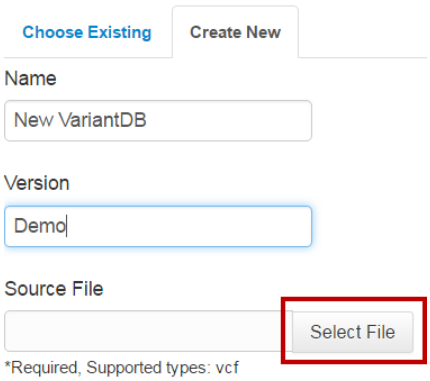


4. In the **Create New** tab, enter a **Name**, and **Version** for the new variantDB.

5. Click **Select File**.

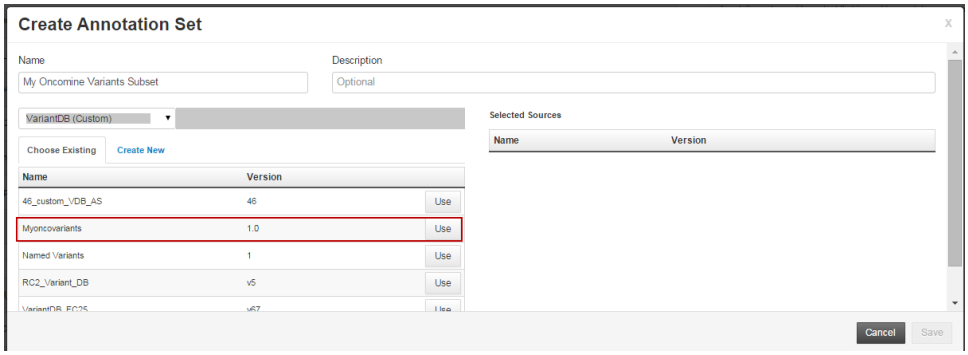
6. Navigate to the <assay name> _variantDB.vcf file, click **Open**, then click **Upload**.

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**.

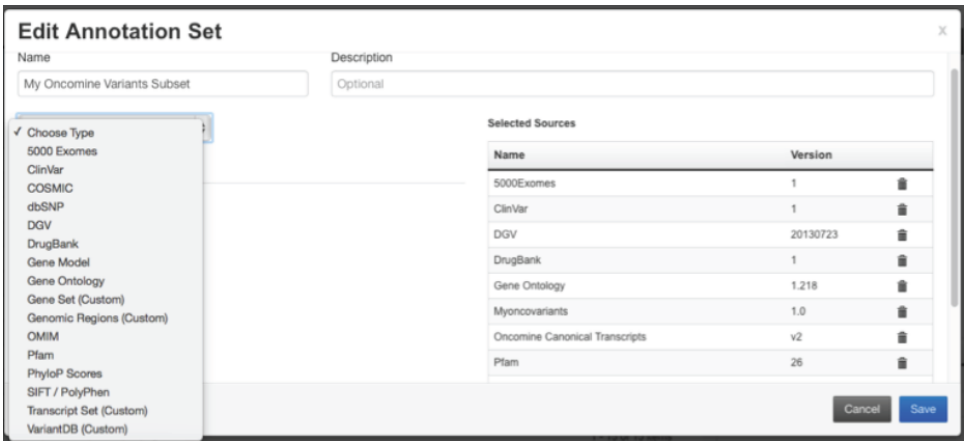


5. In the **Choose Type** dropdown list, select **5000Exomes**, then click **Use**. Repeat for the following sources:
   - ClinVar
   - dbSNP
   - DGV
   - DrugBank
   - Gene Ontology
   - Pfam
   - PhyloP Scores
   - RefGene Functional Canonical Transcripts Scores
   - RefSeq GeneModel
   - Oncomine™ 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)**

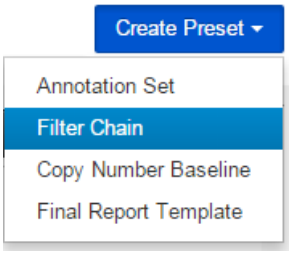


6. Click **Save**.

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**.

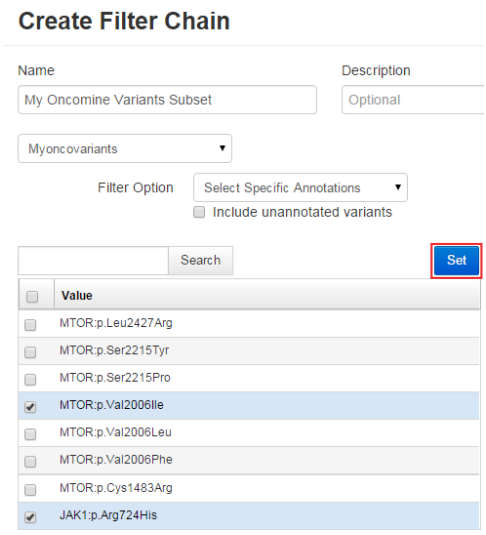


2. In the **Create Filter Chain** dialog, enter a **Name** and **Description** for your new **Filter Chain**.

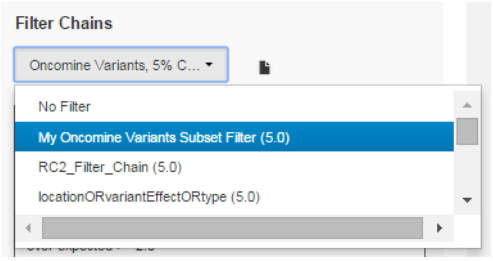
3. Click **Choose Filter**, then select the variantDB you created in the dropdown list.

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

5. Click **Save**.



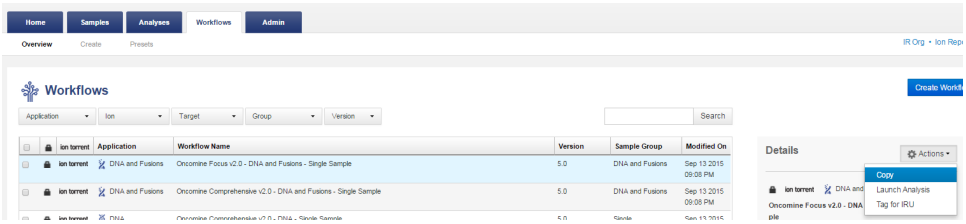
You can now apply your new variant subset filter to analyses.



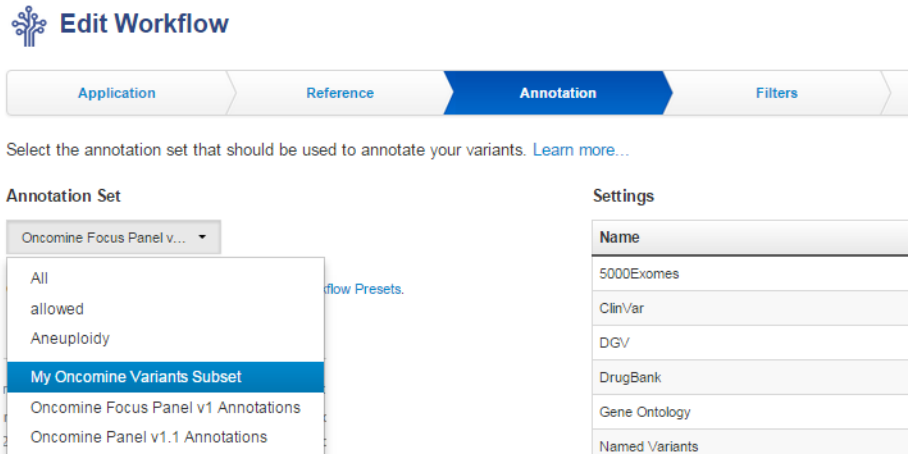
Create a copied workflow with the new annotation set and filter chain

Now you need to create a new workflow to use the annotation set and filter chain that you created.

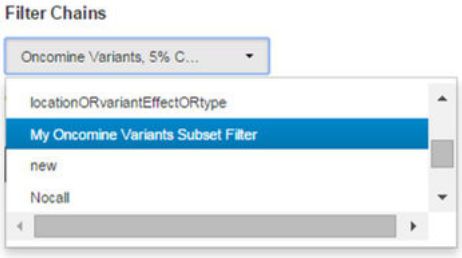
1. In the **Workflows** tab, in the **Overview** screen, select the workflow to be copied, then click **Actions** ▸ **Copy** in the **Details** pane.



2. In the **Annotation** step, add the new annotation set.



3. In the **Filters** step, add the new filter chain.



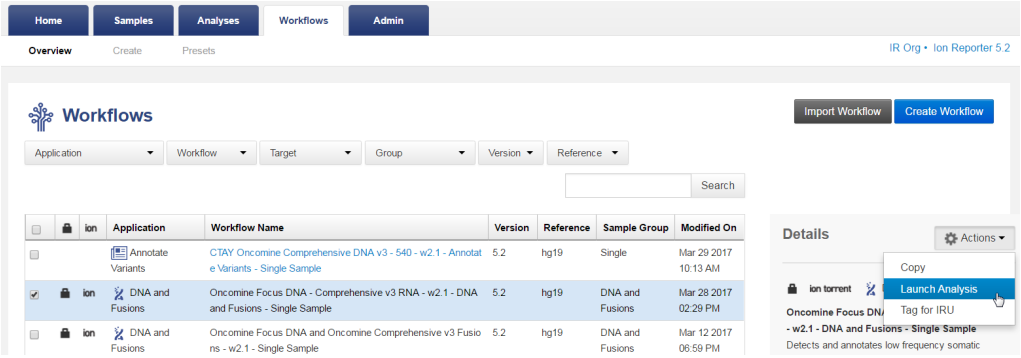
4. In the Confirm step, name the workflow.

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**.



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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

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

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

Documentation and Support

Related documentation

Document	Description
Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide (Pub. No. MAN0013432)	Describes the automated preparation and templating of Oncomine™ Comprehensive Assay 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 540™ Kit – Chef User Guide (Pub. No. MAN0010851)	Describes the automated template preparation of Oncomine™ Comprehensive Assay 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.

Note: For additional documentation, see "Customer and technical support" on page 78.

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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