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Oncomine™ Cell-Free Research Assay

USER GUIDE

for use with:
Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay
Oncomine™ Breast cfDNA Research Assay v2

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ThermoFisher SCIENTIFIC

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Revision	Date	Description
A.0	7 September 2017	User guide for the Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay, and Oncomine™ Breast cfDNA Research Assay v2. Provides instruction for library preparation, templating, sequencing, and data analysis of Oncomine™ Cell-Free Research Assay libraries.

Contents

1 Product information

Product description

IMPORTANT! Store all consumables under the recommended conditions and in an upright position.

The Ion Torrent™ Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay and Ion Torrent™ Oncomine™ Breast cfDNA Research Assay v2 each contain sufficient reagents for preparing 8 libraries from research samples. The kits are intended to be used with the Tag Sequencing BC Set 1–24 (Cat. No. A31830) and Tag Sequencing BC Set 25–48 (Cat. No. A31847) to generate barcoded libraries for multiplexed templating and sequencing.

IMPORTANT! This library preparation method is not compatible with other Ion barcode kits.

We recommend sequencing the libraries on the Ion S5™ or Ion S5™ XL Systems. To identify variants at 0.1% frequency with maximum sensitivity and specificity in cell-free total nucleic acid (cfNA) or cell-free DNA (cfDNA) samples, multiplex up to:

Sequencing chip	Number of multiplexed libraries	
	Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay	Oncomine™ Breast cfDNA Research Assay v2
Ion 530™ Chip	6	5
Ion 540™ Chip	24	20

Contents and storage

Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay

The Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay (Cat. No. A35864) contains sufficient reagents to prepare 8 libraries from cell-free total nucleic acid.

Component	Amount	Storage
cfDNA Library PCR Master Mix	320 µL	–25°C to –15°C
Low TE Buffer	832 µL	
Lung cfTNA Panel	16 µL	
cfDNA Library Primer P1	8 µL	
Tag Sequencing BC1	8 µL	
SuperScript™ VILO™ Master Mix	22 µL	

Oncomine™ Breast cfDNA Research Assay v2

The Oncomine™ Breast cfDNA Research Assay v2 (Cat. No. A35865) contains sufficient reagents to prepare 8 libraries from cell-free total nucleic acid.

Component	Amount	Storage
cfDNA Library PCR Master Mix	320 µL	–25°C to –15°C
Low TE Buffer	832 µL	
Breast cfDNA Panel v2	16 µL	
cfDNA Library Primer P1	8 µL	
Tag Sequencing BC1	8 µL	

Required materials not provided

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

Item	Source
Required for library preparation	
Tag Sequencing Barcode Set	• BC Set 1–24 (A31830) • BC Set 25–48 (A31847)
Agencourt™ AMPure™ XP, 5 mL	Beckman Coulter A63880
Veriti™ 96-Well Thermal Cycler (or equivalent)	4375786
7900HT Fast Real-Time PCR System (or equivalent)	4351405
Microcentrifuge[1]	MLS
Vortex mixer with a rubber platform	MLS
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
MicroAmp™ Splash-Free 96-Well Base	4312063
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
DynaMag™–96 Side Skirted Magnet	12027
Ion Library TaqMan® Quantitation Kit	4468802
Nuclease-free water, molecular biology grade	MLS
Ethanol, 96–100%	MLS
Pipettors, 2–200 µL, and low-retention filtered pipette tips	MLS
Required for template preparation and sequencing[2]	
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef, or Ion 520™ & Ion 530™ Kit – Chef, or Ion 540™ Kit – Chef	A34019 A27757 A27759
Ion 530™ Chip Kit (2 × 4-pack), or Ion 540™ Chip Kit (2 × 4-pack)	A27764 A27766
RNase-Free Microfuge Tubes (1.5 mL)	AM12400
Wipes, disposable lint-free	MLS

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

[2] The assays have been validated using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef, which requires Torrent Suite™ Software 5.4 or higher. Performance has been demonstrated using the Ion 520™ & Ion 530™ Kit – Chef, and the Ion 540™ Kit – Chef which require Torrent Suite™ Software 5.2 or higher.

2 Procedural guidelines

- When preparing DNA libraries for use, make certain to observe sterile laboratory procedures at all times to ensure minimal contamination.
- When you are sealing a plate or removing the sealing film from a plate, you must place the plate in a MicroAmp™ Splash-Free 96-Well Base to minimize unintended mixing of contents from neighboring wells.
- Prepare the 80% ethanol the same day you will use it.
- Use cell-free total nucleic acid (cfNA) extracted using a method optimized for cfNA isolation from plasma. We recommend the MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit (A36716). You can expect 5–50 ng of cfDNA and 5–100 pg of cfRNA from 10-mL blood research sample collected in a K_2EDTA blood collection tube.
- Use cell-free DNA (cfDNA) extracted using a method optimized for cfDNA isolation from plasma. We recommend the MagMAX™ Cell-Free DNA Isolation Kit (Cat. No. A29319) which was included during the Oncomine™ cfDNA workflow verification testing. Follow the "Alternate protocol for isolation of higher concentration cfDNA" in Appendix B of the *MagMAX™ Cell-Free DNA Isolation Kit User Guide* (Pub. No. MAN0014327). You can expect from 5–50 ng of cfDNA from a 10-mL blood research sample collected in a K_2EDTA blood collection tube.
- For ease of processing through the magnetic bead steps, you may prefer to process samples in the 96-well plate in columns as opposed to rows.
- For best results, use plasma samples with minimal to low level of hemolysis. To prevent hemolysis during blood collection follow guidelines provided in the **http://blog.fisherbioservices.com/avoiding-hemolysis-in-blood-sample-collection-and-processing** blog.
- We recommend the Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851) for quantification of cfNA and cfDNA samples. Spectrophotometric quantification methods are not recommended, because they are not reliable when the nucleic acid concentration is low. Use of these methods can lead to gross overestimation of the concentration of sample, under-seeding of the target amplification reaction, and low library yields.
- The recommended input amount of 20 ng cfDNA enables the detection of rare variants present at 0.1% frequency. This represents detection of one variant containing DNA template in the background of 999 DNA templates with wild type reference sequence. For best results, use as much cfDNA (up to 50 ng) as you have from the cell free fraction of a blood research sample. Successful libraries can be generated from 1–50 ng of cfDNA.

3 Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay library preparation

Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay workflow

Reverse transcribe cell-free total nucleic acid

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Amplify the target amplicons with barcoded primers

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Prepare 80% ethanol

Prepare sufficient 80% ethanol for the entire procedure immediately before use.

Number of Samples	Volume of ethanol	Volume of nuclease-free water
1	800 µL	200 µL
n	n × 800 µL	n × 200 µL

Reverse transcribe cell-free total nucleic acid

Reverse transcription is only required for the Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay. If you are preparing libraries for the Oncomine™ Breast cfDNA Research Assay v2 proceed to "Amplify targets" on page 22 and start from there.

1. 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™ VILO™ Master Mix	2.6 µL
Cell-free total nucleic acid (20 ng)[1]	⩽10.4 µL
Nuclease-free Water	to 13 µL
Total volume per well	**13 µL**

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

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

   Note: Move the sealed plate around the vortex mixer platform to ensure thorough mixing of each well in the plate.

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

Temperature	Time
42°C	30 minutes
85°C	5 minutes
10°C	Indefinite

STOPPING POINT Samples can be stored at 10°C for up to 2 hours in the thermal cycler. For longer term, store at –20°C.

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

Amplify targets

1. Thaw the following components on ice:
   - cfDNA Library PCR Master Mix
   - Oncomine™ Lung Cell-Free Total Nucleic Acid Panel

   IMPORTANT! Confirm both components thaw completely with no visible ice remaining. Vortex to mix, then centrifuge briefly to collect the contents before pipetting the required volumes.

2. Place the plate on the splash-free 96-well base, then carefully remove the seal.

3. Set up the target amplification reaction by adding the following components in the order listed to each sample well:

Component	Volume
Cell-free total nucleic acid reverse transcription products	13 µL
Nuclease-free water	—
Lung cfTNA Panel	2 µL
cfDNA Library PCR Master Mix	15 µL
Total volume	**30 µL**

   Note: Add cfDNA Library PCR Master Mix last to minimize the time the reaction mixture is at room temperature, or set up the PCR reaction on ice or in a pre-chilled (4°C) cold block.

4. Seal the plate with a new MicroAmp™ Clear Adhesive Film.

5. Vortex the 96-well plate to mix, then centrifuge briefly (300 × *g* for 30 seconds) to collect the contents.

   Note: Move the plate around the flat adaptor of the vortex mixer to ensure thorough mixing of each well in the plate.

6. Pre-heat the Veriti™ 96-well Thermal Cycler to 90°C, place a compression pad on the plate, then load the plate and run the following program:

Stage	Temperature	Time
Hold	98°C	1 minute
2 Cycles	98°C	15 seconds
	64°C	2 minutes
	62°C	2 minutes
	60°C	4 minutes
	58°C	2 minutes
	72°C	30 seconds
Hold	72°C	2 minutes
Hold	4°C	up to 1 hour

Note: There are slight changes in PCR conditions compared to the existing lung, breast, and colon Oncomine™ cfDNA Assays. Follow the conditions listed in the table exactly for PCR setup.

STOPPING POINT PCR products may be stored at 4°C for up to one hour.

Purify the target amplicons

IMPORTANT! Incubate the AMPure™ XP reagent at room temperature for at least 30 minutes, then vortex thoroughly to disperse the beads before use. Pipet the solution slowly. We recommend using low-retention pipette tips.

1. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents.
2. Place the plate on the MicroAmp™ Splash-Free 96-Well Base, then carefully remove the plate seal.
3. Use a 100 µL pipette to measure the reaction volume in each well. If the volume is <30 µL, add nuclease-free water to bring the volume in each well to 30 µL.
4. Add 45 µL (1.5 × sample volume) of Agencourt™ AMPure™ XP Reagent to each sample, seal the plate with a new MicroAmp™ Clear Adhesive Film.
5. Vortex for 15 seconds, then incubate at room temperature for 5 minutes.

   IMPORTANT! Thorough mixing of beads with samples is very important. After vortexing, check that the contents of each well is homogeneous in color.

6. Repeat Step 5, then centrifuge briefly (1 minute at 300 × *g*) to collect the contents.

   Note:
   - Total incubation time, with repeating the step, ~10 minutes.
   - Do not centrifuge the plate too fast with beads in the wells, because this can damage the beads.

7. Place the plate on the splash-free 96-well base, then carefully remove the seal.

8. Place the plate in the magnetic stand, incubate for 5 minutes or until the solution clears, then remove and discard the supernatant without disturbing the pellet.

9. Wash the magnetic beads with freshly prepared 80% ethanol.
   a. Add 150 µL of 80% ethanol to each sample.

   b. Incubate at room temperature for 30 seconds until the solution clears, then remove and discard the supernatant without disturbing the pellet.

      Note: Do not disturb the pellet or move the plate side-to-side between positions on the magnet during the wash steps.

10. Repeat step 9.

11. Keeping the plate in the magnetic stand, use a smaller pipette (10- or 20-µL) to remove any remaining ethanol, then air-dry the beads at room temperature for 5 minutes.

12. Transfer the plate to the splash-free 96-well base, then add 24 µL of Low TE buffer to the pellet.

13. Seal the plate with a new adhesive film, vortex thoroughly to disperse the beads, then incubate at room temperature for 5 minutes.

14. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, return the plate to the splash-free 96-well base, then carefully remove the seal.

15. Transfer the plate to the magnetic stand, incubate for at least 2 minutes, then transfer 23 µL of the supernatant to new wells on the same plate.

    Note: We recommend low-retention pipette tips to avoid sample loss during transfer.

IMPORTANT! PCR amplification can be inhibited by small amounts of carry-over beads. Remove any beads before proceeding to the second round of PCR. If you see beads in the pipette tip containing supernatant, slowly pipet the supernatant and beads back into their respective well on the side of the well next to the magnet so that the beads pass over the magnet. Then repeat step 15, repelleting the beads for an additional 1 minute.

Amplify the target amplicons with barcode adapted primers

1. Thaw the following components on ice:
   - cfDNA Library PCR Master Mix
   - cfDNA Library Primer P1
   - Tag Sequencing BC#

   Note: Tag Sequencing barcodes are required. This library method is not compatible with other library barcoding kits. Use a different Tag Sequencing BC# from the Tag Sequencing Barcode Set for each sample to be sequenced on the same chip in a multiplexed sequencing run.

2. Set up the second round of PCR with the plate still on the magnetic stand. Add the following components to each 23-µL sample:

Component	Volume
DNA from step 15 in the previous procedure	23 µL
Tag Sequencing BC (#1–48)	1 µL
cfDNA Library Primer P1	1 µL
cfDNA Library PCR Master Mix	25 µL
Total volume	**50 µL**

   Note: cfDNA Library PCR Master Mix should be added last to minimize the amount of time the reaction mixture spent at room temperature.

3. Seal the plate with a new MicroAmp™ adhesive film, vortex thoroughly to mix, then centrifuge the plate briefly (1 minute at 300 × *g*) to collect the contents.

4. Pre-heat the Veriti™ 96-well Thermal Cycler to 90°C, place a MicroAmp™ Optical Film Compression Pad on the plate, then load the plate in the thermal cycler and run the following program:

Stage	Temperature	Time
Hold	98°C	1 minute
18 cycles	98°C	15 seconds
	64°C	15 seconds
	72°C	15 seconds
Hold	72°C	5 minutes
Hold	4°C	Indefinite

   Note: There are slight changes in PCR conditions compared to the existing lung, breast, and colon Oncomine™ cfDNA Assays. Follow the conditions listed in the table exactly for PCR setup.

STOPPING POINT PCR products may be stored overnight at –20°C.

Purify the barcoded library

IMPORTANT! Incubate the AMPure™ XP reagent at room temperature for at least 30 minutes, then vortex thoroughly to disperse the beads before use. Pipet the solution slowly.

1. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, place the plate on the splash-free 96-well base, then carefully remove the plate seal.
2. Use a 100-µL pipette to measure the reaction volume in each well. If the volume is <50 µL, add nuclease-free water to bring the volume in each well to 50 µL.
3. Add 57.5 µL (1.15 × sample volume) of Agencourt™ AMPure™ XP Reagent to each sample, then seal the plate with a new MicroAmp™ Clear Adhesive Film.
4. Vortex for 15 seconds, then incubate at room temperature for 5 minutes.
5. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
6. Transfer the plate to the magnetic stand, incubate for 5 minutes or until the solution clears, then remove and discard the supernatant without disturbing the pellet.
7. Wash the magnetic beads with freshly prepared 80% ethanol.
   a. Add 150 µL 80% ethanol to each sample.
   b. Incubate at room temperature for 30 seconds until the solution clears, then remove and discard the supernatant without disturbing the pellet.

      Note: Do not disturb the pellet or move the plate side-to-side between positions on the magnet during the wash steps.
8. Repeat step 7.
9. Keeping the plate in the magnetic stand, use a smaller pipette (10- or 20-µL) to remove any remaining ethanol, then air-dry the beads at room temperature for 5 minutes.
10. Transfer the plate to the splash-free 96-well base, add 50 µL of Low TE buffer to the pellet, then seal the plate with a new adhesive film.
11. Vortex thoroughly to disperse the beads, incubate at room temperature for 5 minutes.
12. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
13. Transfer the plate to the magnetic stand and incubate for at least 2 minutes, then transfer 50 µL of the supernatant to new wells on the same plate.

    Note: Occasionally, carry-over of a small amount of beads occurs. This does not inhibit the procedures that follow.

Size select the barcoded library

1. Transfer the plate to the splash-free 96-well base, then add 50 µL (1.0 × sample volume) of Agencourt™ AMPure™ XP Reagent to each sample.
2. Seal the plate with a new MicroAmp™ Clear Adhesive Film, vortex thoroughly to disperse the beads, then incubate at room temperature for 5 minutes.
3. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
4. Place the plate in the magnetic stand, incubate for 5 minutes or until the solution clears, then remove and discard the supernatant without disturbing the pellet.
5. Wash the magnetic beads with freshly prepared 80% ethanol.
   a. Add 150 µL 80% ethanol to each sample.
   b. Incubate at room temperature for 30 seconds until the solution clears, then remove and discard the supernatant without disturbing the pellet.

      Note: Do not disturb the pellet or move the plate side-to-side between positions on the magnet during the wash steps.
6. Repeat step 5.
7. Keeping the plate in the magnetic stand, use a smaller pipette (10- or 20-µL) to remove any remaining ethanol, then air-dry the beads at room temperature for 5 minutes.
8. Transfer the plate to the 96-well base, then add 30 µL of Low TE buffer to the pellet.
9. Seal the plate with a new adhesive film, vortex thoroughly to disperse the beads, then incubate at room temperature for 5 minutes.
10. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
11. Transfer the plate to the magnetic stand for at least 2 minutes, then transfer 28 µL of the supernatant to new wells on the same plate.

    Note: Transfer a slightly smaller volume (28 µL instead of 30 µL) to avoid bead carry-over during this step. Keep the final library on ice (or store at –20°C) during set-up for "Quantify the library by qPCR".

Quantify the library by qPCR

Determine the concentration of each Oncomine™ cfDNA library by qPCR with the Ion Library TaqMan® Quantitation Kit. Analyze each sample, standard, and no template control (NTC) in triplicate reactions.

1. Prepare five 10-fold serial dilutions of the E. coli DH10B Ion Control Library (~68 pM; from the Ion Library TaqMan® Quantitation Kit) at 6.8 pM, 0.68 pM, 0.068 pM, 0.0068 pM, and 0.00068 pM.

2. Mark the five dilutions as standards (1–5), then use these concentrations in the qPCR instrument software.

3. Dilute each sample library as follows:
   a. Make a 1:100 dilution by combining 2 µL library with 198 µL nuclease-free water, vortex to mix, then centrifuge briefly to collect the contents.

   b. Add 3 µL of the 1:100 diluted library to 27 µL nuclease-free water to make a 1:1000 dilution, vortex to mix, then centrifuge briefly to collect the contents.

4. Calculate, then prepare the required volume of PCR master mix for triplicate reactions of each library sample, standard, and NTC using the following table. Include a 5–10% overage to accommodate pipetting errors.

Component	Volume per reaction	
	96-well plate	384-well plate
2X TaqMan® Master Mix	10 µL	5 µL
20X Ion TaqMan® Assay	1 µL	0.5 µL
Total	**11 µL**	**5.5 µL**

5. In an Optical 96-well Fast PCR plate set up the PCR for each sample, standard, and NTC by adding the following components:

Component	Volume per reaction	
	96-well plate	384-well plate
PCR Master Mix	11 µL	5.5 µL
1:1000 dilution of the sample[1]	9 µL	4.5 µL

[1] Substitute E. coli DH10B standards prepared in steps 1 and 2 for standards. Substitute nuclease-free water for NTC.

6. Load the plate in the 7900HT Fast Real-Time PCR Instrument, then run the following program:

Stage	Temperature	Time
Hold	50°C	2 minutes
Hold	95°C	20 seconds
40 cycles	95°C	1 second
	60°C	20 seconds

7. Calculate the average concentration of the undiluted library by multiplying the determined concentration × 1000.

Dilute the library

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

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

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

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

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

Combine libraries

Multiple barcoded libraries can be sequenced on a single chip by combining equal volumes of each library before template preparation. We recommend combining libraries up to a maximum of X libraries per Ion sequencing chip as indicated in the following table:

Ion sequencing chip	Maximum number of libraries (X)	
	Lung cfNA Libraries	Breast cfDNA v2 Libraries
Ion 530™ Chip	6	5
Ion 540™ Chip	24	20

Prepare a combined library as follows.

1. Dilute all individual barcoded libraries to 50 pM concentration.
2. Combine 10 µL of each library in a single 1.5-mL Eppendorf LoBind™ tube.
3. After adding the last library, pipet up and down 5 times to mix, then briefly centrifuge to collect in the contents.

STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C.

Guidelines for templating and sequencing

Proceed to template preparation and sequencing using the following kits.

Chip	Template System	Sequencer	Kit	User Guide
Ion 530™ Chip	Ion Chef™	Ion S5™	Ion 520™ & Ion 530™ Kit – Chef[1] (Cat. Nos. A27757, A30010)	*Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0010846)
			Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A34461, A34019)	*Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854)
Ion 540™ Chip	Ion Chef™	Ion S5™	Ion 540™ Kit – Chef (Cat. Nos. A27759, A30011)	*Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)

[1] The assays have been validated using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef, which requires Torrent Suite™ Software 5.4 or higher. Performance has been demonstrated using the Ion 520™ & Ion 530™ Kit – Chef, and the Ion 540™ Kit – Chef which require Torrent Suite™ Software 5.2 or higher.

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

4 Oncomine™ Breast cfDNA Research Assay v2 library preparation

Oncomine™ Breast cfDNA Research Assay v2 workflow

Amplify targets

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Purify the target amplicons

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Amplify the target amplicons with barcoded primers

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Purify the barcoded library

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Size select the barcoded library

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Quantify the library by qPCR

Prepare 80% ethanol

Prepare sufficient 80% ethanol for the entire procedure immediately before use.

Number of Samples	Volume of ethanol	Volume of nuclease-free water
1	800 µL	200 µL
n	n × 800 µL	n × 200 µL

Amplify targets

1. Thaw the following components on ice:
   - cfDNA Library PCR Master Mix
   - Ion Torrent™ Oncomine™ Breast cfDNA Panel v2

   IMPORTANT! Ensure both components thaw completely with no visible ice remaining. Vortex to mix, then centrifuge briefly to collect the contents before pipetting the required volumes.

2. Place the plate on the splash-free 96-well base, then carefully remove the seal.

3. Set up the target amplification reaction by adding the following components in the order that is listed to each sample well:

Component	Volume
cfDNA, 1–50 ng[1]	X µL
Nuclease-free water	13 – X µL
Breast cfDNA Panel v2	2 µL
cfDNA Library PCR Master Mix	15 µL
Total volume	**30 µL**

[1] We recommend ≥20 ng for 0.1% LOD.

   Note: For input, use as much cfDNA (1–50 ng) as you have after extraction from your research sample for best results.

   Note: Add cfDNA Library PCR Master Mix last to minimize the time the reaction mixture is at room temperature, or set up the PCR reaction on ice or in a pre-chilled (4°C) cold block.

4. Seal the plate with a new MicroAmp™ Clear Adhesive Film.

5. Vortex the 96-well plate to mix, then centrifuge briefly (300 × *g* for 30 seconds) to collect the contents.

   Note: Move the plate around the flat adaptor of the vortex mixer to ensure thorough mixing of each well in the plate.

6. Pre-heat the Veriti™ 96-well Thermal Cycler to 90°C, place a compression pad on the plate, then load the plate and run the following program:

Stage	Temperature	Time
Hold	98°C	1 minutes
2 Cycles	98°C	15 seconds
	64°C	2 minutes
	62°C	2 minutes
	60°C	4 minutes
	58°C	2 minutes
	72°C	30 seconds
Hold	72°C	2 minutes
Hold	4°C	up to 1 hour

Note: There are slight changes in PCR conditions compared to the existing lung, breast, and colon Oncomine™ cfDNA Assays. Follow the conditions that are listed in the table exactly for PCR setup.

STOPPING POINT PCR products can be stored at 4°C for up to one hour.

Purify the target amplicons

IMPORTANT! Incubate the AMPure™ XP reagent at room temperature for at least 30 minutes, then vortex thoroughly to disperse the beads before use. Pipet the solution slowly. We recommend using low-retention pipette tips.

1. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents.
2. Place the plate on the MicroAmp™ Splash-Free 96-Well Base, then carefully remove the plate seal.
3. Use a 100 µL pipette to measure the reaction volume in each well. If the volume is <30 µL, add nuclease-free water to bring the volume in each well to 30 µL.
4. Add 45 µL (1.5 × sample volume) of Agencourt™ AMPure™ XP Reagent to each sample, seal the plate with a new MicroAmp™ Clear Adhesive Film.
5. Vortex for 15 seconds, then incubate at room temperature for 5 minutes.

   IMPORTANT! Thorough mixing of beads with samples is very important. After vortexing, check that the contents of each well is homogeneous in color.

6. Repeat Step 5, then centrifuge briefly (1 minute at 300 × *g*) to collect the contents.

   Note:
   - Total incubation time, with repeating the step, ~10 minutes.
   - Do not centrifuge the plate too fast with beads in the wells, because this can damage the beads.

7. Place the plate on the splash-free 96-well base, then carefully remove the seal.

8. Place the plate in the magnetic stand, incubate for 5 minutes or until the solution clears, then remove and discard the supernatant without disturbing the pellet.

9. Wash the magnetic beads with freshly prepared 80% ethanol.
   a. Add 150 µL of 80% ethanol to each sample.

   b. Incubate at room temperature for 30 seconds until the solution clears, then remove and discard the supernatant without disturbing the pellet.

      Note: Do not disturb the pellet or move the plate side-to-side between positions on the magnet during the wash steps.

10. Repeat step 9.

11. Keeping the plate in the magnetic stand, use a smaller pipette (10- or 20-µL) to remove any remaining ethanol, then air-dry the beads at room temperature for 5 minutes.

12. Transfer the plate to the splash-free 96-well base, then add 24 µL of Low TE buffer to the pellet.

13. Seal the plate with a new adhesive film, vortex thoroughly to disperse the beads, then incubate at room temperature for 5 minutes.

14. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, return the plate to the splash-free 96-well base, then carefully remove the seal.

15. Transfer the plate to the magnetic stand, incubate for at least 2 minutes, then transfer 23 µL of the supernatant to new wells on the same plate.

    Note: We recommend low-retention pipette tips to avoid sample loss during transfer.

IMPORTANT! PCR amplification can be inhibited by small amounts of carry-over beads. Remove any beads before proceeding to the second round of PCR. If you see beads in the pipette tip containing supernatant, slowly pipet the supernatant and beads back into their respective well on the side of the well next to the magnet so that the beads pass over the magnet. Then repeat step 15, repelleting the beads for an additional 1 minute.

Amplify the target amplicons with barcode adapted primers

1. Thaw the following components on ice:
   - cfDNA Library PCR Master Mix
   - cfDNA Library Primer P1
   - Tag Sequencing BC#

   Note: Tag Sequencing barcodes are required. This library method is not compatible with other library barcoding kits. Use a different Tag Sequencing BC# from the Tag Sequencing Barcode Set for each sample to be sequenced on the same chip in a multiplexed sequencing run.

2. Set up the second round of PCR with the plate still on the magnetic stand. Add the following components to each 23-µL sample:

Component	Volume
DNA from step 15 in the previous procedure	23 µL
Tag Sequencing BC (#1–48)	1 µL
cfDNA Library Primer P1	1 µL
cfDNA Library PCR Master Mix	25 µL
Total volume	**50 µL**

   Note: cfDNA Library PCR Master Mix should be added last to minimize the amount of time the reaction mixture spent at room temperature.

3. Seal the plate with a new MicroAmp™ adhesive film, vortex thoroughly to mix, then centrifuge the plate briefly (1 minute at 300 × *g*) to collect the contents.

4. Pre-heat the Veriti™ 96-well Thermal Cycler to 90°C, place a MicroAmp™ Optical Film Compression Pad on the plate, then load the plate in the thermal cycler and run the following program:

Stage	Temperature	Time
Hold	98°C	1 minute
18 cycles	98°C	15 seconds
	64°C	15 seconds
	72°C	15 seconds
Hold	72°C	5 minutes
Hold	4°C	Indefinite

   Note: There are slight changes in PCR conditions compared to the existing lung, breast, and colon Oncomine™ cfDNA Assays. Follow the conditions listed in the table exactly for PCR setup.

STOPPING POINT PCR products may be stored overnight at –20°C.

Purify the barcoded library

IMPORTANT! Incubate the AMPure™ XP reagent at room temperature for at least 30 minutes, then vortex thoroughly to disperse the beads before use. Pipet the solution slowly.

1. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, place the plate on the splash-free 96-well base, then carefully remove the plate seal.
2. Use a 100-µL pipette to measure the reaction volume in each well. If the volume is <50 µL, add nuclease-free water to bring the volume in each well to 50 µL.
3. Add 57.5 µL (1.15 × sample volume) of Agencourt™ AMPure™ XP Reagent to each sample, then seal the plate with a new MicroAmp™ Clear Adhesive Film.
4. Vortex for 15 seconds, then incubate at room temperature for 5 minutes.
5. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
6. Transfer the plate to the magnetic stand, incubate for 5 minutes or until the solution clears, then remove and discard the supernatant without disturbing the pellet.
7. Wash the magnetic beads with freshly prepared 80% ethanol.
   a. Add 150 µL 80% ethanol to each sample.
   b. Incubate at room temperature for 30 seconds until the solution clears, then remove and discard the supernatant without disturbing the pellet.

      Note: Do not disturb the pellet or move the plate side-to-side between positions on the magnet during the wash steps.
8. Repeat step 7.
9. Keeping the plate in the magnetic stand, use a smaller pipette (10- or 20-µL) to remove any remaining ethanol, then air-dry the beads at room temperature for 5 minutes.
10. Transfer the plate to the splash-free 96-well base, add 50 µL of Low TE buffer to the pellet, then seal the plate with a new adhesive film.
11. Vortex thoroughly to disperse the beads, incubate at room temperature for 5 minutes.
12. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
13. Transfer the plate to the magnetic stand and incubate for at least 2 minutes, then transfer 50 µL of the supernatant to new wells on the same plate.

    Note: Occasionally, carry-over of a small amount of beads occurs. This does not inhibit the procedures that follow.

Size select the barcoded library

1. Transfer the plate to the splash-free 96-well base, then add 50 µL (1.0 × sample volume) of Agencourt™ AMPure™ XP Reagent to each sample.
2. Seal the plate with a new MicroAmp™ Clear Adhesive Film, vortex thoroughly to disperse the beads, then incubate at room temperature for 5 minutes.
3. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
4. Place the plate in the magnetic stand, incubate for 5 minutes or until the solution clears, then remove and discard the supernatant without disturbing the pellet.
5. Wash the magnetic beads with freshly prepared 80% ethanol.
   a. Add 150 µL 80% ethanol to each sample.
   b. Incubate at room temperature for 30 seconds until the solution clears, then remove and discard the supernatant without disturbing the pellet.

      Note: Do not disturb the pellet or move the plate side-to-side between positions on the magnet during the wash steps.
6. Repeat step 5.
7. Keeping the plate in the magnetic stand, use a smaller pipette (10- or 20-µL) to remove any remaining ethanol, then air-dry the beads at room temperature for 5 minutes.
8. Transfer the plate to the 96-well base, then add 30 µL of Low TE buffer to the pellet.
9. Seal the plate with a new adhesive film, vortex thoroughly to disperse the beads, then incubate at room temperature for 5 minutes.
10. Briefly centrifuge the PCR plate (1 minute at 300 × *g*) to collect the contents, transfer the plate to the splash-free 96-well base, then carefully remove the seal.
11. Transfer the plate to the magnetic stand for at least 2 minutes, then transfer 28 µL of the supernatant to new wells on the same plate.

    Note: Transfer a slightly smaller volume (28 µL instead of 30 µL) to avoid bead carry-over during this step. Keep the final library on ice (or store at –20°C) during set-up for "Quantify the library by qPCR".

Quantify the library by qPCR

Determine the concentration of each Oncomine™ cfDNA library by qPCR with the Ion Library TaqMan® Quantitation Kit. Analyze each sample, standard, and no template control (NTC) in triplicate reactions.

1. Prepare five 10-fold serial dilutions of the E. coli DH10B Ion Control Library (~68 pM; from the Ion Library TaqMan® Quantitation Kit) at 6.8 pM, 0.68 pM, 0.068 pM, 0.0068 pM, and 0.00068 pM.

2. Mark the five dilutions as standards (1–5), then use these concentrations in the qPCR instrument software.

3. Dilute each sample library as follows:
   a. Make a 1:100 dilution by combining 2 µL library with 198 µL nuclease-free water, vortex to mix, then centrifuge briefly to collect the contents.

   b. Add 3 µL of the 1:100 diluted library to 27 µL nuclease-free water to make a 1:1000 dilution, vortex to mix, then centrifuge briefly to collect the contents.

4. Calculate, then prepare the required volume of PCR master mix for triplicate reactions of each library sample, standard, and NTC using the following table. Include a 5–10% overage to accommodate pipetting errors.

Component	Volume per reaction	
	96-well plate	384-well plate
2X TaqMan® Master Mix	10 µL	5 µL
20X Ion TaqMan® Assay	1 µL	0.5 µL
Total	**11 µL**	**5.5 µL**

5. In an Optical 96-well Fast PCR plate set up the PCR for each sample, standard, and NTC by adding the following components:

Component	Volume per reaction	
	96-well plate	384-well plate
PCR Master Mix	11 µL	5.5 µL
1:1000 dilution of the sample[1]	9 µL	4.5 µL

[1] Substitute E. coli DH10B standards prepared in steps 1 and 2 for standards. Substitute nuclease-free water for NTC.

6. Load the plate in the 7900HT Fast Real-Time PCR Instrument, then run the following program:

Stage	Temperature	Time
Hold	50°C	2 minutes
Hold	95°C	20 seconds
40 cycles	95°C	1 second
	60°C	20 seconds

7. Calculate the average concentration of the undiluted library by multiplying the determined concentration × 1000.

Dilute the library

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

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

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

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

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

Combine libraries

Multiple barcoded libraries can be sequenced on a single chip by combining equal volumes of each library before template preparation. We recommend combining libraries up to a maximum of X libraries per Ion sequencing chip as indicated in the following table:

Ion sequencing chip	Maximum number of libraries (X)	
	Lung cfNA Libraries	Breast cfDNA v2 Libraries
Ion 530™ Chip	6	5
Ion 540™ Chip	24	20

Prepare a combined library as follows.

1. Dilute all individual barcoded libraries to 50 pM concentration.
2. Combine 10 µL of each library in a single 1.5-mL Eppendorf LoBind™ tube.
3. After adding the last library, pipet up and down 5 times to mix, then briefly centrifuge to collect in the contents.

STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C.

Guidelines for templating and sequencing

Proceed to template preparation and sequencing using the following kits.

Chip	Template System	Sequencer	Kit	User Guide
Ion 530™ Chip	Ion Chef™	Ion S5™	Ion 520™ & Ion 530™ Kit – Chef[1] (Cat. Nos. A27757, A30010)	*Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0010846)
			Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A34461, A34019)	*Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854)
Ion 540™ Chip	Ion Chef™	Ion S5™	Ion 540™ Kit – Chef (Cat. Nos. A27759, A30011)	*Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)

[1] The assays have been validated using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef, which requires Torrent Suite™ Software 5.4 or higher. Performance has been demonstrated using the Ion 520™ & Ion 530™ Kit – Chef, and the Ion 540™ Kit – Chef which require Torrent Suite™ Software 5.2 or higher.

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

5 Create an assay-specific Planned Run

IMPORTANT!

- These kits are compatible with Torrent Suite™ Software 5.2 or later and Ion Reporter™ Software 5.6 or later. Before proceeding, check for updates to the Torrent Suite™, Ion Reporter™, and Ion Chef™ System software, and install the updates if available.
- If running Torrent Suite™ Software 5.2 or 5.4 you must install the Ion Reporter™ Uploader 5.6 plugin to allow data transfer to the Ion Reporter™ Server. Ion Reporter™ Uploader 5.6 plugin is compatible with Torrent Suite™ Software 5.2 or later. See "Install the Ion Reporter™ Uploader plugin on your Torrent Server" on page 43 for more information.

About planned runs

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

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

Application	Torrent Suite™ Software template	Description
Oncology - Liquid Biopsy	• Oncomine™ Lung Liquid Biopsy DNA • Oncomine™ Breast Liquid Biopsy DNA • Oncomine™ TagSeq Liquid Biopsy[1] • Oncomine™ TagSeq S540 Liquid Biopsy[2]	Planned run template for use with cell-free DNA (cfDNA) or cell-free total nucleic acid (cfNA) research samples. Analysis parameters are optimized for the sensitive and specific detection of rare somatic variants (SNPs, InDels) present at 0.1% frequency in cfDNA.
	• Oncomine™ Lung Tumor DNA • Oncomine™ Breast Tumor DNA • Oncomine™ TagSeq Tumor[1] • Oncomine™ TagSeq S540 Tumor[2]	Planned run template for use with solid tumor research samples from FFPE as well as fresh frozen tumor tissue. Analysis parameters are optimized for the sensitive and specific detection of rare somatic variants (SNPs, InDels) present at 0.5% frequency. Analysis parameters are optimized to eliminate false positives due to DNA damage resulting from formalin fixation.

[1] Available in Torrent Suite™ Software 5.4 or later.

[2] Available in Torrent Suite™ Software 5.6 or later.

Create a custom Planned Run template

IMPORTANT!

- Before creating a template file the **Target Regions** BED files must be uploaded to the Torrent Server. Required files can be found on the **thermofisher.com** product page.
- If using the Torrent Suite™ Software variantCaller 5.4 or later plugin to perform SNV and small indel variant analysis a Hotspot Regions BED file and tissue specific parameter file (JSON) must be installed. Required files can be found on the **thermofisher.com** product page.
- The assays have been validated using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef, which requires Torrent Suite™ Software 5.4 or higher. Performance has been demonstrated using the Ion 520™ & Ion 530™ Kit – Chef, and the Ion 540™ Kit – Chef which require Torrent Suite™ Software 5.2 or higher.

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

1. Sign in to the Torrent Browser for the Torrent Server connected to your Ion Chef™ System.

2. Under the **Plan** tab, in the **Templates** screen, click **Oncology - Liquid Biopsy** in the research application list.

3. Find the appropriate **Oncomine™ Liquid Biopsy DNA** (Lung or Breast) template in the **Oncology - Liquid Biopsy** list, click ⚙ then select **Copy**.

Torrent Suite™ Software version	Templates available
5.2	• Oncomine Lung Liquid Biopsy DNA • Oncomine Breast Liquid Biopsy DNA
5.4	• Oncomine Lung Liquid Biopsy DNA • Oncomine Breast Liquid Biopsy DNA • Oncomine TagSeq Liquid Biopsy[1]
5.6	• Oncomine Lung Liquid Biopsy DNA • Oncomine Breast Liquid Biopsy DNA • Oncomine TagSeq Liquid Biopsy[1] • Oncomine TagSeq S540 Liquid Biopsy[1,2]

[1] Recommended.
[2] Compatible with Ion 540™ Chip only.

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

The copy template wizard will open to the **Save** tab.

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

Field	Action...
Template Name	Enter a name for the Planned Run template.
Reference Library	Select **hg19(Human (hg19))**.
Target Regions[1]	Select the appropriate Target Region file: • **Oncomine_Lung_cfNA.08212017.Designed.bed** • **Oncomine_Breast_cfDNA_v2.08212017.Designed.bed**
Hotspot Regions[1,2]	Select the appropriate Hotspot Region file: • **Oncomine_Lung_cfNA.08212017.Hotspots.bed** • **Oncomine_Breast_cfDNA_v2.08212017.Hotspots.bed**

[1] Check with your service representative for updates to ensure the most current files are being used. See "Download and install BED files" on page 44 for BED file installation instructions.

[2] A Hotspot Regions BED file is only required if using the Torrent Suite™ Software variantCaller plugin to perform SNV and small indel variant analysis.

5. *(Optional)* If you are using Torrent Suite™ Software 5.2, update the **Alignment** parameter.
   a. Select the **Custom Analysis Parameters** radio button.
   b. Scroll down to the **Alignment** pane, then replace the existing text string with " `tmap mapall ... -J 25 --end-repair 15 --do-repeat-clip --context stage1 map4`".

6. In the **Create Plan** workflow bar, click the **Ion Reporter** step, then:
   - To export files for analysis using Ion Reporter, select your **Ion Reporter Account**, select **Automatically upload to Ion Reporter after run completion** as **Sample Grouping**, then click **Next**.
   - If performing analysis using only the variantCaller 5.4 (or later) plugin of the Torrent Suite™ Software, ensure **None** and **Self** are selected for the **Ion Reporter Account** and **Sample Grouping** respectively, then click **Next**.

7. In the **Create Plan** workflow bar, click the **Research Application** step, verify that **Oncology–Liquid Biopsy** and **Tag Sequencing** are selected for **Application** and **Target Technique** respectively, then click **Next**.

8. In the **Kits** step, verify the **Ion Chef Template Kit** radio button is selected, and the following fields are completed:

Field	Selection	
	Ion 530™ Chip	**Ion 540™ Chip**
Instrument	Ion S5™ System	
Library Kit Type	Oncomine cfDNA Assay	
Template Kit[1]	Ion 520™ & Ion 530™ Kit – Chef or Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef	Ion 540™ Kit – Chef
Read Length	200	

Field	Selection	
Sequencing Kit	Ion S5™ Sequencing Kit	
Base Calibration Mode	Default Calibration	
Chip Type	Ion 530™ Chip	Ion 540™ Chip
Barcode Set[2]	TagSequencing	
Flows	500	

[1] Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef available in Torrent Suite™ Software 5.4 orlater.

[2] If running Torrent Suite™ Software 5.2 select IonCode - TagSequencing

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

10. In the **Plugins** step:

    - *(If running Torrent Suite™ Software 5.2)*, ensure **variantCaller** is **deselected**. Variant calls are provided through the Ion Reporter™ Software 5.6.
    - *(If running Torrent Suite™ Software 5.4 or later)*, running the variantCaller plugin is optional and intended for SNV and small indel calls only. Download and install the Hotspot Regions BED file and tissue specific parameter file (JSON) from the **thermofisher.com** product page.

    IMPORTANT! Variant calls are provided through the Ion Reporter™ Software in Torrent Suite™ Software 5.4 or later.

11. In the **Projects** step, select the projects to receive data from this run, then click **Next**.

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

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

Create a Planned Run

1. Sign in to the Torrent Browser for the Torrent Server connected to your Ion Chef™ System.

2. Under the **Plan** tab, in the **Templates** screen, click **Oncology - Liquid Biopsy** in the left navigation menu.

3. In the Oncology - Liquid Biopsy list, click on your customized Planned Run template name or the provided Oncomine™ Liquid Biopsy DNA template, alternatively click ⚙ ▼ then select **Plan Run**.
   The create plan wizard will open to the **Plan** tab.

4. Enter a **Run Plan Name**.

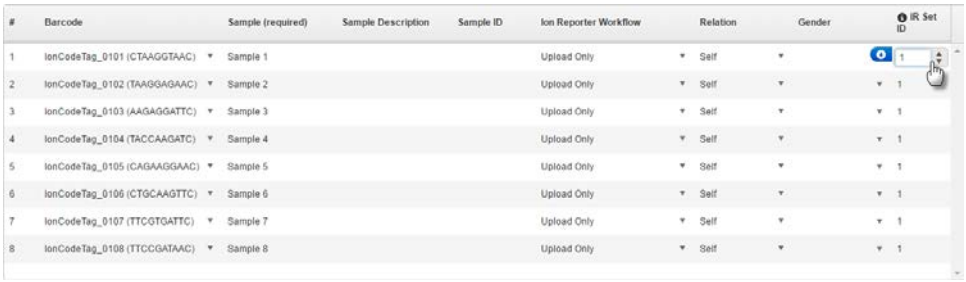
5. Verify the **Use same reference & BED files for all barcodes** radio button is selected.

6. In the **Number of barcodes** field, enter the number of barcodes that will be used in this run, then click the check mark ✔ button to the right of this field.

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

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

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



#	Barcode	Sample (required)	Sample Description	Sample ID	Ion Reporter Workflow	Relation	Gender	IR Set ID
1	IonCodeTag_0101 (CTAAGGTAAC)	Sample 1			Upload Only	Self		1
2	IonCodeTag_0102 (TAAGGAGAAC)	Sample 2			Upload Only	Self		1
3	IonCodeTag_0103 (AAGAGGATTC)	Sample 3			Upload Only	Self		1
4	IonCodeTag_0104 (TACCAAGATC)	Sample 4			Upload Only	Self		1
5	IonCodeTag_0105 (CAGAAGGAAC)	Sample 5			Upload Only	Self		1
6	IonCodeTag_0106 (CTGCAAGTTC)	Sample 6			Upload Only	Self		1
7	IonCodeTag_0107 (TTCGTGATTC)	Sample 7			Upload Only	Self		1
8	IonCodeTag_0108 (TTCCGATAAC)	Sample 8			Upload Only	Self		1

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

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

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

    IMPORTANT! **Sample Names** must be unique to each sample. BAM files from samples with the same **Sample Name** will be grouped together by the Ion Reporter™ Software and analyzed together. This feature can be used to increase sequencing read depth by combining results from separate sequencing runs if desired.

11. Click **Plan Run**.

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

6 Ion Reporter™ Variant Analysis

Analysis workflows in Ion Reporter™ Software

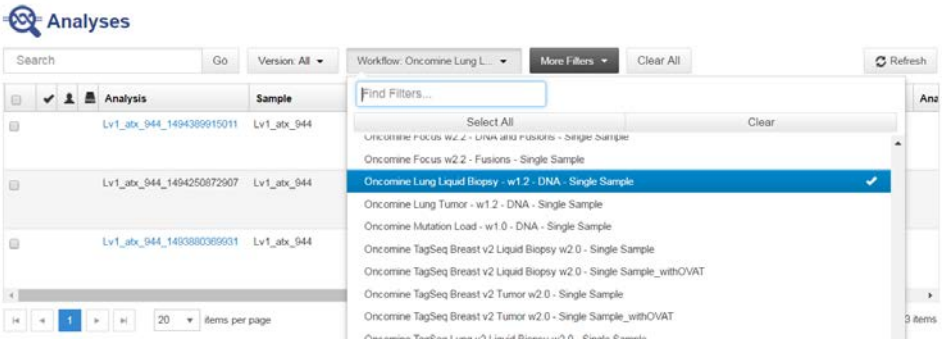
In Torrent Suite™ Software, you can plan your run to transfer data automatically to the appropriate Ion Reporter™ server and be analyzed through one of the available Oncomine™ analysis workflows.

Analysis Workflow	Description
Oncomine™ TagSeq Lung v2 Liquid Biopsy - w2.0 -Single Sample	Detects and annotates low frequency variants including SNPs/InDels (down to 0.1% limit of detection), Fusions, and CNVs from targeted nucleic acid libraries (DNA & RNA) from the Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay. This is compatible with DNA & RNA purified from cell-free total nucleic acids.
Oncomine™ TagSeq Lung v2 Tumor - w2.0 - Single Sample	Detects and annotates low frequency variants including SNPs/InDels (down to 0.5% limit of detection), Fusions, and CNVs from targeted nucleic acid libraries (DNA & RNA) from the Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay. Due to deamination events caused by the FFPE process, the minimum alternative allele frequency is set to 0.3%. This makes it compatible with DNA & RNA purified from FFPE tumor tissue as well as fresh frozen tumor tissue.
Oncomine™ TagSeq Breast v2 Liquid Biopsy - w2.0 -Single Sample	Detects and annotates low frequency variants including SNPs/InDels (down to 0.1% limit of detection), and CNVs from targeted DNA libraries from the Oncomine™ Breast cfDNA Research Assay v2. This is compatible with DNA purified from cell-free DNA.
Oncomine™ TagSeq Breast v2 Tumor - w2.0 - Single Sample	Detects and annotates low frequency variants including SNPs/InDels (down to 0.5% limit of detection), and CNVs from targeted DNA libraries from the Oncomine™ Breast cfDNA Research Assay v2. Due to deamination events caused by the FFPE process, the minimum alternative allele frequency is set to 0.3%. This makes it compatible with DNA purified from FFPE tumor tissue as well as fresh frozen tumor tissue.

Visualize identified variants

Ion Reporter™ Software analyses are performed automatically on uploading of the data files from the Torrent Suite™ Software. The **Visualize** pathway of results analysis allows the side-by-side comparison of multiple samples. To view the results:

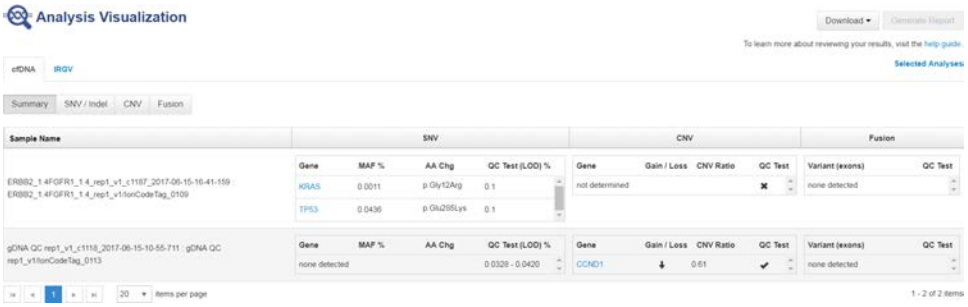
1. Sign in to the Ion Reporter™ Software.
2. Click the **Analyses** tab.
3. Click the column headers to sort the results, or use the available filters to limit the list of analyses (for example, select one or more workflows from the **Workflow** dropdown list).



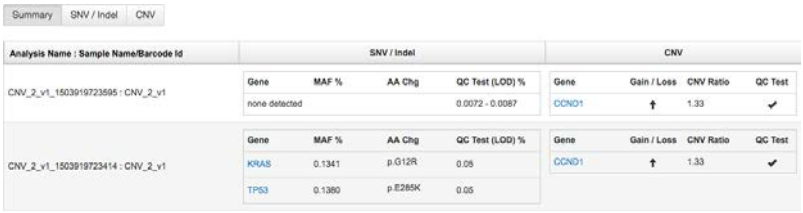
4. Click the checkbox adjacent to each analysis of interest, then click **Visualize**.
   Alternatively, select the analyses, then in the **Details** pane, click ⚙ **Actions** ▸ **Visualize**.

   Note: Select two or more analyses to visualize a side-by-side comparison of multiple results. Advanced users, click the hyperlinked sample name in the **Analysis** column, to view and manage additional Oncomine™ annotation details for a single sample (see Appendix C, "View and manage extended analysis results for a single sample" for more information).

5. The **Analysis Visualization** screen opens to the **Summary** tab and lists all the identified SNVs, CNVs, and Fusions (Lung only). Click a hyperlinked **Gene** name to be redirected to the HGNC report for that gene.



Example visualization of the Oncomine™ Lung cfNA Assay.

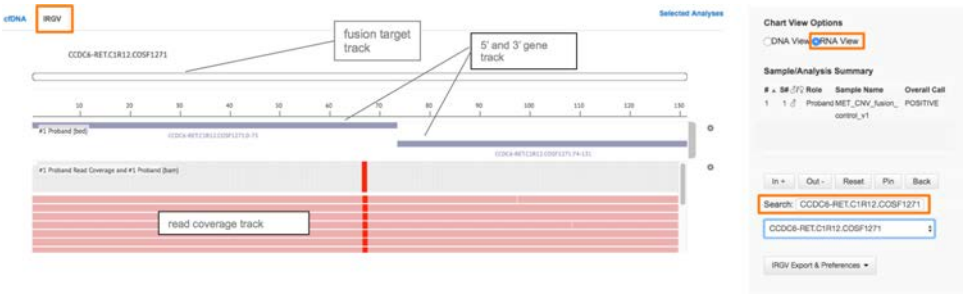


Example visualization of the Oncomine™ Breast cfDNA Assay v2.

A none detected result indicates that down to the displayed limit of detection (LOD), no variants were observed in the sample within or above the LOD range. See "Quality control (QC) thresholds" on page 56 for more information.

6. Click the **SNV/Indel**, **CNV**, or **Fusion** (Lung only) tab to view detailed analysis metrics. See Appendix E, "Detailed analysis metrics" for a description of each metric.

7. In the **SNV/Indel**, **CNV**, or **Fusion** detailed view, click the link in the **Locus** column to view specific variants in the Ion Reporter™ Genomic Viewer (IRGV).



Example Fusion variant result in the Ion Reporter™ Genomic Viewer.

Note: The IRGV viewer displays CNVs as ploidy assuming 100% tumor cellularity whereas we report CNVs as fold difference.

Visualization interpretation guidance

Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay

Metric	Description
Copy Number Variation	
CNV Ratio	Should be interpreted as the fold amplification (gain) as detected by the assay. CNV specific amplicon (MET) coverage levels are compared to non-CNV amplicon coverage.
P-value	Significance of CNV Ratio measurement based on amplicon coverage variability (MAPD level) and magnitude of the pairwise coverage differences between the CNV and non-CNV amplicons. High coverage variability will result in less significant p-values. See page 56 for QC and CNV calling rules.
Fusion detection	
Nomenclature	Each reported fusion target follows a specific naming convention such that the 5'- and 3'-genes are reported along with donor and acceptor exon numbers. Lastly, a COSMIC ID or NCBI transcript accession number is added to the end of each target name. For example, EML4-ALK.E13A20.COSF463 identifies the EML4-ALK fusion variant with exon 13 of EML4 fused to exon 20 of ALK.
Fusion QC genes	Two non-fused process control genes (HMBS and TBP) that have been shown to be consistently detected in cell-free nucleic acid extracts are included in the assay to inform quality of fusion variant calls.
Analysis detail	• Fusion targets are reported as **FUSION** in the **Type** column. • Fusion QC genes are reported as **ProcControl** in the **Type** column. • See page 56 for QC and Fusion calling rules.
MET Exon 14 Skipping Assay	
Nomenclature	• There is one assay specific to the exon 14 skipping detection in the MET gene called MET-MET.M13M15. • Two additional wild type assays are provided to inform the quality of a MET exon 14 skipping variant call. These are named MET.E6E7.WT and MET.E11E12.WT.
Analysis detail	• MET exon 14 targets are reported as **RNAExonVariant** in the **Type** column. • See page 56 for QC and MET exon 14 skipping calling rules.

Oncomine™ Breast cfDNA Research Assay v2

Metric	Description
Copy Number Variation	
CNV Ratio	Should be interpreted as the fold amplification (gain) as detected by the assay. CNV specific amplicon (CCND1, ERBB2, FGFR1) coverage levels are compared to non-CNV amplicon coverage.

Metric	Description
P-value	Significance of CNV Ratio measurement based on amplicon coverage variability (MAPD level) and magnitude of the pairwise coverage differences between the CNV and non-CNV amplicons. High coverage variability will result in less significant p-values. See page 56 for QC and CNV calling rules.
***De novo* (non-hotspot) variant calling in TP53**	
Analysis detail	• Panel includes approximately 80% coverage of the TP53 gene. • These variants are reported as **PN** (potentially novel) in the **Info** column. If the variant is reported as **HS** in the **Info** column, this variant is a hotspot specifically targeted by the breast panel. • These variant calls must be at a frequency of ≥0.5% to be reported in the analysis visualization. To view *de novo* TP53 variants at lower frequencies, download a VCF file from the visualization pages.

Troubleshooting

For troubleshooting information refer to the appropriate user guide:

- *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854)
- *Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)

B

Supplemental procedures

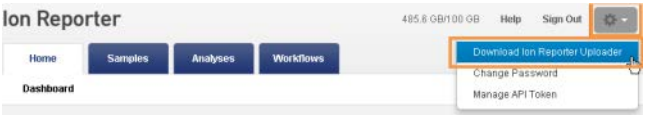
Install the Ion Reporter™ Uploader plugin on your Torrent Server

The IonReporterUploader 5.6 plugin is automatically installed on Torrent Server when you update to a new release.

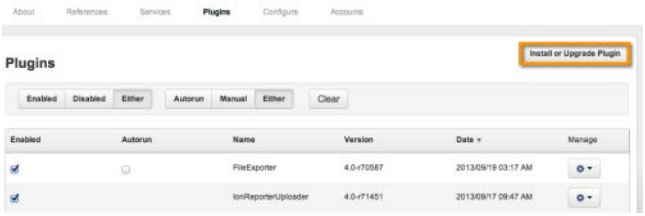
To reinstall or update IonReporterUploader 5.6 plugin for Torrent Suite™ Software 5.2 or later, go to **http://iru.ionreporter.thermofisher.com/**. If you do not have an internet connection, then download and install the latest file named `IonReporterUploader_<version>.deb` from **http://iru.ionreporter.thermofisher.com/**.

Note: An administrative **ionadmin** account is not required for this procedure.

1. Sign in to Ion Reporter™ Software, then click **Settings (⚙) ▸ Download Ion Reporter Uploader**.



2. Click the filename **IonReporterUploader.zip**, then download the file to your local machine.



3. Sign in to Torrent Suite™ Software, then click **Settings (⚙) ▸ Plugins**.

4. Click **Install or Upgrade Plugin**.

5. Click **Upload a Plugin file**, then browse to the **IonReporterUploader.zip** file. Click **Open**, click **Upload**, then **Install**.

Download and install BED files

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

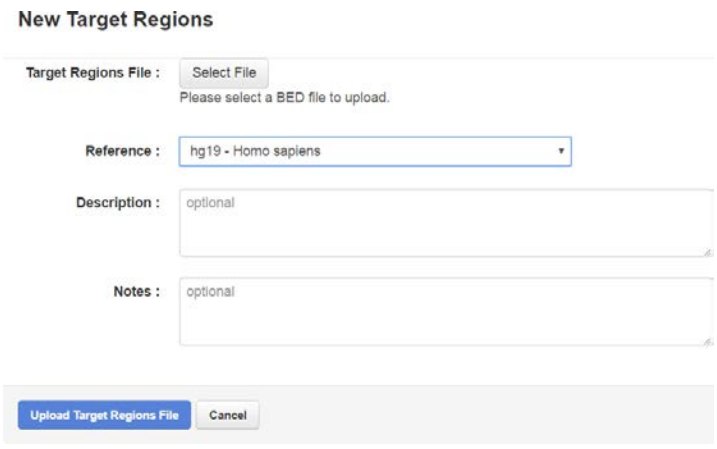
1. In the Thermo Fisher Scientific homepage, enter "Oncomine™ cfDNA Assay" (or the product catalog number) into the search field, then press **Enter**.
2. Navigate to the appropriate assay product page, scroll down to **Product literature**, then click on the desired **Target Regions** or **Hotspots**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

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

   a. In the left navigation menu, click **Target Regions**, then click the **Add Target Regions** button.

   b. Select **hg19 - Homo sapiens** from the **Reference** dropdown list.

   c. Click **Select File**, then navigate to and select the downloaded Target Regions .zip file:
      - **Oncomine_Lung_cfNA.08212017.Designed.bed**
      - **Oncomine_Breast_cfDNA_v2.08212017.Designed.bed**



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

6. Upload the Hotspots .BED file:

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

   a. In the left navigation menu, click **Hotspots**, then click the **Add Hotspots** button.

   b. Select **hg19 - Homo sapiens** from the **Reference** dropdown list.

c. Click **Select File**, then navigate to and select the downloaded Hotspots .zip file:
   - **Oncomine_Lung_cfNA.08212017.Hotspots.bed**
   - **Oncomine_Breast_cfDNA_v2.08212017.Hotspots.bed**

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

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

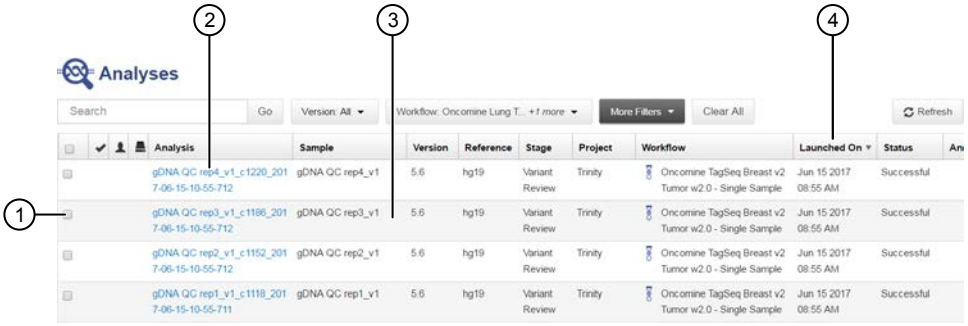
C

View and manage extended analysis results for a single sample

Ion Reporter™ Software analyses are performed automatically on uploading of the data files from the Torrent Suite™ Software. To view and manage the extended analysis results of a single sample:

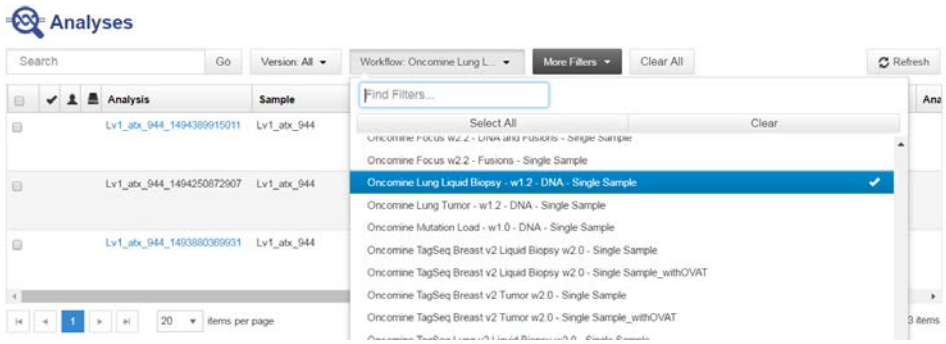
1. Sign in to the Ion Reporter™ Software.
2. Click the **Analyses** tab.
3. In the **Analyses** screen you can:

To...	Action
Select an analysis	Click the checkbox
Open an Analysis Results screen.	Click the hyperlink (in the Analysis column).
View details	Click anywhere in the analysis' row, except on the hyperlink.
Sort	Click column headers to sort the analyses based on the column contents.



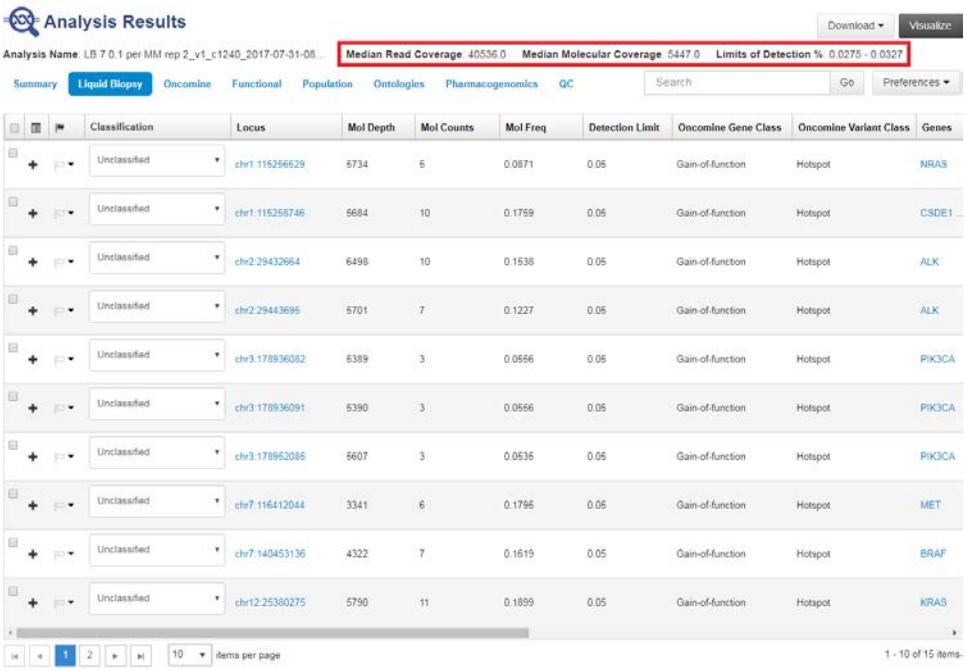
① Select analysis
② Open Analysis Results screen
③ View details
④ Sort

4. To view and manage the extended Oncomine™ Cell-Free Research Assay results, use the available filters to limit the list of analyses (e.g., select your workflow from the **Workflow** dropdown list), then click the hyperlink in the **Analysis** column.



5. In the **Analysis Results** screen sort or filter the data using the Oncomine™-specific annotations. See the software help menu for more options.

6. Review the results in the **Median Read Cov**, **Median Mol Cov**, and **LOD %** columns.



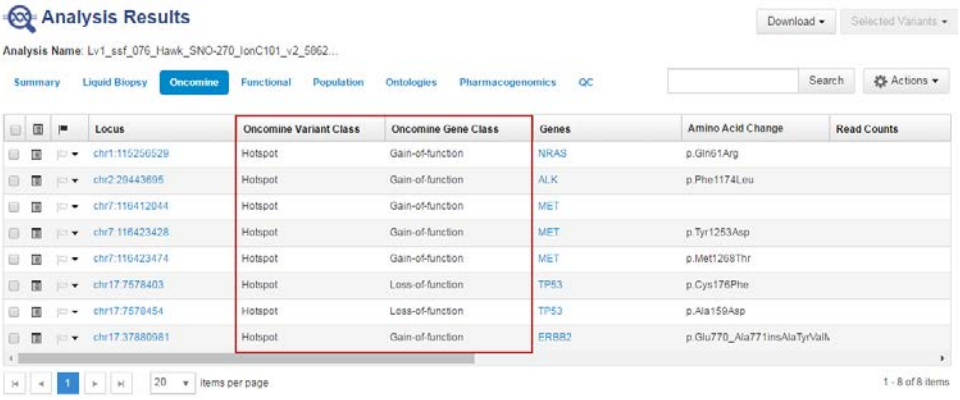
Column	Description
Median Read Coverage	Reports median coverage across targets. Median Molecular Coverage reports median number of individual interrogated DNA molecules across targets.
Median Molecular Coverage	Directly influences the limit of detection in a sample run. We always require two independent molecular families to identify a variant for it to be called. Lower median molecular coverage values result in less sensitive detection of variants at 0.1% frequency, although still sufficient for sensitive detection of variants with higher frequency. For example, Median Molecular Coverage of 700 is sufficient for accurate detection of variants at 0.5% frequency.
LOD %	A segment (e.g., 0.02–0.03) where 0.02 represents the median value across all targets, and 0.03 represents the limit of detection (LOD) for the 80th percentile targets. If both numbers are ≤0.1% then the sequencing run is of acceptable quality for 0.1% LOD.

For sensitive variant detection down to 0.1% frequency, we see optimal results when targeting a Median Read Coverage >25,000, Median Molecular Coverage >2,500, and both numbers of the LOD % segment are ≤0.1.

7. In the **Liquid Biopsy** tab, view **Mol Depth**, **Mol Counts**, and other columns.

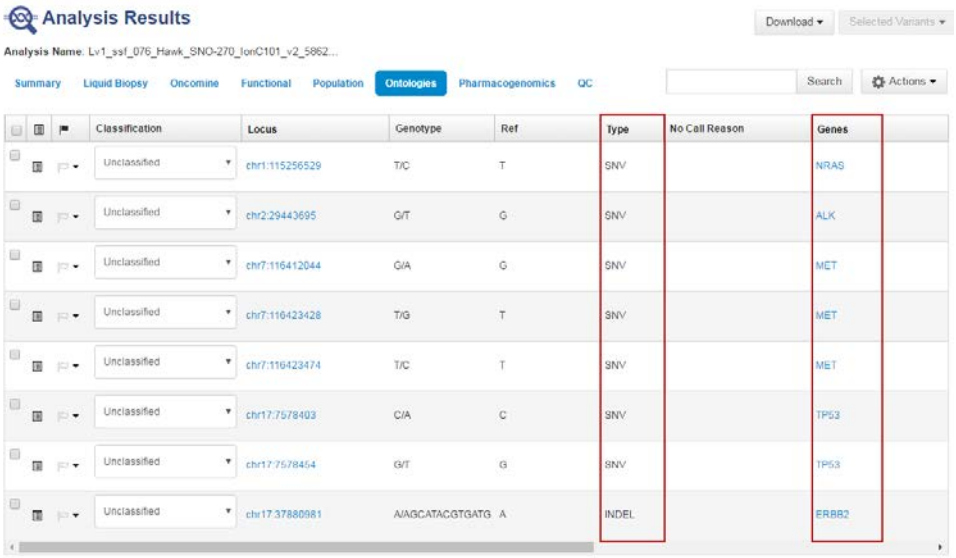
Column	Description
Molecular Depth	Reports number of interrogated DNA molecules containing target. It defines limit of detection at hotspot position in a particular run and sample. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at 0.2%. If molecular depth is ≥2,500, you can have high confidence that no variant is present down to 0.1% LOD. For reference calls, Molecular Depth provides measurable metric that serves as confirmation for variant absence among a large number of interrogated molecules.
Molecular Counts	Reports the number of detected DNA molecules containing variant allele.

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



Reference calls display chromosomal position with empty value in amino acid change field.

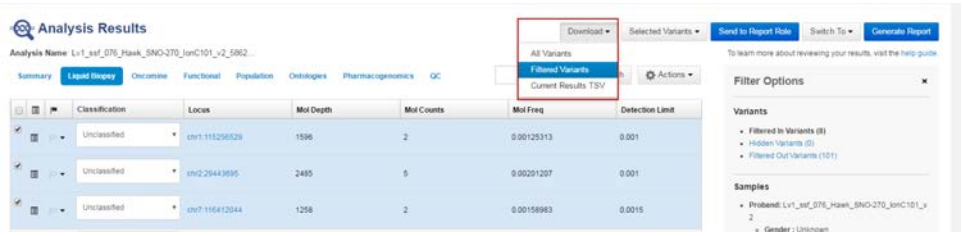
9. In the **Ontologies** tab, click the column headers to sort the list by variant **Type** or **Genes** to analyze your results.



Export results

To export a report:

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



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

   The software generates a ZIP file with three folders: QC, Variants, and Workflow_Settings. Within the Variants folder, you'll find the Oncomine™ annotated VCF file, which is used by the Oncomine™ Knowledgebase Reporter.

3. Open the annotated VCF file, then scroll to the Oncomine™ annotations.


```
chr2    29432664    p.R1275L;p.R1275Q    C    T    18.0    PASS    AF=0.00109827;AO=21;DP=19121;FR=.;LOD=5.0E-4;MAF=6.34115E-
4;MAO=2;MDP=3154;OALT=T;OID=p.R1275Q;OMAPALT=T;OPOS=29432664;OREF=C;FUNC=[{'origPos':'29432664','origRef':'C','normalizedRef':
'C','gene':'ALK','normalizedPos':'29432664','normalizedAlt':'T','polyphen':'1.0','gt':'pos','codon':'CAA','coding':'c.3824G>A',
'sift':'0.0','grantham':'43.0','transcript':'NM_004304.4','function':'missense','protein':'p.Arg1275Gln','location':'exonic',
'origAlt':'T','exon':'25','oncomineGeneClass':'Gain-of-Function','oncomineVariantClass':'Hotspot'}]
GT:DP:AO:AF:FS    0/1:19121:21:0.00109827:1,0,0,0,1,0,0,0,0,0
```


Oncomine™ annotations

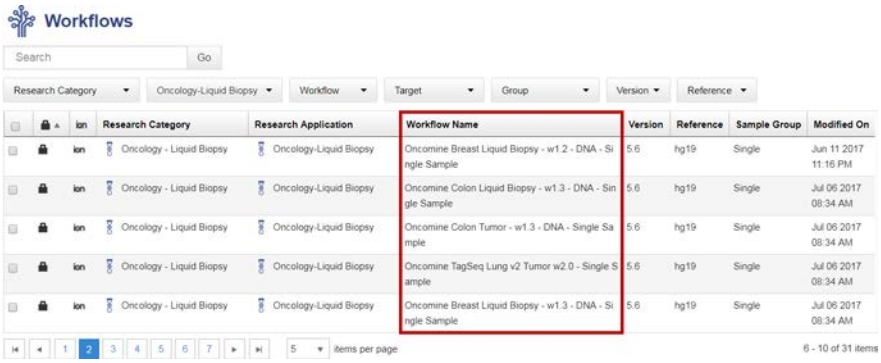
Variant Type	Oncomine™ Gene Class	Oncomine™ Variant Class	Annotation Criteria
Gain of Function Missense Hotspot Mutation	Gain-of-Function	Hotspot	• Variant's functional impact is missense • Variant occurs in Gain of Function gene • Variant's transcript and codon position occur in predefined missense hotspot list
Gain of Function In Frame Hotspot Mutation	Gain-of-Function	Hotspot	• Variant occurs in Gain of Function gene • Variant's function, transcript and coding syntax occur in pre-defined in-frame hotspot list
Loss of Function Missense Hotspot Mutation	Loss-of-Function	Hotspot	• Variant's functional impact is missense • Variant occurs in Loss of Function gene • Variant's transcript and codon position occur in predefined missense hotspot list

Variant Type	Oncomine™ Gene Class	Oncomine™ Variant Class	Annotation Criteria
Loss of Function In Frame Hotspot Mutation	Loss-of-Function	Hotspot	• Variant occurs in Loss of Function gene • Variant's function, transcript and coding syntax occur in pre-defined in-frame hotspot list
Gain of Function Splice Site Hotspot Mutation	Gain-of-Function	Hotspot	• Variant occurs in Gain of Function gene • Variant's transcript, location, and exon occur in pre-defined splice site hotspot list
Loss of Function Synonymous Hotspot Mutation	Loss-of-Function	Hotspot	• Variant occurs in Loss of Function gene • Variant's function, transcript, and coding syntax occur in pre-defined synonymous hotspot list

Manually launch an analysis

If your analysis did not automatically launch, you can launch it manually.

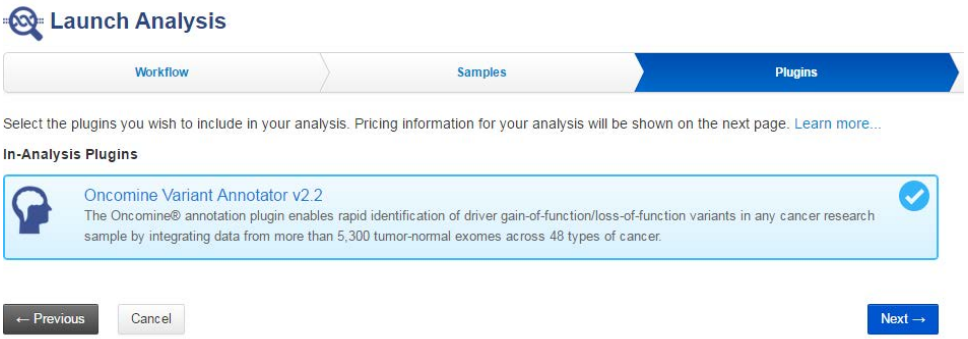
1. Click the **Workflows** tab (or Analyses tab).
2. In the **Research Application** dropdown list, filter for **Oncology-Liquid Biopsy**.
3. In the **Workflow Name** column, click on the workflow appropriate to your assay.



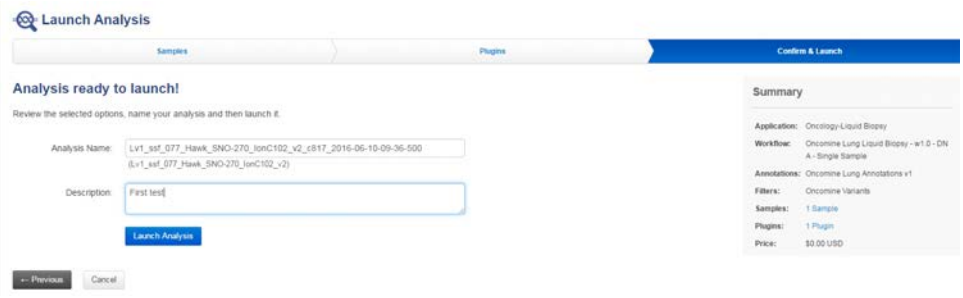
4. In the **Details** pane, click **Actions** ▸ **Launch Analysis**.
5. Search, filter, or sort the list to find your sample of interest, then select one or more DNA samples.

   Note: If you select multiple samples on this page, the software creates a separate analysis for each sample.

   The samples populate a field on the right side of the screen.
6. Click **Next**.
7. Confirm that the **Oncomine Variant Annotator** plugin is selected, then click **Next**.



8. *(Optional)* Edit the **Analysis Name**, then add a **Description**.



9. Click **Launch Analysis**.

10. Follow the steps in the Appendix C, “View and manage extended analysis results for a single sample“ and “Export results“ on page 50 of this appendix to sort, filter, and generate reports of your results.

E Detailed analysis metrics

Metric	Description
Sample ID	Name of the sequenced Sample imported from a sequencing run.
SNV/Indel	
Gene	HGNC reviewed official gene symbol.
AA Chg	Amino acid change resulting from non-synonymous DNA variant.
Mutant Frequency %	Frequency of mutant allele expressed as a percentage.
Oncomine Variant Class	Variant class annotation as defined using Oncomine™ Variant Annotator (OVAT).
Oncomine Gene Class	Variant gene functional annotation as defined using Oncomine™ Variant Annotator (OVAT).
Info	HS (targeted hotspot) or PN (potentially novel TP53 variant). *De novo* variant calls available for the breast panel only.
Genotype	Genotype measured associated with a DNA variant call.
Ref Allele	Reference allele as defined in the human genome reference (hg19).
Mut Molecular Cov.	Molecular coverage of the mutant allele.
WT Molecular Cov.	Molecular coverage of the wild type allele from the reference genome.
Amplicon Coverage	Total read coverage across amplicon containing SNV/Indel hotspot locations.
QC Test (LOD) %	Quality control check for SNV/Indel target regions based on molecular coverage.
Transcript ID	NCBI accession number for the transcript representing the gene target being measured.
Locus	Chromosome and position of detected variant. Click the hyperlink to open the Ion Reporter™ Genomic Viewer to the specified locus.
CNV	
Gene	Gene locus targeted for CNV measurement.
Gain/Loss	Detected copy number gain or loss.
CNV ratio	Ratio of measured CNV gene locus coverage relative to coverage on non-CNV loci.
p-value	Significance of CNV ratio measurement.
Med. Mol Cov. Gene	Median molecular coverage of targeted CNV gene.
Med. Mol Cov. Ref	Median molecular coverage of non-CNV reference loci.

Metric	Description
Med. Read Cov. Gene	Median read coverage of targeted CNV gene.
Med. Read Cov. Ref	Median read coverage of non-CNV reference loci.
QC Test	Assay quality control as determined by amplicon coverage uniformity and number of amplicons remaining after outlier removal.
Valid CNV Amplicons	Number of CNV amplicons remaining after outlier removal.
CNV Locus	Chromosomal location of CNV gene being targeted.
Fusion	
Variant (exons)	Name of fusion targeted and respective acceptor and donor exons.
Oncomine Driver Gene	Cancer driver gene descriptions as reported by Oncomine™ Variant Annotator (OVAT).
COSMIC/NCBI	COSMIC mutation or NCBI accession number.
Mol Cov. Mutant	Median molecular coverage across fusion amplicon.
Read Cov. Mutant	Median read coverage across fusion amplicon.
Detection	Detection status from assay.
QC Test	Assay quality control measured from expression detection of housekeeping genes.
Type	Assay type (e.g., Fusion, RNA exon variant (exon skipping), Proc Control).
Locus	Chromosomal locations of targets included in assay.
Ratio To Wild Type	Ratio molecular for exon skipping assay relative to wild type control amplicons.
Norm count Within Gene	Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons. (Lung panel only)

Quality control (QC) thresholds

QC Test	Detection threshold
SNV/Indel	
A limit of detection (LOD) is calculated and displayed for each variant call. LOD is determined by the level of molecular amplicon coverage. If no variant call is detected, the LOD range is displayed across entire amplicon.	Molecular coverage must be at least 2 with a minimum detection cutoff frequency of 0.035% and 0.05% for lung and breast panels, respectively.
CNV	
The MAPD metric is a measure of read coverage noise detected across all amplicons in a panel. Higher MAPD typically translates to lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. MAPD score is viewable in downloadable VCF file or review of the **Analysis Results** of a single sample extended analysis.	To make a CNV call the following criteria must be met: • MAPD <0.4 • P-value $<10^{-5}$ • CNV Ratio for a copy number gain must be >1.15 • CNV Ratio for a copy number loss must be <0.85 **Note:** The CNV Ratio call thresholds were derived empirically using plasma samples from healthy donors with normal CNV status.
Fusions/Exon Skipping[1]	
• Fusions—Panel includes 2 process control target genes, TBP and HMBS. At least 1 control must have a molecular count of >2 to pass QC. • MET Exon Skipping—Panel includes 2 MET Wild Type control amplicons (gene name has WT at the end). At least 1 of these controls must have a molecular count >2 to pass QC.	Fusion and Exon Skipping amplicons must have >2 molecular counts to be reported.

[1] These variant types are included in the Oncomine™ Lung cfNA Assay, derived from RNA reverse-transcribed into cDNA during library preparation.

F

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). 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:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

G Documentation and support

Obtain information from the Help system

The Torrent Suite™ Software has a Help system that describes how to use each feature of the user interface.

In the toolbar of the Torrent Suite™ Software window, click **Help ▸ Software Help**.

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

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

Customer and technical support

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

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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