iontorrent

Ion Torrent™ Ion CarrierSeq™ ECS Kits

USER GUIDE

For manual library preparation

for use with:
IonCode™ Barcode Adapters
Ion 530™ Chip
Ion 540™ Chip
Ion Chef™ System
Ion GeneStudio™ S5 Systems
Ion S5™ Systems

Catalog Numbers A43585, A48022, A43586, A48023, A43471, and A48036

Publication Number MAN0018483

Revision D.0

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, California 92008 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0018483 C.0 (English)

Revision	Date	Description
D.0	19 September 2023	• Include instruction to denature sample DNA before amplification of the targets (page 18). • Updated Ion S5™ Chef Supplies to include the PCR Plate Frame.
C.0	9 November 2022	• Update to "Check the sequencing run" on page 40. • Update to "Create a Planned Run" on page 33. • Chapters reorganized.
B.0	24 August 2021	• Support added for supplemental panels. • Chip Quantity for Ion 530™ Chip Kit corrected in kit summary table. • Added Chapter 2, "Before you begin". • Updates for Ion Reporter™ Software 5.16 release added. • Troubleshooting topics replaced with links to thermofisher.com. • Content reorganization. • Product description updated.
A.0	16 April 2020	New document for the Ion Torrent™ Ion CarrierSeq™ ECS Kits. Provides instruction for the preparation, templating, and sequencing of libraries with the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel.

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

Contents

1 Product information

Product description

The Ion Torrent™ Ion CarrierSeq™ ECS Kits provide all the reagents and materials necessary for a comprehensive, seamless, and flexible next-generation sequencing (NGS) workflow for expanded carrier screening (ECS). When used with an Ion GeneStudio™ S5 Series System or Ion S5™ System, the kits enable a simple, end-to-end workflow for the detection of carrier-positive samples by research labs interested in maximizing the identification of carrier status using genomic DNA isolated from blood or saliva samples. The Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel included in the kits enables the detection of single nucleotide variants (SNVs), insertion/deletions (INDELs), and copy number variants (CNVs) associated with 418 inherited disorders in a single assay.

The kit includes the following key features:

- Targeting of all coding regions and intron/exon boundaries of 420 genes implicated in 418 inherited disorders.
- Incorporation into a single NGS assay difficult-to-characterize genes due to homology as a result of paralogues: spinal muscular atrophy (SMN1 and SMN2), pseudogenes (Gaucher disease—GBA and GBAP1), 21-hydroxylase deficient congenital adrenal hyperplasia (CYP21A2 and CYP21A1P), and loci (alpha-thalassemia—HBA1 and HBA2).
- Flexible throughput options—kit formats available for 3, 4, 15, or 16 samples per chip or 6, 8, 30, or 32 samples per run.

The Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel, included in the kits, provides comprehensive coverage of common and rare variants to help achieve a higher per-disorder detection rate. The panel targets >14,000 amplicons that cover all coding regions of 420 target genes, including intron/exon boundaries, to genotype more than 36,000 SNVs and INDELs from the ClinVar archive of human variation.

Instrument compatibility

The Ion Torrent™ Ion CarrierSeq™ ECS Kits are compatible with the following instruments.

- Ion GeneStudio™ S5 Systems
- Ion S5™ Systems

Software compatibility

The Ion Torrent™ Ion CarrierSeq™ ECS Kits are compatible with Torrent Suite™ Software 5.16 and later. We recommend updating your Torrent Server to the latest available version of Torrent Suite™ Software before using these kits.

The Ion Torrent™ Ion CarrierSeq™ ECS Kits are compatible with Ion Reporter™ Software 5.16 and later. We recommend updating your Ion Reporter™ Server to the latest available version of Ion Reporter™ Software before using these kits.

Kit contents and storage

IMPORTANT!

- Do not substitute components between sequencing kits. We have verified this protocol using these specific materials. Substitution can adversely affect system performance.
- Store all consumables and cartridges under the recommended conditions and in an upright position. Do NOT store the Ion S5™ Sequencing Reagents (Part No. A27768) on dry ice or in a closed environment where dry ice is present.

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

Kit summary

The Ion Torrent™ Ion CarrierSeq™ ECS Kits (Cat. Nos. A43585, A43586, A48022, and A48023) consist of the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel, the Ion AmpliSeq™ Library Kit Plus, and reagents for templating and sequencing barcoded sample libraries on either the Ion 530™ Chip or the Ion 540™ Chip.

Component	Quantity per kit			
	A43585[1]	A48022[2]	A43586 [3]	A48023[4]
Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel (Cat. Nos. A43471 and A48036)	1	1	4	5
Ion AmpliSeq™ Library Kit Plus (Cat. Nos. A35907 and 4488990)	1	1	4	5
Ion 530™ Chip Kit (Cat. No. A27763)	6	2	NA	NA
Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef[5] (Cat. No. A34461)	3	1	NA	NA
Ion 540™ Chip Kit (Cat. No. A27765)	NA	NA	6	2
Ion 540™ Kit – Chef[5] (Cat. No A30011)	NA	NA	3	1

[1] 96 samples, 4 samples per chip
[2] 24 samples, 3 samples per chip
[3] 384 samples, 16 samples per chip
[4] 120 samples, 15 samples per chip
[5] This kit consists of Ion Chef™ Supplies, Ion Chef™ Solutions, Ion Chef™ Reagents, Ion S5™ Sequencing Solutions, and Ion S5™ Sequencing Reagents.

Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel

The Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel targets 14,044 amplicons covering the coding regions (CDS) of 420 genes including ±25 bp flanking intron/exon boundaries, as well as selected intergenic and intronic regions.

Note: Each CDS region is defined either by a specific transcript or a combination of multiple transcripts.

The Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel is a 2–pool Ion AmpliSeq™ panel at a 2X concentration.

Contents	Reactions	Storage
Cat. No. A43471, 96 reactions		
Pool 1	96	–30°C to –10°C
Pool 2	96	
Cat. No. A48036, 24 reactions		
Pool 1	24	–30°C to –10°C
Pool 2	24	

Ion AmpliSeq™ Library Kit Plus

The Ion AmpliSeq™ Library Kit Plus (Cat. No. A35907 or 4488990) provides reagents for manually preparing 96 libraries.

Component	Amount		Storage
	Cat. No. 4488990 (24 reactions)	Cat. No. A35907 (96 reactions)	
5X Ion AmpliSeq™ HiFi Mix (red cap)	120 µL	480 µL	–30°C to –10°C
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 –30°C to –10°C.

Sequencing chips

Chip kit	Cat. No.	Amount	Storage
Ion 530™ Chip Kit	A27763	4 chips	15°C to 30°C
Ion 540™ Chip Kit	A27765		

Templating and sequencing reagents

IMPORTANT!

- Do not substitute components from any other sequencing kits. We have verified this protocol using these specific materials. Substitution can adversely affect system performance.
- Store all consumables and cartridges under the recommended conditions and in an upright position. Do NOT store the Ion S5™ Sequencing Reagents (Part No. A27768) on dry ice or in a closed environment where dry ice is present.

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

- Each Ion 540™ Kit – Chef contains all the supplies that are required to prepare and sequence 8 Ion 540™ Chips. Catalog No. A30011 supports 4 Ion Chef™ runs (2 chips/Ion Chef™ run) and 4 sequencer initializations (2 sequencing run/sequencer initialization). Select for templating and sequencing of 200-base–read libraries only.
- Each Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef contains all the supplies that are required to prepare and sequence 8 Ion 510™ Chips, Ion 520™ Chips, or Ion 530™ Chips. Catalog No. A34461 supports 4 Ion Chef™ runs (2 chips/Ion Chef™ run) and 4 sequencer initializations (2 sequencing runs/sequencer initialization) for up to 200-base-read libraries.

Component	Quantity per kit	
	A34461	A30011
Ion S5™ Chef Supplies	4 boxes	4 boxes
Ion S5™ Chef Solutions	1 box	1 box
Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents	1 box	—
Ion 540™ Chef Reagents	—	1 box
Ion S5™ Sequencing Solutions	1 box	1 box
Ion S5™ Sequencing Reagents	1 box	1 box

Ion Chef™ reagents and materials

Contents	Amount / box	Storage
Ion S5™ Chef Supplies		
Chip Adapter	2	15°C to 30°C
Enrichment Cartridge v2	1	
Tip Cartridge v2	1	
PCR Plate	1	
PCR Plate Frame	1	
Frame Seal v2	1	
Recovery Station Disposable Lid v2	2	
Recovery Tube v2	12	
Ion S5™ Chef solutions		
Ion S5™ Chef Solutions	4 cartridges	15°C to 30°C
Ion S5™ Chef reagents		
One of the following: • Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents • Ion 540™ Chef Reagents	4 cartridges	–30°C to –10°C

Ion S5™ sequencing reagents and materials

IMPORTANT! Do not store the Ion S5™ Sequencing Reagents on dry ice or in a closed environment containing dry ice.

Contents	Amount / box	Storage
Ion S5™ Sequencing Solutions		
Ion S5™ Wash Solution	4 × 1.5 L	15°C to 30°C
Ion S5™ Cleaning Solution	250 mL	
Ion S5™ Sequencing Reagents		
Ion S5™ Sequencing Reagents	4 cartridges	–30°C to –10°C[1]

[1] Cartridges ship at 2°C to 8°C. Store as indicated, do not store on dry ice.

Required materials not supplied

In addition to a library kit and panel, you need the following materials and equipment. Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
One of the following, or equivalent: • SimpliAmp™ Thermal Cycler • 2720 Thermal Cycler • Veriti™ Thermal Cycler • ProFlex™ 96-well PCR System • GeneAmp™ PCR System 9700[1] or Dual 96-well Thermal Cycler	See web product pages
One of the following: • Ion Library TaqMan™ Quantitation Kit and real-time PCR instrument • Qubit™ 4 Fluorometer[2] and the Qubit™ dsDNA HS Assay Kit (DNA). • Agilent™ 2100 Bioanalyzer™ and Agilent™ High Sensitivity DNA Kit	4468802 Q33238 G2939AA, 5067–4626 Agilent
IonCode™ Barcode Adapters 1–384 Kit	A29751
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	N8010560, 4306737
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes, 1.5-mL	13-698-791 **fisherscientific.com**
Agencourt™ AMPure™ XP Kit	NC9959336, NC9933872 **fisherscientific.com**
DynaMag™–96 Side Magnet, or other plate magnet	12331D
Nuclease-free Water	AM9932
Ethanol, Absolute, Molecular Biology Grade	BP2818500 **fisherscientific.com**
Pipettors, 2–200 µL, and low-retention filtered pipette tips	**MLS**

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

Recommended materials and equipment

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Additional equipment	
Real-time PCR instrument (e.g., Applied Biosystems™ 7900HT, 7500, StepOne™, StepOnePlus™, ViiA™ 7, QuantStudio™ 3, QuantStudio™ 5, QuantStudio™7 , or QuantStudio™ 12K Flex Real–Time PCR Systems)	Various
Fisher Scientific™ Mini Plate Spinner Centrifuge, or equivalent 96-well plate centrifuge	14–100–143 **fisherscientific.com**
MicroAmp™ Adhesive Film Applicator	4333183
Nucleic acid isolation	
MagMAX™ Saliva gDNA Isolation Kit	A39059
MagMAX™ DNA Multi-Sample Ultra 2.0 Kit	A36570
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Nucleic acid quantification	
TaqMan™ RNase P Detection Reagents Kit	4316831
Qubit™ 4 Fluorometer[1] and the Qubit™ dsDNA HS Assay Kit	Q33238

[1] Qubit™ 2.0 Fluorometer and later are supported.

Supplemental Panels for the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel

Panels to supplement the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel (supplemental panels) can be ordered from **AmpliSeq.com**. Contact support for assistance.

Workflow

Ion Torrent™ Ion CarrierSeq™ workflow

Amplify targets (page 18)

Amplify target regions from DNA to generate libraries.

Partially digest amplicons (page 21)

Partially digest libraries to enable adapter ligation.

Ligate adapters and purify (page 21)

Ligate barcode adapters to libraries and purify.

Quantify the libraries (page 23 or page 26)

Quantify the libraries using qPCR, Qubit™ Fluorometer, or Agilent™ 2100 Bioanalyzer™ Instrument.

Plan a run (page 31)

Use Torrent Suite™ Software to create a planned run for templating and sequencing.

Template and sequence libraries (page 38)

Perform templating and sequencing using the Ion Chef™ Instrument and Ion GeneStudio™ S5 Series Sequencer.

Analyze variants and generate report (page 39 and page 42)

Use Ion Reporter™ Software to analyze variants. Use Carrier Reporter Software to further analyze results and generate a report.

2 Before you begin

Procedural guidelines

Reagent preparation:

- Thaw components that contain enzymes—such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, and DNA Ligase—on ice, and keep on ice during procedure.
- Thaw kit components other than enzymes, including genomic DNA and primer panels, at room temperature until no ice is present in the tubes. Vortex all reagents for 5 seconds (EXCEPT for enzymes, which should be flick-mixed) and pulse-centrifuge before use. A pulse-centrifugation is a 5-second centrifugation at maximum speed.
- 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 dissolve.
- Pipet viscous solutions slowly and ensure complete mixing by vigorous vortexing or pipetting up and down at least 5 times.

Laboratory and PCR setup:

- Use good laboratory practices to minimize cross-contamination of products. If possible, perform PCR setup in an area or room that is separate from template preparation. Always change pipette tips between samples.
- Before starting and after use, wash the working surface with 10% bleach followed by two water rinses.
- Use inner wells of PCR plate if possible, and skip wells or columns to prevent cross-contamination between samples.
- Use a calibrated thermal cycler that is specified in “Required materials not supplied” on page 11.
- Ensure that the correct cycling protocol is being used before starting the thermal cycler.
- Store one 96-well cold block at –30°C to –10°C and one 96-well cold block at 2°C to 8°C before use.
- Use the heated lid (105°C) for all thermal cycling conditions.
- Use the default ramp rate for your thermal cycler.

Pipetting recommendations:

- Use aerosol-barrier pipette tips. Change pipette tips between samples.
- Pipet viscous solutions (enzymes, beads, Switch Solution) slowly and ensure complete mixing in the MicroAmp™ 96-well plate.
- Avoid introducing air bubbles when pipetting by keeping the pipette tip at the bottom of the solution in the wells.
- Set pipette to the recommended volume for up and down mixing and insert tip into solution with pipette plunger that is depressed to avoid introducing air bubbles.
- Visually check tips to ensure that volumes are equivalent if using a multi-channel pipette.
- Touch tip to side of well and slowly dispense reagent on the side of the well to form a droplet. This practice enables you both to pipet small volumes accurately and to see that you added reagent to the well.
- Ensure that reagent is adequately dispensed from the pipette tip.

Guidelines for DNA isolation and quantification:

- For each target amplification reaction, use 3,000 copies (10 ng of genomic DNA in ≤3 µL per pool) from peripheral blood or saliva. As there are two pools (target amplification reactions) per sample, 20 ng of genomic DNA is required per sample.
- We recommend the TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831) for quantifying amplifiable human genomic DNA. The Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) can also be used with the Qubit™ Fluorometer.
- We do not recommend methods such as densitometry (for example, NanoDrop™ Spectrophotometers), because these methods do not discriminate between DNA and RNA and therefore are sensitive to small fragments of hydrolyzed RNA. Use of densitometry can lead to overestimation of the concentration of sample DNA, under-seeding of the target amplification reaction, low-quality libraries, and low library yields.
- See “Recommended materials and equipment” on page 12 for recommended kits for isolating gDNA.

Tips

- Target amplification reaction master mixes can be made with 5X Ion AmpliSeq™ HiFi Mix and primer pools, transferred to a 96-well plate, and sample DNA added. However, be careful to add equal amounts of DNA to avoid pool imbalance.
- Arrange samples in columns on the plate for easier pipetting with multichannel pipettes during purification with the DynaMag™ Side Magnet.
- If you observe evaporation in target amplification reactions, avoid using outside wells.
- Plate seals can be firmly applied using 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.
- When using a Qubit™ Fluorometer or the Agilent™ 2100 Bioanalyzer™ instrument, amplified libraries with little or no detectable product can still be quantified with qPCR.
- When transfer to a new plate is specified, solutions can be transferred to a clean well in the same plate instead, if desired.

- If library yield is below 50 pM, libraries can still be sequenced by adding a proportionally larger volume to a combined library or template preparation.
- When amplifying multiple samples in a single PCR plate, ensure that the input across the samples is roughly equivalent so that the selected cycle number is optimal for all the samples in the run.
- When setting up sample-specific master mixes for panels with two or more primer pools, master mixes can be set up in 96-well plates instead of tubes.

3 Prepare Ion AmpliSeq™ Ion CarrierSeq™ libraries

IMPORTANT! If you are using a supplemental panel for the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel, contact support for recommendations for preparing Ion AmpliSeq™ Ion CarrierSeq™ libraries.

Before each use of the kit

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

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

Amplify the targets

IMPORTANT! Primer pools and 5X Ion AmpliSeq™ HiFi Mix are viscous. Pipet slowly and mix thoroughly. We recommend PCR setup on ice or a prechilled cold block.

Ensure the thermal cycler is preheated to 90°C on hold with the lid heated to 105°C. A preheated lid helps to reduce evaporation.

1. For each sample, add 10 ng of DNA in a volume ≤3 µL to 2 adjacent wells of a 96-well plate using a low volume pipettor.

   Note:
   - One well is used to amplify the targets for Pool 1 and the other for Pool 2, see Figure 1.
   - Add 10 ng of control DNA in a volume ≤3 µL to 2 adjacent wells if running a control.

2. Bring the volume of each well to 3 µL with Low TE. Alternatively, the samples can be prediluted to a concentration of 3.3 ng/µL before adding to the plate.

3. Mix by pipetting up and down, then seal the plate with a MicroAmp™ Clear Adhesive Film. Alternatively, vortex the plate after sealing, then centrifuge briefly at 100 × *g* for 30 seconds to collect the contents.

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

5. Incubate the samples at 90°C for 5 minutes, then immediately transfer the plate onto ice or a prechilled 4°C cold block.

6. Incubate on ice or at 4°C for 1 minute, then centrifuge briefly at 100 × *g* for 30 seconds to collect the contents.

7. For each sample, add components to each pair of wells using a low volume pipettor, as described in the following table. See the plate layout example in Figure 1.

Note:

- If preparing multiple libraries, master mixes without DNA are recommended.
- When preparing master mixes, include a 10% overage.

IMPORTANT! Ion AmpliSeq™ Sample ID Panel primer pairs are included in the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel. Do not add more Ion AmpliSeq™ Sample ID Panel primers to target amplification reactions.

Component	Volume per well	
	Pool 1	Pool 2
Denatured DNA Sample, 10 ng	3 µL	3 µL
5X Ion AmpliSeq™ HiFi Mix (red cap)	2 µL	2 µL
Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel Pool 1 (red cap)	5 µL	—
Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel Pool 2 (purple cap)	—	5 µL

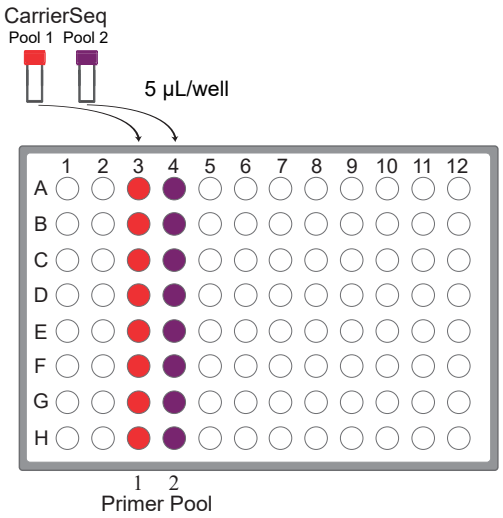


Figure 1

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

8. Mix thoroughly by pipetting up and down, then seal the plate with MicroAmp™ Clear Adhesive Film. Alternatively, vortex the plate after sealing, then centrifuge briefly at 100 × *g* for 30 seconds to collect the contents.

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

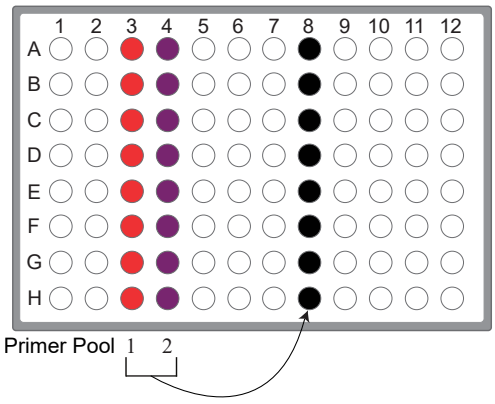
Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
Cycling—12 cycles	Denature	99°C	15 seconds
	Anneal and extend	60°C	16 minutes
Hold	—	10°C	Hold (16 hours maximum)

STOPPING POINT Amplification products can be stored at 10°C overnight (12–16 hours) in the thermal cycler. For longer-term storage, store at –20°C.

Combine the target amplification reactions

1. Centrifuge the plate at 100 × *g* for 30 seconds in a plate centrifuge to collect contents at the bottom of the wells.
2. Carefully remove the plate seal from the plate.
3. Combine the two 10-µL target amplification reactions for each sample by transferring them to an empty well of a new column in the plate, or in a new plate.

IMPORTANT! Pool samples in a new well. Combining libraries in the same well could result in pool imbalance.



Partially digest amplicons

Note: FuPa Reagent is viscous. Flick to mix, then pulse-centrifuge. To avoid carrying over excess enzyme, do not submerge the whole tip in the FuPa Reagent solution. Aspirate solution from the surface.

1. Add 2 µL of FuPa Reagent (brown cap) to each amplified sample. The total volume of each reaction is now ~22 µL.
2. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge at 100 × *g* for 30 seconds to collect droplets.
3. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program:

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

4. Centrifuge the plate at 100 × *g* for 30 seconds before proceeding to the next step.

Ligate adapters to the amplicons and purify

When sequencing multiple libraries on a single chip, you *must* ligate a different IonCode™ Barcode Adapter to each library.

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

Perform the ligation reaction

IMPORTANT! The IonCode™ Adapters must be used.

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. Briefly centrifuge the plate to collect the contents.

3. Carefully remove the plate seal, then add the following components in the order that is listed to each well containing digested amplicons.

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	IonCode™ Adapters	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 library

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

1. Prepare the Agencourt™ AMPure™ XP Reagent.
   a. Bring the Agencourt™ AMPure™ XP Reagent to room temperature.
   b. After the Agencourt™ AMPure™ XP Reagent is warmed to room temperature, vortex thoroughly to disperse the beads, then briefly centrifuge to collect the contents.

2. Prepare 70% Ethanol by combining 231 µL of 100% Ethanol with 99 µL of Nuclease–free water per sample.

   Carefully pipette the 100% Ethanol to ensure accurate volumes.

3. Briefly centrifuge the plate (from "Perform the ligation reaction" on page 21) to collect the contents in the bottom of the wells.

4. 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.

   IMPORTANT! Pipet the solution slowly an ensure that the properly volume of beads has been added.

   Note: Visually inspect each well to ensure that the mixture is homogeneous.

5. Incubate the mixture for 5 minutes at room temperature.

6. 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.

7. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet to wash the beads. Carefully remove, then 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.

8. Repeat step 7 for a second wash.

9. 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. Under conditions of low relative humidity the beads air-dry rapidly. Do not overdry.

Proceed immediately to “Quantify the library by qPCR” on page 23. Alternatively, quantify the libraries using a Qubit™ Fluorometer, see “Quantify the amplified library with a Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument” on page 26.

IMPORTANT! Do not use the Ion Library Equalizer™ Kit.

Quantify the library by qPCR

Elute the library, then determine the concentration by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802). Libraries that have not undergone a second round of amplification typically have yields of 100–500 pM. However, yield is not indicative of library quality. After quantification, determine the dilution factor that results in a concentration of ~100 pM, which is suitable for template preparation using an Ion template kit.

Elute and dilute the library

1. Remove the plate with purified libraries from the plate magnet, then add 50 µL of Low TE to the pellet to disperse the beads.
2. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
3. Incubate at room temperature for at least 2 minutes.
4. Place the plate on the magnet for at least 2 minutes.
5. Prepare a 100-fold dilution for quantification. Remove 2 µL of supernatant, containing the library, then combine with 198 µL of Nuclease-free Water.

Proceed immediately to "Quantify library by qPCR and calculate the dilution factor" on page 24.

Quantify library by qPCR and calculate the dilution factor

Determine the concentration of each Ion AmpliSeq™ library by qPCR with the Ion Library TaqMan™ Quantitation Kit using the following steps. Analyze each sample, standard, and negative control in duplicate 20-µL reactions.

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Control Library (~68 pM; from the Ion Library TaqMan™ Quantitation Kit) at 6.8 pM, 0.68 pM, and 0.068 pM. Mark these tubes as standards, then use these concentrations in the qPCR instrument software.
2. Prepare reaction mixtures. For each sample, control, and standard, combine 20 µL of 2X Ion Library qPCR Master Mix and 2 µL of Ion Library TaqMan™ Quantitation Assay, 20X , then mix thoroughly. Dispense 11-µL aliquots into the wells of a PCR plate.
3. Add 9 µL of the diluted (1:100) Ion AmpliSeq™ library or 9 µL of each control dilution to each well (two wells per sample as noted before), for a total reaction volume of 20 µL.
4. Seal the plate with a MicroAmp™ Optical Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets.
5. Program your real-time instrument.
    a. Enter the concentrations of the control library standards.
    b. Select ROX™ Reference Dye as the passive reference dye.
    c. Select a reaction volume of 20 µL.
    d. Select FAM™ dye/MGB as the TaqMan™ probe reporter/quencher.
    e. The Ion Library qPCR Master Mix can be used on various instruments, as listed in the following table. The fast cycling program was developed using the StepOnePlus™ System in Fast mode.

IMPORTANT! When quantifying Ion CarrierSeq™libraries made from panels with 275-bp designs, use standard qPCR cycling. Fast cycling can result in inaccurate quantification.

Real-time PCR System	Reaction plate	Run mode	Stage	Temperature	Time
7500 Fast	96-well Fast	Fast	Hold (UDG incubation)	50°C	2 minutes
7900 HT 7900 HT Fast	96-well Fast 384-well Standard		Hold (polymerase activation)	95°C	20 seconds
ViiA™ 7			Cycle (40 cycles)	95°C	1 second
QuantStudio™ 3, 5, or 7					
StepOne™ StepOnePlus™	48-/96-well Fast			60°C	20 seconds
7300	96-well Standard	Standard	Hold (UDG incubation)	50°C	2 minutes
7500			Hold (polymerase activation)	95°C	2 minutes
7900 HT 7900 HT Fast			Cycle (40 cycles)	95°C	15 seconds
ViiA™ 7				60°C	1 minute
QuantStudio™ 3, 5, or 7					

6. Following qPCR, calculate the average concentration of the undiluted Ion AmpliSeq™ library by multiplying the concentration that is determined with qPCR by 100.

7. Based on the calculated library concentration, determine the dilution that results in a concentration of 50–100 pM.

   For example:

   - The undiluted library concentration is 300 pM.
   - The dilution factor is 300 pM/100 pM = 3.
   - Therefore, 10 µL of library mixed with 20 µL of Low TE (1:3 dilution) yields approximately 100 pM.

8. Dilute an aliquot of each library to 50 pM, combine libraries, then proceed to template preparation, or store libraries as described below.

Note: Although we recommend using libraries at 50 pM, libraries can be titrated up or down based on sequencing results. We do not recommend using a library concentration above 100 pM.

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

Combine libraries prepared with one panel for equal depth of coverage (for different samples)

You can prepare barcoded libraries from different samples using IonCode™ Barcode Adapters.

For example, if 16 libraries prepared with the same Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel are combined in a single templating and sequencing reaction:

1. Dilute all individual libraries to 50 pM concentration.
2. Add 10 µL of each of the 16 libraries to a single tube.
3. Mix the combined libraries, then proceed to templating and sequencing.

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

Quantify the amplified library with a Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument

Ion AmpliSeq™ libraries must be amplified before quantification to enrich amplifiable material and obtain sufficient material for accurate quantification. Amplify the library using 1X Library Amp Mix, then purify. Quantify the library using a Qubit™ Fluorometer or the Agilent™ 2100 Bioanalyzer™ instrument. Amplified libraries typically have yields of 2,000–10,000 pM. Yield is not indicative of library quality. After quantification, determine the dilution factor that results in a concentration of ~100 pM, which is appropriate for template preparation using an Ion template kit.

Alternatively, the Ion Library TaqMan™ Quantitation Kit can be used to quantify unamplified libraries.

Amplify the library

1. Remove the plate with purified libraries from the plate magnet, then add 50 µL of 1X Library Amp Mix and 2 µL of 25X Library Amp Primers to each bead pellet.

   Note:
   - The 1X Library Amp Mix is used to elute the libraries from the beads.
   - The 1X Library Amp Mix and 25X Library Amp Primers can be combined before addition.

2. Seal the plate with MicroAmp™ 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.
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 MicroAmp™ Clear Adhesive Film, place a MicroAmp™ Optical Film Compression Pad on the plate, load in the thermal cycler, then run the following program:

Stage	Temperature	Time
Hold	98°C	2 minutes
5 cycles	98°C	15 seconds
	64°C	1 minute
Hold	10°C	Hold

STOPPING POINT Samples can be stored at –20°C.

Purify the amplified library

Perform a two-round purification process with the Agencourt™ AMPure™ XP Reagent:

- First round at 0.5X bead-to-sample-volume ratio: High molecular-weight DNA is bound to beads, while amplicons and primers remain in solution. Save the supernatant.
- Second round at 1.2X bead-to-original-sample-volume ratio: Amplicons are bound to beads, and primers remain in solution. Save the bead pellet, and elute the amplicons from the beads.

IMPORTANT!

- Bring Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the solution slowly.
- Use freshly prepared 70% ethanol for the next steps. Combine 231 µL of 100% Ethanol with 99 µL of Nuclease-free Water per sample.
- Do NOT substitute a Dynabeads™-based purification reagent for the Agencourt™ Agencourt™ AMPure™ XP Reagent.

First-round purification

1. Prepare the Agencourt™ AMPure™ XP Reagent.
   a. Bring the Agencourt™ AMPure™ XP Reagent to room temperature.
   b. After the Agencourt™ AMPure™ XP Reagent is warmed to room temperature, vortex thoroughly to disperse the beads, then briefly centrifuge to collect the contents.
2. Prepare 70% Ethanol by combining 231 µL of 100% Ethanol with 99 µL of Nuclease–free water per sample.

   Carefully pipette the 100% Ethanol to ensure accurate volumes.
3. Tap the plate with the amplified library gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells, then remove the plate seal.

4. Add 25 µL (0.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each plate well containing ~50 µL of sample. Mix the bead suspension with the DNA thoroughly by pipetting up and down 5 times.

   IMPORTANT! Pipet the solution slowly an ensure that the properly volume of beads has been added.

   Note: Visually inspect each well to ensure that the mixture is homogeneous.

5. Incubate the mixture for 5 minutes at room temperature.

6. Place the plate in a magnet such as the DynaMag™–96 Side Magnet for at least 5 minutes, or until the solution is clear.

7. Carefully transfer the supernatant from each well to a new well of the 96-well PCR plate without disturbing the pellet.

   IMPORTANT! The supernatant contains the desired amplicons. Do not discard!

Second-round purification

1. To the supernatant from step 4 above, add 60 µL (1.2X original sample volume) of Agencourt™ AMPure™ XP Reagent. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.

2. Incubate the mixture for 5 minutes at room temperature.

3. Place the plate in the magnet for 3 minutes or until the solution is clear. Carefully remove, then discard the supernatant without disturbing the pellet.

   IMPORTANT! The amplicons are bound to the beads. Save the bead pellet.

4. Add 150 µL of freshly prepared 70% ethanol to each well, then move the plate side to side in the magnet to wash the beads. Remove, then 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 five 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.

5. Repeat step 4 for a second wash.

6. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 2–5 minutes. Do not overdry.

7. Remove the plate from the magnet, then add 50 µL of Low TE to the pellet to disperse the beads.

8. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by setting a pipettor to 40 µL, then pipet the mixture up and down at least 5 times before sealing the plate.

9. Incubate at room temperature for at least 2 minutes.

10. Place the plate in the magnet for at least 2 minutes, then analyze an aliquot of the supernatant as described in:
    - "Qubit™ Fluorometer: Quantify the library and calculate the dilution factor" on page 29 or
    - "Agilent™ 2100 Bioanalyzer™ instrument: Quantify the library and calculate the dilution factor" on page 30.

IMPORTANT! The supernatant contains the desired amplicons. Do not discard!

Qubit™ Fluorometer: Quantify the library and calculate the dilution factor

Analyze 10 µL of each amplified library using a Qubit™ Fluorometer and the Qubit™ dsDNA HS Assay Kit. Amplified libraries typically have concentrations of 300–1500 ng/mL. Libraries below 300 ng/mL can still provide good quality sequences. For more information, see the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326).

1. Determine the amplified library concentration:
    a. Make a 1:200 working dilution of Qubit™ dsDNA HS reagent using the Qubit™ dsDNA HS Buffer.
    b. Combine 10 µL of the amplified Ion AmpliSeq™ library with 190 µL of dye reagent, mix well, then incubate for at least 2 minutes.
    c. Prepare each Qubit™ standard as directed in the user guide.
    d. Measure the concentration on the Qubit™ Fluorometer.
    e. *(Qubit™ 2.0 Fluorometer only)* Calculate the concentration of the undiluted library by multiplying by 20. Alternatively, use the "Calculate Stock Conc." feature on your instrument.
2. Based on the calculated library concentration, determine the dilution that results in a concentration of 50–100 pM. A concentration of 19.5 ng/mL (average amplicon size 300 bp) is equivalent to a concentration of 100 pM.
   For example, to determine the dilution that is required to generate a library concentration of 100 pM, perform the following steps.
    - The library concentration is 450 ng/mL.
    - The dilution factor is 450 ng/mL divided by 19.5 ng/mL = 23.
    - Therefore, 10 µL of library that is mixed with 220 µL of Low TE (1:23 dilution) yields approximately 19.5 ng/mL (~100 pM).
3. Dilute an aliquot of each library to 50 pM, combine, then proceed to template preparation, or store libraries as described below.

Note: Although we recommend using libraries at 50 pM, libraries can be titrated up or down based on sequencing results. We do not recommend using a library concentration above 100 pM.

Agilent™ 2100 Bioanalyzer™ instrument: Quantify the library and calculate the dilution factor

Analyze 1 µL of amplified library on the Agilent™ 2100 Bioanalyzer™ instrument with the Agilent™ High Sensitivity DNA Kit (Cat. No. 5067–4626). Amplicon libraries should have multiple peaks in the 120–400 bp size range. Amplified libraries typically have concentrations of 2000–10,000 pM. Yield is not indicative of library quality, and libraries below 1,000 pM can still provide good quality sequences. If the library concentration is over 20,000 pM, dilute the library 1:10 and repeat the quantification to obtain a more accurate measurement.

1. Determine the molar concentration of the amplified library using the Bioanalyzer™ software. Ensure that the upper and lower marker peaks are identified and assigned correctly. Follow the manufacturer's instructions to perform a region analysis (smear analysis). Briefly:
   a. Select the **Data** icon in the Contexts panel, then view the electropherogram of the sample to be quantified.
   b. Select the **Region Table** tab below, then create a region spanning the desired amplicon peaks. Correct the baseline if needed.
   c. The molarity is automatically calculated, then displayed in the table in pmol/L (pM).
2. Based on the calculated library concentration, determine the dilution that results in a concentration of 50–100 pM.
   For example, to determine the dilution that is required to generate a library concentration of 100 pM, perform the following steps.
   - The library concentration is 3,000 pM.
   - The dilution factor is 3,000 pM/100 pM = 30.
   - Therefore, 10 µL of library mixed with 290 µL of Low TE (1:30 dilution) yields approximately 100 pM.
3. Dilute an aliquot of each library to 50 pM, combine, then proceed to template preparation, or store libraries as described below.

Note: Although we recommend using libraries at 50 pM, libraries can be titrated up or down based on sequencing results. We do not recommend using a library concentration above 100 pM.

Store libraries

Store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at –30°C to –10°C.

4 Create a Planned Run

IMPORTANT! If you are using a supplemental panel for the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel, see "Planned Run—Supplemental panels for the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel" on page 46.

Note: For additional information, see the help system for your version of Torrent Suite™ Software.

Before first use—download the required files

The Ion Torrent™ Ion CarrierSeq™ ECS Kits require a target regions file.

1. Before using the panel for the first time, contact support to obtain the target regions file.
   Target regions file: Ion_AmpliSeq_CarrierSeq_ECS_Panel_v1.7.bed
2. Ensure that the checkbox is deselected for hotspot information.
   The Ion Torrent™ Ion CarrierSeq™ ECS Kits do not detect hotspots.
3. Follow the instructions in the help system for your version of Torrent Suite™ Software to upload the target regions file to the Torrent Suite™ 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 GeneStudio™ S5 Series Sequencer and subsequent data analysis.

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.

Create a custom Planned Run template

IMPORTANT! Before creating a custom Planned Run template ensure that your Ion Reporter™ account is configured, and that the most current **Reference Library**, **Target Regions** BED files are installed. See "Before first use—download the required files" on page 31 for more information. Contact your local service representative to obtain the most current BED files.

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

1. Sign in to the Torrent Suite™ Software for the Torrent Server connected to your Ion Chef™ System.
2. Under the **Plan** tab, in the **Templates** screen, click **CarrierSeq** in the research application list.
3. In the **CarrierSeq** list, click a template name.
4. Enter or select the required information in each field:

Field	Action…
Run Plan Name	Enter a name for the Planned Run template.
Analysis Parameters	Ensure **Default (Recommended)** is selected.
DNA Reference Library	Select **GRCh38.p2.mask1**.
DNA Target Regions	Select **Ion_AmpliSeq_CarrierSeq_ECS_Panel_v1.7**.
DNA Hotspot Regions	Leave blank.

5. In the **Kits** step, select the **Ion Chef Template Kit** radio button, then complete the following fields.

Field	Selection
Instrument	Select **Ion GeneStudio™ S5 System**.
Sample Preparation Kit	*(Optional)* Select the sample preparation kit used.
Library Kit Type	Select one of the following kits. • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 530™ Chips** • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 540™ Chips**
Template Kit	Select **IonChef**, then click in the text box and select one of the following kits. • **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef** • **Ion 540™ Kit – Chef**
Sequencing Kit	Select **Ion S5™ Sequencing Kit**.
Chip Type	Select one of the following chips. • Ion 530™ Chip • Ion 540™ Chip

(continued)

Field	Selection
Control Sequence	Select Ion AmpliSeq™ Sample ID Panel.
Barcode Set	Select **IonCode™**.
Flows	550

6. Click **Next**.
7. In the **Plugins** step, select the **coverageAnalysis** and **sampleID** plugin, then click **Next**.
8. For the **coverageAnalysis** click **Configure**, ensure that the **Sample Tracking** checkbox is checked, click **Save Changes**, then click **Next**.
9. In the **Projects** step, select the project or projects that receive data from the runs that use this template, then click **Next**.
10. In the **Save** step, click **Copy Template** to save the new run template.

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

Create a Planned Run

IMPORTANT!

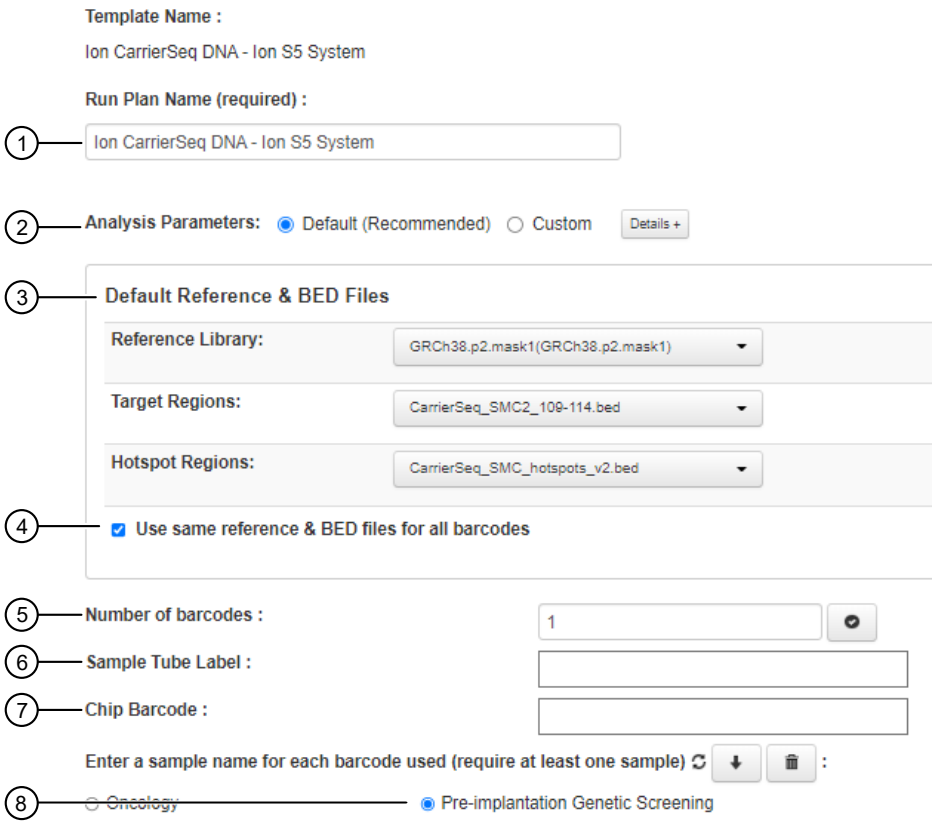
- Before creating a Planned Run ensure that your Ion Reporter™ account is configured, and that the **Reference Library** and **Target Regions** files are installed.
- Ensure that Ion Reporter™ parameters are selected in the **Ion Reporter Uploader** step (step 8). Do not manually upload files from Torrent Suite™ Software to Ion Reporter™ Software.

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

Callout	Field	Action
1	Run Plan Name	Enter a Run Plan name.
2	Analysis Parameters	Ensure the Default (Recommended) is selected.

(continued)

Callout	Field	Action
3	Default Reference & BED Files	• DNA Reference Library: **GRCh38.p2.mask1** • DNA Target Regions: **Ion_AmpliSeq_CarrierSeq_ECS_Panel_v1.7** • DNA Hotspot Regions: None
4		Select one of the following. • If all samples on the same chip are using the same Target Regions file, ensure that the checkbox is selected. • If the samples on the same chip are using different Target Regions files, ensure that the checkbox is deselected. The Target Regions and Hotspot Regions files are selected in step 12.
5	Number of barcodes	Enter the number of barcodes to be used in this run, then click ✔, which is found to the right of this field.
6	Sample Tube Label	*(Optional)* Enter or scan the barcode of the Ion Chef™ sample tube to be used in the run.
7	Chip Barcode	No entry required.
8		Select **Pre-Implantation Genetic Screening**.



5. **(Optional)** in the **Add a note box**, enter notes for the run.
6. **(Optional)** in the **Add LIMS Meta Data**, enter notes for the run.
7. Ensure that all **Monitoring Thresholds** are set to 30.
8. In the **Ion Reporter** step, complete the following fields.

Field	Selection
Ion Reporter Account	Select your Ion Reporter™ account. You must select an account.
Sample grouping	Select **Self**.
Ion Reporter Upload Options	Select **Automatically upload to Ion Reporter after run completion**.
Existing workflow	Select one of the following. • **CarrierSeq ECS - 530 - w1.4 - Single Sample** • **CarrierSeq ECS - 540 - w1.4 - Single Sample**

9. In the **Kits** step, complete the following fields.

Field	Selection
Instrument	Select **Ion GeneStudio™ S5 System**.
Chip Type	Select one of the following chips. • Ion 530™ Chip • Ion 540™ Chip
(Optional) Sample Preparation Kit	*(Optional)* Select the sample preparation kit used.
Control Sequence	Select Ion AmpliSeq™ Sample ID Panel.
Library Kit Type	Select one of the following kits. • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 530™ Chips** • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 540™ Chips**
Barcode Set	Select **IonCode™**.
Template Kit	Select **IonChef**, then click in the text box and select one of the following kits. • **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef** • **Ion 540™ Kit – Chef**
Flows	550
Sequencing Kit	Select **Ion S5™ Sequencing Kit**.
Mark as Duplicate Reads	Leave deselected.
Enable Realignment	Leave deselected.
Advanced Settings	Select **Use Recommended Defaults**.

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

Note: Ensure that the sampleID is enabled in Torrent Suite™ Software. For more information, see the help system for your version of Torrent Suite™ Software.

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

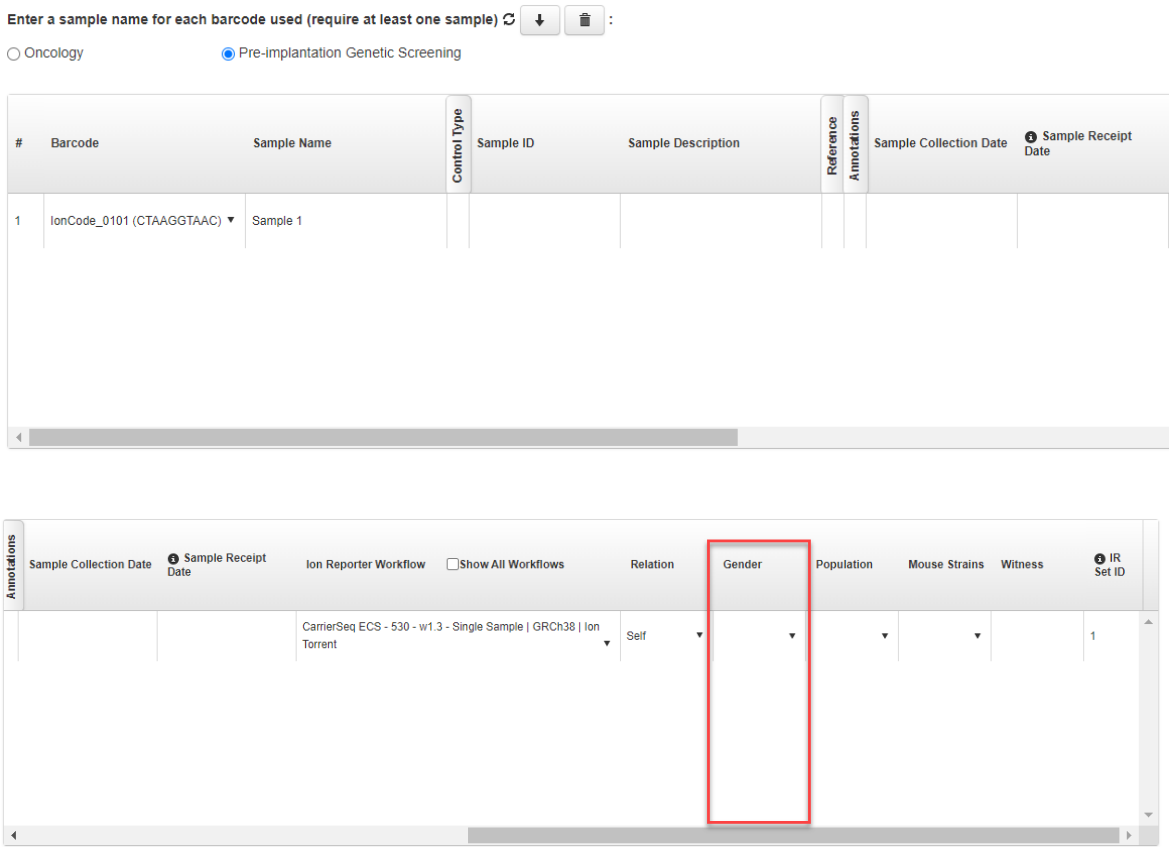
12. In the **Plan** step, for each sample, enter sample information.

IMPORTANT! Omitting gender information may result in incorrect X-linked copy number variant calling.

Field[1]	Action
Barcode	For each sample select the **Barcode** from the dropdown list.
Sample Name	Accept the auto-populated sample names or click in a field, then enter a unique sample name. We recommend sample names be unique even between runs.
Control Type (expanded)	Select No Template Control from the dropdown list to designate a sample as a no template control.
Sample ID	*(Optional)* Click in the field, then enter a sample ID.
Sample Description	*(Optional)* Click in the field, then enter a sample description.
Reference[2]	*(Optional)* Select the reference file for the barcode.
Annotations (expanded)	• *(Optional)* **Biopsy Days** • *(Optional)* **Cell Number** • *(Optional)* **Couple ID** • *(Optional)* **Embryo ID**
Sample Collection Date	*(Optional)* The date the sample was collected.
Sample Receipt Date	*(Optional)* The date when the sample was received.
Ion Reporter Workflow	Select the workflow from the dropdown. Select **Show All Workflows** to display more workflows in the dropdown.
Relation	*(Optional)* Select the relation from the dropdown menu.
Gender	Select the gender from the dropdown menu.
Population	*(Optional)* Select the population from the dropdown menu.
Mouse Strains	Leave blank.
Witness	*(Optional)*
IR Set ID	Leave blank.

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

[2] The Reference, Target Regions, and Hotspots Regions fields are displayed only if the checkbox for **Use same Reference & BED files for all barcodes** is selected (see step 4).



13. Click **Plan Run**.

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

Note: If you have not entered the sample tube label (see step 4), you must select a run plan. You are prompted to do so when you set up the Ion Chef™ Instrument.

---

5 Guidelines for templating and sequencing

Proceed to template preparation and sequencing using one of the following kits.

IMPORTANT!

- You can multiplex up to 4 libraries on a Ion 530™ Chip.
- You can multiplex up to 16 libraries on a Ion 540™ Chip.

Template System	Sequencer	Kit	User Guide
Ion Chef™	Ion GeneStudio™ S5 Sequencers	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™ Kit – Chef (Cat. No. A30011)	*Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)

6 Analyze variants

Note: For additional information, see the help system for your version of the Ion Reporter™ Software.

Analysis workflows in Ion Reporter™ Software

If the appropriate Ion Reporter™ Software workflow was selected in your Planned Run in the Torrent Suite™ Software, automated analysis has already been performed and you can view the analysis results in the Ion Reporter™ Software.

For instructions about manually launching an analysis, see “Manually launch an analysis” on page 46.

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

IMPORTANT! Do not use custom baselines for analysis. The workflows listed here use the correct baseline.

The following workflows are available in Ion Reporter™ Software 5.16 or later.

Analysis Workflow	Description
CarrierSeq ECS - 530 - w1.4 - Single Sample	Detects and analyzes somatic variants (SNPs, INDELs), in targeted DNA libraries from the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel run on the Ion 530™ Chip.
CarrierSeq ECS - 540 - w1.4 - Single Sample	Detects and analyzes somatic variants (SNPs, INDELs), in targeted DNA libraries from the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel run on the Ion 540™ Chip.

Check the sequencing run

Use the parameters in the following table to check the sequencing run. Follow the recommended actions if a parameter is below the acceptance value.

Parameter	Acceptance	Source	Recommended action
Sample level metrics			
Mean Depth (coverage)	≥200x	Torrent Suite™ Software: coverageAnalysis Plugin	Resequence the library.
Uniformity	≥93%	Torrent Suite™ Software: coverageAnalysis Plugin	One of the following: • Remake the library. • Check pool imbalance[1]
MAPD[2]	≤0.3	Ion Reporter™ Software	Remake the library.
Chip level metric			
aq20 Mean Read Length[3]	≥155 bp	Torrent Suite™ Software	Re–run all libraries from the chip.

[1] A pool imbalance can occur for incorrectly pooled samples. See “Combine the target amplification reactions” on page 20.

[2] MAPD values reflect the sensitivity of CNV calling. Higher MAPD values could be an indicator of lower data quality. For values between 0.25 and 0.3, the accuracy of CNV calling can be reduced.

[3] The single aq20 Mean Read Length value produced for the entire chip should be evaluated to determine if read lengths were sufficient for the run.

Download Ion Reporter™ annotation VCF files

VCF (variant call format), or TSV (tab separated value) files of the complete or filtered results can be downloaded from the **Analysis Results** page.

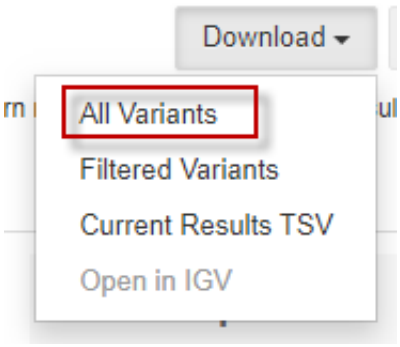
1. In the Ion Reporter™ Software **Home** tab, click **View analyses** or click the **Analyses** tab. Search, filter, or scroll to find your analysis in the list of **Analyses**, then click the Analysis link.

2. In the **Analyses** screen, select the analyses to be included in the CarrierReporter report, then click **Visualize**. To select an analysis, click the checkbox to the left of an analysis.

3. In the Analysis Visualization screen, click **Download** ▸ **All Variants**.

   Note: Selecting **Filtered Variants** results in an error.

   After the files are ready for download, you are sent an email notification.



4. Click **Home** ▸ **Notifications** to open the **Notifications** screen, then click ⬇ next to the **Notification** to download your results. Alternatively, select the checkbox next to one or more **Notifications**, then click **Download** to download multiple results files.
   The software generates a ZIP file with three folders: QC, Variants, and Workflow_Settings for each result. The annotated VCF file is found in the Variants folder.

5. Save the ZIP file to your local storage.

IMPORTANT! Do not extract the ZIP folder.

7 Generate a report using the Carrier Reporter Software

The Carrier Reporter Software is a web–based software that supports variant review and report generation.

Software compatibility

The Carrier Reporter Software is only compatible with the Chrome™ web browser.

Before first use—Access Carrier Reporter

Contact Support to receive access to Carrier Reporter. See Appendix D, “Documentation and support”.

Note: You will need two sets of login credentials, one set to access Carrier Reporter and one set to access the Carrier Reporter help.

Carrier Reporter guidelines

- A VCF file cannot be uploaded to Carrier Reporter more than once.
- In order to partner samples, the partner must be in the Carrier Reporter system. Carrier Reporter enables samples to be partnered with other samples for variant review of the potential offspring of the two partners. For details see the Carrier Reporter help system (“Access the Carrier Reporter help” on page 43).
- The name of the sample in the Ion Reporter™ Software (callout 1 in the following figure) is entered into the Sample No. field when setting up a sample in Carrier Reporter (callout 2 in the following figure).

① Ion Reporter™ Software **Analyses** screen—Name of sample
② Carrier Reporter **Sample Details** screen—Sample No. field

Access the Carrier Reporter help

1. Sign in to Carrier Reporter.
2. In the **Samples Management** screen, click your username, then select **Igentify Help Center**.
3. In the next screen, enter your sign in username for Carrier Reporter and the password for the Igentify help center.

   Note: The password for help center access may be different from the password for signing in to Carrier Reporter.

4. Search for the topic using the topic title or keywords.

Carrier Reporter workflow

Step	Workflow step	Topics in the Carrier Reporter help
	Carrier Reporter navigation overview	Samples Management Screen
	Define system panel[1]	Create System Panel
1	Create Samples	Add Sample to the system

(continued)

Step	Workflow step	Topics in the Carrier Reporter help
2	Upload data	• Upload CarrierSeq Results • Upload Genetic Results to the System
3	Review uploaded results	Samples management
4	Pair and review samples	Samples Details Screen
5	Generate report	• Report Generation Tab—lab • Report Generation Tab—clinic

[1] Before using Carrier Reporter for the first time, you must define a system panel.

Troubleshooting and FAQs

Visit our online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For the Next–Generation Sequencing Support Center: **thermofisher.com/ngssupport**
- For FAQs for this product: **http://thermofisher.com/A43585faqs**
- To browse the FAQ database and search using keywords: **thermofisher.com/faqs**

B

Supplemental procedures

Manually launch an analysis

To launch an Ion Reporter™ Software analysis manually:

1. Sign in to the Ion Reporter™ Software.
2. Select **Analyses** ▸ **Launch**
3. In the **Workflows** step, select **Carrier Screening** from the **Research Category** dropdown list.
4. In the **Workflow Name** column, click the appropriate workflow (see "Analysis workflows in Ion Reporter™ Software" on page 39), then click **Next**.
5. In the **Samples** step, click the checkbox next to a sample to select that sample, then click **Next**.
6. In the **Plugins** step, click **Next**.
7. Enter an **Analysis Name** and **Description**, then click **Launch Analysis**.

Planned Run—Supplemental panels for the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel

Before you begin

Create custom BED files

The Ion Torrent™ Ion CarrierSeq™ ECS Kits require a Target Regions file. If you are using a supplemental panel, you must modify the Target Regions file from the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel.

Contact support for assistance.

1. Before using the panel for the first time, download the Target Regions file to your local computer from **AmpliSeq.com**.

   Note: Some supplemental panels may also require a Hotspots Regions BED file. Contact support for assistance.

2. Follow the instructions in the help system for your version of Torrent Suite™ Software to upload the Target Regions BED file to the Torrent Suite™ Software.

3. Contact support to create one or more custom BED files for your supplemental panel.

Create a custom analysis workflow for the supplemental panel

You must create a custom analysis workflow for a supplemental panel.

Contact support for assistance to create the custom analysis workflow.

1. Sign in to the Ion Reporter™ Software.

2. In the **Workflows** tab, click **Overview**.

3. In the **Workflows** table, click the row for **CarrierSeq ECS - 530 - w1.4 - Single Sample** or **CarrierSeq ECS - 540 - w1.4 - Single Sample**, then click **(Actions) ▸ Copy**.
   The workflow bar opens to the **Research Application** step.

4. For each step (**Research Application**, **Reference**, **Annotation**, **Filters**, **Copy Number**, **Plugins**, **Final Report**, and **Parameters**), enter the information as directed by support. For detailed instructions, see the help system for your version of Ion Reporter™ Software.

5. In the **Confirm** step, name the analysis workflow, enter an optional description, then click **Confirm** and **Save Workflow**.

To verify that the analysis workflow was copied, click the **Workflows** tab, then click **Overview**, and search for the analysis workflow name to confirm that the custom analysis workflow is listed in the **Workflows** table.

Create a custom Planned Run template—Supplementary panel

IMPORTANT! Before creating a custom Planned Run template ensure that your Ion Reporter™ account is configured, the most current **Reference Library** file is uploaded, and correct **Target Regions** and **Hotspots Regions** BED files are created and installed. See “Before you begin” on page 46 for more information.

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

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

2. Under the **Plan** tab, in the **Templates** screen, click **CarrierSeq** in the research application list.

3. In the **CarrierSeq** list, click a template name.

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

Field	Action…
Run Plan Name	Enter a name for the Planned Run template.
Analysis Parameters	Ensure **Default (Recommended)** is selected.
DNA Reference Library	Select **GRCh38.p2.mask1**.
DNA Target Regions	Select the custom Target Regions BED file for the supplemental panel (see “Create custom BED files” on page 46).
DNA Hotspot Regions	*(Optional)* Select the custom Hotspots Regions BED file for the supplemental panel (see “Create custom BED files” on page 46).

5. In the **Kits** step, select the **Ion Chef Template Kit** radio button, then complete the following fields.

Field	Selection
Instrument	Select **Ion GeneStudio™ S5 System**.
Sample Preparation Kit	*(Optional)* Select the sample preparation kit used.
Library Kit Type	Select one of the following kits. • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 530™ Chips** • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 540™ Chips**
Template Kit	Select **IonChef**, then click in the text box and select one of the following kits. • **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef** • **Ion 540™ Kit – Chef**
Sequencing Kit	Select **Ion S5™ Sequencing Kit**.
Chip Type	Select **one of the following:** • Ion 530™ Chip • Ion 540™ Chip
Control Sequence	Select Ion AmpliSeq™ Sample ID Panel.
Barcode Set	Select **IonCode™**.
Flows	550

6. Click **Next**.

7. In the **Plugins** step, select the **coverageAnalysis** and **sampleID** plugin, then click **Next**.

8. For the **coverageAnalysis** click **Configure**, ensure that the **Sample Tracking** checkbox is checked, click **Save Changes**, then click **Next**.

9. In the **Projects** step, select the relevant project, then click **Next**.

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

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

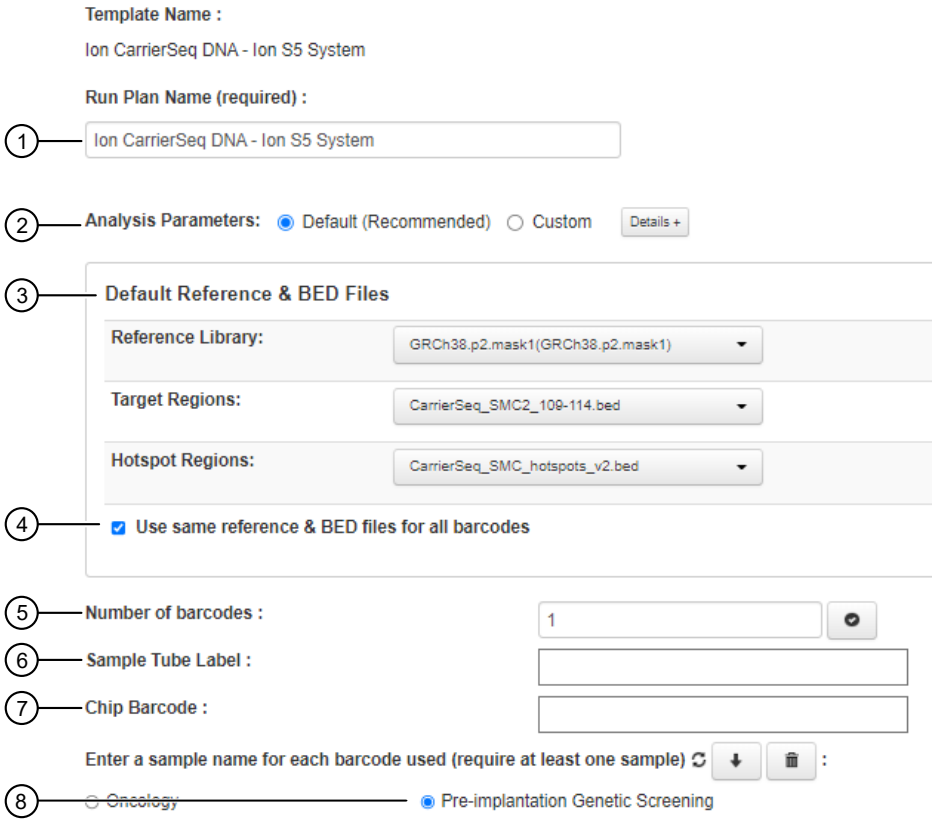
Create a Planned Run—Supplementary panel

IMPORTANT!

- Before creating a Planned Run ensure that your Ion Reporter™ account is configured, and that the **Reference Library** and **Target Regions** files are installed.
- Ensure that Ion Reporter™ parameters are selected in the **Ion Reporter Uploader** step (step 8). Do not manually upload files from Torrent Suite™ Software to Ion Reporter™ Software.

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

Callout	Field	Action
1	Run Plan Name	Enter a Run Plan name.
2	Analysis Parameters	Ensure the Default (Recommended) radio button is selected.
3	Default Reference & BED Files	• DNA Reference Library: **GRCh38.p2.mask1** • DNA Target Regions: **Ion_AmpliSeq_CarrierSeq_ECS_Panel_v1.7** • DNA Hotspot Regions: None
4		Select one of the following. • If all samples on the same chip are using the same Target Regions file, ensure that the checkbox is selected. • If the samples on the same chip are using different Target Regions files, ensure that the checkbox is deselected. The Target Regions and Hotspot Regions files are selected in step 12.
5	Number of barcodes	Enter the number of barcodes to be used in this run, then click ✓, which is found to the right of this field.
6	Sample Tube Label	*(Optional)* Enter or scan the barcode of the Ion Chef™ sample tube to be used in the run.
7	Chip Barcode	No entry required.
8		Select **Pre-Implantation Genetic Screening**.



5. **(Optional)** in the **Add a note box**, enter notes for the run.
6. **(Optional)** in the **Add LIMS Meta Data**, enter notes for the run.
7. Ensure that all **Monitoring Thresholds** are set to 30.
8. In the **Ion Reporter** step, complete the following fields.

Field	Selection
Ion Reporter Account	Select your Ion Reporter™ account. You must select an account.
Sample grouping	Select **Self**.
Ion Reporter Upload Options	Select **Automatically upload to Ion Reporter after run completion.**
Existing workflow	Select the custom workflow for the supplementary panel. (“Create a custom analysis workflow for the supplemental panel” on page 47)

9. In the **Kits** step, complete the following fields.

Field	Selection
Instrument	Select **Ion GeneStudio™ S5 System**.
Chip Type	Select **one of the following:** • Ion 530™ Chip • Ion 540™ Chip
(Optional) Sample Preparation Kit	*(Optional)* Select the sample preparation kit used.
Control Sequence	Select Ion AmpliSeq™ Sample ID Panel.
Library Kit Type	Select one of the following kits. • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 530™ Chips** • **Ion Torrent™ Ion CarrierSeq™ ECS Kit with Ion 540™ Chips**
Barcode Set	Select **IonCode™**.
Template Kit	Select **IonChef**, then click in the text box and select one of the following kits. • **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef** • **Ion 540™ Kit – Chef**
Flows	550
Sequencing Kit	Select **Ion S5™ Sequencing Kit**.
Mark as Duplicate Reads	Leave deselected.
Enable Realignment	Leave deselected.
Advanced Settings	Select **Use Recommended Defaults**.

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

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

12. In the **Plan** step, for each sample, enter sample information.

IMPORTANT! Omitting gender information can result in incorrect X-linked copy number calling.

Field[1]	Action
Barcode	For each sample select the **Barcode** from the dropdown list.
Sample Name	Accept the auto-populated sample names or click in a field, then enter a unique sample name. We recommend sample names be unique even between runs.
Control Type (expanded)	Select No Template Control from the dropdown list to designate a sample as a no template control.

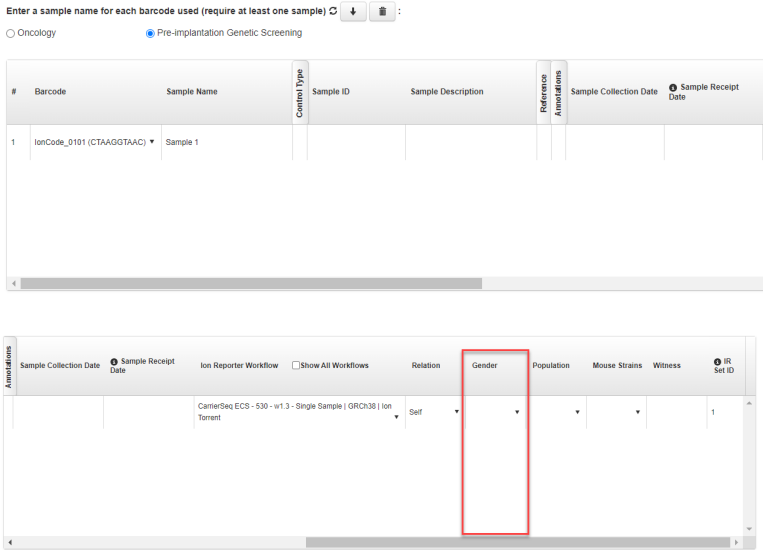
(continued)

Field[1]	Action
Sample ID	*(Optional)* Click in the field, then enter a sample ID. **Note:** Ensure that the sampleID is enabled in Torrent Suite™ Software. For additional information, see the help system for your version of Torrent Suite™ Software.
Sample Description	*(Optional)* Click in the field, then enter a sample description.
Reference[2]	*(Optional)* Select the reference file for the barcode.
Annotations (expanded)	• *(Optional)* **Biopsy Days** • *(Optional)* **Cell Number** • *(Optional)* **Couple ID** • *(Optional)* **Embryo ID**
Sample Collection Date	*(Optional)* The date the sample was collected.
Sample Receipt Date	*(Optional)* The date when the sample was received.
Ion Reporter Workflow	Select the workflow from the dropdown. Select **Show All Workflows** to display additional workflows in the dropdown.
Relation	*(Optional)* Select the relation from the dropdown menu.
Gender	Select the gender from the dropdown menu.
Population	*(Optional)* Select the population from the dropdown menu.
Mouse Strains	Leave blank.
Witness	*(Optional)*
IR Set ID	Leave blank.

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

[2] The Reference, Target Regions, and Hotspots Regions fields are displayed only if the checkbox for **Use same Reference & BED files for all barcodes** is selected (see step 4).

① Sample information



13. Click **Plan Run**.

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

Note: If you have not entered the sample tube label (see step 4), you must select a run plan. You are prompted to do so when you set up the Ion Chef™ Instrument.

After the sequencing run and analysis are complete, proceed to “Download Ion Reporter™ annotation VCF files” on page 40.

C Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

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

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

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

Biological hazard safety

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

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

D Documentation and support

Customer and technical support

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

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

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