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Ion AmpliSeq™ Microbiome Health Research Kits

USER GUIDE

For manual library preparation

for use with:
Ion AmpliSeq™ Microbiome Health Research 540 Kit
Ion AmpliSeq™ Microbiome Health Research 550 Kit

Catalog Numbers A46495, A46496, and A46497
Publication Number MAN0018602
Revision D.0

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

Revision history: MAN0018602 D.0 (English)

Revision	Date	Description
D.0	18 September 2023	Updated Ion S5™ Chef Supplies to include the PCR Plate Frame.
C.0	10 March 2023	• Added support for Ion AmpliSeq™ Microbiome Health Research Kit, Assay Only Bundle. • Updated Guidance for Bacterial Marker field. • Number of possible false positive species corrected. • Troubleshooting content replaced with links to **thermofisher.com**. • Content reorganization.
B.0	14 May 2020	• Support added for Ion AmpliSeq™ Microbiome Health Research 550 Kit. • Support added for Ion Reporter™ Software analysis workflows.
A.0	10 December 2019	New document for the Ion AmpliSeq™ Microbiome Health Research 540 Kit. Provides instruction for the preparation, templating, and sequencing of libraries with the Ion AmpliSeq™ Microbiome Health Research – 16S rRNA Gene Pool and Ion AmpliSeq™ Microbiome Health Research – Target Species Pool.

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

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1 Product information

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

Product description

The Ion AmpliSeq™ Microbiome Health Research Kit is a comprehensive, targeted next-generation sequencing (NGS) assay for characterizing the human gut microbiome. The assay consists of two pools of Ion AmpliSeq™ oligonucleotide primers and associated reagents to generate amplicon libraries for NGS on Ion Torrent platforms. Using the kit, microbial DNA isolated from human stool samples is used to generate libraries for templating and sequencing on the Ion Chef™ and Ion GeneStudio™ systems.

The kit includes the following key features:

- Up to 64 paired libraries (32 samples) can be combined and loaded onto a single Ion 540™ Chip in a single workflow.
- Up to 96 paired libraries (48 samples) can be combined and loaded onto a single Ion 550™ Chip in a single workflow.
- Typically, the entire workflow can be completed in 3 days for a single chip including the analysis.
- The minimum input is 1 ng/pool for the Ion AmpliSeq™ Microbiome Health Research – 16S rRNA Gene Pool.
- The minimum input is 10 ng/pool for the Ion AmpliSeq™ Microbiome Health Research – Target Species Pool.

Sequencer compatibility

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

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

The Ion S5™ Sequencer and the Ion S5™ XL Sequencer are no longer available for purchase and are replaced by the Ion GeneStudio™ S5 Sequencer and Ion GeneStudio™ S5 Prime Sequencer, respectively. Both of the Ion S5™ and Ion S5™ XL Sequencers continue to be supported by Thermo Fisher Scientific. The Ion AmpliSeq™ Microbiome Health Research Kits are compatible with both the Ion S5™ and Ion GeneStudio™ S5 Series Sequencers.

Contents and storage

Kit summary

The Ion AmpliSeq™ Microbiome Health Research Kits (Cat. Nos. A46496 and A46497) consist of the Ion AmpliSeq™ Microbiome Health Research – 16S rRNA Gene Pool, the Ion AmpliSeq™ Microbiome Health Research – Target Species Pool, the Ion AmpliSeq™ Library Kit Plus, IonCode™ Barcode Adapters, and reagents for templating and sequencing barcoded sample libraries from bacterial DNA on the Ion 540™ and Ion 550™ Chips. Sufficient reagents are provided to prepare uniquely barcoded DNA-libraries from 256 or 384 research samples.

Component	Quantity per kit		
	A46496[1]	A46497[2]	A46495[3]
Ion AmpliSeq™ Microbiome Health Research Panel	6	8	1
Ion AmpliSeq™ Library Kit Plus (Cat. No. A35907)	6	8	1
IonCode™ Barcode Adapter 0101–0196	1	1	1
Ion 540™ Kit – Chef (Cat. No. A30011)	1	N/A	N/A
Ion 540™ Chip Kit (Cat. No. A27766)	1	N/A	N/A
Ion 550™ Kit – Chef (Cat. No. A34541)	N/A	1	N/A
Ion 550™ Chip Kit (Cat. No. A34537)	N/A	1	N/A

[1] Ion 540™bundle, 256 samples (32/chip)
[2] Ion 550™bundle, 384 samples (48/chip)
[3] 48 samples, assay bundle only

Ion AmpliSeq™ Microbiome Health Research Panel

Contents	Amount	Storage
5X Ion AmpliSeq™ Microbiome Health Research – 16S rRNA Gene Pool (green cap)	1 × 260 µL	–30°C to –5°C
5X Ion AmpliSeq™ Microbiome Health Research – Target Species Pool (purple cap)	1 × 260 µL	

Note: For the Ion AmpliSeq™ Microbiome Health Research – Target Species Pool, with the following exceptions, all the primers in this pool are designed to be targeted to a single bacterial species.

- *Escherichia coli* primers—Also detect and amplify DNA from *Shigella*.
- *Streptococcus infantarius* primers—Also detect and amplify DNA from *Streptococcus equinus*, due to extremely high biological identity between strains of the above species.

Ion AmpliSeq™ Library Kit Plus

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

Component	Amount	Storage
5X Ion AmpliSeq™ HiFi Mix (red cap)	480 µL	–30°C to –10°C
FuPa Reagent (brown cap)	192 µL	
Switch Solution (yellow cap)	384 µL	
DNA Ligase (blue cap)	192 µL	
25X Library Amp Primers (pink cap)	192 µL	
1X Library Amp Mix (black cap)	4 × 1.2 mL	
Low TE	2 × 6 mL	15°C to 30°C[1]

[1] Can be stored at –30°C to –10°C.

IonCode™ Barcode Adapters

Contents	Amount	Storage
IonCode™ Barcode Adapters 0101–0196	1 × 96-well plate (20 µL/well)	–30°C to –5°C

Sequencing chips

Chip kit	Cat. No.	Amount	Storage
Ion 540™ Chip Kit	A27765	4 chips	15°C to 30°C
Ion 550™ Chip Kit	A34537		

Ion Chef™ reagents and materials

Contents	Amount / box		Storage
	Cat. No. A30011	Cat. No. A34541	
Ion S5™ Chef Supplies			
Chip Adapter	2	2	15°C to 30°C
Enrichment Cartridge v2	1	1	
Tip Cartridge v2	1	1	
PCR Plate	1	1	
PCR Plate Frame	1	1	
Frame Seal v2	1	1	
Recovery Station Disposable Lid v2	2	2	
Recovery Tube v2	12	12	
Ion S5™ Chef solutions			
Ion S5™ Chef Solutions	4 cartridges	NA	15°C to 30°C
Ion 550™ Chef Solutions	NA	4 cartridges	
Ion S5™ Chef reagents			
Ion 540™ Chef Reagents	4 cartridges	NA	–30°C to –10°C
Ion 550™ Chef Reagents	NA	4 cartridges	

Ion S5™ sequencing reagents and materials

IMPORTANT! Do not store the Ion S5™ Sequencing Reagents (Part No. A30011) on dry ice or in a closed environment containing dry ice.

Contents	Amount / box	Storage
Ion S5™ Sequencing Solutions (Part No. A27767)		
Ion S5™ Wash Solution	4 × 1.5 L	15°C to 30°C
Ion S5™ Cleaning Solution	250 mL	
Ion S5™ Sequencing Reagents (Part No. A30011)		
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.

Workflow

The following workflow summarizes the procedure for generating Ion AmpliSeq™ DNA libraries using the Ion AmpliSeq™ Microbiome Health Research Kits for templating and sequencing on the Ion Chef™ and Ion GeneStudio™ S5 Systems.

Ion AmpliSeq™ Microbiome Health Research Kits workflow

Prepare libraries (page 17)

Prepare DNA amplification reactions. Amplify the targets. Partially digest amplicons.

Ligate adapters to the amplicons and purify (page 19)

Dilute adapters. Perform the ligation reaction. Purify and elute the library.

Quantify the library (page 22)

Determine the library concentration by qPCR and calculate the dilution factor that results in a concentration of ~50 pM.

Combine libraries (page 25)

Combine libraries into a single library pool.

Template and sequence (page 25)

Proceed to templating and sequencing on the Ion Chef™ Instrument and Ion GeneStudio™ S5 Series Sequencer.

Create a Planned Run (page 29)

Create a planned run by entering run parameters for templating and sequencing your samples. Planned Runs are digital instructions that are created in Torrent Suite™ Software for controlling the template preparation and sequencing instruments.

Visualize Ion Reporter™ analysis (page 36 or page 41)

Analyze data using Ion Reporter™ Software.

2 Reagents, supplies, and required materials

This chapter lists the reagents, supplies, and materials needed to operate the Ion GeneStudio™ S5 Series Sequencer, and provides consumables ordering and storage information. Reagents and supplies can be ordered as kits and starter packs, but most consumables can also be ordered individually as your needs require.

Note: Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.

Required materials not supplied

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

Item	Source
Agencourt™ AMPure™ XP Kit	A63880 or A63881 Beckman Coulter
One of the following thermal cyclers: • AB™ 2720 Thermal Cycler • Veriti™ Dx 96-well Thermal Cycler, 0.2 mL • ProFlex™ 96-well PCR System • GeneAmp™ PCR System 9700[1] or Dual 96-well Thermal Cycler	**thermofisher.com**
One of the following Real-Time PCR instruments: • QuantStudio™ Real-Time PCR System • Applied Biosystems™ 7900HT, or 7500 • StepOne™, or StepOnePlus™ • ViiA™ 7 Real-Time PCR System	**thermofisher.com**
Microcentrifuge (must accommodate standard 1.5-, 0.5-, and 0.2-mL microcentrifuge tubes)	**MLS**
Vortex mixer	**MLS**

(continued)

Item	Source
96-well plate centrifuge	**MLS**
Ion Library TaqMan™ Quantitation Kit	4468802
MicroAmp™ Optical 96-well Reaction Plate	N8010560 4306737 (with barcode)
MicroAmp™ Fast Optical 96-Well Reaction Plate	4346907
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Adhesive Film	4306311
MicroAmp™ Compression Pad	4312639
DynaMag™-96 Side Magnet, or other plate magnet	12331D
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes, 1.5 mL	13-698-791 **fisherscientific.com**
Teknova DNA Suspension Buffer	50-843-203 **fisherscientific.com**
Nuclease-free Water	AM9932
Absolute ethanol	**MLS**
Pipettors, 2–200 µL, and low-retention filtered pipette tips	**MLS**
(Optional) IonCode™ Barcode Adapters 1–384 Kit[2]	A29751

[1] Supported but no longer available for purchase.
[2] The kits contain IonCode™ Barcode Adapters 1-96. However, you can use additional IonCode™ Barcode Adapters with the kits.

Recommended materials

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

Item	Source
Recommended additional equipment	
Qubit™ 4 Fluorometer[1]	Q33238
Recommended for nucleic acid isolation	
MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit	A42358 and A42357

(continued)

Item	Source
Recommended for nucleic acid quantification	
Qubit™ dsDNA HS Assay Kit	Q32851/Q32854
Qubit™ dsDNA BR Assay Kit	Q32853 /Q32850
Recommended controls	
Gut Microbiome Genomic Mix	MSA-1006 American Type Culture Collection (ATCC)
Nuclease-free Water	See “Required materials not supplied” on page 11.

[1] Qubit™ 2.0 & Qubit™ 3.0 Fluorometers are supported but no longer available for purchase.

3 Before you begin

Procedural guidelines

- Minimize freeze-thaw cycles of Ion AmpliSeq™ Microbiome Health Research pools by aliquoting as needed for your experiments. Pools can be stored at 4°C for up to one year.
- Use good laboratory practices to minimize cross-contamination of products. If possible, perform PCR setup in an area or room that is free of amplicon contamination. Always change pipette tips between samples.
- Use a calibrated thermal cycler specified in "Required materials not supplied" on page 11.
- Pipet viscous solutions, such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, Switch Solution, DNA Ligase, and panels, slowly and ensure complete mixing by vortexing or pipetting up and down several times.

Before each use of the kit

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

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

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 applicator in the MicroAmp™ Adhesive Film Applicator. Plate seals can be removed with less effort when hot. Try removing seals right after taking the plate out of the thermal cycler.

- When using the 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.
- 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.
- During target amplification, 98°C can be used for the enzyme activation and denaturation steps for some panels, especially those targeting cDNA. This change can improve underperforming amplicons.

4 Library Preparation

Guidelines for DNA isolation, quantification, and input

- We recommend the MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (Cat. Nos. A42358 or A42357) for isolating bacterial DNA from research stool samples.
- We recommend the Qubit™ dsDNA HS Assay Kit (Cat. Nos. Q32851 or Q32854) or Qubit™ dsDNA BR Assay Kit (Cat. Nos. Q32850 or Q32853) for quantification of DNA samples.
- Quantification methods such as spectrophotometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA. Use of these methods can lead to gross overestimation of the concentration of sample DNA, under-seeding of the target amplification reaction, low library yields, and poor chip loading.
- Each Ion AmpliSeq™ Microbiome Health Research – 16S rRNA Gene Pool library target amplification reaction requires 1.0 ng (≥0.167 ng/µL) of bacterial DNA.
- Each Ion AmpliSeq™ Microbiome Health Research – Target Species Pool library target amplification reaction requires 10 ng (≥1.67 ng/µL) of bacterial DNA.
- Increasing the amount of DNA can result in higher-quality libraries, especially when DNA quality is poor or unknown.

Prepare libraries

Prepare DNA target amplification reactions

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

1. Place a 96-well plate on ice or in a pre-chilled 4°C cold block.
2. Dilute each purified template DNA in Low TE or nuclease-free water.

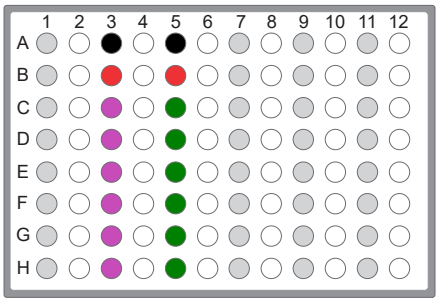
Pool	Description
Target Species	Dilute template DNA to 1.67 ng/µL.
16S	Dilute template DNA to 0.167 ng/µL.

3. Prepare separate target amplification reactions for the Target Species and 16S primer pools. Add the following components to individual wells of a 96-well PCR plate.

Note: If processing multiple samples, prepare a reaction master mix (+ 5–10% overage) without DNA template for each primer pool. Pipet 14.0 µL of each primer pool-specific reaction master mix into an individual well, then add 6.0 µL of the sample-specific DNA template dilution (1.67 ng/µL or 0.167 ng/µL) to each well.

Component	Volume per sample	
	Species Pool	16S Pool
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL	4 µL
5X Ion AmpliSeq™ Microbiome Health Research – Target Species Pool	4 µL	—
5X Ion AmpliSeq™ Microbiome Health Research – 16S rRNA Gene Pool	—	4 µL
10 ng DNA (≥1.67 ng/µL)[1]	X µL	—
1.0 ng DNA (≥0.167 ng/µL)[1]	—	X µL
Nuclease-free Water	12–X µL	12–X µL
Total Volume	20 µL	20 µL

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



- Ion AmpliSeq™ Microbiome Health Research – Target Species Pool
- *(Optional)* No template control (NTC)
- Ion AmpliSeq™ Microbiome Health Research – 16S rRNA Gene Pool
- *(Optional)* Positive control (PC)

Note:

- The NTC and PC wells that are shown in this example plate layout can be included as controls for each pool.
- We recommend using Nuclease–free Water as the NTC. See “Required materials not supplied” on page 11.

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

IMPORTANT! When mixing, use a new tip for each well. Do not cross contaminate the target amplification reactions.

Amplify the DNA targets

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

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 min
20 Cycles	Denature	99°C	15 sec
	Anneal and extend	60°C	4 min
Hold	—	10°C	Hold

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

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

Partially digest amplicons

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

1. Keep the FuPa Reagent (brown cap) on ice, gently vortex to mix, then briefly centrifuge to collect.
2. Carefully remove the adhesive film from the plate.

   IMPORTANT! Be careful when removing the film to minimize contamination.
3. Add 2 µL of FuPa Reagent to each amplified sample. The total volume is ~22 µL.
4. Seal the plate with a new 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.
5. Place a compression pad on the plate, load in the thermal cycler, then run the following program:

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

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

Proceed immediately to "Perform the ligation reaction" on page 20. Do not store the partially digested amplicons overnight.

Ligate adapters to the amplicons and purify

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

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

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

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

Ion Xpress™ Barcode Adapters only: Combine and dilute adapters

For each barcode X selected, prepare a mix of Ion P1 Adapter and Ion Xpress™ Barcode X at a final dilution of 1:4 for each adapter. Scale volumes as necessary. Use 2 µL of this barcode adapter mix in the ligation reaction.

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

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

[1] X = barcode chosen

Note: Store diluted adapters at –20°C.

Perform the ligation reaction

1. If there is visible precipitate in the Switch Solution or the tube cap after thawing, vortex or pipet up and down at room temperature to resuspend before pipetting.
2. 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 *or* diluted Ion Xpress™ barcode adapter mix (for barcoded libraries)	2 µL
3	DNA Ligase (blue cap)	2 µL
—	**Total volume** (including ~22 µL of digested amplicon)	**~30 µL**

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

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

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

STOPPING POINT Samples can be stored for up to 24 hours at 10°C on the thermal cycler. For longer term, store at –20°C.

Purify the library

IMPORTANT!

- Bring Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the solution slowly.
- Do NOT substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

1. Prepare 70% ethanol (350 µL × # of samples) fresh daily.
2. Briefly centrifuge the plate to collect the contents in the bottom of the wells.
3. Carefully remove the plate seal, then add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.

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

4. Incubate the mixture for 5 minutes at room temperature.
5. 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.
6. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side 3 times 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, 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.

7. Repeat step 6 for a second wash.

8. 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! 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.

Elute 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. Carefully remove the plate seal, then transfer the supernatant (~50 µL) to a new 96-well plate or 1.5-mL Eppendorf LoBind™ tube.

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

Quantify the library by qPCR

Determine library concentration by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802). After quantification, determine the dilution factor that results in a concentration of ~50 pM.

For instructions, see the *Ion Library TaqMan™ Quantitation Kit User Guide* (Pub. No. MAN0015802).

Prepare library dilutions

Before qPCR quantification, the libraries must be diluted to a concentration that falls inside the range of the standards. We recommend diluting the libraries 500-fold.

1. Combine 2 µL of supernatant, containing the library, with 998 µL of Nuclease-free Water in a 96-well deep-well plate or 1.5-mL Eppendorf LoBind™ tube.
2. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

Quantify library by qPCR and calculate dilution factor

Determine the concentration of each Ion AmpliSeq™ Microbiome Health Research Kit library by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802). Analyze each sample, standard, and negative control in duplicate reactions. Sample libraries typically have yields of 100–2,500 pM. NTC libraries typically yield below 50 pM. However, library yield is not indicative of library quality. After quantification and determination of the dilution factor that results in a concentration of ~50 pM, dilute the libraries to 50 pM.

1. Prepare three 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, and 0.068 pM. Mark these tubes as standards, then use these concentrations in the qPCR instrument software.

Standard	Control Library	Nuclease-free water[1]	Dilution factor	Concentration
1	5 µL undiluted Control Library	45 µL	1:10	6.8 pM
2	5 µL Std 1	45 µL	1:100	0.68 pM
3	5 µL Std 2	45 µL	1:1000	0.068 pM

[1] Not DEPC-treated.

Note: When you program the qPCR instrument, enter the concentration of each standard in the "Amount" field.

2. Calculate, then prepare the required volume of PCR Master Mix for duplicate 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**

3. In an Optical PCR plate setup duplicate PCR reactions for each sample, standard, and NTC. To each well add the following components:

Component	Volume per reaction	
	96-well plate	384-well plate
PCR Master Mix	11 µL	5.5 µL
1:500 dilution of the sample[1]	9 µL	4.5 µL
Total	**20 µL**	**10 µL**

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

4. Program your real-time instrument.
   a. Enter the following thermal cycling parameters.

Stage	Temperature	Time
Hold (UDG incubation)	50°C	2 min
Hold (polymerase activation)	95°C	20 sec
Cycle (40 cycles)	95°C	1 sec
	60°C	20 sec

   b. Enter the concentrations of the control library standards.

   c. Select ROX™ Reference Dye as the passive reference dye.

   d. Select a reaction volume of 20 µL for a 96–well plate or 10 µL for a 384–well plate.

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

   f. Set the analysis parameters:
      - Threshold—deselect **Automatic Threshold**, then enter a Threshold value of 0.2.
      - Automatic Baseline—ensure that Automatic Baseline is selected.

5. Following qPCR, calculate the average concentration of each library by multiplying the determined concentration by the dilution factor used (1:500 recommended).

6. Based on the calculated library concentration, determine the dilution factor for the undiluted library that results in a concentration of ~50 pM.
   For example:
   - The undiluted library concentration is 300 pM.
   - The dilution factor is 300 pM/50 pM = 6.
   - Therefore, 10 µL of library that is mixed with 50 µL of Low TE (1:6 dilution) yields approximately 50 pM.

7. Dilute library to 50 pM as described, then combine all libraries to be sequenced on a single chip into a single pool (see “Combine libraries” on page 25).

Combine libraries

When combining Target Species and 16S pool libraries into a single library pool, from the same or from different samples, we recommend combining all libraries in equal proportions. At least 25 µL of library pool is required to perform the templating reaction on the Ion Chef™ Instrument.

IMPORTANT! Prepare a fresh library dilution before each Ion Chef™ Instrument run. Use the diluted library within 48 hours.

1. Combine 5 µL of each uniquely barcoded, 50 pM diluted library together into a 1.5-mL Eppendorf LoBind™ tube.

   Note:
   - If combining fewer than 5 libraries together into a library pool, combine enough volume of each library to ensure that you have a final volume of 25 µL. You must have 25 µL to perform the templating reaction.
   - We recommend combining up to 64 libraries into a single pool for sequencing on a single Ion 540™ Chip. Libraries can be paired (from the same sample) or unpaired (from different samples), as long as each library has a unique barcode.
   - Do not use a library with a concentration less than 50 pM.
   - For runs that include a no-template control (NTC), dilute the NTC library 1:10 in Low TE buffer and then add in the same volume of diluted NTC library as is added for DNA sample libraries.

2. Vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 10 times.

Guidelines for templating and sequencing

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

Chip	Template System	Sequencer	Kit	User Guide
Ion 540™ Chip	Ion Chef™	Ion GeneStudio™ S5 Series Sequencer[1]	Ion 540™ Kit – Chef (Cat. Nos. A27759, A30011)	*Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851)
Ion 550™ Chip	Ion Chef™	Ion GeneStudio™ S5 Series Sequencer[1]	Ion 550™ Kit – Chef (Cat. No. A34541)	*Ion 550™ Kit – Chef User Guide* (Pub. No. MAN0017275)

[1] The Ion S5™ Sequencer and the Ion S5™ XL Sequencer are supported but no longer available for purchase. Ion AmpliSeq™ Microbiome Health Research kits are compatible with both the Ion S5™ and Ion GeneStudio™ S5 Series Sequencers.

To create a specific Run Plan for use in templating and sequencing, see Chapter 5, “Create a Planned Run”. For additional information, see the user guide for the Ion 540™ Chip or Ion 550™ Chip.

5 Create a Planned Run

IMPORTANT!

- This kit is compatible with Torrent Suite™ Software version 5.12 or later. Before proceeding, check for updates to the Torrent Suite™, Ion Reporter™, and Ion Chef™ System software. Contact your service representative for help with upgrading the software.
- Before creating a Planned Run you must enable the Ion AmpliSeq™ Microbiome Health Research template, and upload the **Reference Library** on the Torrent Server. See Appendix B, "Supplemental information" for more information. Contact your local service representative to obtain the most current file.

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. Each chip that is prepared in an Ion Chef™ run requires its own Planned Run.

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

In Torrent Suite™ Software 5.12 or later, use the **Ion AmpliSeq Microbiome Health Research Panel** template as the primary Planned Run template.

Application	Torrent Suite™ Software template	Description
16S Target Sequencing	Ion AmpliSeq Microbiome Health Research Panel	DNA-only Planned Run template

Confirm the IonReporterUploader plugin version

Ensure that the version of the IonReporterUploader plugin is 5.12.0.4 or later.

Previous versions of the plugin do not support the upload of 2 barcodes per sample using the same sample name.

If you do not have the correct version of the plugin, contact support.

Create a Planned Run template

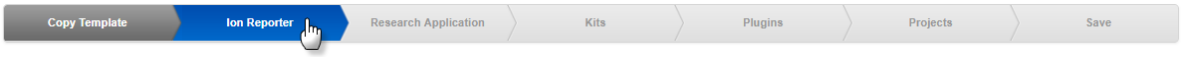
We recommend creating a Planned Run template for reuse when the same conditions are used for multiple runs. For a complete description of each field in the **Create Plan** workflow bar, see the *Torrent Suite™ Software Help*.

Note: If you are using Torrent Suite™ Software version 5.12 or later and are using the Ion AmpliSeq Microbiome Health Research Panel template, proceed to "Create a Planned Run" on page 29.

1. Sign in to the Torrent Suite™ Software.
2. In the **Plan** tab, in the **Templates** screen, click **16S rRNA Profiling** in the research application list.
3. In the **16S rRNA Profiling** list, **Ion AmpliSeq Microbiome Health Research Panel** row, click **(Settings)** ▸ **Copy**.
   The **Copy Template** wizard opens to the **Save** step.
4. In the **Template Name** field, enter a name for the Planned Run template.
5. For **Analysis Parameters**, ensure Default (Recommended) is selected.
6. Ensure that the correct **Default Reference & BED Files** are selected.

Field	File
Reference Library:	Microbiome_health_panel_reference
Target Regions:	None
Hotspots Regions:	None

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



Ion Reporter™ Software version	Selection
5.12.1 or earlier	Select **None**.
5.14 or later	• Your Ion Reporter™ 5.14 account. • **AmpliSeq Microbiome Health - w1.0 - Single Sample** under **Existing Workflow**. • **Self** under **Sample Grouping**. • **Automatically upload to Ion Reporter after run completion** under **Ion Reporter Upload Options**.

Note: If the Ion Reporter™ 5.14 account is not configured, configure it through Ion Reporter Configure settings (see "Configure the IonReporterUploader plugin in Torrent Suite™ Software" on page 48 for more information).

8. Click **Next**.

9. In the **Research Application** step, select **Metagenomics** and **16S Targeted Sequencing** for **Research Application** and **Target Technique** respectively, then click **Next**.

10. In the **Kits** step, make the following selections, then click **Next**.

Item	Selection
Instrument	Ion GeneStudio™ S5 System
Library Kit Type	Ion AmpliSeq™ Library Kit Plus
Template Kit	Select the **Ion Chef** radio button, then select one of the following: • Ion 540™ Kit – Chef • Ion 550™ Kit – Chef
Sequencing Kit	Ion S5™ Sequencing Kit
Base Calibration Mode	Default Calibration
Chip Type	One of the following: • Ion 540™ Chip • Ion 550™ Chip
Barcode Set	IonCode
Flows	500

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

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

13. In the **Save** step.
    a. *(Optional)* Edit the **Template Name**.
    b. Ensure that **Microbiome_health_panel_reference** is selected.
    c. Click **Save Template**.

    The Planned Run template is added to the list on the **Templates** screen.

Create a Planned Run

The following procedure requires the use of a Planned Run template. To create a template see "Create a Planned Run template" on page 27.

1. Sign in to the Torrent Suite™ Software.

2. In the **Plan tab**, in the **Templates** screen, click **16S Targeted Sequencing** in the left navigation menu.

3. Select one of the following actions.
    - For Torrent Suite™ Software version 5.12.1 or later, in the 16S Targeted Sequencing list, select **Ion AmpliSeq Microbiome Health Research Panel**.
    - In the 16S Targeted Sequencing list, click your customized Planned Run template name or click ⚙ ▸ **Plan Run**.

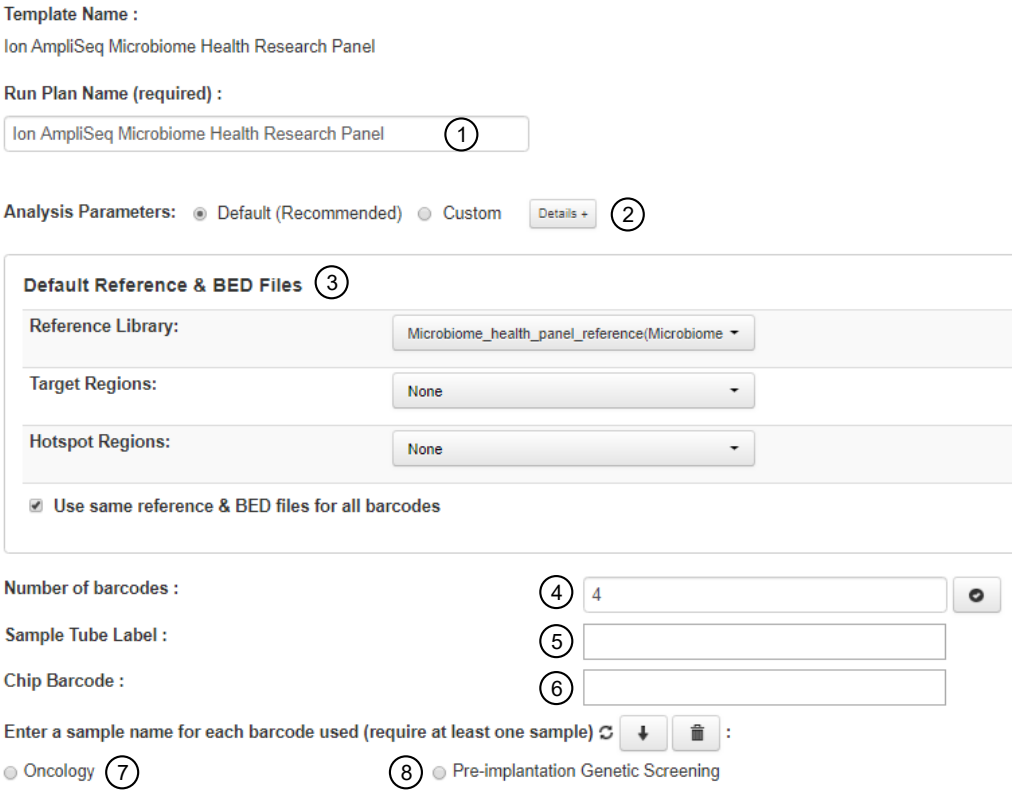
    The **Create Plan** workflow opens to the **Plan** step.

4. Make the following selections.

Field	Selection
Run Plan Name	Enter a Planned Run name.
Analysis Parameters	Ensure the **Default (Recommended)** radio button is selected.
Reference Library	Microbiome_health_panel_reference
Target Regions	None
Hotspots Regions	None
Use same reference & BED files for all barcodes	Ensure that the checkbox is selected.
Number of barcodes	Enter the number of barcodes used in this run, then click ✓ to the right of this field. The default value is 64 barcodes.
Sample Tube Label	Enter or scan the barcode of the Ion Chef™ Library Sample Tube that is used in the run.
Chip Barcode	No entry required.

(continued)

Field	Selection
Oncology	Ensure that the radio button is deselected.
Pre–implantation Genetic Screening	Ensure that the radio button is deselected.



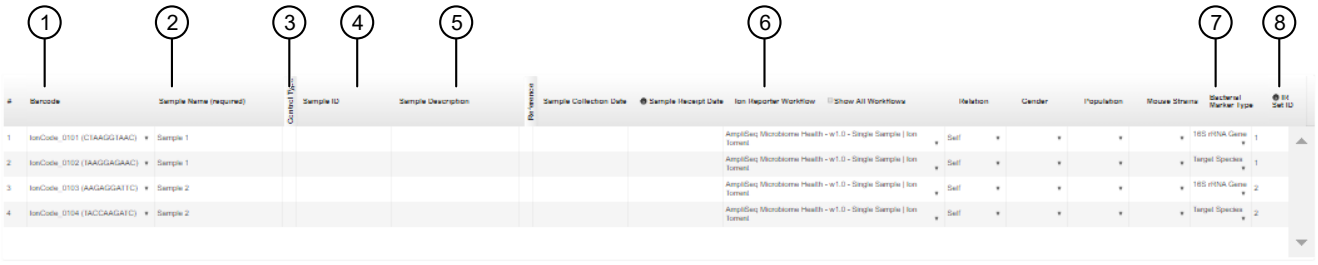
1. Run Plan Name
2. Analysis Parameters
3. Default Reference & BED files
4. Number of barcodes
5. Sample Tube Label
6. Chip Barcode
7. Oncology
8. Pre–implantation Genetic Screening

5. Complete the samples table by entering the following information.

   - To populate the **Samples Table** automatically, click **Load Samples Table**, then select an appropriate CSV file containing sample information specific for this Planned Run. (To create and save a **Samples Table** CSV file, see "(Optional) Create a Sample Table template file" on page 32.)
   - To populate the **Samples Table** manually, enter sample information into the samples table. To reveal additional columns, click vertical column headers (Control Type, Reference, Annotations).

Note: Fields that are listed in the following table are required. Fields that are not listed are not required to be populated to create a Planned Run.

Field	Action
Barcode	For each sample select the **Barcode** that identifies it 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 that the sample names (either auto-populated or user defined) that you pick are unique even between runs. **IMPORTANT!** For barcodes from the same biological sample, use exactly the same **Sample Name**. In the downstream workflow, the sample name is used to pair libraries from the 16S and Target Species pools.
Control Type (collapsed)	Click to expand, then 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.
Ion Reporter Workflow	Ensure the correct workflow is selected. **IMPORTANT!** If you have Ion Reporter™ Software version 5.12.1 or earlier, leave deselected.
Bacterial Marker Type	1. Select **16S rRNA Gene** or **Target Species** from the dropdown list. 2. *(Optional)* Click  to copy the entry to all the rows. **Note:** For Torrent Suite™ Software version 5.12.1 or later, the **Bacterial Marker Type** column is displayed only when the planned run is generated from the **AmpliSeq Microbiome Research Panel** template.
IR Set ID	The IR Set ID links individual samples for analysis. Ensure the correct value is auto-populated. Select from the dropdown list to change. **IMPORTANT!** For barcodes from the same biological sample, use exactly the same **IR Set ID**. In the downstream workflow, samples with the same the **IR Set ID** are considered related samples. The **IR Set ID** is used to pair libraries from the 16S and Targeted Species pools. Do not give unrelated samples the same IR Set ID value (even if that value is zero or blank).



① Barcode
② Sample Name
③ Control Type (collapsed)
④ Sample ID
⑤ Sample Description
⑥ Ion Reporter Workflow
⑦ Bacterial Marker Type
⑧ IR Set ID

6. If you are using Ion Reporter™ Software version 5.12.1 or earlier, in the **Ion Reporter** step, ensure that **None** is selected for **Ion Reporter Account** and **Self** is selected for **Sample Grouping**.

7. Click **Plan Run**.

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

(Optional) Create a Sample Table template file

1. In the **Plan** step of a Planned Run, Click **Save Samples Table** to save the CSV file to your computer.

2. Edit the CSV file by entering all required sample information into the appropriate sample information columns, then save the CSV file to your computer.

6 Data Analysis using Ion Reporter™ Software

IMPORTANT! For barcodes from the same biological sample, use exactly the same Sample Name. In the downstream workflow, the sample name is used to pair libraries from the 16S and Target Species pools. If libraries are incorrectly named, see "Edit sample attributes in Ion Reporter™ Software" on page 49.

IMPORTANT!

- You must use Ion Reporter™ Software version 5.14 or later. We recommend updating to the latest available version of Ion Reporter™ Software.
- If you are using Ion Reporter™ Software version 5.12.1 or earlier, skip this chapter and follow the instructions in Appendix C, "AmpliSeq_Microbiome_Health_Analysis plugin for Torrent Suite™ Software".

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

Note: For the Ion AmpliSeq™ Microbiome Health Research – Target Species Pool, with the following exceptions, all the primers in this pool are designed to be targeted to a single bacterial species.

- *Escherichia coli* primers—Also detect and amplify DNA from *Shigella*.
- *Streptococcus infantarius* primers—Also detect and amplify DNA from *Streptococcus equinus*, due to extremely high biological identity between strains of the above species.

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 35.

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

Analysis workflow	Description
AmpliSeq Microbiome Health – w1.0 – Single Sample	Detects 73 bacterial species that are associated with disease, identifies bacterial species taxonomy of the microbial species present, and displays analysis results of the sequencing run.

(Optional) Create a custom analysis workflow for the Ion AmpliSeq™ Microbiome Health Research Panel

We recommend that you start with a predefined analysis workflow or a custom analysis workflow and begin with an optimized set of parameters.

When you create a custom analysis workflow, you can change parameter settings.

You can copy predefined analysis workflows and custom analysis workflows from the current software and from previous versions of the software. When you copy analysis workflows from an earlier version of the software, you must use target regions files, hotspots files, and fusion panel files from the same version of the software. You can view the analysis workflow version in the **Details** pane. For more information, see the help system for your version of the Ion Reporter™ Software.

1. Sign in to the Ion Reporter™ Software.
2. In the **Workflows** tab, click **Overview**.
3. In the **Workflows** table, click the row for the analysis workflow that you want to copy, then click **(Actions)** ▸ **Copy**.
   The workflow bar opens to the **Research Application** step.
   When you copy an analysis workflow, some settings and fields are defined by the analysis workflow and remain selected.
4. In the **Research Application** step, ensure that the **Research Application** is **Microbiome Analysis** and **Sample Groups** is **Single**, then click **Next**.
5. In the **Reference** step, confirm that the required files are selected, then click **Next**.
   - For target species libraries, the reference is packaged with the software.
   - For the 16S rRNA Gene libraries, select one of the following libraries. For help in selecting a reference library, contact support (Appendix E, “Documentation and Support”).
     - Custom SILVA SSU Database 138
     - Curated MicroSEQ(R) Reference Library v2013.1 (Microbiome)
     - Curated Greengenes v13.5 (Microbiome)
6. *(Optional)* In the **Parameters** step, edit parameters, then click **Next**.
   For more information about parameters, contact support (Appendix E, “Documentation and Support”).
7. In the **Confirm** step, enter an analysis **Workflow Name** and **Description** *(Optional)*, then click **Confirm** ▸ **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.

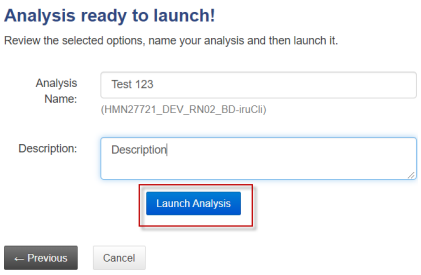
Manually launch an analysis

Note: If you are using a planned run created from a template with **Automatically upload to Ion Reporter after run completion** under **Ion Reporter Upload Options** selected (see "Create a Planned Run template" on page 27), the analysis is performed automatically. You can manually launch an analysis to reanalyze a run.

1. Sign in to the Ion Reporter™ Software.
2. In the **Analyses** tab, select one of the following.
   - Select the **Launch** sub tab.
   - Click **Launch Analysis**, then select **Manual** from the dropdown list.
3. In the **Launch Analysis** screen, in the **Workflow** tab, click the **Research Application** button, then select **Microbiome Analysis** from the dropdown list.
4. Select *AmpliSeq Microbiome Health – w1.0 – Single Sample* or a custom workflow, then click **Next**.
5. In the **Launch Analysis** window, in the **Samples** tab, click a sample to select that sample, then click **Next**.

   Note: You can enter a term in the search box to find a sample.
6. In the **Launch Analysis** window, in the **Plugins** tab, ensure that no plugins are listed, then click **Next**.

   The workflow for the Ion AmpliSeq™ Microbiome Health Research Panel does not require any plugins.
7. *(Optional)* In the **Launch Analysis** window, in the **Confirm & Launch** tab, enter an **Analysis Name** and **Description**.
8. Click **Launch Analysis**.



Microbiome health research analysis results

Analyses that are performed in Ion Reporter™ Software with the AmpliSeq Microbiome Health – w1.1 – Single Sample analysis workflow show analysis results from the detection of 73 bacterial species, and identification of bacterial species taxonomy for the microbial species that are present.

Microbiome health research analysis results are designed to help you study the potential impacts of the microbiome on human health, such as research on disease and chronic conditions, and the maintenance of a healthy immune system.

View microbiome health research analysis results—Single Sample

If you use the AmpliSeq Microbiome Health analysis workflow, you can view the analysis results in Ion Reporter™ Software.

To visualize multiple analyses, see "View microbiome health research analysis results—Multiple samples" on page 41.

1. In the **Analyses** tab, click **Overview**.
2. Click **Workflow** filter, then select an AmpliSeq Microbiome Health analysis workflow to narrow the list to microbiome health research analysis results.

   You can further refine the list of analyses with other filters, or click column headings to sort the list.
3. Click the analyses name link.

   The **Analysis Results** screen opens.

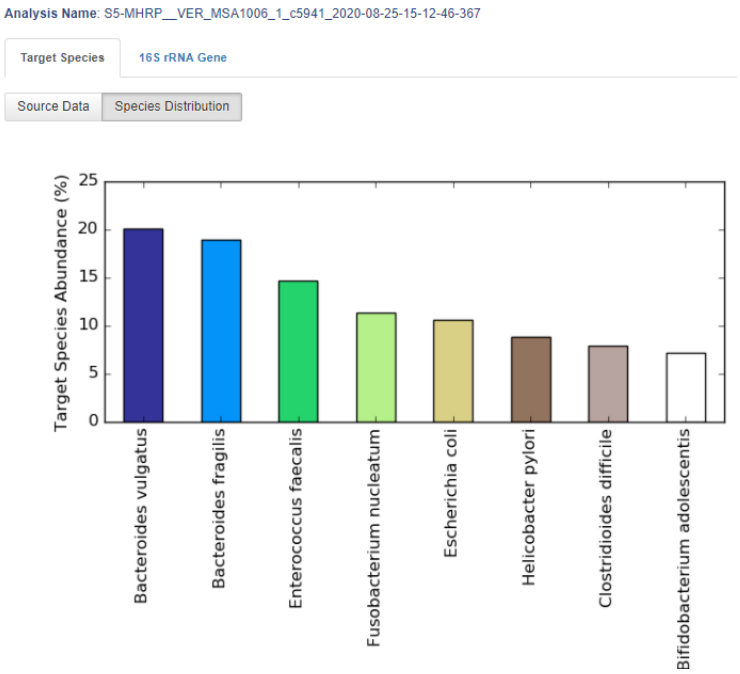
   The following parameters are displayed.

   - Pool QC—Pool QC is determined to be either PASS, FAIL, or NO_DATA. If the total number of valid mapped reads is greater than the threshold applied and the mean read length of all the reads from the BAM file is greater than the threshold, pool QC is determined to be PASS, otherwise FAIL. If pool QC is determined to be FAIL, it means that library pool failed and the results are not valid. If pool QC is NO_DATA, it means that pool did not contain any data.
   - Total Reads—The total number of filtered and trimmed reads independent of length reported in the output BAM file.
   - Total Valid Mapped Reads—Total number of reads mapped to the reference sequences with good mapping quality (reads with alignment score greater than the min local alignment score threshold).
   - Mean read length—The average length of all reads from the BAM file.
4. Select the **Target Species** tab, then click **Source Data** or **Species Distribution**.

   - The Ion AmpliSeq™ Microbiome Health Research – Target Species Pool detects 73 bacterial species that are associated with human disease. Click **Download Results** to download a file with a list of the 73 species.

Note: Some of the primer pairs designed to detect the 73 targeted species can sometimes amplify strains of other non-targeted species. As a result, an additional 16 species can be reported as well. When such species are detected the species names are reported with (*), indicating that it is one of the non-targeted species.

- The **Species Distribution** view displays a bar chart of the abundance of the species that are detected.

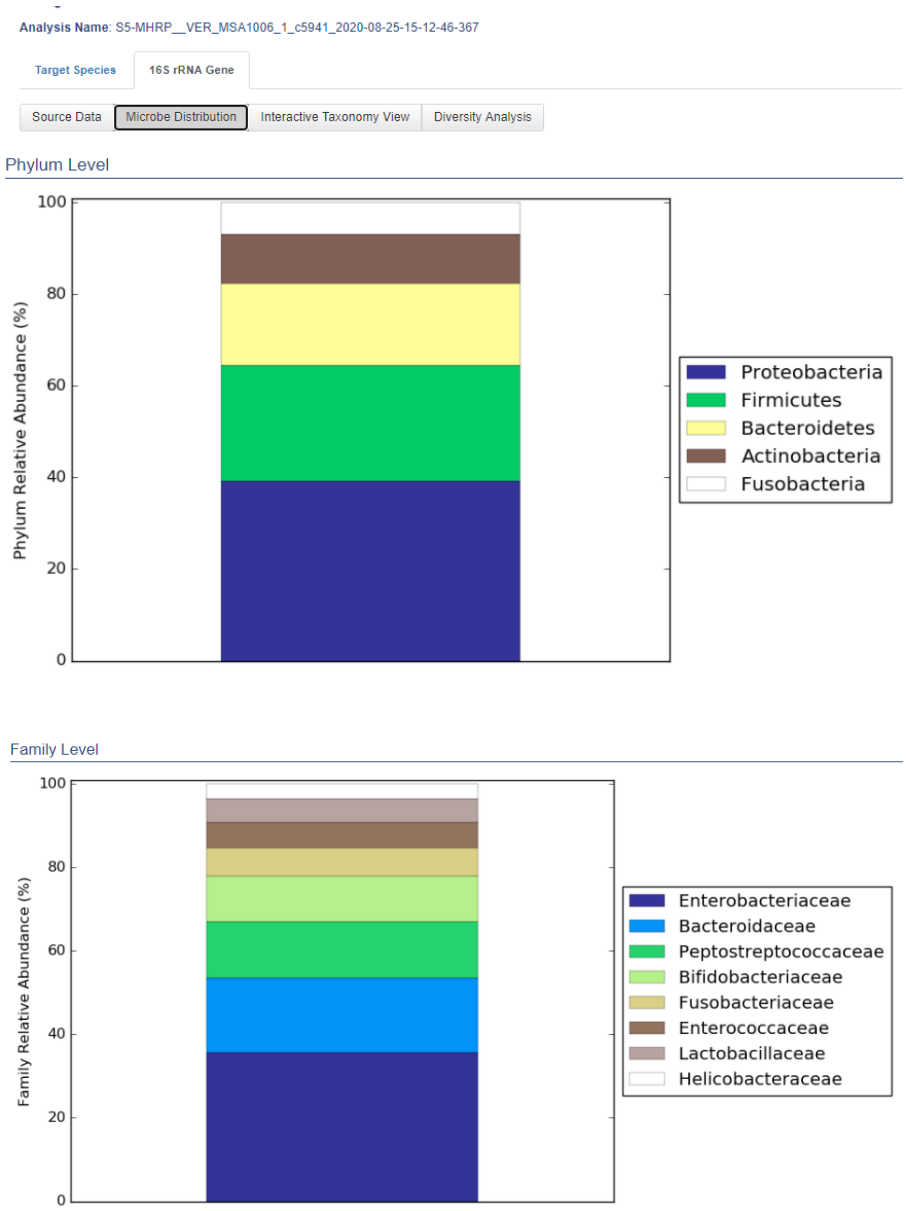


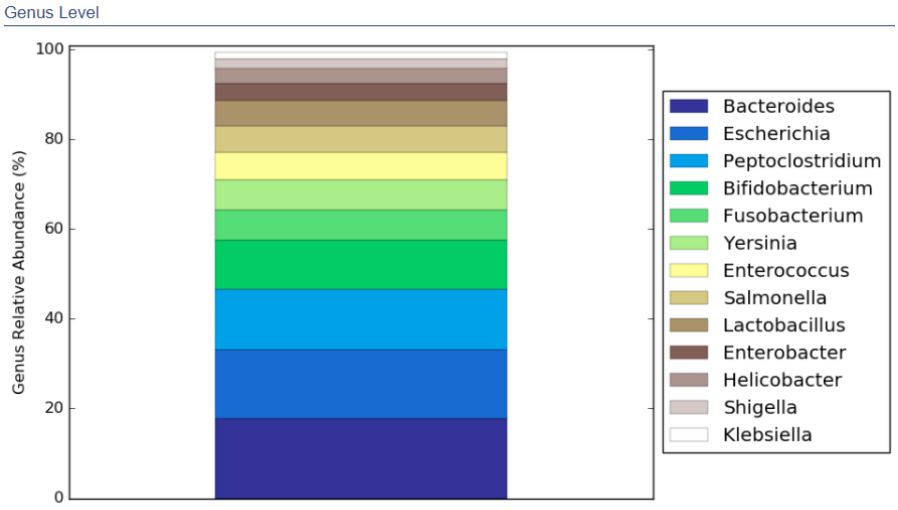
- The **Source Data** view displays the parameters in the following table for each detected species.

Parameter	Description
Species name	Bacterial species.
Detected Target species abundance	Percentage abundance of the species in the sample.
Read Count	Raw read count.
Normalized read count	Read counts are normalized by the number of amplicons per sequence and the sampling depth per sample. Normalized read counts are comparable across different microbes and samples.

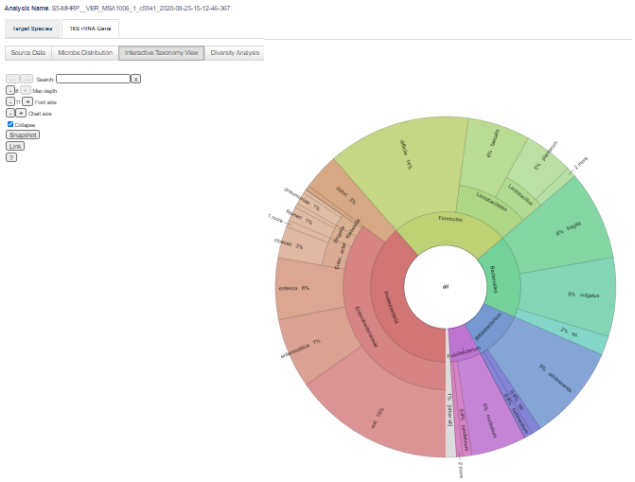
5. Click **16s rRNA Gene tab**, then select an option to view taxonomy data.
   - To view a summary of the taxonomy data, click **Source Data**.

- To view bar charts of the abundance of detected species at the phylum, family and genus levels, click **Microbe Distribution**.





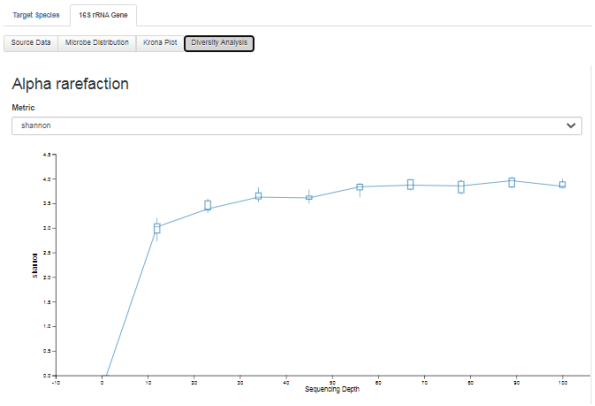
- To view a interactive taxonomy view of the detected species, click **Interactive Taxonomy View**.



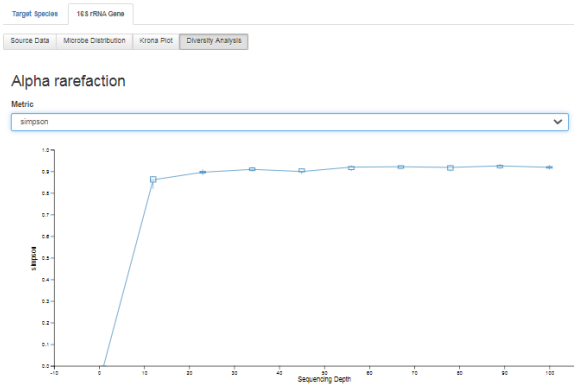
You can adjust the taxonomy view as follows.

- Enter a species to highlight that species in the **Search** field.
- Click **Max depth** to adjust the number of taxonomy classes that are displayed.
- Click **Font size** to change the font size of the characters.
- Click **Chart size** to change the size of the plot.
- Click **Collapse** to toggle the number of displayed taxonomy classes between 4 and 6.
- Click **Snapshot** to display the plot in a new window to enable saving the plot as a graphic.
- Click **Link** to display a link that can be copied for sharing or bookmarking
- Click **?** to open the help system for generating the interactive taxonomy view

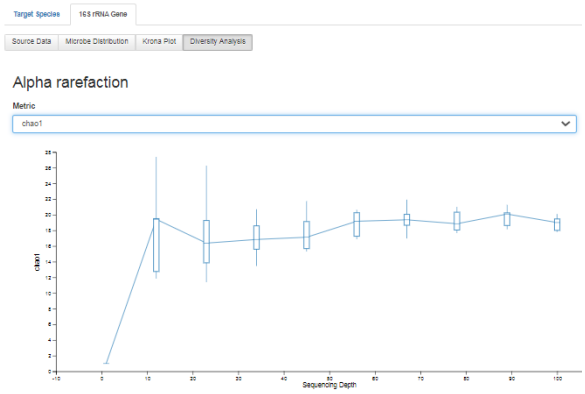
- To view an Alpha rarefaction plot, click **Diversity Analysis** then select an option from the **Alpha Rarefaction Metric**dropdown menu.
  - **shannon**



  - **simpson**



  - **chao1**



6. To download data files, click **Download Results**.
   A ZIP file containing the following files is downloaded to your local drive.

File	File extension	Description
Target Species Panel	XLSX	A list of the 73 bacterial species detected by Ion AmpliSeq™ Microbiome Health Research – Target Species Pool. Not all of the species are detected in a particular sample.
Taxonomy plot	HTML	The Krona plot for the sample.
Folders for alpha diversity tests	various	Data for Alpha diversity tests.

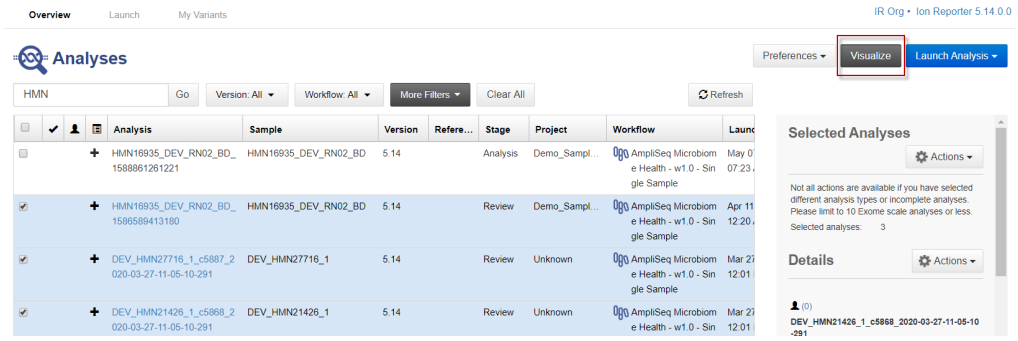
View microbiome health research analysis results—Multiple samples

If you use the AmpliSeq Microbiome Health analysis workflow, you can visualize analysis results in Ion Reporter™ Software.

To view the analysis results from a single analysis, see "View microbiome health research analysis results—Single Sample" on page 36.

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

2. Select the check box for each analysis to be used for analysis, then click **Visualize**.
   You must select at least three analyses. A minimum of three analyses are required for statistical analysis.



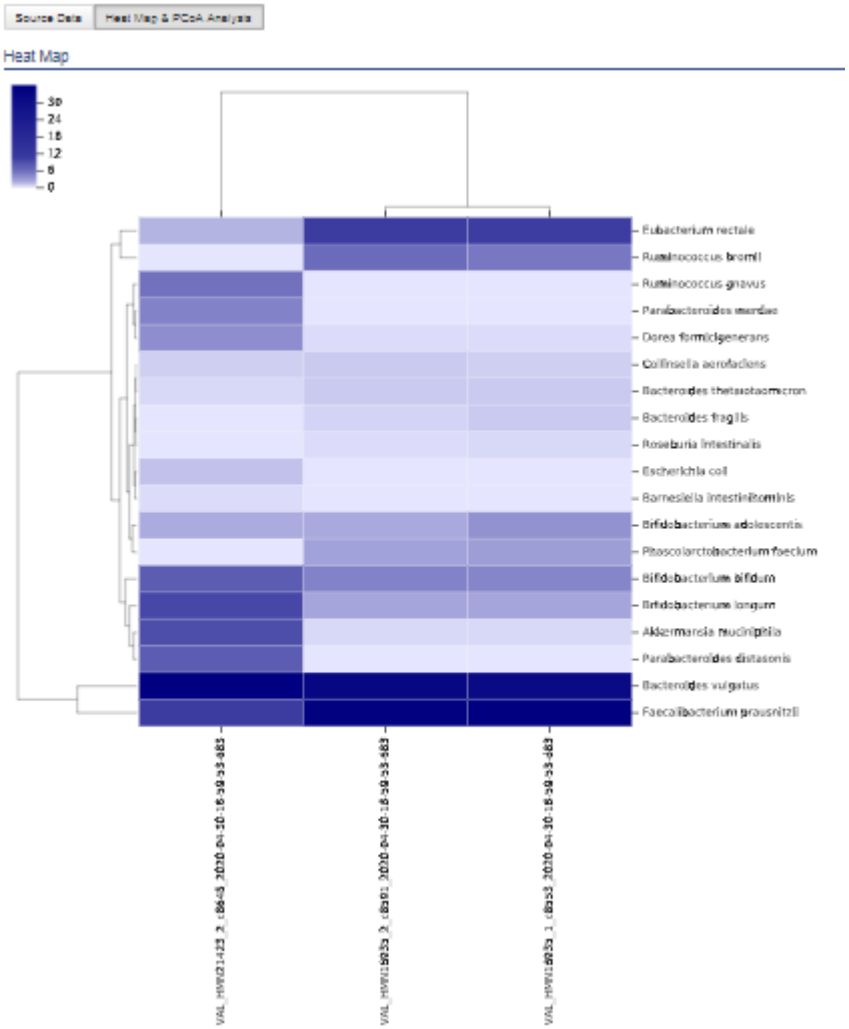
   The **Analysis Results** screen opens to the **Summary** tab.

   The **Summary** tab lists data for both the target species and the 16S rRNA gene. The **Target Species Markers** lists the total number of detected species. The **16S rRNA Gene Markers** lists taxonomic data that is identified in the sample, and a value for **Alpha Diversity**. The alpha diversity

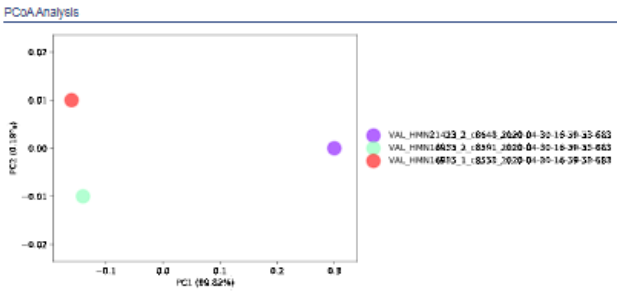
results describe the diversity in a single sample at the species, genus, and family levels, and show the following metrics for each analyses.

- Pool QC—Pool QC is determined to be either PASS or FAIL. If the total number of valid mapped reads is greater than the threshold applied and the mean read length of all the reads from the BAM file is greater than the threshold, pool QC is determined to be PASS, otherwise FAIL. If pool QC is determined to be FAIL, it means that library pool failed and the results are not valid.
- Total Reads—The total number of filtered and trimmed reads independent of length reported in the output BAM file.
- Total Valid Mapped Reads—Total number of reads mapped to the reference sequences with good mapping quality (reads with alignment score greater than the minimum local alignment score threshold).
- Mean read length—The average length of all reads from the BAM file.

3. You can view results for individual samples, or view graphical representations of the selected analyses.
   - To view data for an individual sample, click **View Results** in the **Results** column of the **Target Species Markers** or the **16S rRNA Gene Markers** sections. Click **Back to Summary** to return to the **Summary**.
4. To view target species data, select the **Target Species** tab, then click the **Source Data** or **Heat Map & PCoA Analysis** sub tab.
   - The Ion AmpliSeq™ Microbiome Health Research – Target Species Pool detects 73 bacterial species that are associated with human disease. Click **Download Results** to download a file with a list of the 73 species.
   - Click **Source Data** to view data on the abundance of each species in each of the selected samples.
   - Click **Heat Map & PCoA Analysis** for a heat map of the abundance of the identified target species for each sample and a PCoA analysis plot, which shows Beta diversity results to describe similarities and dissimilarities between samples.



Species distribution heat map



PCoA analysis plot

5. To view taxonomy data, in the **16S rRNA Gene Markers** section, click **View Results** in row of a sample, then select one of the following sub tabs.
   a. To view a summary of the taxonomy data, click **Source Data**.

b. To view a bar chart of the abundance of detected species at the phylum, family and genus levels and a heat map, click **Microbe Distribution**. The following figures shows example charts of the family and genus levels.

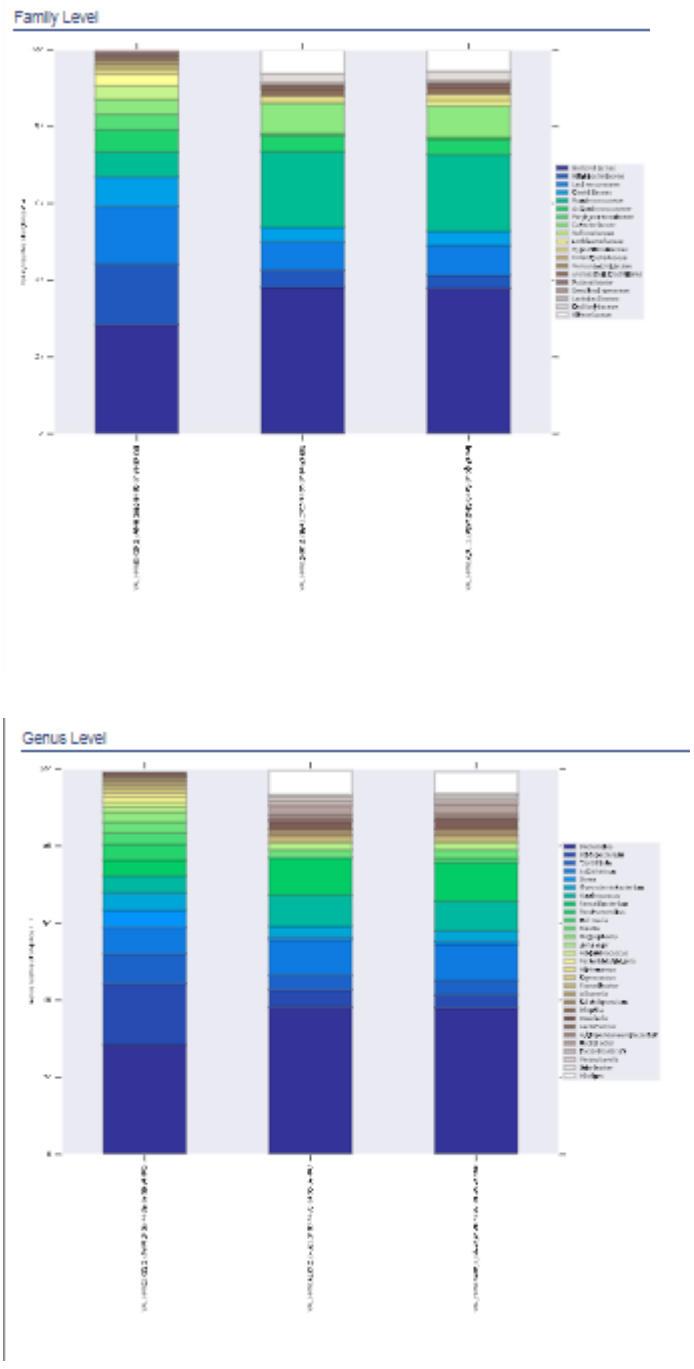
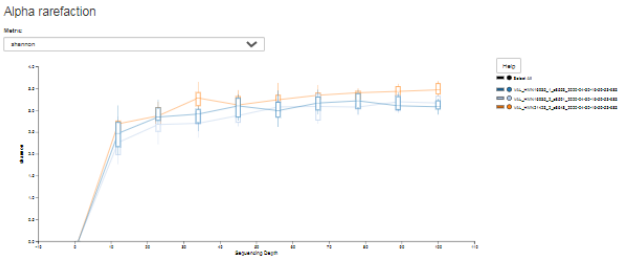


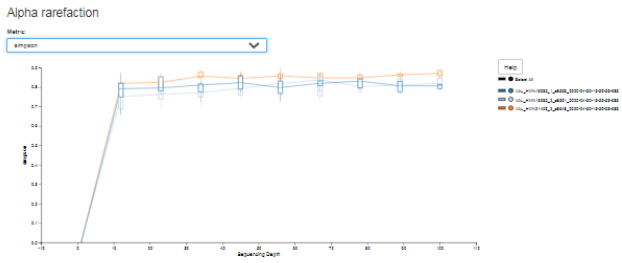
Figure 1 Bar charts for family and genus levels. Family level bar charts are also available.

c. To view the Alpha diversity values for each sample, click **Alpha Diversity Analysis**. Select any of the following options.

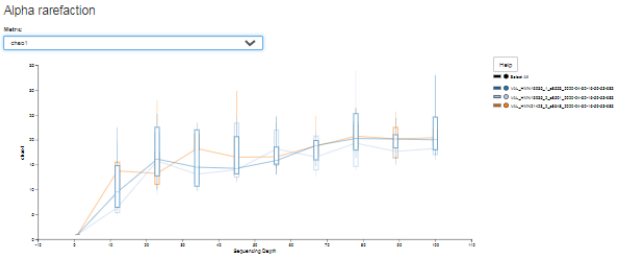
- **shannon**



- **simpson**



- **chao1**



d. To view Beta Diversity analyses of the selected samples, click **Beta Diversity Analysis**, then click a matrix or plot.

Note:

- Matrix files in TSV format are downloaded to your local drive.
- Plots are shown in a new window.
- Click **Download Results** to download a ZIP file that includes a list of the 73 species in the target species panel.

---

6. To download data files, click **Download Results**.

Note: The Alpha and Beta diversity data sets take longer to load. Click to save or open the file, depending on your browser settings.

A ZIP file containing the following files is downloaded to your local drive.

File	File extension	Description
Target Species Panel	XLSX	A list of the 73 bacterial species detected by Ion AmpliSeq™ Microbiome Health Research – Target Species Pool. Not all of the species are detected in a particular sample.
Various files	BIOM, QZA, TXT, SVG, XLSX	Files for the taxonomic data, matrices, bar plots and heat maps that are available for the analysis results.
Folders for diversity tests	Includes TSV, QZA, JSONP, QZV, HTML, XLXS.	Files for all (alpha and beta) diversity test results.

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: **http://thermofisher.com/ngssupport**
- For FAQs for this product: **http://thermofisher.com/A46496faqs**
- To browse the FAQ database and search using keywords: **thermofisher.com/faqs**

B Supplemental information

Update Ion AmpliSeq™ Microbiome Health Research Kit templates in Torrent Suite™ Software

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

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

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

Configure the IonReporterUploader plugin in Torrent Suite™ Software

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

Field	Directions
Server Type	Select a server type. [1]
Display Name	Enter a meaningful name of your choice. This name is used in the Planned Run template wizard and is shown to other Torrent Suite™ Software users. Use only alphanumeric characters, spaces, and underscores.
Server	Enter:[1]
Port	Enter: 443

(continued)

Field	Directions
Username	Enter your Ion Reporter™ Software username (your email address)
Password	Enter your Ion Reporter™ Software password

[1] Ask your Ion Reporter™ Server administrator for this value.

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

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

6. Click **Get Versions**, select **Ion Reporter 5.14 or later**, then click **✓Add**.

Edit sample attributes in Ion Reporter™ Software

Sequence results from multiple libraries prepared from the same sample or multiple sequencing results from the same library can be combined for increased analytical power.

1. In the **Samples** tab, in the **Overview** screen, click the sample with a single BAM file.
   The **Define Samples** screen opens to the **Samples** tab. On the right side, the sample is displayed along with a box for creating new samples.

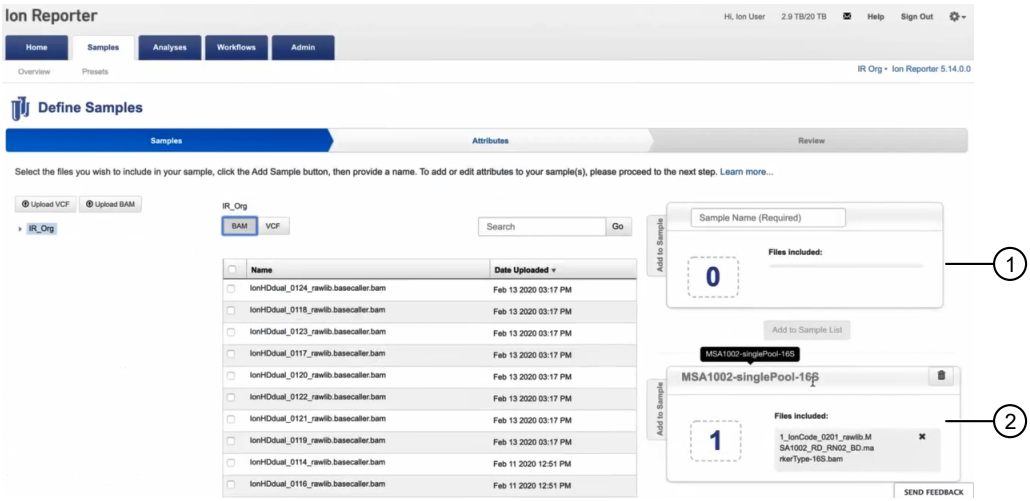


Figure 2 Define samples screen

(1) Add sample screen—This box is used only if the BAM files were not uploaded to Ion Reporter™ Software. For additional information, see the help system for your version of Ion Reporter™ Software.

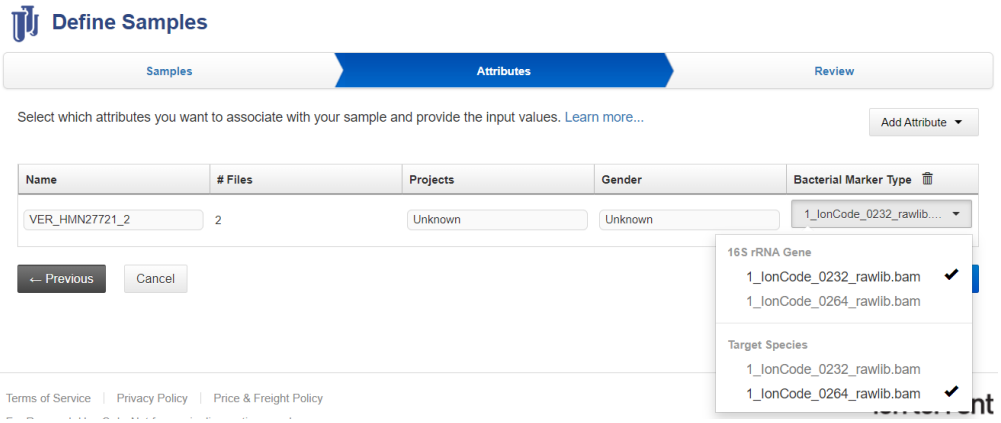
(2) Current sample box—The number of BAM files associated with the sample are indicated in the dotted box.

2. Click the checkbox of the barcode from the list to be paired with the sample that was selected in step 1, then click the **Add to Sample** tab of the current sample box (callout 2 Figure 2).

3. Click **Next**.

4. In the **Attributes** step, ensure that the correct **Bacterial Marker Type** (16S rRNA or Target Species) has been selected for each barcode.

   A sample can have 1 or 2 barcodes. Only one bacterial marker type can be associated with a barcode.



5. *(Optional)* In the **Attributes** step, add or edit an attribute.

Choice	Action
Add an attribute	Click **Add Attribute**, then select an attribute from the dropdown list.
Edit an attribute	Click the displayed attribute, then edit.

6. *(Optional)* In the **Attributes** step, to add or edit another attribute, repeat step 5.

7. Click **Next**.

8. To save the sample, click **Save**.

9. Reanalyse the sample.
   Proceed to "Manually launch an analysis" on page 35.

Sample analysis and visualization

The AmpliSeq Microbiome Health – w1.0 – Single Sample workflow performs the following analyses.

Analysis	Visualization	Sample type	Notes
Full taxonomic profiling	Krona plot	Single sample	—
Microbiome composition analysis	Stacked bar plots	Single and multisample	—

(continued)

Analysis	Visualization	Sample type	Notes
Microbiome diversity analysis[1]	Alpha diversity analysis	Single and multisample	The following metrics are available. • Shannon • Simpson • Chao1
	Beta diversity analysis	Multisample	In addition to Beta rarefaction plots, the following matrices are available. • Bray-Curtis distance matrix • Euclidean distance matrix • Jaccard distance matrix
Comparative analysis	Heat Map	Multisample	—
	PCoA	Multisample	—

[1] For additional details, see the Qiime 2 website (**https://qiime2.org/**).

C

AmpliSeq_Microbiome_Health_Analysis plugin for Torrent Suite™ Software

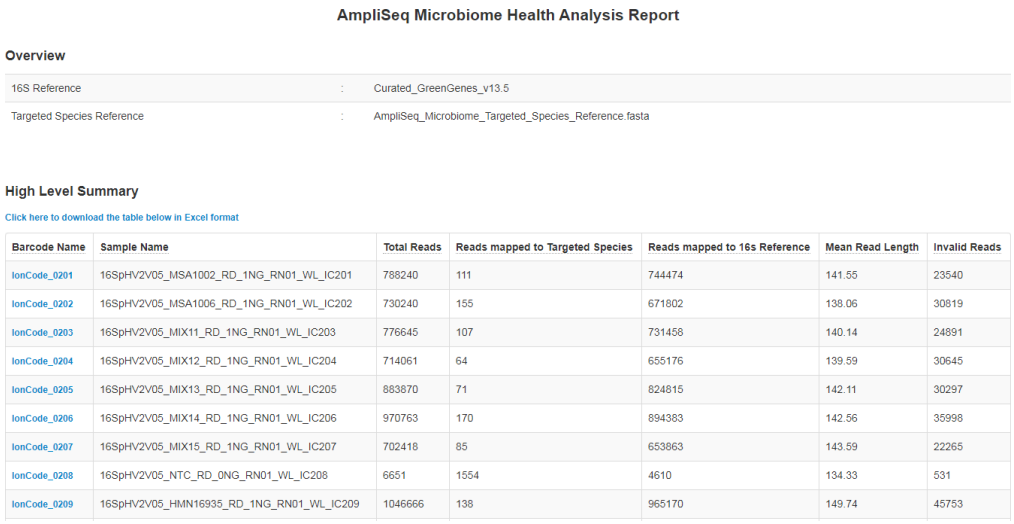
IMPORTANT! If you are using Ion Reporter™ Software version 5.14 or later, follow the procedure in Chapter 6, "Data Analysis using Ion Reporter™ Software".

If you are unable to use Ion Reporter™ Software version 5.14 or later, you can use the AmpliSeq_Microbiome_Health_Analysis plugin to analyze sequencing runs and view the analysis results in Torrent Suite™ Software.

Note: The AmpliSeq_Microbiome_Health_Analysis plugin does not have some of the advanced capabilities of Ion Reporter™ Software version 5.14 or later, including analysis and visualization of data using the Qiime 2™ Software platform.

Review AmpliSeq_Microbiome_Health_Analysis plugin results in Torrent Suite™ Software

1. Sign in to the Torrent Suite™ Software.
2. In the **Data** tab, click **Completed Runs & Reports**.
3. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
4. In the left navigation menu, click **AmpliSeq_Microbiome_Health_Analysis** to view the plugin summary.
5. In the plugin summary, click the name of the run.
   The **AmpliSeq Microbiome Health Analysis Report** opens in a new screen.
   The **Overview** section lists the 16S Reference and Targeted Species Reference files.



AmpliSeq Microbiome Health Analysis Report

Overview

16S Reference	:	Curated_GreenGenes_v13.5
Targeted Species Reference	:	AmpliSeq_Microbiome_Targeted_Species_Reference.fasta

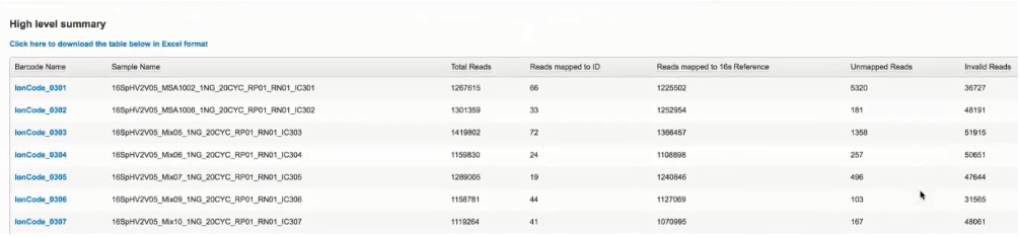
High Level Summary

Click here to download the table below in Excel format

Barcode Name	Sample Name	Total Reads	Reads mapped to Targeted Species	Reads mapped to 16s Reference	Mean Read Length	Invalid Reads
IonCode_0201	16SpHV2V05_MSA1002_RD_1NG_RN01_WL_IC201	788240	111	744474	141.55	23540
IonCode_0202	16SpHV2V05_MSA1006_RD_1NG_RN01_WL_IC202	730240	155	671802	138.06	30819
IonCode_0203	16SpHV2V05_MIX11_RD_1NG_RN01_WL_IC203	776645	107	731456	140.14	24891
IonCode_0204	16SpHV2V05_MIX12_RD_1NG_RN01_WL_IC204	714061	64	655176	139.59	30645
IonCode_0205	16SpHV2V05_MIX13_RD_1NG_RN01_WL_IC205	883870	71	824815	142.11	30297
IonCode_0206	16SpHV2V05_MIX14_RD_1NG_RN01_WL_IC206	970763	170	894383	142.56	35998
IonCode_0207	16SpHV2V05_MIX15_RD_1NG_RN01_WL_IC207	702418	85	653863	143.59	22265
IonCode_0208	16SpHV2V05_NTC_RD_0NG_RN01_WL_IC208	6651	1554	4610	134.33	531
IonCode_0209	16SpHV2V05_HMN16935_RD_1NG_RN01_WL_IC209	1046666	138	965170	149.74	45753

View the High level summary table

The **High level summary** section provides summary information for each barcoded sample.



High level summary

Click here to download the table below in Excel format

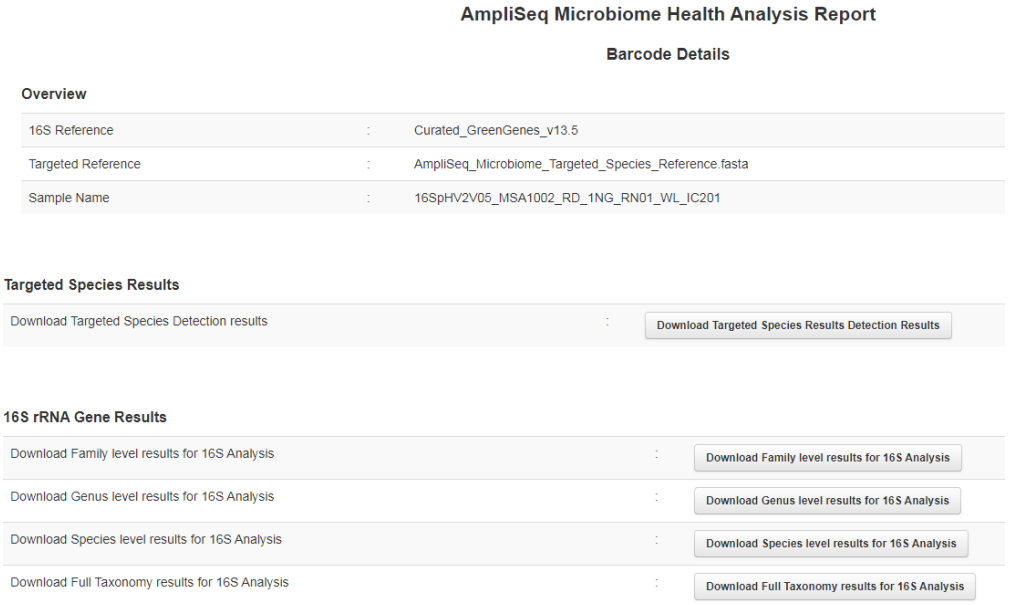
Barcode Name	Sample Name	Total Reads	Reads mapped to ID	Reads mapped to 16s Reference	Unmapped Reads	Invalid Reads
IonCode_0301	16SpHV2V05_MSA1002_1NG_20CYC_RP01_RN01_IC301	1267615	66	1225502	5320	36727
IonCode_0302	16SpHV2V05_MSA1006_1NG_20CYC_RP01_RN01_IC302	1301359	33	1252954	181	48191
IonCode_0303	16SpHV2V05_Mix05_1NG_20CYC_RP01_RN01_IC303	1419802	72	1366457	1358	51915
IonCode_0304	16SpHV2V05_Mix06_1NG_20CYC_RP01_RN01_IC304	1159830	24	1108898	257	50651
IonCode_0305	16SpHV2V05_Mix07_1NG_20CYC_RP01_RN01_IC305	1289005	19	1240846	496	47644
IonCode_0306	16SpHV2V05_Mix09_1NG_20CYC_RP01_RN01_IC306	1158781	44	1127069	103	31565
IonCode_0307	16SpHV2V05_Mix10_1NG_20CYC_RP01_RN01_IC307	1119264	41	1070995	167	48061

Parameter	Description
Barcode Name	The individual barcode in the barcode set. The row labeled **No barcode** reports on unclassified barcodes, which are reads that could not be classified as a match for one of the expected barcodes in the barcode set.
Sample Name	Name of the sample that was sequenced.
Total Reads	Total number of filtered and trimmed library reads (independent of length). This number is reported in the barcode BAM file.
Reads mapped to Targeted Species Reference	The total number of reads mapped to the Target Species reference.
Reads mapped to 16S Reference	The total number of reads mapped to the 16S reference.

(continued)

Parameter	Description
Mean Read Length	The average read length, in base pairs (bp), of all filtered and trimmed library reads reported in the BAM file for the barcoded run.
Invalid Reads	The total number of reads with lengths outside of the selected range. For details, see “Microbiome Analysis plugin parameters” on page 57.

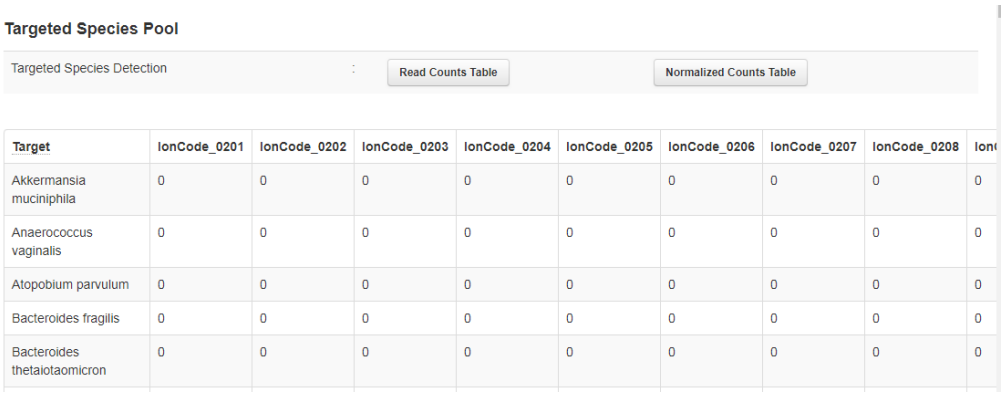
1. In the **AmpliSeq Microbiome Health Analysis Report** screen, scroll to the **High level summary** section.
2. *(Optional)* To download the table, click **Click here to download the table below in Excel format**.
3. *(Optional)* To see the details for a barcode, click a barcode name in the **Barcode Name** column. The barcode details report opens in a new screen.



- Click **Download Targeted Species Detection Results** to download results for the Targeted Species report.
- Click any of the buttons in the **16S rRNA Gene Results** section to view taxonomy results.

View the species detected by the assay

The **Targeted Species Pool** provides a list of the species that are detected for each barcoded library.



Targeted Species Pool

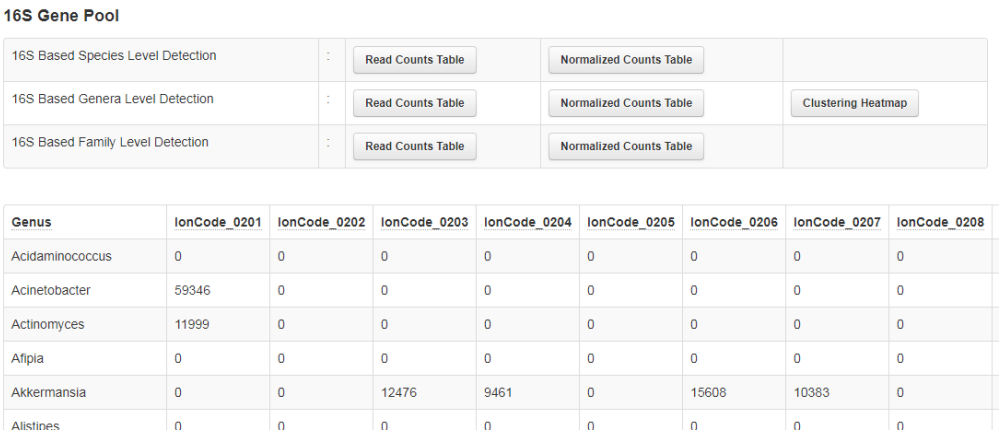
Targeted Species Detection : Read Counts Table Normalized Counts Table

Target	IonCode_0201	IonCode_0202	IonCode_0203	IonCode_0204	IonCode_0205	IonCode_0206	IonCode_0207	IonCode_0208	Ion
Akkermansia muciniphila	0	0	0	0	0	0	0	0	0
Anaerococcus vaginalis	0	0	0	0	0	0	0	0	0
Atopobium parvulum	0	0	0	0	0	0	0	0	0
Bacteroides fragilis	0	0	0	0	0	0	0	0	0
Bacteroides thetaiotaomicron	0	0	0	0	0	0	0	0	0

1. In **AmpliSeq Microbiome Health Analysis Report** screen, scroll to the **Targeted Species Pool** section.
2. To download the results, select one of the following actions.
   - To download a table of raw read counts per species, click **Read Counts Table**. This table lists the absolute read counts that map to individual annotations of microbes.
   - To download a table of normalized read counts per species, click **Normalized Counts Table**. This table lists absolute read counts that map to individual annotations of microbes, normalized by barcode read depth and the number of amplicons.

View taxonomy results

The **16S Gene Pool** section provides taxonomy information for each barcoded library.



16S Gene Pool

16S Based Species Level Detection :	Read Counts Table	Normalized Counts Table	
16S Based Genera Level Detection :	Read Counts Table	Normalized Counts Table	Clustering Heatmap
16S Based Family Level Detection :	Read Counts Table	Normalized Counts Table	

Genus	IonCode_0201	IonCode_0202	IonCode_0203	IonCode_0204	IonCode_0205	IonCode_0206	IonCode_0207	IonCode_0208
Acidaminococcus	0	0	0	0	0	0	0	0
Acinetobacter	59346	0	0	0	0	0	0	0
Actinomyces	11999	0	0	0	0	0	0	0
Afipia	0	0	0	0	0	0	0	0
Akkermansia	0	0	12476	9461	0	15608	10383	0
Alistipes	0	0	0	0	0	0	0	0

1. In **AmpliSeq Microbiome Analysis Report** screen, scroll to the **16S Gene Pool** section.
2. To download the table, select one of the following actions.
   - To download the raw read counts table for a taxonomy level (family, genus, or species), click **Read Counts Table** button for that taxonomy level. This table lists the absolute read counts that map to individual annotations of microbes.
   - To download the normalized counts table for a taxonomy level (family, genus, or species), click **Normalized Counts Table** button for a taxonomy level. This table lists absolute read counts that map to individual annotations of microbes, normalized by barcode read depth and the number of amplicons.
   - To download the clustering heatmap for the genus taxonomy level, click **Clustering Heatmap**. The clustering heat map provides a chip level view of relative abundance, clustered by both barcodes and genus level annotation of microbes.

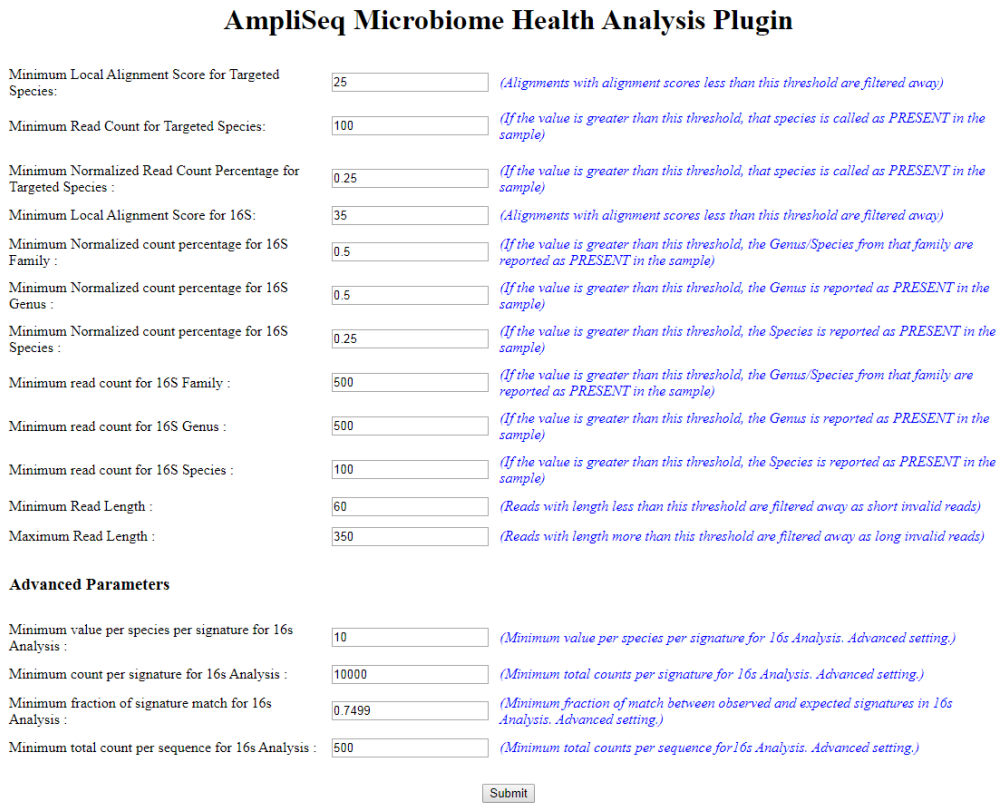
Manually run the AmpliSeq_Microbiome_Health_Analysis plugin

The following procedure is used to rerun the AmpliSeq_Microbiome_Health_Analysis plugin. Rerunning the plugin enables you to redo the analysis of a run using different parameters for the plugin.

1. Sign in to the Torrent Suite™ Software.
2. In the **Data** tab, click **Completed Runs & Reports**.
3. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
4. Click **Plugins** ▸ **Select Plugins to Run** ▸ **AmpliSeq_Microbiome_Health_Analysis**.

5. In the next dialog, configure the **AmpliSeq_Microbiome_Health_Analysis** plugin, then click **Submit**.

   The following figure lists the default settings, which have been optimized for 64 barcodes (samples) per chip. Optimization may be required if you are running a different number of barcodes per chip.



Microbiome Analysis plugin parameters

Parameter	Default value	Range	Value type	Description
Main Parameters				
Minimum Local Alignment Score for Targeted Species	25	>0	Integer	Alignments with alignment scores less than this threshold are filtered away.
Minimum Read Count for Targeted Species	100	≥0	Integer	If the value is greater than this threshold, that species is called as PRESENT in the sample.
Minimum Normalized Read Count Percentage for Targeted Species	0.25	1≥value≥0	Decimal	If the value is greater than this threshold, that species is called as PRESENT in the sample.
Minimum Local Alignment Score for 16S	35	>0	Integer	Alignments with alignment scores less than this threshold are filtered away.

(continued)

Parameter	Default value	Range	Value type	Description
Minimum Normalized count percentage for 16S Family	0.5	1≥value≥0	Decimal	If the value is greater than this threshold, the Genus/Species from that family are reported as PRESENT in the sample.
Minimum Normalized count percentage for 16S Genus	0.5	1≥value≥0	Decimal	If the value is greater than this threshold, the Genus is reported as PRESENT in the sample.
Minimum Normalized count percentage for 16S Species	0.25	1≥value≥0	Decimal	If the value is greater than this threshold, the Species is reported as PRESENT in the sample.
Minimum read count for 16S Family	500	>0	Integer	If the value is greater than this threshold, the Genus/Species from that family are reported as PRESENT in the sample.
Minimum read count for 16S Genus	500	>0	Integer	If the value is greater than this threshold, the Genus is reported as PRESENT in the sample.
Minimum read count for 16S Species	100	>0	Integer	If the value is greater than this threshold, the Species is reported as PRESENT in the sample.
Minimum Read Length	60	>0	Integer	Reads with length less than this threshold are filtered away as short invalid reads.
Maximum Read Length	350	>0	Integer	Reads with length more than this threshold are filtered away as long invalid reads.
Advanced parameters				
Minimum value per species per signature for 16S Analysis	10	>0	Integer	Minimum value per species per signature for 16S Analysis.
Minimum count per signature for 16S Analysis	10,000	>0	Integer	Minimum total counts per signature for 16S Analysis.
Minimum fraction of signature match for 16S Analysis	0.7499	1≥value≥0	Decimal	Minimum fraction of match between observed and expected signatures in 16S Analysis.
Minimum total count per sequence for 16S Analysis	500	>0	Integer	Minimum total counts per sequence for 16S Analysis.

Download and install the AmpliSeq_Microbiome_Health_Analysis plugin in Torrent Suite™ Software

IMPORTANT! You must install this plugin only if you have Ion Reporter™ Software version 5.12.1 or earlier.

The AmpliSeq_Microbiome_Health_Analysis plugin is available on Connect at **https://apps.thermofisher.com/apps/publiclib/#/plugins**. This plugin is not preinstalled in the Torrent Suite™ Software.

On Connect, an administrator can install or upgrade the following:

1. Sign in to **https://apps.thermofisher.com/apps/publiclib/#/plugins**.
2. Select the AmpliSeq_Microbiome_Health_Analysis plugin, select the checkbox to indicate that you agree to the terms and conditions, then click **Download Plugin**.
   Either a compressed directory or a debian file that contains the plugin is downloaded to your local machine.
3. In Torrent Suite™ Software, click ⚙ **(Settings)** ▸ **Plugins** ▸ **Install or Upgrade Plugin**.
4. Click **Select File**, browse to the location where you downloaded the plugin file, select the file, then click **Open**.
5. In the **Install or Upgrade Plugin** dialog box, click **Upload and Install**.

The plugin is now visible in Torrent Suite™ Software.

D

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.

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

Documentation and Support

Related documentation

Document	Description
Ion AmpliSeq™ Library Kit Plus User Guide (Pub. No. MAN0017003)	Comprehensive instruction for the preparation of Ion AmpliSeq™ libraries and provides detailed instruction and troubleshooting for use of the Ion Library Equalizer™ Kit.
Ion Library TaqMan™ Quantitation Kit User Guide (Pub. No. MAN0015802)	Provides detailed instruction and troubleshooting for use of the Ion Library TaqMan™ Quantitation Kit

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

Customer and technical support

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

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

Limited product warranty

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