
Qiita-GNPS-workshop

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Materials below are intended for CMI Qiita/GNPS workshop participants. They include all information covered during days 1 and 2 of the workshop.

For more information on Qiita, including Qiita philosophy and documentation, please visit [Qiita website](#).

A description of many of the terms used in this tutorial can be found in this [glossary](#)

For general information about workshops, please visit the [Center for Microbiome Innovation website](#) or [contact the CMI directly](#).

This tutorial will walk you through creation of your account and a test study in Qiita.

1.1 Getting CMI Workshop example data

There are two separate example datasets made available to you - a *processing dataset* containing raw sequencing files which we will process to generate information about the identity and relative amounts of microbes in our samples (n=14), and an *analysis dataset* which contains a unique set of pre-processed samples (n=30) which we will use for statistical and community analyses.

1.1.1 Processing dataset

You can download the [processing dataset](#) directly from GitHub. These files contain 16S rRNA microbiome data for 14 human skin samples. It is a subset of data that we will use later for analysis. Real sequencing data can be tens of gigabytes in size!

The files are:

- CMI_workshop_lane1_S1_L001_R1_001.fastq.gz # 16S sequences - forward reads
- CMI_workshop_lane1_S1_L001_R2_001.fastq.gz # 16S sequences - reverse reads
- CMI_workshop_lane1_S1_L001_I1_001.fastq.gz # 16S sequences - barcodes
- sample_info.txt # The sample information file
- prep_info_16S.txt # The preparation information file

1.1.2 Analysis dataset

Example data that you can use for analysis are available to you directly on Qiita. You don't need to download anything to your hard drive. Instructions how to access these data are provided in the [analysis tutorial](#).

1.2 Setting up Qiita

1.2.1 Signing up for a Qiita account

Open your browser (it must be Chrome or Firefox) and go to Qiita (<https://qiita.ucsd.edu>).

Click on “Sign Up” on the upper-right-hand corner.



The “New User” link brings you to a page on which you can create a new account. Optional fields are indicated explicitly, while all other fields are required.

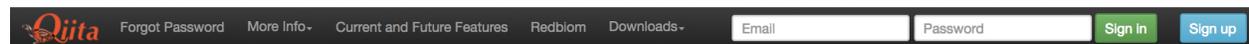
Enter User Information

Email	<input type="text" value="Email"/>
Password	<input type="password" value="password"/>
Confirm password	<input type="password" value="password"/>
Name (Optional)	<input type="text" value="name"/>
Affiliation (Optional)	<input type="text" value="affiliation"/>
Address (Optional)	<input type="text" value="address"/>
Phone # (Optional)	<input type="text" value="phone"/>

Once the form is submitted, an email will be sent to you containing instructions on how to verify your email address.

1.2.2 Logging into your account and resetting a forgotten password

Once you have created your account, you can log into the system by entering your email and password.



If you forget your password, you will need to reset it. Click on “Forgot Password”.

This will take you to a page on which to enter your email address; once you click the “Reset Password” button, the system will send you further instructions on how to reset your lost password.

1.2.3 Updating your settings and changing your password

If you need to reset your password or change any general information in your account, click on your email at the top right corner of the menu bar to access the page on which you can perform these tasks.

<p>User Information</p> <p>Name <input type="text"/></p> <p>Affiliation <input type="text"/></p> <p>Address <input type="text"/></p> <p>Phone <input type="text"/></p> <p>Save Edits</p>	<p>Change Password</p> <p>Old Password <input type="password"/></p> <p>New Password <input type="password"/></p> <p>Repeat New Password <input type="password"/></p> <p>Change Password</p>
---	--

<p>User Information</p> <p>Name <input type="text"/></p> <p>Affiliation <input type="text"/></p> <p>Address <input type="text"/></p> <p>Phone <input type="text"/></p> <p>Save Edits</p>	<p>Change Password</p> <p>Old Password <input type="password"/></p> <p>New Password <input type="password"/></p> <p>Repeat New Password <input type="password"/></p> <p>Change Password</p>
---	--

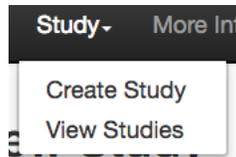
1.3 Studies in Qiita

Studies are the source of data for Qiita. Studies can contain only one set of samples but can contain multiple sets of raw data, each of which can have a different preparation – for example, 16S, shotgun metagenomics, and metabolomics, or even multiple preparations of the same type (e.g., a plate rerun, biological and technical replicates, etc).

In the *analysis tutorial*, our study contains 30 samples, each with two types of data: 16S and metabolomic. To represent this project in Qiita, we created a single study with a single sample information file that contains all 30 samples. Then, we linked separate preparation files for each data type.

1.4 Creating an example study

To create a study, click on the “Study” menu and then on “Create Study”. This will take you to a new page that will gather some basic information to create your study.



The “Study Title” has to be unique system-wide. Qiita will check this when you try to create the study, and may ask you to alter the study name if the one you provide is already in use.

A principal investigator is required, and a list of known PIs is provided. If you cannot find the name you are looking for in this list, you can choose to add a new one.

Select the environmental package appropriate to your study. Different packages will request different specific information about your samples. For more details, see the [publication](#). For this test study for the *processing tutorial*, choose **human-skin**.

There is also an option to specify time series type (“Event-Based Data”) if you have such data. In our case, the samples come from a time series study design, so you should select “multiple intervention, real”. For more information on time

Create a new Study

* = Required Field

Study Title	<input type="text" value="[user's_name]"/>	*
Study Alias	<input type="text" value="[user's_name]"/>	*
DOI Just values, no links, comma separated values	<input type="text"/>	
PUBMED ID Just values, no links, comma separated values	<input type="text"/>	
Study Abstract	<input type="text" value="[user's abstract]"/>	*
Study Description	<input type="text" value="[description of user's study]"/>	*
Principal Investigator	<input type="text" value="Select an Option"/>	*
Lab Person	<input type="text" value="Select an Option"/>	*
	Can't find the person you're looking for? Add a person	
Environmental Packages You can select multiple entries by control-clicking (mac: command-clicking)	<ul style="list-style-type: none">airbuilt environmenthost-associatedhuman-amniotic-fluidhuman-associatedhuman-bloodhuman-guthuman-oralhuman-skinhuman-urinehuman-vaginalmicrobial mat/biofilmmiscellaneous natural or artificial environmentplant-associatedsedimentsoilwastewater/sludgewater	*
Event-Based Data	<input type="text" value="No timeseries"/>	
<input type="button" value="Create Study"/>		

series types, you can check out the [in-depth tutorial](#) on the Qiita website.

Once your study has been created, you will be informed by a green message; click on the study name to begin adding your data.

Study [user's_name] successfully created

1.5 Adding sample information

Sample information is the set of metadata that pertains to your biological samples: these are the measured variables that are motivating you to look for response variables in the microbiome. **IMPORTANT:** your metadata are your study; it is imperative that those data are consistent, correct, and sufficiently detailed. (To learn more, including how to format your own sample info file, check out the [in-depth documentation](#) on the Qiita website.)

The first point of entrance to a study is the study description page. Here you will be able to edit the study info, upload files, and manage all other aspects of your study.

The screenshot shows the Qiita web interface. At the top, there is a navigation bar with the Qiita logo and several menu items: Analysis, Study, More Info, Current and Future Features, Redbiom, Downloads, Welcome cmiinfo@ucsd.edu, Log Out, and icons for mail, home, and a menu. Below the navigation bar, there are three buttons: 'Study Information', 'Sample Information', and 'Upload Files'. A message below the 'Upload Files' button says 'Issues opening the downloaded zip?'. To the right of these buttons, the study title is '[user's_name] - ID 11568'. Below the title, the study name '[user's_name]' is displayed. The 'Abstract' section contains the text '[user's abstract]'. The 'Study ID' is 11568. The 'Publications' section lists 'PI: Rob Knight (UCSD)'. The 'Lab Contact' is 'None'. The 'Shared With' is 'None'. The 'Samples' count is 0. The 'EBI' status is 'not submitted'. There are three buttons: 'Share', 'Edit', and 'Delete'. To the right of the 'Abstract' section, there is a 'Study Tags' section with the text 'Previously admin, Previously assigned, New' and an input field 'Add more tags'. Below the input field, there is a note: 'New tags are linked to the user that created them. Report abuse.' and a 'Save tags' button. At the bottom of the page, there is a footer with the text: 'Thank you for using Qiita. For citations point to <http://qiita.microbio.me>. Questions? qiita.help@gmail.com. Read our [terms and conditions](#).'

Since we are using a practice set of data, under “Study Tags” write “Tutorial” and select “Save Tags”. As part of our routine clean up efforts, this tag will allow us to find and remove studies and analyses generated using the template data and information.

The first step after study creation is uploading files. Click on the “Upload Files” button: as shown in the figure below, you can now drag-and-drop files into the grey area or simply click on “select from your computer” to select the fastq, fastq.gz or txt files you want to upload.

Note: Per our Terms of Condition for use, by uploading files to Qiita you are certifying that they do not contain: 1) Protected health information within the meaning of 45 Code of Federal Regulations part 160 and part 164, subparts A and E; [see checklist](#) 2) Whole genome sequencing data for any human subject; [HMP human sequence removal protocol](#) 3) Any data that is copyrighted, protected by trade secret, or otherwise subject to third party proprietary rights, including privacy and publicity rights, unless you are the owner of such rights or have permission from the rightful owner(s) to transfer the data and grant it to Qiita, on behalf of the Regents of the University of California, all of the license rights granted in our [Terms](#).

Uploads can be paused at any time and restarted again, as long as you do not refresh, navigate away from the page, or log out of the system from another browser window.

Study Tags

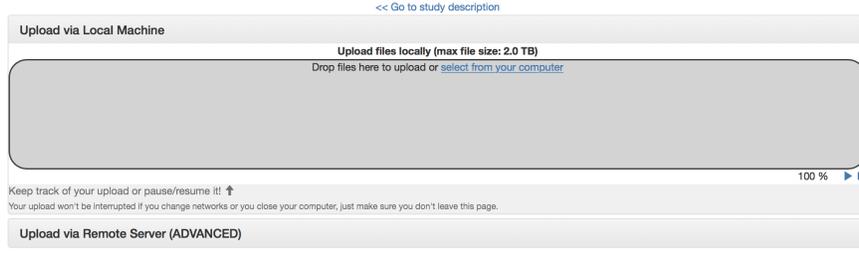
Previously [admin](#), [Previously assigned](#), [New](#)

Add more tags

New tags are linked to the user that created them. Report [abuse](#).

Save tags

To proceed, drag the file named “sample_info.txt” into the upload box. It should upload quickly and appear below “Files” with a checkbox next to it below.



Once your file has uploaded, click on “Go to study description” and, once there, click on the “Sample Information” tab. Select your sample information from the dropdown menu next to “Upload information” and click “Create”.

If something is wrong with the sample information file, Qiita will let you know with a red banner at the top of the screen.

If the file processes successfully, you should be able to click on the “Sample Information” tab and see a list of the imported metadata fields.

Note: The warning is to let you know this study is missing columns that are required for EBI-ENA submission. For more information you can visit the [Send data to EBI-ENA](#) information page.

To check out the different metadata values select the “Sample-Prep Summary” tab. On this page, select a metadata column to visualize in the “Add sample column information to table” dropdown menu and click “Add column.”

Next, we’ll add 16S raw data and process it.

Next: *Adding a preparation template and linking it to raw data*

Now, we’ll upload some actual microbiome data to explore. To do this, we need to add the data themselves, along with some information telling Qiita about how those data were generated.

[user's_name] - ID 11568

[user's_name]

Sample Information

Select sample information file:

sample_info.txt

If uploading a QIIME mapping file, select the data type of the prep information:

Choose a data type...

Create

! The 'sample_name' column is missing from your template, this file cannot be parsed. ×
Need help? Send us an [email](#).

1.6 Adding a preparation template and linking it to raw data

Where the *sample info file* has the biological metadata associated with your samples, the *preparation info file* contains information about the specific technical steps taken to go from sample to data. Just as you might use multiple data-generation methods to get data from a single sample – for example, target gene sequencing and shotgun metagenomics – you can have multiple prep info files in a single study, associating your samples with each of these data types. You can learn more about prep info files at the [Qiita documentation](#).

Go back to the “Upload Files” interface. In the [example data](#), find and upload the 3 “**fastq.gz files**” and the “**prep_info_16S.txt**” file.

These files will appear under “Files” when they finish uploading.

Then, go to the study description. Now you can click the “Add New Preparation” button. This will bring up the following dialogue:

Select “prep_info_16S.txt” from the “Select file” dropdown, and “16S” as the data type. Optionally, you can also select one of a number of investigation types that can be used to associate your data with other like studies in the database. Click “Create New Preparation”.

You should now be brought to a “Processing” tab of your preparation info:

By clicking on the “Summary” tab on this page you can see the preparation info that you uploaded.

As the owner of the study, you will also have the options to delete or deprecate the preparation. Once an analysis has been created from any object in a preparation, you will be unable to delete the prep. Deprecating the preparation lets others know it is an older version.

In addition, you should see a “16S” button appear under “Data Types” on the menu to left:

[user's name] - ID 13164

[user's name]

Do you want to submit to [EBI-ENA](#)? Review the [submission checklist](#)

Sample Information

 Sample Info

 Delete

Sample Info is missing columns "env_package"

Sample Information

Sample-Prep Summary

Number of samples: 14

Number of columns: 21

Update sample information:

Choose file...

Direct upload file (< 2MB)

Column for the tube identifier: ?

None (not available)

Information summary



anonymized_name:

Values

[user's name] - ID 13164

[user's name]

Do you want to submit to [EBI-ENA](#)? Review the [submission checklist](#)

Sample Information

 Sample Info

 Delete

Sample Info is missing columns "env_package"

Sample Information

Sample-Prep Summary

Sample Summary

Add sample column information to table

 Add column

 Delete Selected

 Delete Non Overlapping Samples

<input type="checkbox"/>	sample_name
<input type="checkbox"/>	13164.1D11
<input type="checkbox"/>	13164.1D12

[<< Go to study description](#)

Upload via Local Machine

Upload files locally (max file size: 2.0 TB)

Drop files here to upload or [select from your computer](#)

10 % ▶ ||

Keep track of your upload or pause/resume !!! ↑
Your upload won't be interrupted if you change networks or you close your computer, just make sure you don't leave this page.

Upload via Remote Server (ADVANCED)

Files

CMI_workshop_lane1_S1_L001_I1_001.fastq.gz (4568505)	<input type="checkbox"/> download
CMI_workshop_lane1_S1_L001_R1_001.fastq.gz (16733712)	<input type="checkbox"/> download
CMI_workshop_lane1_S1_L001_R2_001.fastq.gz (17568260)	<input type="checkbox"/> download
prep_info_16S.txt (2250)	<input type="checkbox"/> download

[<< Go to study description](#)
 Thank you for using Qiita. [Citing Qiita?](#)
 Questions? qiita.help@gmail.com; don't forget to add your study or analysis id.
[Read our terms and conditions.](#)

Add a new preparation file: (* Required fields)

Name:

Select file: *

Select data type: *

Select Investigation Type:

Unsure? [Check](#)

Not sure what to select? [Check](#)

Create New Preparation

Thank you for using Qiita. [Citing Qiita?](#)
 Questions? qiita.help@gmail.com; don't forget to add your study or analysis id.
[Read our terms and conditions.](#)

[user's name] - ID 13164

[user's name]

Do you want to submit to [EBI-ENA](#)? Review the [submission checklist](#)

[user's_prep_info] - ID 8886 (16S)

 Edit name

 Prep info

 QIIME map

 Deprecate

 Delete

Sample Listing

Summary

Processing

No files attached to this preparation

Select type:

Add a name for the file:

[user's name] - ID 13164

[user's name]

Do you want to submit to [EBI-ENA](#)? Review the [submission checklist](#)

[user's_prep_info] - ID 8886 (16S)

 Edit name

 Prep info

 QIIME map

 Deprecate

 Delete

Sample Listing

Summary

Processing

Number of samples: 14

Number of columns: 17

Update prep information:

Select Investigation Type: Unsure? [Check](#)

Information summary



barcode: All the values in this category are different.



extraction_robot

Data Types (click on the tabs)

 16S

You can click this to reveal the individual prep info files of that data type that have been associated with this study:

Data Types (click on the tabs)

☰ **16S**

[user's_prep_info] - ID 4220 - sandbox
 None - ID None
 None

If you have multiple 16S preparations (for example, if you sequenced using several different primer sets), these would each show up as a separate entry here.

Now, you can associate the sequence data from your study with this preparation.

[user's name] - ID 13164

[user's name]

Do you want to submit to [EBI-ENA](#)? Review the [submission checklist](#)

[user's_prep_info] - ID 8886 (16S)

↗ Deprecate
🗑 Delete

✎ Edit name
↓ Prep info
↓ QIIME map

Sample Listing

Summary

Processing

No files attached to this preparation

Select type: Choose a type...

Add a name for the file:

Select the processing tab again. In the prep info dialogue, there is a dropdown menu below the words *No files attached to this preparation*, labeled “Select type”. Click “Choose a type” to see a list of available file types. In our case, we’ve uploaded FASTQ-formatted files for all samples in our study, so we will choose “FASTQ - None”. In some cases outside of this tutorial, you may have per sample FASTQ files, so take care in considering which data type you are handling.

Magically, this will prompt Qiita to associate your uploaded files with the corresponding samples in your preparation info. (Our prep info file has a column named *run_prefix*, which associated the *sample_name* with the file name prefix for that particular sample).

You should see this as filenames showing up in the green: *raw barcodes* (file with *I1* in its name), *raw forward seqs* (*R1* in name) and *raw reverse seqs* (*R2* in name) columns below the import dropdown. You’ll want to give the set of these FASTQ files a name (Add a name for the file field below *Select type: FASTQ - None*), and then click “Add files” below.

That’s it! Your data are ready for processing.

[user's name] - ID 13164

[user's name]

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

[user's_prep_info] - ID 8886 (16S) [Edit name](#) [Prep info](#) [QIIME map](#)

[Deprecate](#) [Delete](#)

[Sample Listing](#) [Summary](#) [Processing](#)

No files attached to this preparation

Select type: FASTQ - None

Add a name for the file: [user's_name]

Now, you can import files from other studies [Choose an artifact to import...](#) or click and drag your uploaded files to the correct file type

Please make sure that the correct files are in the correct column.

Note: the system will try to auto select the files based on run_prefix, if that doesn't work, either the type you selected doesn't support the use of run_prefix or the run_prefix is wrong

Available Files	raw barcodes	raw forward seqs	raw reverse seqs
<input type="text"/>	CMI_workshop_lane1_S1_L001_I1_	CMI_workshop_lane1_S1_L001_R1_	CMI_workshop_lane1_S1_L001_R2_

[Add files](#)

1.7 Exploring the raw data

Click on the 16S menu on the left. Now that you've associated sequence files with this prep, you'll have a "Processing network" displayed:

[user's_prep_info] - ID 8886 (16S) [Edit name](#) [Prep info](#) [QIIME map](#)

[Deprecate](#) [Delete](#)

[Sample Listing](#) [Summary](#) [Processing](#)

Processing network [Hide](#)

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 3 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

[user's_name]
(FASTQ)

If you see this message:

It means that your files need time to load. Refresh your screen after about 1 minute.

Your collection of FASTQ files for this prep are all represented by a single object in this network, called "[user's_name] (FASTQ)" in the example. Click on the object.

Now, you'll have a series of choices for interacting with this object. You can click "Edit" to rename the object,

 This prep template is currently being updated

“Process” to perform analyses, or “Delete” to delete it. In addition, you’ll see a list of the actual files associated with this object.

[user's_name] (ID: 43489) Visibility: sandbox  Edit name  Process  Delete  Request approval

Available files:

 CMI_workshop_lane1_S1_L001_R2_001.fastq.gz (raw reverse seqs)

 CMI_workshop_lane1_S1_L001_I1_001.fastq.gz (raw barcodes)

 CMI_workshop_lane1_S1_L001_R1_001.fastq.gz (raw forward seqs)

Currently, no summary exists.

 Generate summary

Scroll to the bottom, and you’ll also see an option to generate a summary of the object.

Currently, no summary exists.

 Generate summary

If you click this button, it will be replaced with a notification that the summary generation has been added to the processing queue.

To check on the status of the processing job, you can click the rightmost icon at the top of the screen:

This will open a dialogue that gives you information about currently running jobs, as well as jobs that failed with some sort of error. *Please note*, this dialogue keeps the entire history of errors that Qiita encountered for your jobs, so take notice of dates and times in the *Heartbeat* column.

The summary generation shouldn’t take too long. You may need to refresh your screen. When it completes, you can click back on the FASTQ object and scroll to the bottom of the page to see a short peek at the data in each of the FASTQ files in the object. These summaries can be useful for troubleshooting.

Now, we’ll process the raw data into something more interesting.

1.8 Processing 16S data

Scroll back up and click on the “CMI tutorial - 14 skin samples(FASTQ)” artifact, and select “Process”. Below the files network, you will now see a “Choose command” dropdown menu. Based on the type of object, this dropdown menu will give you a list of available processing steps.

For 16S “FASTQ” objects, the only available command is “Split libraries FASTQ”. The converts the raw FASTQ data into the file format used by Qiita for further analysis (you can read more extensively about this file type [here](#)).

Select the “Split libraries FASTQ” step. Now, you will be able to select the specific combination of parameters to use for this step in the “Choose parameter set” dropdown menu.

✖ Deprecate
🗑 Delete

Sample Listing
Summary
Processing

Processing network
Hide

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 8 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command: Split libraries FASTQ ▼

Required parameters:

input data: [user's_name] (FASTQ) ▼

Optional parameters:

Parameter set: Multiplexed FASTQ; Golay 12 base pair reverse cor ▼

Note: changing default parameter values not allowed

barcode type: golay_12

max bad_run_length: 3

max barcode_errors: 1.5

min per_read_length_fraction: 0.75

phred offset: auto ▼

phred quality_threshold: 3

rev comp:

rev comp_barcode:

rev comp_mapping_barcodes:

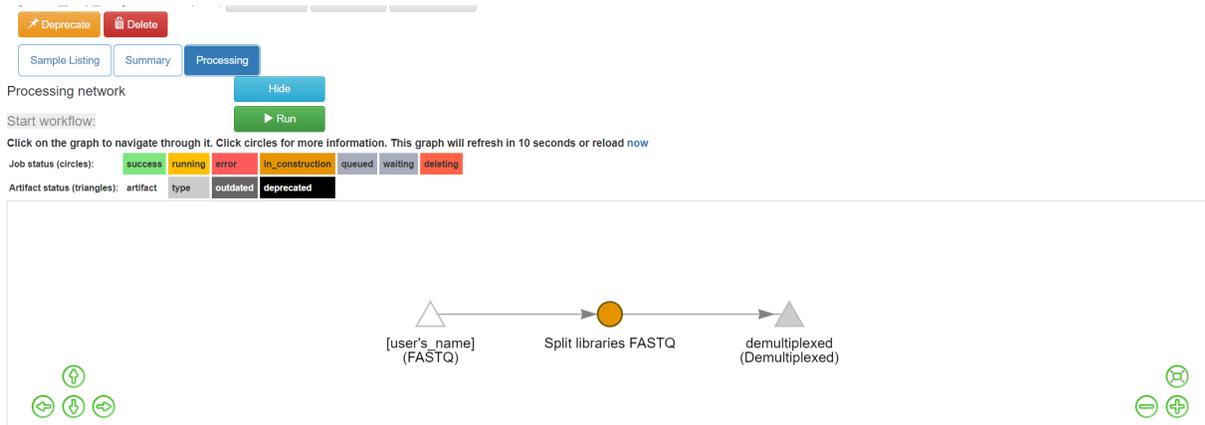
sequence_max_n: 0

Add Command

For our files, choose “Multiplexed FASTQ; Golay 12 base pair reverse complement mapping file barcodes with reverse complement barcodes”. The specific parameter values used will be displayed below. **For most raw data coming out of the Knight Lab you will use the same setting.**

Click “Add Command”.

You’ll see the files network update. In addition to the original white object, you should now see the processing command (represented in yellow) and the object that will be produced from that command (represented in grey).

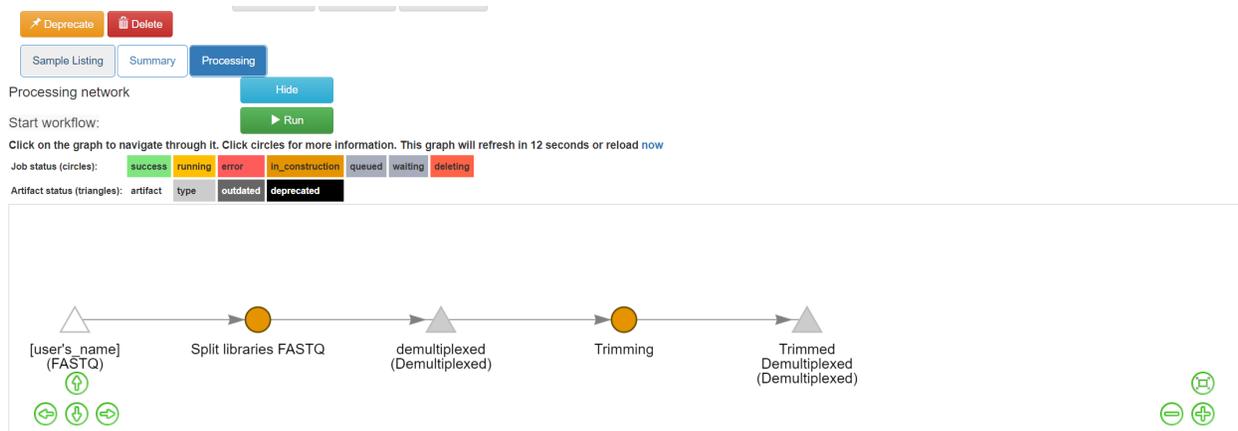


You can click on the command to see the parameters used, or on an object to perform additional steps.

Next we want to trim to a particular length, to ensure our samples will be comparable to other samples already in the database. Click back on the “demultiplexed (Demultiplexed)”. This time, select the Trimming operation. Currently, there are seven trimming length options. Let’s choose “100 basepairs”, which trims to the first 100bp, for this run, and click “Add Command”.

Click “Add Command”, and you will see the network update:

Note that the commands haven’t actually been run yet! (We’ll still need to click “Run” at the top.) This allows us to add multiple processing steps to our study and then run them all together.



We're going to process our sequence files using two different workflows. In the first, we'll use a conventional reference-based OTU picking strategy to cluster our 16S sequences into OTUs. This approach matches each sequence to a reference database, ignoring sequences that don't match the reference. In the second, we will use `deblur`, which uses an algorithm to remove sequence errors, allowing us to work with unique sequences instead of clustering into OTUs. Both of these approaches work great with Qiita, because we can compare the observations between studies without having to do any sort of re-clustering!

1.9 The closed-reference workflow

To do closed reference OTU picking, click on the “Trimmed Demultiplexed (Demultiplexed)” object and select the “Pick closed-reference OTUs” command. We will use the “Defaults” parameter set for our data, which are relatively small. For a larger data set, we might want to use the “Defaults - parallel” implementation.

By default, Qiita uses the GreenGenes 16S reference database. You can also choose to use the Silva 119 18S database, or the UNITE 7 fungal ITS database.

Click “Add Command”, and you will see the network update:

Here you can see the blue “Pick closed-reference OTUs” command added, and that the product of the command is a BIOM-formatted OTU table.

That's it!

1.10 The deblur workflow

The `deblur` workflow is only marginally more complex. Although you can `deblur` the demultiplexed sequences directly, “`deblur`” works best when all the sequences are the same length. By trimming to a particular length, we can also ensure our samples will be comparable to other samples already in the database.

Click back on the “Trimmed Demultiplexed (Demultiplexed)” object. This time, select the `Deblur` operation. Choose “`Deblur`” from the “Choose command” dropdown, and “Defaults” for the parameter set.

Add this command to create this workflow:

Now you can see that we have the same “Trimmed Demultiplexed (Demultiplexed)” object being used for two separate processing steps – closed-reference OTU picking, and `deblur`.

Deprecate Delete

Sample Listing Summary Processing

Processing network Hide

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 10 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command:

Required parameters:

Input data:

Optional parameters:

Parameter set:

Note: changing default parameter values not allowed

reference-seq:

reference-tax:

similarity:

sortmerma coverage:

sortmerma e_value:

sortmerma max_pos:

threads:

Add Command

Deprecate Delete

Sample Listing Summary Processing

Processing network Hide

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 4 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

⚠️ Deprecate 🗑️ Delete

Sample Listing Summary Processing

Processing network Hide

Start workflow: ▶ Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 10 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command:

Required parameters:

Demultiplexed sequences:

Optional parameters:

Parameter set:

Note: changing default parameter values not allowed

Error probabilities for each Hamming distance:

Indexed negative filtering database:

Indexed positive filtering database:

Insertion/deletion (indel) probability:

Jobs to start:

Maximum number of insertion/deletion (indel):

Mean per nucleotide error rate:

Minimum dataset-wide read threshold:

Minimum per-sample read threshold:

Negative filtering database:

Positive filtering database:

Reference phylogeny for SEPP:

Sequence trim length (-1 for no trimming):

Threads per sample:

Add Command

⚠️ Deprecate 🗑️ Delete

Sample Listing Summary Processing

Processing network Hide

Start workflow: ▶ Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 11 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

As you can see, “deblur” produces two BIOM-formatted OTU tables as output. The “deblur reference hit table (BIOM)” contains deblurred sequences that have been filtered to try and exclude things like organellar mitochondrial reads, while “deblur final table (BIOM)” has all the sequences.

1.11 Running the workflow

Now, we can see the whole set of commands and their output files:

Processing network

Start workflow: ▶ Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 11 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Click “Run” at the top of the screen, and Qiita will start executing all of these jobs. You’ll see a “Workflow submitted” banner at the top of your window.

The full workflow can take time to load depending on the amount of samples and Qiita workload. You can keep track of what is running by looking at the colors of the command artifacts. If yellow, the commands are being run now. If green, the commands have successfully been run. If red, the commands have failed.

Once objects have been generated, you can generate summaries for them just as you did for the original “FASTQ” object.

The summary for the “demultiplexed (Demultiplexed)” object gives you information about the length of sequences in the object:

The summary for a BIOM-format OTU table gives you a table summary, details regarding the frequency per sample, and a histogram of the number of features per sample:

Next: *Analysis of Closed Reference Process*

1.12 Analysis of Closed Reference Process

To create an analysis, hover over “Analysis” on the top menu and select “Create new analysis” from the drop down menu.

Deprecate Delete

Sample Listing Summary Processing

Processing network Hide

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 7 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Open summary in a new window

Features

Total: 304000
Max: 150
Mean: 149
Standard deviation: 150
Median: 0

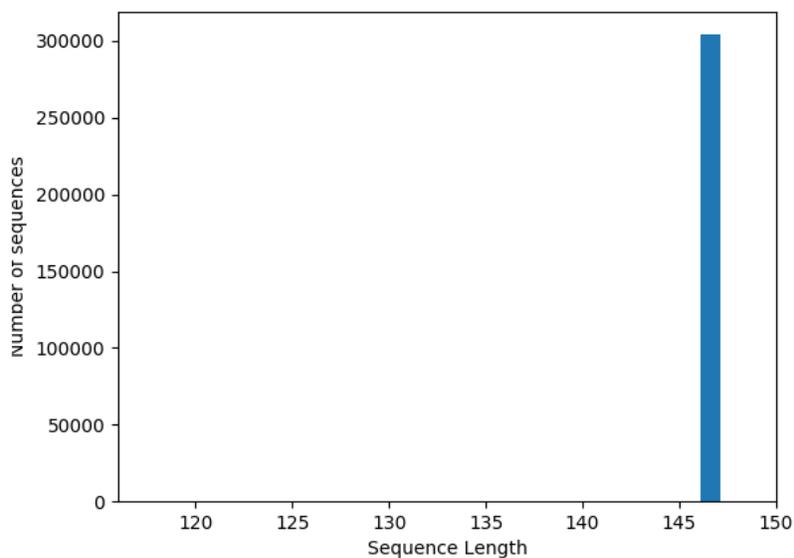


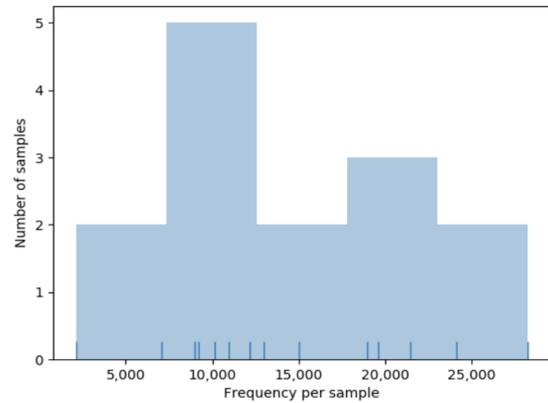
Table summary

Metric	Sample
Number of samples	14
Number of features	1,049
Total frequency	201,137

Frequency per sample

	Frequency
Minimum frequency	2,099.0
1st quartile	9,447.75
Median frequency	12,588.0
3rd quartile	19,446.75
Maximum frequency	28,285.0
Mean frequency	14,366.92857142857

Frequency per sample detail ([csv](#) | [html](#))



This will take you to a list of studies with samples available to you for analysis, divided between your studies and publicly available studies (“Public Studies”).

Filter by column data (Title, abstract, PI, etc):

Filter studies by tags: (Admin, User)

Your Studies

Show 5 entries

Expand for analysis	Title	Study ID	Samples	Shared With These Users	Principal Investigator	Publications	Status	Qiita EBI submission
1	CMI workshop analysis CMIWorkshop	11269	30	Modify Owner: Tomasz Austin Swafford, Alison Vrbanc, CMI, fernando.vargas0341@gmail.com, Justine Debellus, mlw023@ucsd.edu, Robert Quinn, Yoshiki	Tomasz Kosciolok		public	not submitted
1	[user's_name]	11574	14	Modify Owner: CMI	Rob Knight		sandbox	not submitted
No BIOMs	Microbiome and metabolome in opioid and methamphetamine addicted patients	11480	124	Modify Owner: CMI Austin Swafford, Bryn Taylor, Carolina S. Carpenter, fernando.vargas0341@gmail.com, Greg Humphrey, sorochanian@eng.ucsd.edu	Karsten Zengler		sandbox	not submitted

Public Studies

Expand for analysis	Title	Study ID	Samples	Principal Investigator	Publications	Qiita EBI submission
1	Seasonal restructuring of the ground squirrel gut microbiota over the annual hibernation cycle	926	46	Hannah Carey	23152108, 10.1152/ajpregu.00387.2012	not submitted

Find the “CMI workshop analysis” study in Public Studies. You can use the search window at the top right, or filter by tags (“CMIWorkshop” tag). Click the green plus sign at the left of the row under “Expand for analysis”. This will expand the study to expose all the objects from that study that are available to you for analysis.

To look more closely at the details of the artifact, select “Per Artifact (1).” Here you can add each of these objects to the analysis by selecting the “Add” button. We will just add the Closed Reference OTU table object by clicking “Add” in that row.

Now, the second-right-most icon at the top bar should turn green, indicating that there are samples selected for analysis.

Clicking on the icon will take you to a page where you can refine the samples you want to include in your analysis. Here, all 30 of our samples are currently included:

Expand for analysis	Title	Study ID	Samples	Shared With These Users	Principal Investigator	Publications	Status	Qiita EBI submission
1	CMI workshop analysis CMIWorkshop	11269	30	Modify Owner: Tomasz Austin Swafford, Alison Vrbanc, CMI, fernando.vargas0341@gmail.com, Justine Debellus, miw023@ucsd.edu, Robert Quinn, Yoshiki	Tomasz Kosciolok		public	not submitted

Artifacts	Processing method	Data type
Add all	Per Artifact (1)	16S (0)

Artifacts	Processing method	Data type
Add all	Per Artifact (1)	16S (0)

	Name	Samples in Prep Info	Files
Add	raw_biom_closed-reference (33738 - 2017-08-17 13:40:15)	30	test.biom

Log Out

1 Studies

Processed Data

30 Samples

Selected Samples

Create Analysis Clear Selected

CMI workshop analysis

Processed Data

id	Datatype	Processed Date	Algorithm	Parameters	Samples selected from Prep Info
33738	16S	2017-08-17 13:40:15.365324	None		30 Show/Hide samples Remove

You could optionally exclude particular samples from this set by clicking on “Show/Hide samples”, which will show each individual sample name along with a “remove” option. (Removing them here will mask them from the analysis, but will not affect the underlying files in any way.)

This should be good for now. Click the “Create Analysis” button, enter a name and description, then click “Create Analysis”.

Create new analysis ×

Analysis name

Description

This brings you to the processing network page. This may take 2 to 5 minutes to load. When it has loaded, you can analyze the data.

Processing network

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 5 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

df1t_name
(BIOM)

⬅ ⬆ ➡

⊖ ⊕

Before we process the data, let’s have a look at the summary of the contents of the biom file. Select the “df1t_name (BIOM)” artifact to see a summary of this file displaying a table summary, details regarding the frequency per sample, histogram of the number of features per sample:

As you can see, this file contains 30 samples with roughly 36,000 features, in our case, picked-OTUs (or operational taxonomic unit).

Now we can begin analyzing these samples. Let’s go ahead and select “df1t_name (BIOM)” then select “Process”. This will take us to the commands selection page. Once there, the commands pull down tab can be accessed which will display thirty-eight actions.

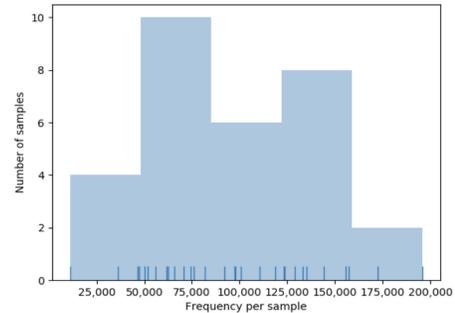
Table summary

Metric	Sample
Number of samples	30
Number of features	35,723
Total frequency	2,879,112

Frequency per sample

	Frequency
Minimum frequency	11,030.0
1st quartile	61,778.25
Median frequency	94,809.0
3rd quartile	127,517.25
Maximum frequency	196,075.0
Mean frequency	95,970.4

[Frequency per sample detail \(csv | html\)](#)



The text in brackets is the actual underlying commands from QIIME2. We will now go through the use of some of the most-used commands which will enable you to generate summaries, plot your data, and calculate statistics to help you get the most out of your data.

1.12.1 Rarefying Data

For certain analyses such as those we are about to conduct, the data should be *rarefied*. This means that all the samples in the analysis will have their features, in this case OTUs, randomly subsampled to the same desired number, reducing potential alpha and beta diversity biases. Samples with fewer than this number of features will be excluded, which can also be useful for excluding low abundance samples like blanks. To choose a good cutoff for your data, view the histogram that was made when we generated the summary of the data.

An appropriate cutoff would exclude clear outliers, but retain most of the samples. Here we have already removed blanks from our data and eliminated the outliers prior to analysis so we will just use the minimum number of features observed in our samples (11030) as the cutoff.

To rarefy the data, select “Rarefy table” from the drop-down menu. The parameters will appear below the workflow diagram:

Several parameters will have only one option which will be automatically selected for you. In the field, “The total frequency that each sample should be rarefied to. . . (sampling depth)”, we will specify the number of features to rarefy our samples to. Enter “11030” in this box, and click “Add Command”.

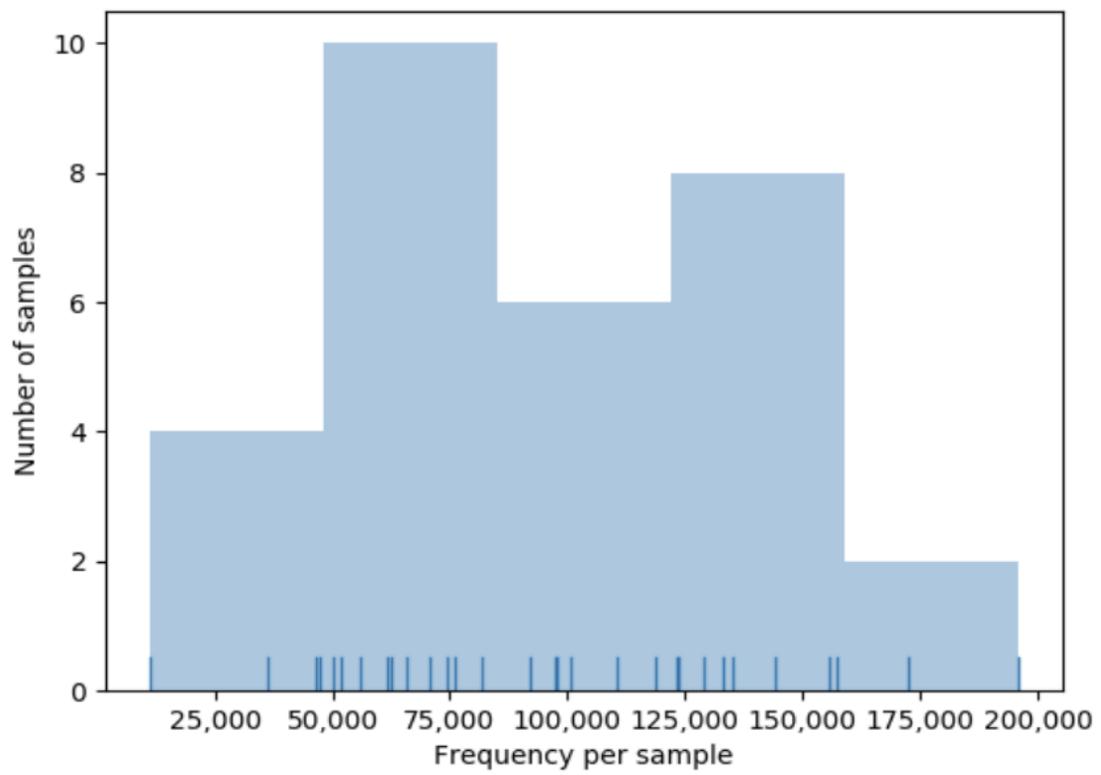
Click the “Run” button above the workflow network to start the process of rarefaction. You can now view your jobs that are running by clicking on the server button in the top-right corner of the screen:

The view will return to the original screen, while the rarefied feature-table generation job runs. Your browser will automatically refresh every 15 seconds until the “rarefied table (BIOM)” artifact appears:

Select the newly generated “rarefied_table (BIOM)” artifact. This time instead of seeing a histogram of the rarefied samples, you instead see a brief summary confirming that your samples have all be rarefied to the same depth. Now that the data are rarefied, we can begin the analysis.

✓ Choose command...

Add pseudocount to table [add_pseudocount]
Alpha diversity (phylogenetic) [alpha_phylogenetic]
Alpha diversity [alpha]
Alpha rarefaction curves [alpha_rarefaction]
Beta diversity (phylogenetic) [beta_phylogenetic]
Beta diversity [beta]
Beta diversity rarefaction [beta_rarefaction]
Collapse features by their taxonomy at the specified level [collapse]
Compute first differences or difference from baseline between sequential states [first_differences]
Convert (and merge) positive numeric metadata (in)to feature table. [metatable]
Convert to presence/absence [presence_absence]
Convert to relative frequencies [relative_frequency]
Core diversity metrics (non-phylogenetic) [core_metrics]
Core diversity metrics (phylogenetic and non-phylogenetic) [core_metrics_phylogenetic]
Filter features from table [filter_features]
Filter samples from table [filter_samples]
Generate a heatmap representation of a feature table [heatmap]
Generate heatmap of important features. [heatmap]
Generate interactive volatility plot [volatility]
Group samples or features by a metadata column [group]
Identify core features in table [core_features]
Linear mixed effects modeling [linear_mixed_effects]
Nested cross-validated supervised learning classifier. [classify_samples_ncv]
Nested cross-validated supervised learning regressor. [regress_samples_ncv]
Nonparametric microbial interdependence test [nmit]
Paired difference testing and boxplots [pairwise_differences]
Plot longitudinal feature volatility and importances [plot_feature_volatility]
Pre-fitted sklearn-based taxonomy classifier [classify_sklearn]
Rarefy table [rarefy]
Remove features from table if they're not present in tree. [filter_table]
Split a feature table into training and testing sets. [split_table]
Subsample table [subsample]
Summarize table [summarize]
Taxonomy-based feature table filter. [filter_table]
Transpose a feature table. [transpose]
Visualize taxonomy with an interactive bar plot [barplot]



Choose command:

Rarefy table [rarefy]

Required parameters:

The feature table to be rarefied.:

dfft_name (BIOM)

Optional parameters:

Parameter set:

Default

Rarefy with replacement by sampling from the multinomial distribution instead of rarefying without replacement. (with replacement):

The total frequency that each sample should be rarefied to. Samples where the sum of frequencies is less than the sampling depth will be not be included in the resulting table unless subsampling is performed with replacement. (sampling depth):

11030

Add Command

Shared with: Hide

Processing network ▶ Run

Start workflow:

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 4 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

```

graph LR
    A[dfft_name (BIOM)] --> B((Rarefy table))
    B --> C[rarefied_table (BIOM)]
    style A fill:#ccc,stroke:#ccc
    style B fill:#FFD700,stroke:#ccc
    style C fill:#ccc,stroke:#ccc
    
```



Shared with: Hide

Processing network ▶ Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 5 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

```

graph LR
    A[dfft_name (BIOM)] --> B((Rarefy table))
    B --> C[rarefied_table (BIOM)]
    style A fill:#ccc,stroke:#ccc
    style B fill:#90EE90,stroke:#ccc
    style C fill:#ccc,stroke:#ccc
    
```

1.12.2 Taxa Bar Plots

When creating a 16S closed-reference BIOM table in Qiita, each sequence is matched to the Greengenes database using a 97% sequence identity threshold, and assigned a taxonomy (See this section for a [refresher on 16S data](#)). This enables us to display this data to view the percentage of each taxa within each sample.

When using “Deblurred” data, there is no taxa assignment since features are kept as individual error-corrected sequences, so if you are referencing this tutorial with your own deblurred data you can skip to the next section “Alpha Diversity Analysis”.

To display the taxonomic profiles of our samples, we will select our rarefied table artifact, and click “Process”. The same processing view we saw previously now appears, so click on “Visualize taxonomy with an interactive bar plot” from the drop-down menu to arrive at the following view:

Shared with:

Processing network Hide

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 4 seconds or reload [now](#)

Job status (circles): success running error in construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command: Visualize taxonomy with an interactive bar plot

Required parameters:

Feature table to visualize at various taxonomic levels.: rarefied_table (BIOM)

Optional parameters:

Parameter set: Default

Add Command

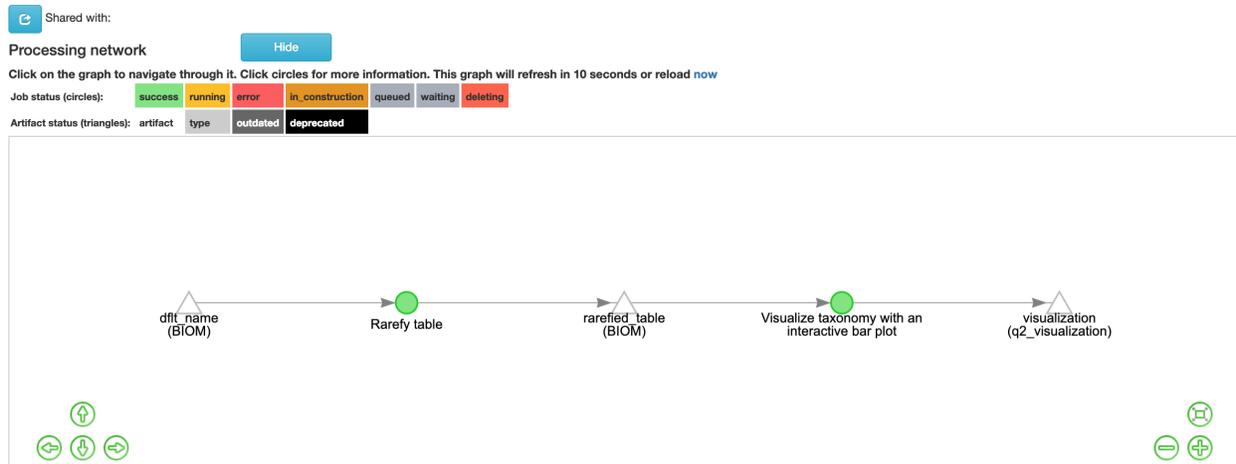
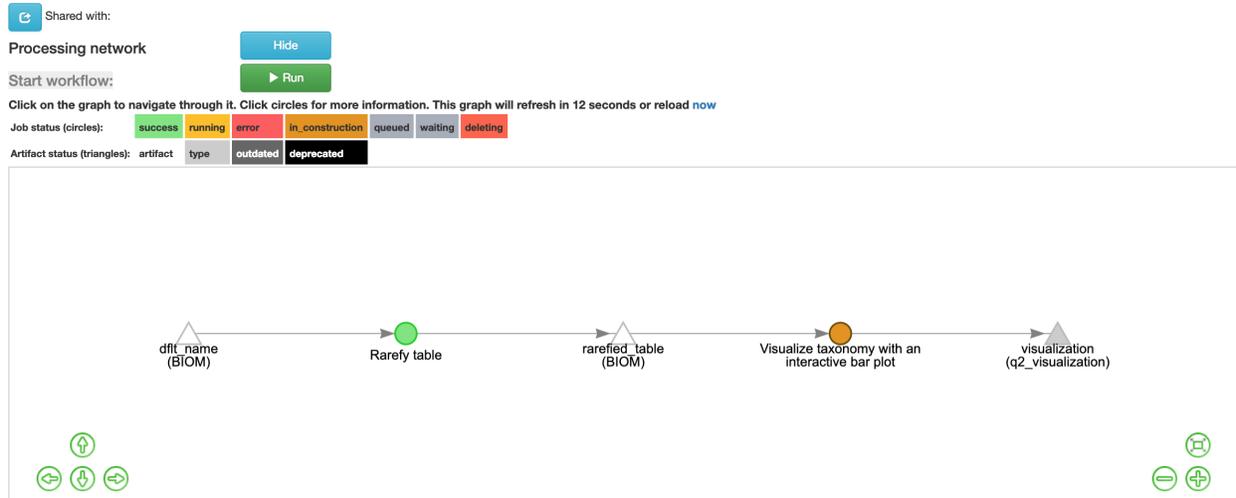
All of the parameters for this command are fixed so simply click “Add Comand” to continue. Once the command is added the workflow will appear:

Click the run button to start the process. Once the “visualization (q2_visualization)” artifact is generated you should see this screen:

Once the q2 visualization artifact is chosen in the network, the taxa barplot will appear below. The taxa plots offers visualization of the makeup of each sample. Each color will represent a different taxon and each column a different sample. It will have four pull-down menus: “Taxonomic Level,” “Color Palette,” and two “Sort Samples By” options.

The “Taxonomic Level” menu allows you to view the taxa within your samples at different specificities. There are 7 level options: 1- Kingdom, 2- Phylum, 3- Class, 4- Order, 5- Genus, 6- Species, 7- Subspecies.

The “Color Palette” menu allows you to change the coloring of your taxa barplot. You can select through “Discrete” palettes in which each taxa is a different color or “Continuous” palettes in which each taxa is a different shade of one





color.

The “Sort Sample By” menus allow you to sort your data either by sample metadata or taxonomic abundance and either by ascending or descending order.

1.12.3 Alpha Diversity Analysis

Now, let’s analyze the alpha diversity of your samples. Alpha diversity metrics describe the diversity of features within a sample or a group of samples. This is used to analyze the diversity within rather than between samples or a group of samples.

Observed Operational Taxonomic Units

One type of analysis for alpha diversity, and the simplest, is looking at the number of observed, unique features, or OTUs in this example, also known as feature richness. This type of analysis will provide the number of unique OTUs found in a sample or group of samples.

To perform an alpha diversity analysis of feature richness, select the rarefied “rarefied table (BIOM)” artifact in the processing network and select “Process”. Select “Alpha diversity [alpha]” from the drop-down menu. The parameters will appear below the workflow diagram:

Several parameters have been automatically selected for you since these options cannot be changed. In the field, “The alpha diversity metric... (metric)”, we will specify the alpha diversity metric to run in our analysis. Select “Number of distinct features” from the drop-down menu in this box, and click “Add Command”.

Once the command is added the workflow should appear as follows:

Click the run button to start the process of the alpha diversity analysis. The view will return to the original screen, while the alpha diversity analysis job runs.

Shared with: Hide

Processing network

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 13 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command:

Required parameters:

The feature table containing the samples for which alpha diversity should be computed.:

Optional parameters:

Parameter set:

The alpha diversity metric to be computed. (metric):

Add Command

Shared with: Hide

Processing network

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 5 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

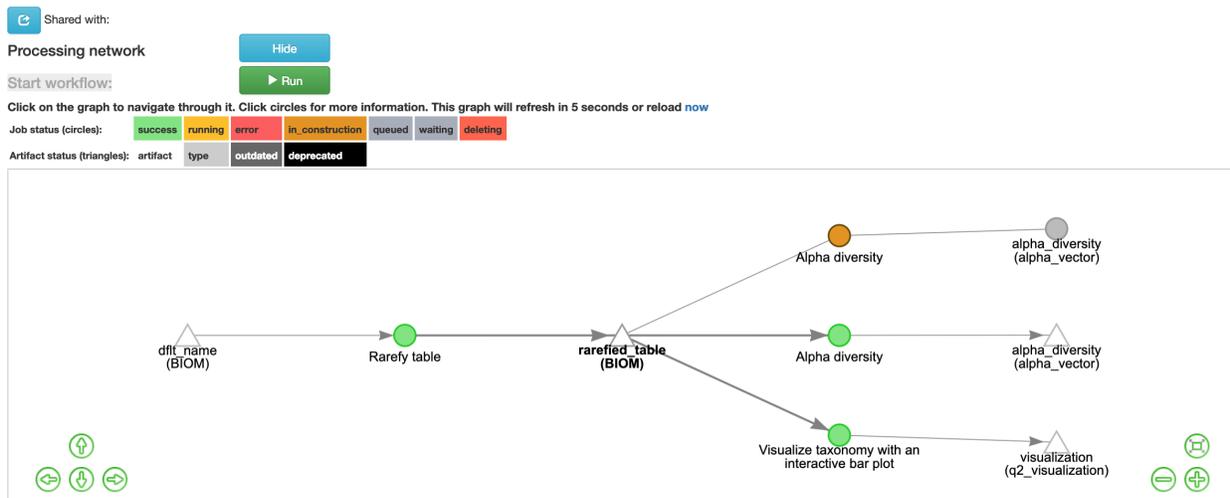
Artifact status (triangles): artifact type outdated deprecated

Shannon Diversity Index

Another alpha diversity metric commonly used is the Shannon diversity index. In addition to feature richness, this metric considers the abundance of each taxon relative to the total abundance across all taxa in a sample. Therefore, this metric takes into account both feature richness and abundance.

To perform an alpha diversity analysis using the Shannon diversity index, select the “rarefied table (BIOM)” artifact in the processing network and select “Process”. Select “Alpha diversity [alpha]” from the drop-down menu. The parameters will appear below the workflow diagram as previously. Also as before, several parameters have been automatically selected for you. In the field, “The alpha diversity metric... (metric)”, select “Shannon’s index” from the drop-down menu in this box, and click “Add Command”.

Once the command is added the workflow should appear as follows:



Click the run button to start the process of the alpha diversity analysis. The view will return to the original screen, while the alpha diversity analysis job runs.

Faith’s Phylogenetic Diversity Index

The final alpha diversity analysis in this tutorial uses Faith’s phylogenetic diversity index. This index also measured abundance and diversity but considers the phylogenetic distance spanning all features in a sample. The results can also be displayed as a phylogeny, rather than as a plot.

To perform an alpha diversity analysis using Faith’s phylogenetic diversity index, select the “rarefied table (BIOM)” artifact in the processing network and select “Process”. Select “Alpha diversity (phylogenetic)” with the Qiime2 command of “alpha phylogenetic” not “alpha phylogenetic old” from the drop-down menu. The parameters will appear below the workflow diagram:

Several parameters have been automatically selected for you. For example, in the field, “The alpha diversity metric... (metric)”, “Faith’s Phylogenetic Diversity” has already been chosen from the drop-down menu in this box. In the “Phylogenetic tree” field select “/databases/gg/13_8/trees/97_otus_no_none.tree” then click “Add Command”.

Once the command is added the workflow should appear as follows:

Click the run button to start the process of the alpha diversity analysis. The view will return to the original screen, while the alpha diversity analysis job runs.

Processing network Hide

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 7 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command:

Required parameters:

The feature table containing the samples for which alpha diversity should be computed.:

Optional parameters:

Parameter set:

Phylogenetic tree:

The alpha diversity metric to be computed. (metric):

Add Command

Shared with:

Processing network Hide

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 1 seconds or reload [now](#)

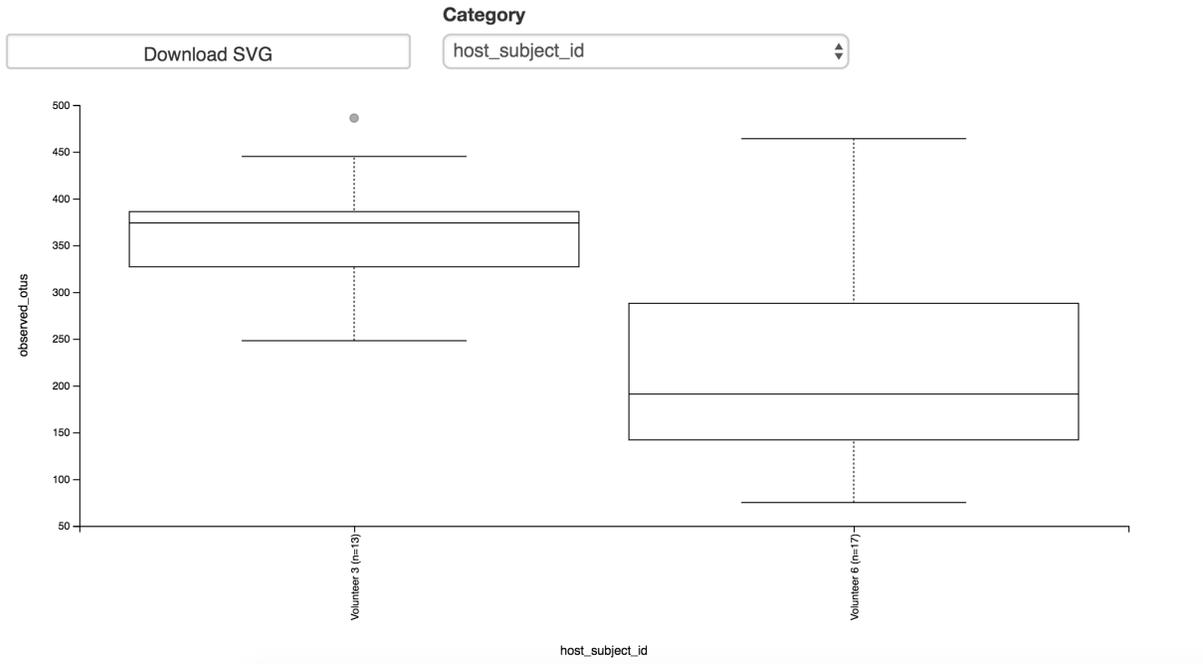
Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Alpha Diversity Outputs

Each alpha diversity analysis will output an interactive boxplot that shows how that alpha diversity metric correlates with different metadata categories:

Alpha Diversity Boxplots



To change the category, choose the “Category” pull-down menu and choose the metadata category you would like to analyze:

You will also be given the outcomes to Kruskal-Wallis tests:

1.12.4 Beta Diversity Analysis

One can also measure beta diversity in Qiita. Beta diversity measures feature turnover among samples (i.e., the diversity between samples rather than within each sample). This is used to compare samples to one another.

Bray-Curtis Dissimilarity

One commonly used beta diversity metric is Bray-Curtis dissimilarity. This metric quantifies how dissimilar samples are to one another.

To perform an analysis of beta diversity using the Bray-Curtis dissimilarity metric, select the “rarefied table (BIOM)” artifact in the processing network and select “Process”. Then select “Beta diversity” from the drop-down menu. The parameters will appear below the workflow diagram:

Several parameters have been automatically selected for you. In the field, “The beta diversity metric. . . (metric), we will specify the beta diversity analysis to run. Select “Bray-Curtis dissimilarity” from the drop-down menu in this box, and click “Add Command”.

To create a principal coordinates plot of the Bray-Curtis dissimilarity distance matrix, select the “distance matrix (distance matrix)” artifact and select “Process”. Select “Perform Principal Coordinate Analysis (PCoA)” from the drop-down menu. The parameters will appear below the workflow diagram:

Category

- ✓ host_subject_id
- sex
- subject
- extraction_robot
- side
- product_metabolites
- extractionkit_lot
- collection_date
- timepoint
- well_id
- lane
- processing_robot
- common_body_product_chemicals_present
- LinkerPrimerSequence
- BarcodeSequence
- phase
- sample_plate
- tm50_8_tool
- tm300_8_tool

Kruskal-Wallis (all groups)

	Result
H	9.723809725184108
p-value	0.0018189608066191715

Kruskal-Wallis (pairwise)

[Download CSV](#)

Group 1	Group 2	H	p-value	q-value
Volunteer 3 (n=13)	Volunteer 6 (n=17)	9.72381	0.001819	0.001819

Choose command:

Beta diversity [beta]

Required parameters:

The feature table containing the samples over which beta diversity should be computed.:

Rarefied 11030 (BIOM)

Optional parameters:

Parameter set:

Default

A pseudocount to handle zeros for compositional metrics. This is ignored for other metrics. (pseudocount):

1

The beta diversity metric to be computed. (metric):

Aitchison distance

The number of jobs to use for the computation. This works by breaking down the pairwise matrix into n jobs even slices and computing them in parallel. If -1 all CPUs are used. If 1 is given, no parallel computing code is used at all, which is useful for debugging. For n_jobs below -1, (n_cpus + 1 + n_jobs) are used. Thus for n_jobs = -2, all CPUs but one are used. (Description from sklearn.metrics.pairwise_distances) (n_jobs):

1

Add Command

Choose command:

Principal Coordinate Analysis [pcoa]

Required parameters:

The distance matrix on which
PCoA should be computed.:

distance_matrix (distance_matrix)

Optional parameters:

Parameter set:

Default

Dimensions to reduce the distance matrix to. This number determines how many eigenvectors and eigenvalues are returned, and influences the choice of algorithm used to compute them. By default, uses the default eigendecomposition method, SciPy's `eigh`, which computes all eigenvectors and eigenvalues in an exact manner. For very large matrices, this is expected to be slow. If a value is specified for this parameter, then the fast, heuristic eigendecomposition algorithm `fsvd` is used, which only computes and returns the number of dimensions specified, but suffers some degree of accuracy loss, the magnitude of which varies across different datasets. (number of_dimensions):

Add Command

All of the parameter have automatically selected for you just click “Add Command”.

Once the command is added the workflow should appear as follows:



Click the run button to start the process of the beta diversity analysis. The view will return to the original screen, while the beta diversity analysis job runs.

Unweighted UniFrac Analysis

Another commonly used distance metric for measuring beta diversity is unweighted UniFrac distance. *Unweighted* refers to that the metric considers only feature richness and not abundance, when comparing samples to one another. This differs from the weighted UniFrac distance metric, which takes into account both feature richness and abundance, for each sample.

To perform unweighted UniFrac analysis, select the “rarefied table (BIOM)” artifact in the processing network and select “Process”. Then select “Beta diversity (phylogenetic)” from the drop-down menu. The parameters will appear below the workflow diagram:

Most of the parameters have been automatically selected for you, but you will need to select the phylogenetic tree to use. Click on the dropdown next to “Phylogenetic tree:” and select “/databases/gg/13_8/trees/97_otus_no_none.tree” and then click “Add Command”.

To create a principal coordinates plot of the unweighted Unifrac distance matrix, select the “distance_matrix (distance_matrix)” artifact that will be generated using Unweighted UniFrac distance. Note that, unless you rename each distance matrix (see below: Altering Workflow Analysis Names), they will appear identical until you select them to view their provenance information. Once you have selected the distance matrix artifact, select “Perform Principal Coordinate Analysis (PCoA)” from the drop-down menu. The parameters will appear below the workflow diagram:

All of the parameters have been automatically selected for you just click “Add Command”. Once the command is added the workflow should appear as follows:

Click the run button to start the process of the beta diversity analysis. The view will return to the original screen, while the beta diversity analysis job runs.

Principal Coordinate Analysis

Clicking on the “pcoa (ordination_results)” (Principal Coordinate Analysis) artifact will open an interactive visualization of the similarity among your samples. Generally speaking, the more similar the samples with respect to their features, the closer they are likely to be in the PCoA ordination plot. The Emperor visualization program offers a very useful way to explore how patterns of similarity in your data associate with different metadata categories.

Once the Emperor visualization program loads, the PCoA result will look like:

You will see tabs including “Color”, “Visibility”, “Opacity”, “Scale”, “Shape”, “Axes”, and “Animations”.

Required parameters:

The feature table containing the samples over which beta diversity should be computed.:

Rarefied 11030 (BIOM)

Optional parameters:

Parameter set:

Default

In a bifurcating tree, the tips make up about 50% of the nodes in a tree. By ignoring them, specificity can be traded for reduced compute time. This has the effect of collapsing the phylogeny, and is analogous (in concept) to moving from 99% to 97% OTUs (bypass tips):

Perform variance adjustment based on Chang et al. BMC Bioinformatics 2011. Weights distances based on the proportion of the relative abundance represented between the samples at a given node under evaluation. (variance adjusted):

Phylogenetic tree:

/databases/gg/13_8/trees/97_otus_no_none.tree

The beta diversity metric to be computed. (metric):

Unweighted UniFrac

The number of workers to use. (n jobs):

1

This parameter is only used when the choice of metric is generalized unifrac. The value of alpha controls importance of sample proportions. 1.0 is weighted normalized UniFrac.

Shared with: Hide

Processing network Run

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 6 seconds or reload now

Circle status: success running error in_construction queued waiting deleting

Circle types: artifact type **deprecated**

Choose command: Principal Coordinate Analysis

Required parameters:
 Dimensions to reduce the distance matrix to. This number determines how many eigenvectors and eigenvalues are returned and influences the choice of algorithm used to compute them. By default, uses the default eigendecomposition method, SciPy's eigh, which computes all eigenvectors and eigenvalues in an exact manner. For very large matrices, this is expected to be slow. If a value is specified for this parameter, then the fast, heuristic eigendecomposition algorithm fvdi is used, which only computes and returns the number of dimensions specified, but suffers some degree of accuracy loss, the magnitude of which varies across different datasets. (number of dimensions):

The distance matrix on which PCA should be computed: distance_matrix (distance_matrix)

Optional parameters:
 Parameter set: Default

Add Command

Shared with: Hide

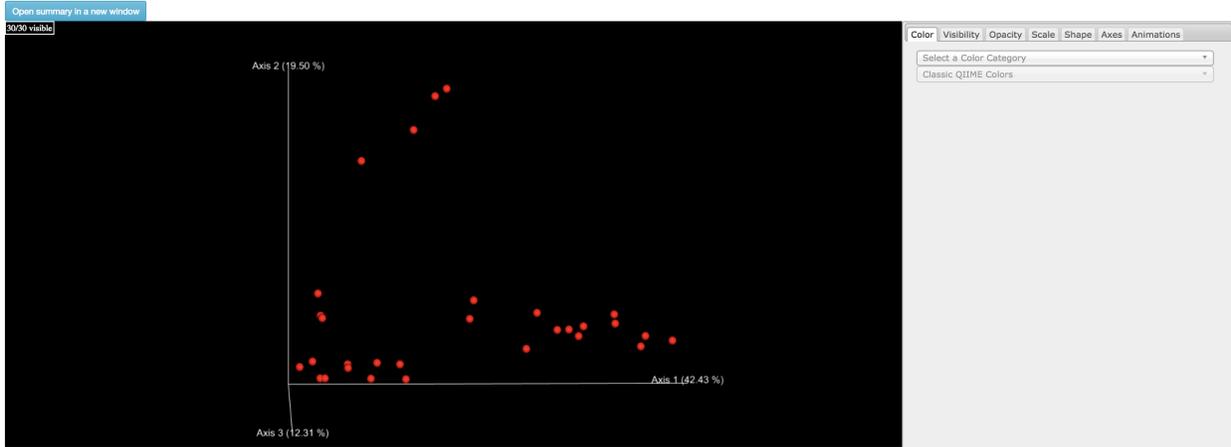
Processing network Run

Start workflow: Run

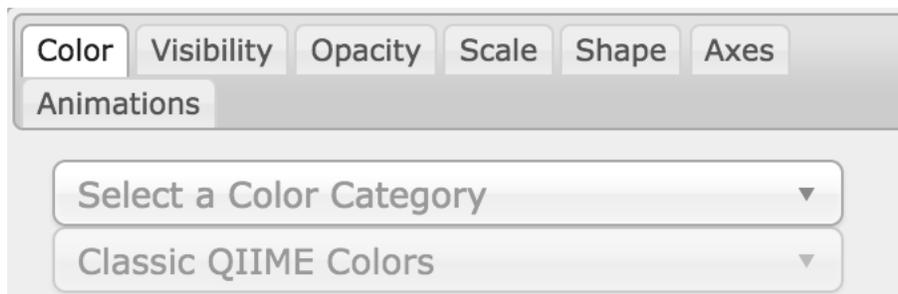
Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 0 seconds or reload now

Circle status: success running error in_construction queued waiting deleting

Circle types: artifact type **deprecated**



Under “Color” you will notice two pull-down menus:



If you click on the pull-down that says “Select a Color Category” you can select a metadata category that will color the samples by the entries in that metadata category.. Under “Classic QIIME Colors”, you can select how each group will be colored.

Under the “Visibility” tab you will notice 1 pull-down menu:



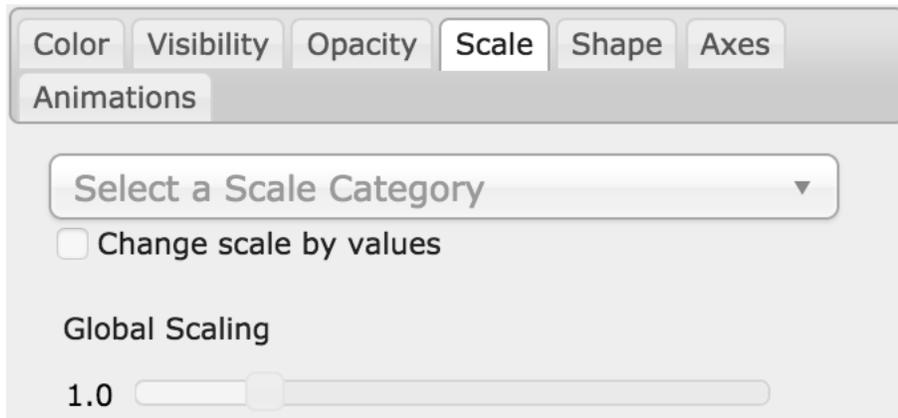
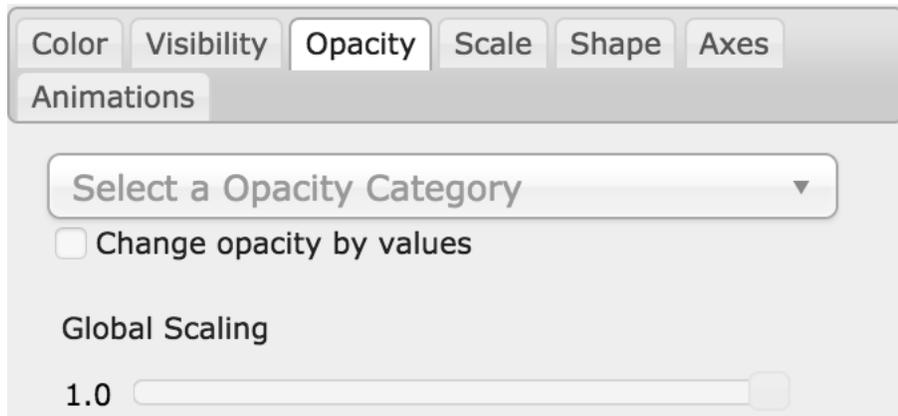
If you click on the pull-down for “Select a Visibility Category” you can select which group or groups will be displayed on the PCoA plot. Please note that if you remove the visibility of any samples this does not recalculate the distances between other samples. Removing samples can result in a plot that is misleading.

Under the “Opacity” tab you will notice 1 pull-down menu:

If you click on the pull-down for “Select an Opacity Category” you can select the categories in which the opacity will change on the PCoA plot. Once chosen, these groups will be displayed under “Global Scaling” and, when selected, you can change the opacity of each group separately. Under “Global Scaling” you can change the opacity of all of the samples.

Under the “Scale” tab you will notice 1 pull-down menu:

If you click on the pull-down for “Select a Scale Category” you can choose the grouping of your samples. Under



“Global Scaling” you can change the point size for each group separately on the PCoA plot, or change the global scaling to change the point size for all of the samples.

Under the “Shape” tab you will notice 1 pull-down menu:



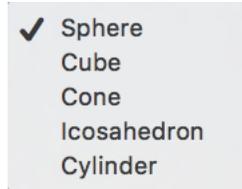
If you click on the pull-down for “Select a Shape Category” and select a metadata category, you can alter the shape of each group on the PCoA plot.

Under the “Axes” tab you will notice 5 pull-down menus:

The first 3 pull-down menus located under “Visible” allow you to change the axis that are being displayed. The “Axis and Labels Color” menu allow you to change the color of your axis and label of the PCoA. The “Background Color” menu allows you to change the color of the background of the PCoA. The % Variation Expanded graph displays how different the most dissimilar samples are by percentage for each axis that can be used.

Under the “Animations” tab you will notice 2 pull-down menus:

If you click the pull-down for “Category to sort samples” you can choose the category that you will be sorting the samples by. By selecting the pull-down menu for “Category to group sample” you can choose the category that you



will be grouping the samples by.

Beta Diversity Group Significance

Another way to study the beta diversity is by measuring the beta diversity group significance. Beta diversity group significance measures whether groups of samples are significantly different from one another using a permutation-based statistical test. Sample groups are designated by metadata variables.

If you have completed the tutorial up to this point, you can begin analysis of beta diversity group significance from one of your beta diversity distance matrices (jump down two paragraphs). Here we begin with the rarefied feature-table. To perform a beta group significance analysis, select the “rarefied table (BIOM)” artifact in the processing network and select “Process”. Select “Beta diversity” from the drop-down menu. The parameters will appear below the workflow diagram:

Several parameters have been automatically selected for you. In the field, “The beta diversity metric... (metric)”, we will specify the beta diversity distance metric to use in our analysis. Note that if you attempt to create a distance matrix that already exists in the Processing network, you will get an error stating such. For example, if you have already created a beta diversity distance matrix using the Bray-Curtis dissimilarity metric, you will have to select a unique metric here (e.g., “Aitchison distance”). In the “Phylogenetic tree” field enter “/databases/gg/13_8/trees/97_otus.tree”, and click “Add Command”.

To create the beta group significance analysis, select the “distance_matrix (distance_matrix)” artifact of interest in the Processing network, and select “Beta diversity group significance” from the drop-down menu. The parameters will appear below the workflow diagram:

Several parameters have been automatically selected for you. In the “Metadata column to use” field we will specify the category from the metadata file to be used for determining significance between groups (e.g., subject). Using the “Perform pairwise tests...” checkbox we can indicate if we would like the group significance to be run “Pairwise”, otherwise the analysis will be done across all groups (i.e., Non-pairwise). Note that for metadata variables for which there are only two groups, this distinction makes no difference. In the field, “The group significance test... (method)”, we will specify the correlation test that will be applied (e.g., [PERMANOVA \[Permutational multivariate analysis of variance\]](#)). Then click “Add Command”. Once the command is added the workflow should appear as follows:

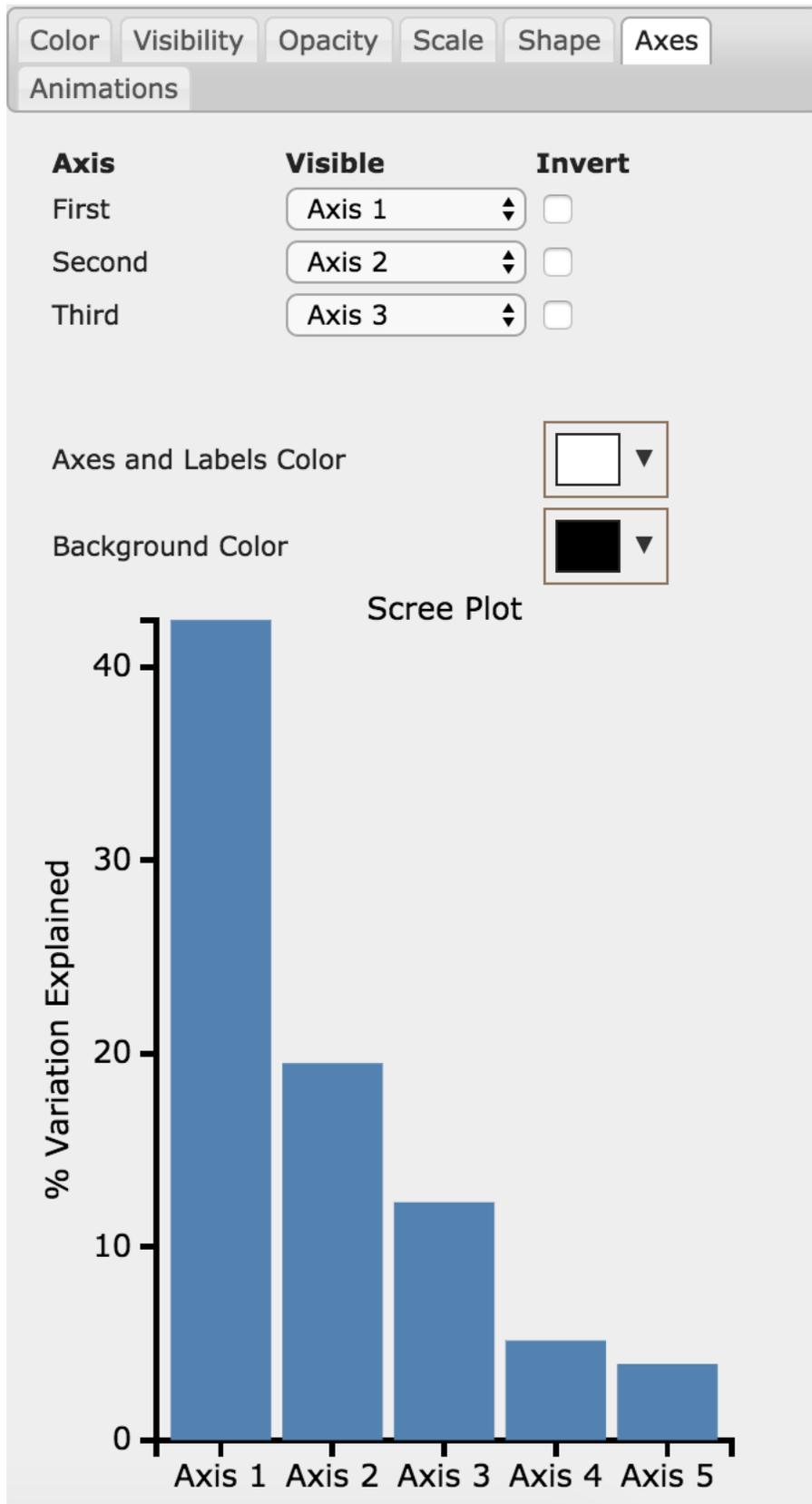
Click the run button to start the process of the beta diversity group significance analysis. The view will return to the original screen, while the beta diversity group significance analysis job runs.

Beta Group Significance Output Analysis

Once the beta group significance “visualization (q2_visualization)” artifact is chosen in the network, the beta diversity group significance Overview, which in our case shows results from the PERMANOVA (i.e., across all groups) and Group significance plots will appear:

The results from pairwise PERMANOVA tests will also be displayed if included in the analysis:

The command ‘Beta diversity group significance’ provides PERMANOVA that can be run on a single categorical metadata variable. If you instead would like to provide multiple terms in the form of an equation, you can use the command ‘adonis PERMANOVA test for beta group significance’. This latter command implements the ‘adonis’ function from the R package, vegan.



Color
Visibility
Opacity
Scale
Shape
Axes

Animations

Gradient

Category to sort samples

Trajectory

Category to group samples

⏪

▶

⏸

Speed: 1x

Radius: 1

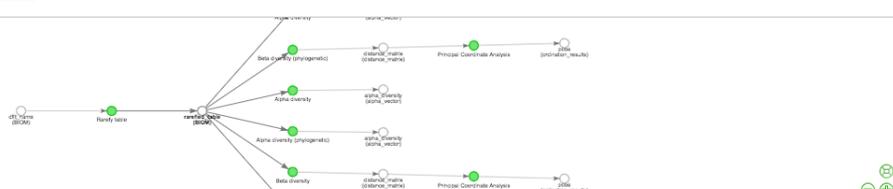
Shared with: Hide

Processing network

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 0 seconds or reload now

Circle status: success running error in_construction queued waiting deleting

Circle types: artifact type deprecated



Choose command:

Required parameters:
 The feature table containing the samples over which beta diversity should be computed:

Optional parameters:
 Parameter set:

A pseudocount to handle zero for compositional metrics. This is ignored for other metrics. (pseudocount):

The beta diversity metric to be computed. (metric):

The number of jobs to use for the computation. This works by breaking down the pairwise matrix into n jobs even slices and computing them in parallel. If >1 all CPUs are used. If 1 is given, no parallel computing code is used at all, which is useful for debugging. For n_jobs below -1, (n_cpus + 1 + n_jobs) are used. Thus for n_jobs = -2, all CPUs but one are used. (Description from sklearn.metrics.pairwise_distances) (n_jobs):

Add Command

Shared with: Hide Run

Processing network

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 1 seconds or reload now

Circle status: success running error in construction queued waiting deleting

Circle types: artifact type **deprecated**

Choose command:

Required parameters:

Matrix of distances between pairs of samples:

Optional parameters:

Parameter set:

Metadata column to use:

Perform pairwise tests between all pairs of groups in addition to the test across all groups. This can be very slow if there are a lot of groups in the metadata column. (pairwise):

The group significance test to be applied. (method):

The number of permutations to be run when computing p-values. (permutations):

Add Command

Shared with: Hide Run

Processing network

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 1 seconds or reload now

Circle status: success running error in construction queued waiting deleting

Circle types: artifact type **deprecated**

Overview

		PERMANOVA results			
method name		PERMANOVA			
test statistic name		pseudo-F			
sample size		30			
number of groups		2			
test statistic		11.2253			
p-value		0.001			
number of permutations		999			

Pairwise permanova results

[Download CSV](#)

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
Volunteer 3	Volunteer 6	30	999	11.225262	0.001	0.001

1.12.5 Filtering Data

Using Qiita you can also filter your data. This allows you to filter out samples.

To filter the data, select the “rarefied table (BIOM)” artifact in the processing network and select “Process”. Then select “Filter samples from table” from the drop-down menu. The parameters will appear below the workflow diagram:

Several parameters have been automatically selected for you. In the “SQLite WHERE-clause” field we are filtering out all samples except for certain samples. In this case we wanted to filter out all samples except those in which `subject = 'Volunteer 3'`, and click “Add Command”. If instead you want to filter out all of Volunteer 3’s samples, either use the SQLite WHERE-clause above while also checking the box “If true, the samples selected... will be excluded”, or alternatively use the SQLite WHERE-clause `subject != 'Volunteer 3'`, and click “Add Command”. If you want to filter for samples containing an apostrophe, write it out in the following format: `subject = \"Volunteer 3's samples\"`. **Keep in mind that all fields are case sensitive.**

Click “Run” to execute the filtering process.

An example of how you can use filtering in your analysis is explained in the following “Filtered Unweighted UniFrac Analysis” section.

Filtered Unweighted UniFrac Analysis

By filtering, you can perform unweighted UniFrac analysis but this time without certain sample.

After filtering your data (shown in the previous “Filtering Data” section), you can perform a beta diversity analysis by selecting the “filtered_table (BIOM)” in the Processing network and clicking “Process”. Select “Beta diversity (phylogenetic)” from the drop-down menu. The parameters will appear below the workflow diagram:

All of the parameters have been automatically selected for you, just click “Add Command”.

To create a principal coordinates plot of the unweighted Unifrac distance matrix, select the “distance_matrix (distance_matrix)” artifact that you set up above, and select “Perform Principal Coordinate Analysis (PCoA)” from the drop-down menu. The parameters will appear below the workflow diagram:

Most of the parameters have been automatically selected for you, but you will need to select the phylogenetic tree to use. Click on the dropdown next to “Phylogenetic tree:” and select “/databases/gg/13_8/trees/97_otus_no_none.tree” and then click “Add Command”. Once the command is added the workflow should appear as follows:

Click the run button to start the process of the beta diversity analysis. The view will return to the original screen, while the beta diversity analysis job runs.

1.12.6 Altering Workflow Analysis Names

To alter the name of a result, click the artifact then use the edit button on the processing network page.

This will cause a window to pop-up where you can input the name you’d like to replace it with.

1.13 Analysis of Deblur Processed Data

Creating an analysis of your deblurred data is virtually the same as the process for the Closed Reference data, but there are a few important differences to note.

First, because the Deblur process creates two separate BIOM tables, you should make a note of the specific object ID number for the specific artifact you want to use. In my case, that’s ID 33331, the deblurred table with “only-16S” reads.

The specific ID for your table will be unique. You can use it to select the correct table for analysis.

Choose command:

Filter samples from table [filter_samples]

Required parameters:

The feature table from which samples should be filtered.:

Rarefied 11030 (BIOM)

Optional parameters:

Parameter set:

Default

If true, the samples selected by `metadata` or `where` parameters will be excluded from the filtered table instead of being retained. (exclude ids):

SQLite WHERE clause specifying sample metadata criteria that must be met to be included in the filtered feature table. If not provided, all samples in `metadata` that are also in the feature table will be retained. (where):

The maximum number of features that a sample can have to be retained. If no value is provided this will default to infinity (i.e., no maximum feature filter will be applied). (max features):

The maximum total frequency that a sample can have to be retained. If no value is provided this will default to infinity (i.e., no maximum frequency filter will be applied). (max frequency):

The minimum number of features that a sample must have to be retained. (min features):

The minimum total frequency that a sample must have to be retained. (min frequency):

Add Command

Required parameters:

The feature table containing the samples over which beta diversity should be computed.:

Rarefied 11030 (BIOM)

Optional parameters:

Parameter set:

Default

In a bifurcating tree, the tips make up about 50% of the nodes in a tree. By ignoring them, specificity can be traded for reduced compute time. This has the effect of collapsing the phylogeny, and is analogous (in concept) to moving from 99% to 97% OTUs (bypass tips):

Perform variance adjustment based on Chang et al. BMC Bioinformatics 2011. Weights distances based on the proportion of the relative abundance represented between the samples at a given node under evaluation. (variance adjusted):

Phylogenetic tree:

/databases/gg/13_8/trees/97_otus_no_none.tree

The beta diversity metric to be computed. (metric):

Unweighted UniFrac

The number of workers to use. (n jobs):

1

This parameter is only used when the choice of metric is generalized unifrac. The value of alpha controls importance of sample proportions. 1.0 is weighted normalized UniFrac.

Shared with: Hide Run

Processing network

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 1 seconds or reload now

Circle status: success running error in_construction queued waiting deleting

Circle types: artifact type **deprecated**

Choose command:

Required parameters:

Dimensions to reduce the distance matrix to. This number determines how many eigenvectors and eigenvalues are returned, and influences the choice of algorithm used to compute them. By default, uses the default eigendecomposition method, SciPy's eigh, which computes all eigenvectors and eigenvalues in an exact manner. For very large matrices, this is expected to be slow. If a value is specified for this parameter, then the fast, heuristic eigendecomposition algorithm `fvml` is used, which only computes and returns the number of dimensions specified, but suffers some degree of accuracy loss, the magnitude of which varies across different datasets. (number of dimensions):

The distance matrix on which PCOA should be computed:

Optional parameters:

Parameter set:

Add Command

Shared with: Hide Run

Processing network

Start workflow: Run

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 12 seconds or reload now

Circle status: success running error in_construction queued waiting deleting

Circle types: artifact type **deprecated**

Edit name Process Delete Show processing information

Updating artifact 33455 name

Introduce the new name:

 Update

dfit_name (ID: 33331) Visibility: sandbox  Edit  Process  Delete

Jobs using this data

Available files:

 12694_analysis_16S_deblurworkflowdeblurv102_referencehitbiom.biom (biom)

 12694_analysis_16S_deblurworkflowdeblurv102_referencehitbiom.biom.html (html summary)

Open summary in a new window

Number of samples: 23
Number of features: 755
Minimum count: 1585
Maximum count: 1915
Median count: 1712
Mean count: 1734

Qiita employs Deblur on trimmed sequences (UC San Diego studies are typically processed with 90, 100, and 150 nt) with no minimum read threshold count, followed by insertion of output sequences into the latest (Aug 2013) 99% OTU tree from GreenGenes using SATÉ-Enabled Phylogenetic Placement (SEPP) as part of the QIIME2 [q2-fragment-insertion plugin](#).

In addition, Deblur in Qiita uses a reduced threshold of 1 for sequence abundance across all samples, rather than the default value of 10 used in Deblur outside of Qiita. The single read threshold was chosen (rather than the default 10 read threshold) to support the best practice of setting a custom minimum read threshold that is suitable to your single-study analysis or meta-analysis. This can be done using the command, ‘Filter features from table’.

1.14 Creating a Meta-Analysis

One of the most powerful aspects of Qiita is the ability to compare your data with hundreds of thousands of samples from across the planet. Right now, there are over 230,000 samples publicly available for you to explore:

(You can get up-to-date statistics by clicking “Stats” under the “More Info” option on the top bar.)

Creating a meta-analysis is just like creating an analysis, except you choose data objects from multiple studies. Let’s start creating a meta-analysis by adding the Closed-Reference OTU table we generated in the *processing tutorial* to a new analysis. Select “Create new analysis” from the Analysis drop-down menu. Find the study you created for the *processing tutorial* under “Your studies”, and click the green circle to show artifacts from that study. There should be three artifacts, one “Pick closed-reference OTUs” and two “Deblur” feature-tables. Select “Per artifact” for the “Pick closed-reference OTUs” to expand the list of artifacts associated - in our case there is only one. Select “Add” to add this feature-table to our meta-analysis.

Next, we’ll look for some additional data to compare against.

You noticed the “Other Studies” table below “Your Studies” when adding data to the analysis. (Sometimes this takes a while to load - please be patient.) These are publicly available data for you to explore, and each should have processed data suitable for comparison to your own (i.e., same trim length and method for calling features, etc.).

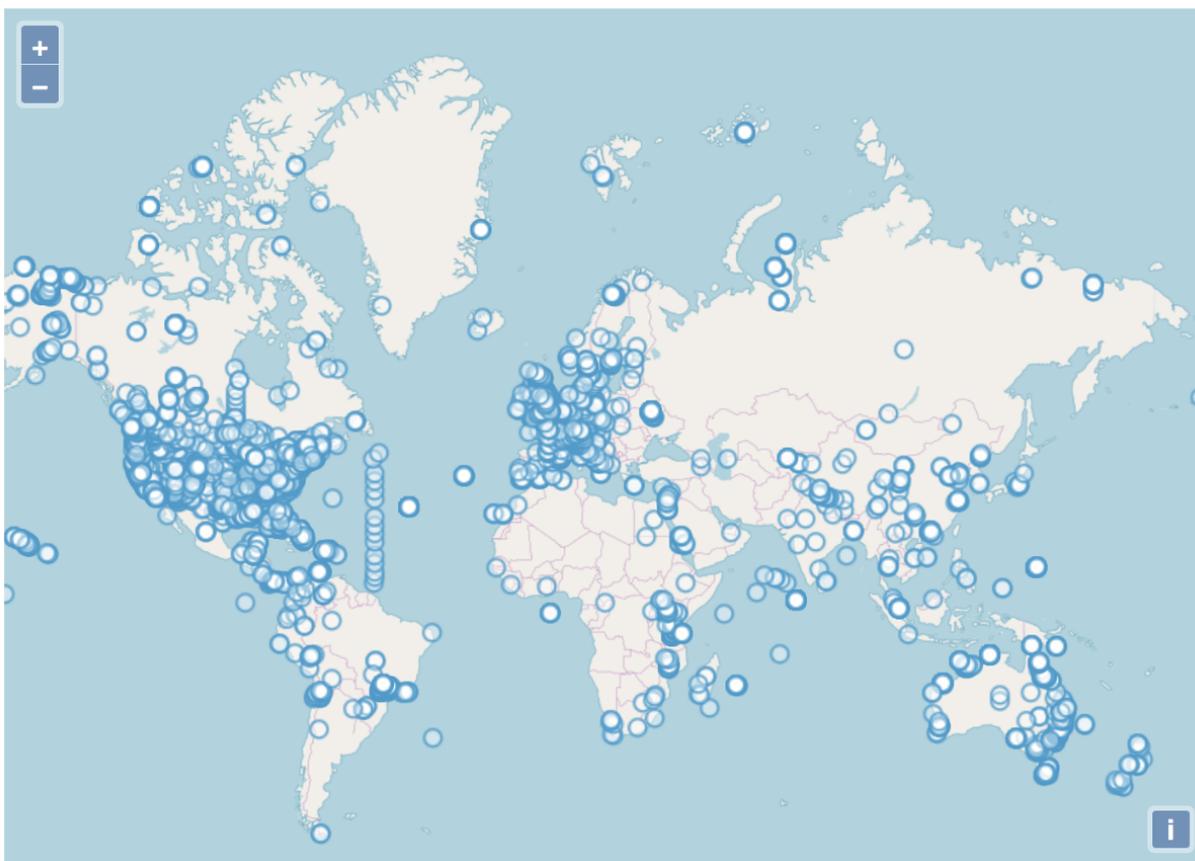
There are a couple tools provided to help you find useful public studies.

First, there are a series of “tags” listed at the top of the window:

There are two types of tags: admin-assigned (yellow), and user-assigned (blue). You can tag your own study with any tag you’d like, to help other users find your data. For some studies, Qiita administrators will apply specific reserved

Generated on: 07-03-19 00:48:37

Studies	Samples	Users	Jobs
<i>public</i> : 484	<i>public</i> : 258,437		
<i>private</i> : 268	<i>private</i> : 188,592		
<i>sandbox</i> : 1,696	<i>sandbox</i> : 368,063	6,563	226,243
<i>submitted to EBI</i> : 313	<i>submitted to EBI</i> : 195,161		
	<i>submitted to EBI (prep)</i> : 149,768		



Filter studies by tags: (Admin, User)

Select tags for filtering

tags to help identify particularly relevant data. The “GOLD” tag, for example, identifies a small set of highly-curated, very well-explored studies. If you click on one of these tags, all studies not associated with that tag will disappear from the tables.

Second, there is a search field that allows you to filter studies in real time. Try typing in the name of a known PI, or a particular study organism – the thousands of publicly available studies will be filtered down to something that is easier to look through.

Filter results by column data (Title, abstract, PI, etc):

Let’s try comparing our data (i.e., from 14 human skin samples) to the “Global Gut” dataset of human microbiomes from the US, Africa, and South America from the study “Human gut microbiome viewed across age and geography” by Yatsunenکو et al. We can search for this dataset using the DOI from the paper: 10.1038/nature11053. Once the study appears, click the green circle as above to view the artifacts from that study; there should be several. Select “Per artifact” for the table that was processed the same way as yours above - “Pick closed-reference OTUs... Trimming (length:100)”.

Public Studies

Expand for analysis (artifact count)	Title	Study ID	Samples	Principal Investigator	Publications	Qiita EBI submission																					
7	 Human gut microbiome differentiation viewed across cultures, ages and families illumina	850	528	Jeff Gordon	22699611, 25974302, 10.1038/nature11053, 10.1016/j.chom.2015.04.009	PRJEB3079 (submitted)																					
<table border="1"> <thead> <tr> <th>Artifacts</th> <th>Processing method</th> <th>Data type</th> </tr> </thead> <tbody> <tr> <td>Add all</td> <td>Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: all.biom) Trimming (length: 100)</td> <td>16S (V4)</td> </tr> <tr> <td>Add all</td> <td>Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: all.biom) Trimming (length: 90)</td> <td>16S (V4)</td> </tr> <tr> <td>Add all</td> <td>Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: reference-hit.biom) Trimming (length: 100)</td> <td>16S (V4)</td> </tr> <tr> <td>Add all</td> <td>Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: reference-hit.biom) Trimming (length: 90)</td> <td>16S (V4)</td> </tr> <tr> <td>Add all</td> <td>Per Artifact (1) Pick closed-reference OTUs (reference-seq/ /databases/gg/13_8/rep_set/97_otus.fasta) Split libraries FASTQ</td> <td>16S (V4)</td> </tr> <tr> <td>Add all</td> <td>Per Artifact (1) Pick closed-reference OTUs (reference-seq/ /databases/gg/13_8/rep_set/97_otus.fasta) Trimming (length: 100)</td> <td>16S (V4)</td> </tr> </tbody> </table>							Artifacts	Processing method	Data type	Add all	Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: all.biom) Trimming (length: 100)	16S (V4)	Add all	Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: all.biom) Trimming (length: 90)	16S (V4)	Add all	Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: reference-hit.biom) Trimming (length: 100)	16S (V4)	Add all	Per Artifact (1) Deblur (Reference phylogeny for SEPP: Greengenes_13.8, BIOM: reference-hit.biom) Trimming (length: 90)	16S (V4)	Add all	Per Artifact (1) Pick closed-reference OTUs (reference-seq/ /databases/gg/13_8/rep_set/97_otus.fasta) Split libraries FASTQ	16S (V4)	Add all	Per Artifact (1) Pick closed-reference OTUs (reference-seq/ /databases/gg/13_8/rep_set/97_otus.fasta) Trimming (length: 100)	16S (V4)
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<table border="1"> <thead> <tr> <th></th> <th>Name</th> <th>Samples in Prep Info</th> <th>Files</th> </tr> </thead> <tbody> <tr> <td>Add</td> <td>OTU table (45113 - 2018-02-05 19:35:28)</td> <td>528</td> <td>otu_table.biom</td> </tr> </tbody> </table>								Name	Samples in Prep Info	Files	Add	OTU table (45113 - 2018-02-05 19:35:28)	528	otu_table.biom													
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Add	OTU table (45113 - 2018-02-05 19:35:28)	528	otu_table.biom																								
Add all	Per Artifact (1) Pick closed-reference OTUs (reference-seq/ /databases/gg/13_8/rep_set/97_otus.fasta) Trimming (length: 90)			16S (V4)																							

Add the closed reference OTU table from this study to your analysis. You should now be able to click the green analysis icon in the upper right and see both your own OTU table and the public study OTU table in your analysis staging area:

You can now click “Create Analysis” just as before to begin specifying analysis steps. This time, let’s jump straight to calculating beta diversity. To try something new, rather than rarefy prior to calculating beta diversity as above, we can do it simultaneously and using an iterative rarefaction process as to get a better estimate of real differences among samples. Select “Beta diversity rarefaction” from the drop-down menu. We will only change a few of the parameters: In the field, “The beta diversity metric... (metric)”, select “Unweighted UniFrac”. For “The total frequency that each sample should be rarefied to... (sampling depth)”, enter a rarefaction depth of 2118 - the observed minimum number of features in your *processing tutorial* data. Then click “Add command”, and “Run”.

Because you’ve now expanded the number of samples in your analysis by more than an order of magnitude, this step will take a little longer to complete. But when it does, a PCoA ordination plot will be generated immediately and you will be able to use Emperor to explore the samples in your test dataset to samples from around the world!

Note that in this plot, each sample point has a cloud that represents the iterative rarefaction process in this analysis. When samples are colored by the metadata variable “geo_loc_name” you can see that the “unknown” or human skin samples from our *processing tutorial* fall to one end of the distribution of gut samples from the larger Yatsunenکو study.

Shotgun metagenomics is an alternative approach to amplicon sequencing that provides information at the genomic

Selected Samples

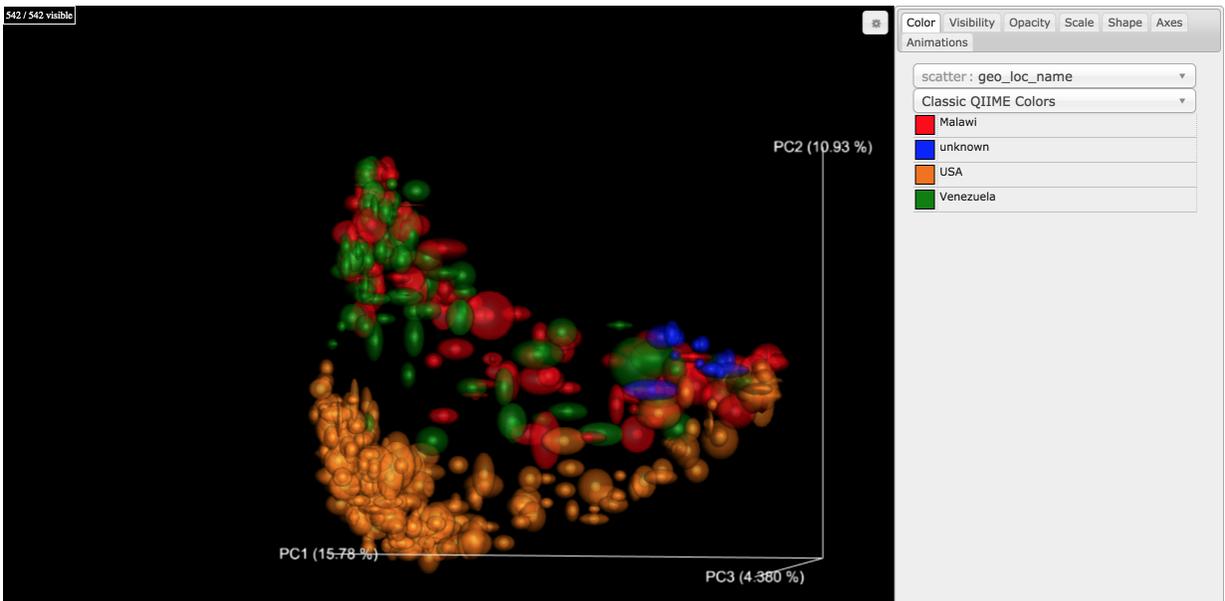
Create Analysis Clear Selected

CMI workshop analysis

Processed Data						
id	Datatype	Processed Date	Algorithm	Parameters	Samples	
33297	16S	2017-08-09 15:47:22.486078	None		30	Show/Hide samples Remove

Human gut microbiome differentiation viewed across cultures, ages and families illumina

Processed Data						
id	Datatype	Processed Date	Algorithm	Parameters	Samples	
2458	16S	2015-10-15 15:14:56.647454	QIIME (Pick closed-reference OTUs)	similarity: 0.97 reference_name: Greengenes sortmerg_n_value: 1 sortmerg_max_pos: 10000 input_data: 171 threads: 5 sortmerg_coverage: 0.97 reference_version: 13.8-97	528	Show/Hide samples Remove



level, rather than for single loci such as 16S, 18S, and the fungal ITS region. In addition to providing finer resolution with respect to taxonomic classification, metagenomics provides functional data that allows for more comprehensive analyses of microbial communities. We are currently working to make available additional metagenomic tools in Qiita. Below we describe how to use [Woltka](#) to produce taxonomic profiles for shotgun metagenomic data, and then move to common downstream analyses of alpha- and beta-diversity.

1.15 Processing Shotgun Metagenomic Data

We will start by creating a new study with shotgun metagenomic data for 23 human fecal samples subsetted from the American Gut Project. As we did for amplicon data, go to the study drop-down menu and select ‘Create Study’. Complete the necessary fields similar as shown below.

<p>Study Title Study titles may only contain ASCII characters</p>	<input type="text" value="CMI Tutorial - Shotgun Metagenomics"/>	*
<p>Study Alias</p>	<input type="text" value="CMI Tutorial - Shotgun Metagenomics"/>	*
<p>DOI Just values, no links, comma separated values</p>	<input type="text"/>	
<p>PUBMED ID Just values, no links, comma separated values</p>	<input type="text"/>	
<p>Study Abstract</p>	<input type="text" value="An example study working through shotgun metagenomics data for the CMI tutorial"/>	*
<p>Study Description</p>	<input type="text" value="A subset of the American Gut Project including 23 human fecal samples"/>	*
<p>Principal Investigator</p>	<p>Can't find the person you're looking for? Add a person</p> <input type="text" value="Qiita Tutorial, UCSD"/>	*
<p>Lab Person</p>	<input type="text" value="Qiita Tutorial, UCSD"/>	
<p>Analytical Notes Any relevant information about the samples or the processing that other users should be aware of (e.g. problematic samples, explaining certain metadata columns, etc) - renders as markdown</p>	<input type="text"/>	
<p>Environmental Packages You can select multiple entries by control-clicking (mac: command-clicking)</p>	<div style="border: 1px solid #ccc; padding: 5px;"> <ul style="list-style-type: none"> air built environment host-associated human-amniotic-fluid human-associated human-blood <li style="background-color: #007bff; color: white;">human-gut human-oral human-skin human-urine human-vaginal microbial mat/biofilm miscellaneous natural or artificial environment plant-associated sediment soil wastewater/sludge water </div>	*
<p>Event-Based Data</p>	<input type="text" value="No timeseries"/>	

Once the study has been created, click on the study name in the green banner to go to the study page. Click on ‘Upload Files’, and upload the contents of the [shotgun data](#).

Uploading files for: CMI tutorial - Shotgun Metagenomics (CMI tutorial - Shotgun Metagenomics)

Currently we can process (fastq, fastq.gz, txt, tsv, sff, fasta, fna, qual, biom, xlsx):

- Note that '.zip' files can not be processed.
- Note that '.fasta' and '.fna' files require '.qual' files for submission.
- Note that '.txt.' files must be tab separated and require the extension '.txt'.

Per our Terms of Condition for use, you certify that the files you are uploading do not contain:

- protected health information within the meaning of 45 Code of Federal Regulations part 160 and part 164, subparts A and E; see [checklist](#)
- whole genome sequencing data for any human subject; [HMP human sequence removal protocol](#)
- any data that is copyrighted, protected by trade secret, or otherwise subject to third party proprietary rights, including privacy and publicity rights, unless you are the owner of such rights or have permission from the rightful owner(s) to transfer the data and grant it to Qiita, on behalf of the Regents of the University of California, all of the license rights granted in our [Terms](#).

[<< Go to study description](#)

Upload via Local Machine

Upload files locally (max file size: 2.0 TB)

Drop files here to upload or [select from your computer](#)

Upload via Remote Server (ADVANCED)

Files

Select All Unselect All

Delete selected files

[<< Go to study description](#)

Thank you for using Qiita. Citing Qiita?

Questions? qiita.help@gmail.com; don't forget to add your study or analysis id.
Read our [terms and conditions](#).

Once the files are uploaded, click on 'Sample Information' on the left side of the screen and select the sample information file 'qiita_shotgun_metagenomic_tutorial_sample_info.txt' and click 'Create'.

Study Information

Sample Information

Upload Files

Issues with downloads or opening the downloaded zip?

No preparation information has been added yet

CMI Tutorial - Shotgun Metagenomic - ID 13633

CMI Tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

Sample Information

Select sample information file:

qiita_shotgun_metagenomic_tutorial_sample_info.txt Direct upload file (< 2MB)

If uploading a QIIME mapping file, select the data type of the prep information:

Metagenomic

Create

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Once complete, you may need to refresh your screen to see the added option of 'Add New Preparation'. Click 'Add New Preparation' and give the preparation a name of your choosing. Select the prep info file, 'qiita_shotgun_metagenomic_tutorial_prep_info.txt', and select 'metagenomic' in the first drop-down menu, and 'WGS' (whole genome sequencing) in the second. Then click 'Create New Preparation'.

This will create a new preparation under 'Data Types'. You can click on the new preparation which will show the name of your preparation. You can click on this preparation or the page will automatically open the preparation once you create it. Of the new options you should select 'per_sample_FASTQ - None', and give the files a name. Then click 'Add Files'.

Once your sequence files are added, you should see a processing network with an artifact representing the sequence data.

We will now process the sequence data using Woltka to create taxa-abundance tables at three hierarchical levels: phylum, family, and genus. Woltka (Web of Life Toolkit App), is a bioinformatics package for shotgun metagenome data analysis that we will use for taxonomic classification of sequences using a reference database. Woltka uses [Web of Life \(WoL\)](#) to align to references.

- Study Information
- Sample Information
- Upload Files
- Add New Preparation

[Issues with downloads or opening the downloaded zip?](#)

No preparation information has been added yet

CMI Tutorial - Shotgun Metagenomic - ID 13633

CMI Tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

Add a new preparation file: (* Required fields)

Name:

Select file: *

Select data type: *

Select Investigation Type: Unsure? [Check](#) Not sure what to select? [Check](#)

[Create New Preparation](#)

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Analysis ▾ Study ▾ Admin ▾ redbiom Qlmp Help ▾ More Info ▾
Welcome justinparkshaffer@gmail.com Log Out (15)

- Study Information
- Sample Information
- Upload Files
- Add New Preparation

[Issues opening the downloaded zip?](#)

Data Types (click on the tabs)

Metagenomic

- per-sample FASTQ - ID 6216 - sandbox
- None - ID None
- None

CMI tutorial - Shotgun Metagenomics - ID 12280

CMI tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 6216 (Metagenomic)
[Edit name](#)
[Prep info](#)
[QIIME map](#)
[Delete](#)

Summary
Processing

No files attached to this preparation

Select type: per_sample_FASTQ - None

Add a name for the file: Shotgun data

Now, you can import files from other studies Choose an artifact to import...

or click and drag your uploaded files to the correct file type

Please make sure that the correct files are in the correct column.

Note: the system will try to auto select the files based on run_prefix, if that doesn't work, either the type you selected doesn't support the use of run_prefix or the run_prefix is wrong

Available Files	raw forward seqs	raw reverse seqs
F32615.shotgun.R1.fastq.gz		
F27690.shotgun.R1.fastq.gz		
F6947.shotgun.R1.fastq.gz		
F30258.shotgun.R1.fastq.gz		
F12313.shotgun.R1.fastq.gz		
F30083.shotgun.R1.fastq.gz		
F18425.shotgun.R1.fastq.gz		
F27689.shotgun.R1.fastq.gz		
F30116.shotgun.R1.fastq.gz		
F30231.shotgun.R1.fastq.gz		
F30313.shotgun.R1.fastq.gz		
F31151.shotgun.R1.fastq.gz		

[Add files](#)

This prep template is currently being updated

Study Information
Sample Information
Upload Files
Add New Preparation
Issues opening the downloaded zip?

Data Types (click on the tabs)
Metagenomic

CMI tutorial - Shotgun Metagenomics - ID 12280

CMI tutorial - Shotgun Metagenomics
Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 6216 (Metagenomic) Edit name Prep info QIIME map

Summary Processing Hide

Processing network Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 8 seconds or reload [now](#)

Job status (circles): success running error in construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Shotgun data (per_sample_FASTQ)

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1.16 Running Woltka on human-filtered per-sample FASTQ data

All shotgun metagenomic data is filtered of human reads prior to being uploaded to Qiita. If you have additional, non-human host reads that you would like to filter from your data, you can see if they are available under the command, 'QC_Filter'. To proceed with processing the data using Woltka, click on the per-sample FASTQ artifact named 'Shotgun data' and click 'Process'.

From the dropdown menu, select 'Woltka'.

Change the 'Parameter set' to 'wol' and click 'Add Command'.

You should see the command added to the processing network:

Click 'Run' to run the command.

Once the command has completed, click on the 'Taxonomic Predictions - species' artifact.

Examine the table summary to see the number of samples that were retained, and the number of features that were assembled.

In the next section we will begin an analysis starting with this feature-table.

1.17 Analysis of Shotgun Metagenomic Data

We will begin our analysis with the Qiita study you created as part of the 'Shotgun metagenomics processing' tutorial. Start by viewing the processing network for the Metagenomic preparation in that study:

Lets take full advantage of the resolution that metagenomic data provides, and analyze our data at the species-level. Select the 'Taxonomic Predictions - species' artifact in the processing network.

Click the button under the processing network labeled 'Add to analysis'. A menu will appear in the top right-hand corner of the screen, and the icon it is derived from will change to be colored green as shown below.

Qiita Analysis Study Admin redbiom Qlump Help More Info - Welcome justinparkshaffer@gmail.com Log Out (15)

Study Information
Sample Information
Upload Files
Add New Preparation
[Issues with downloads or opening the downloaded zip?](#)

Data Types (click on the tabs)
Metagenomic

CMI tutorial - Shotgun Metagenomics - ID 12280

CMI tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 6216 (Metagenomic) [Edit name](#) [Prep info](#) [QIIME map](#)

Summary Processing

Processing network [Hide](#)

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 4 seconds or reload now

Job status (circles): success running error in construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Shotgun data (ID: 67936) Visibility: sandbox [Edit name](#) [Process](#) [Delete](#) [Request approval](#) [Submit to EBI](#)

Available files: [Show/Hide](#)

Currently, no summary exists.

[Generate summary](#)

Thank you for using Qiita. Citing Qiita?
Questions? qiita.help@gmail.com; don't forget to add your study or analysis id.
[Read our terms and conditions.](#)

Study Information
Sample Information
Upload Files
Add New Preparation
[Issues with downloads or opening the downloaded zip?](#)

Data Types (click on the tabs)
Metagenomic

CMI Tutorial - Shotgun Metagenomic - ID 13633

CMI Tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 10345 (Metagenomic) [Edit name](#) [Prep info](#) [Sample info \(only this prep\)](#)

Sample Listing Summary Processing

Processing network [Hide](#)

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 6 seconds or reload now

Job status (circles): success running error in construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command:

- Choose command...
- Adapter and host filtering
- Sortmeza v2.1b
- Split libraries FASTQ
- Wobias v0.1.1

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- Study Information
- Sample Information
- Upload Files
- Add New Preparation

Issues with downloads or opening the downloaded zip?

Data Types (click on the tabs)

Metagenomic

per-sample FASTQ - ID 10345 - sandbox
None - ID None
None

CMI Tutorial - Shotgun Metagenomic - ID 13633

CMI Tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 10345 (Metagenomic) [Edit name](#) [Prep info](#) [Sample info \(only this prep\)](#)

[Deprecate](#) [Delete](#)

[Sample Listing](#) [Summary](#) [Processing](#) [Hide](#)

Processing network

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 11 seconds or reload now

Job status (circles): success running error in construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Taxonomic Predictions - species (ID: 110705) Visibility: sandbox [Edit name](#) [Add to Analysts](#) [Delete](#) [Request approval](#) [Generate Download Link](#) [Show processing information](#)

Available files: [Show/Hide](#)

[Open summary in a new window](#)

[Overview](#) [Interactive Sample Detail](#) [Feature Detail](#)

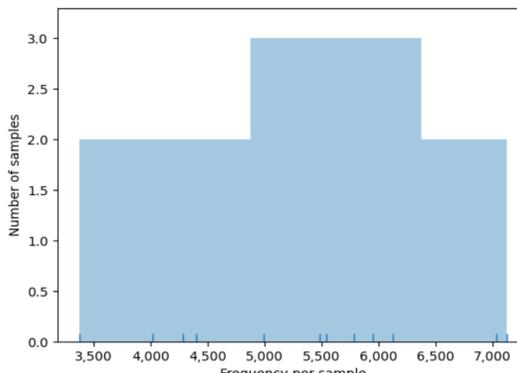
Table summary

Metric	Sample
Number of samples	12
Number of features	679
Total frequency	64,117

Frequency per sample

	Frequency
Minimum frequency	3,370.0
1st quartile	4,367.75
Median frequency	5,515.0
3rd quartile	5,998.25
Maximum frequency	7,132.0
Mean frequency	5,343.083333333333

[Frequency per sample detail \(csv | html\)](#)



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Qiita Analysis ▾ Study ▾ Admin ▾ redbiom Qiimp Help ▾ More Info ▾ Welcome justinparkshaffer@gmail.com Log Out (15)

Study Information
Sample Information
Upload Files
Add New Preparation
Issues opening the downloaded zip?

Data Types (click on the tabs)
Metagenomic

CMI tutorial - Shotgun Metagenomics - ID 12280

CMI tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 6216 (Metagenomic) [Edit name](#) [Prep info](#) [QIIME map](#)

Summary Processing **Hide**

Processing network

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 9 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Taxonomic Predictions - species (ID: 67941) Visibility: sandbox [Edit name](#) [Add to Analysis](#) [Delete](#) [Request approval](#)

[Show processing information](#)

Qiita Analysis ▾ Study ▾ Admin ▾ redbiom Qiimp Help ▾ More Info ▾ Welcome justinparkshaffer@gmail.com Log Out (15)

Study Information
Sample Information
Upload Files
Add New Preparation
Issues opening the downloaded zip?

Data Types (click on the tabs)
Metagenomic

CMI tutorial - Shotgun Metagenomics - ID 12280

CMI tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 6216 (Metagenomic) [Edit name](#) [Prep info](#) [QIIME map](#)

Summary Processing **Hide**

Processing network

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 6 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Taxonomic Predictions - species (ID: 67941) Visibility: sandbox [Edit name](#) [Add to Analysis](#) [Delete](#) [Request approval](#)

[Show processing information](#)

Available files: [Show/Hide](#)

12 samples selected.

Study Information
Sample Information
Upload Files
Add New Preparation
Issues opening the downloaded zip?

Data Types (click on the tabs)
Metagenomic

SELECTED SAMPLES
1 Studies
Processed Data
12 Samples

CMI tutorial - Shotgun Metagenomics - ID 12280

CMI tutorial - Shotgun Metagenomics

Do you want to submit to EBI-ENA? Review the [submission checklist](#)

per-sample FASTQ - ID 6216 (Metagenomic) [Edit name](#) [Prep info](#) [QiIME map](#)

Summary Processing

Processing network [Hide](#)

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 0 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Shotgun data (per_sample_FASTQ) → Shogun → Shogun Alignment Profile (BIOM)
Shogun → Taxonomic Predictions - shylum (BIOM)
Shogun → Taxonomic Predictions - genus (BIOM)
Shogun → Taxonomic Predictions - emperline

Click the green icon to proceed with creating the analysis.

Click 'Create Analysis'. This will bring up a window where you must give the analysis a name and description of your choosing. Click 'Create Analysis'.

Once the analysis has been created, you will see a processing network with a single artifact named 'dft name' - this is the species-level feature-table that we brought over.

Select the artifact and click 'Process', then select 'Visualize taxonomy with an interactive bar plot', and click 'Add Command'.

This will create new artifacts in the analysis processing network as shown below.

Let's add some additional commands before running our job. Select the species-level feature-table again but this time select the command 'Core diversity metrics (non-phylogenetic)'. Enter 1500 into the last field - this is the rarefaction depth - or number of reads that each sample will be subsampled to, and then 'Add Command'.

Now we are ready to run the analysis. Click 'Run' above the processing network. Once the analyses are complete, you can explore the taxonomy barplots and emperor visualizations of principle coordinates analyses of beta-diversity, as well as perform additional analyses such as alpha correlation or comparisons.

1.18 Notes on metabolomics

Edited for the Dorrestein Lab by Louis-Felix Nothias, Daniel Petras and Ricardo Silva on December 2016. Last edit on April 2017.

1.18.1 About the metabolomics workshop

In the following documentation, we are providing step-by-step tutorials to perform basic analysis of liquid chromatography coupled to tandem mass spectrometry data (LC-MS/MS). These tutorials can be employed to process untargeted metabolomics data, such as those generated for seed funded project.

Selected Samples

CMI tutorial - Shotgun Metagenomics

Processed Data

id	Datatype	Processed Date	Algorithm	Parameters	Samples selected from Prep Info
67941	Metagenomic	2019-02-27 15:27:28.439764	qp-shogun (Shogun)	Aligner tool: utree Input: 67936 Number of threads: 5 Database: /opt/genome/qiita_dbs/shogun/rep82	12

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 Questions? qiita.help@gmail.com; don't forget to add your study or analysis id.
[Read our terms and conditions.](#)

Selected Samples

CMI tutorial - Shotgun Meta

Processed Data

id	Datatype	Processed Date	Algorithm	Parameters	Samples selected from
67941	Metagenomic	2019-02-27 15:27:28.439764	qp-shogun (Shogun)	Aligner tool: utree Input: 67936 Number of threads: 5 Database: /opt/genome/qiita_dbs/shogun/rep82	12

Create new analysis

Analysis name

Description

[Create analysis](#)

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[Read our terms and conditions.](#)

CMI tutorial - Shotgun Metagenomics Analysis - ID 21954 (Private) [Make analysis public](#)

CMI tutorial - Shotgun Metagenomics Analysis

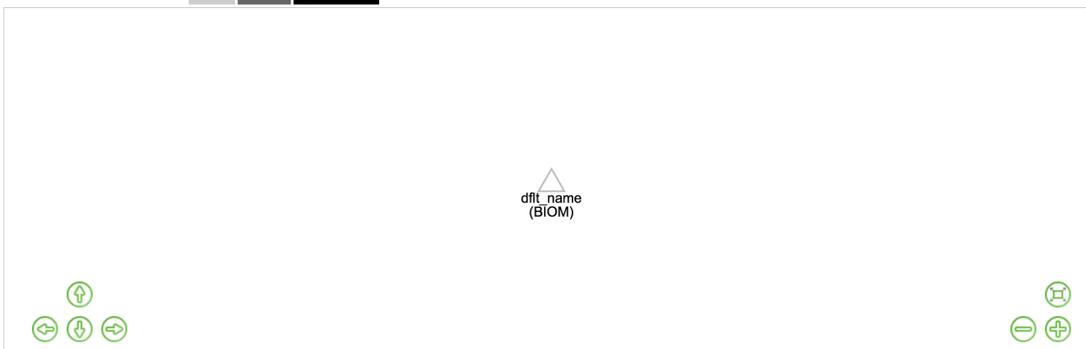
Shared with:

Processing network [Hide](#)

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 7 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated



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CMI tutorial - Shotgun Metagenomics Analysis - ID 21954 (Private) [Make analysis public](#)

CMI tutorial - Shotgun Metagenomics Analysis

Shared with:

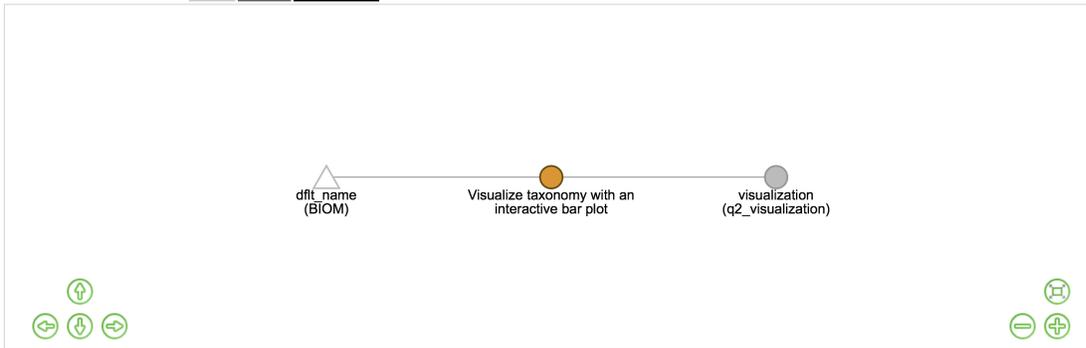
Processing network [Hide](#)

Start workflow: [Run](#)

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 10 seconds or reload now

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated



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Fig. 1: This will bring up several new artifacts in the processing network as shown below.

Qiita Analysis - Study - Admin - redbiom Qilmp Help - More Info - Welcome justinparkshaffer@gmail.com Log Out (15)

CMI tutorial - Shotgun Metagenomics Analysis - ID 21954 (Private) [Make analysis public](#)

CMI tutorial - Shotgun Metagenomics Analysis

Shared with: [Hide](#)

Processing network [Run](#)

Start workflow: [Run](#)

Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 12 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command:

Thank you for using Qiita. Citing Qiita?
Questions? qiita.help@gmail.com; don't forget to add your study or analysis id.
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Qiita Analysis - Study - Admin - redbiom Qilmp Help - More Info - Welcome justinparkshaffer@gmail.com Log Out (15)

Workflow 12990 submitted

CMI tutorial - Shotgun Metagenomics Analysis - ID 21954 (Private) [Make analysis public](#)

CMI tutorial - Shotgun Metagenomics Analysis

Shared with: [Hide](#)

Processing network [Run](#)

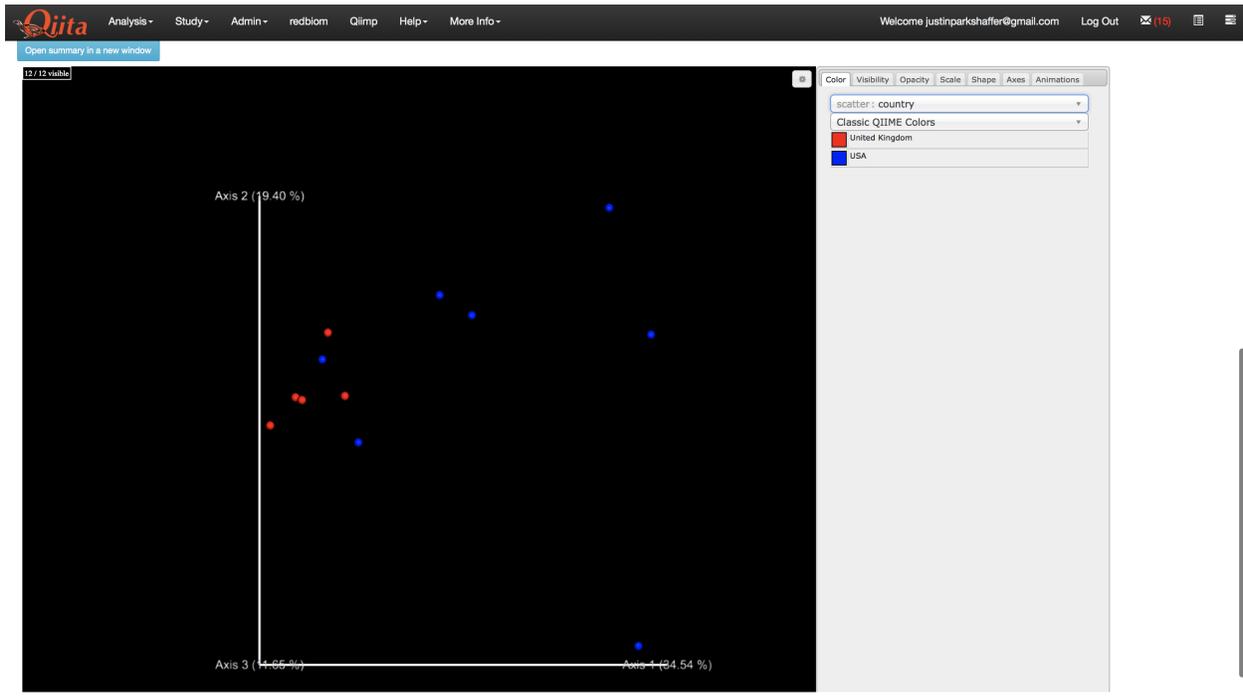
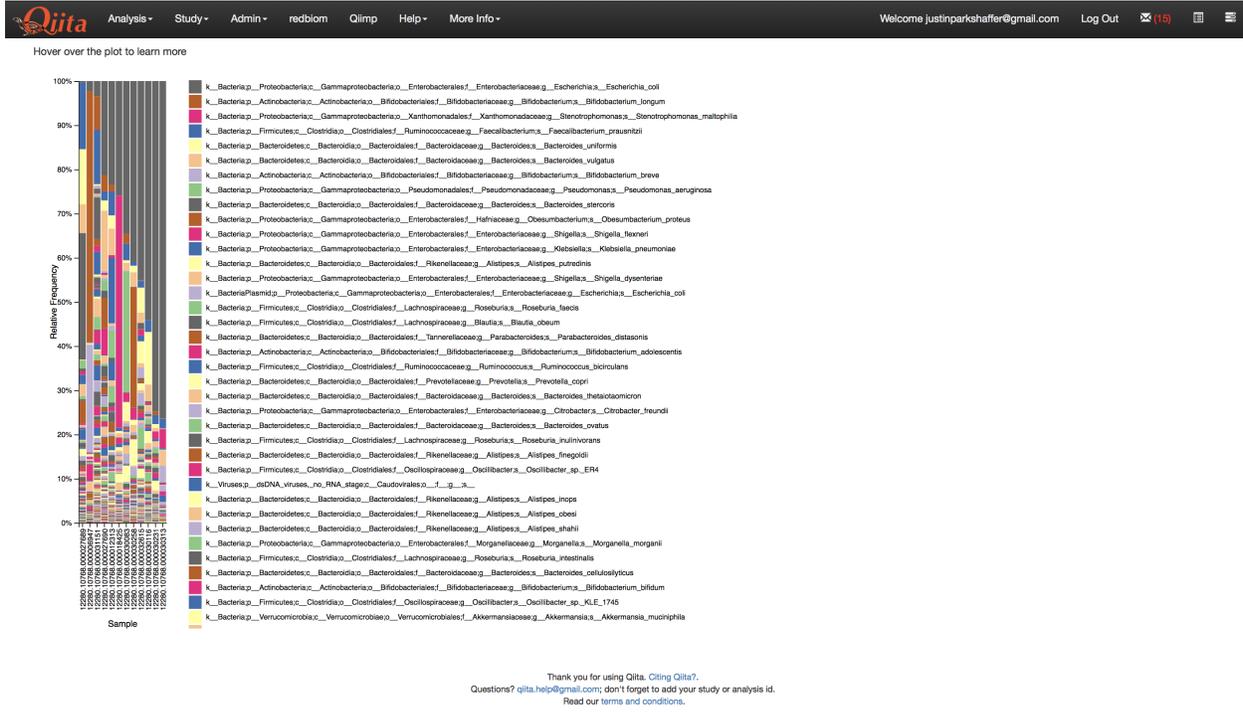
Click on the graph to navigate through it. Click circles for more information. This graph will refresh in 11 seconds or reload [now](#)

Job status (circles): success running error in_construction queued waiting deleting

Artifact status (triangles): artifact type outdated deprecated

Choose command:

Thank you for using Qiita. Citing Qiita?
Questions? qiita.help@gmail.com; don't forget to add your study or analysis id.
[Read our terms and conditions.](#)

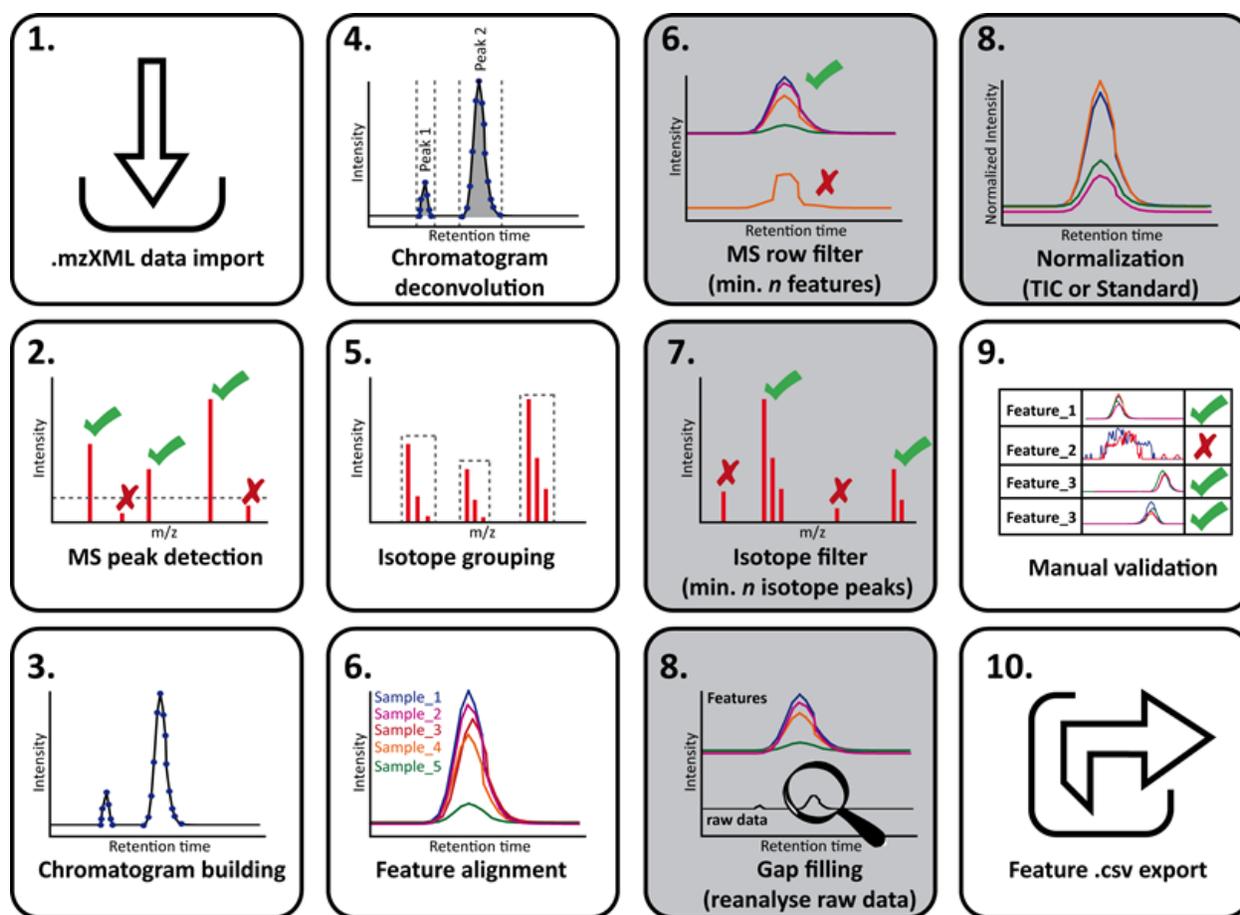


- The GNPS web-platform will be used to generate a qualitative analysis of your sample LC-MS/MS data. Such as the annotation of known compounds (by MS/MS spectral matching with public library), along as annotating unknown compounds by molecular networking (by spectral similarity).
- And we will use MZmine2 to process LC-MS/MS data in order to generate a feature table. This feature table contains the list of detected compounds and their relative distribution across samples. This feature table will be used to generate statistical analysis in Qiita.

1.19 Feature finding with MZmine2

Please follow this (link) to install the software and dependencies.

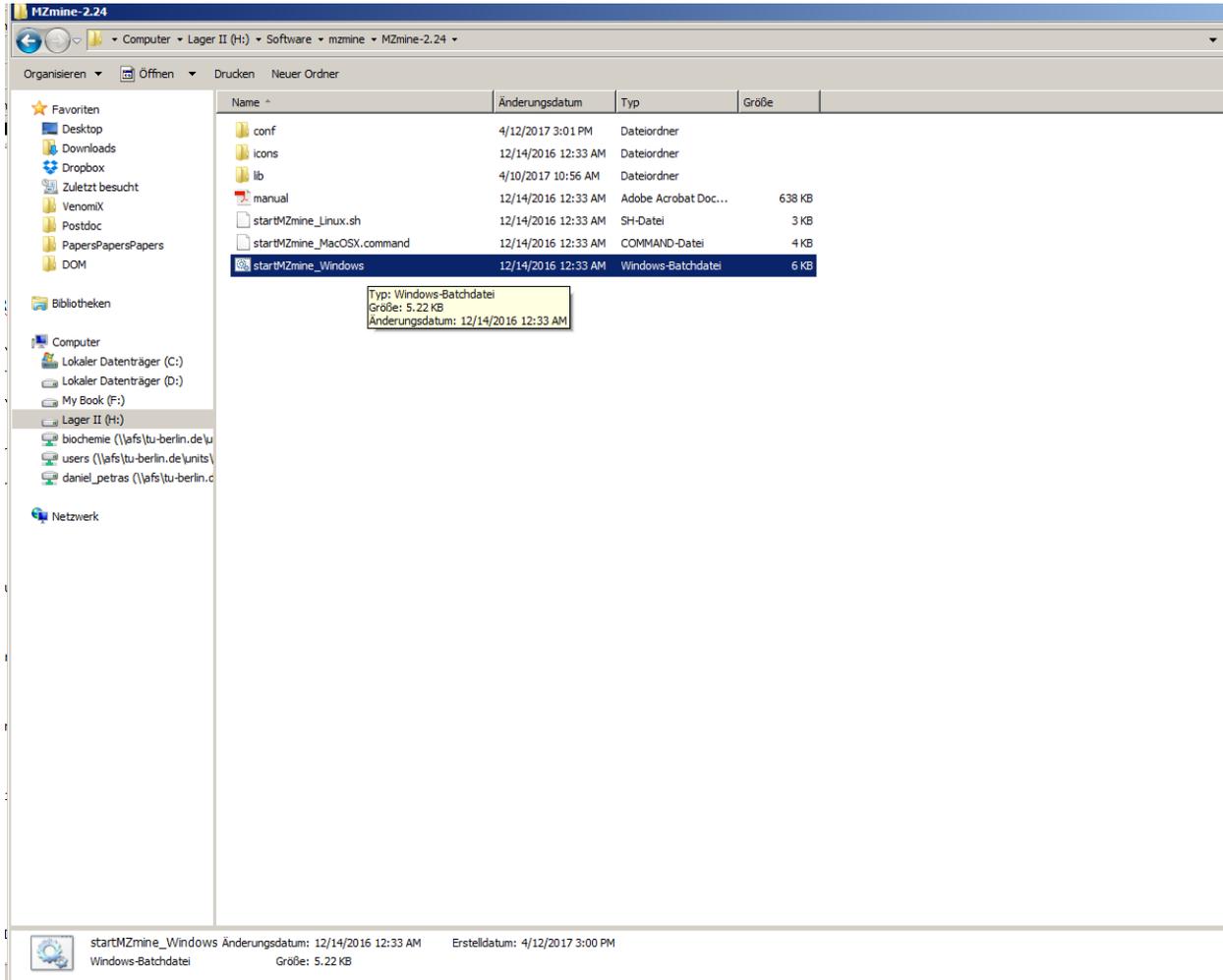
1.19.1 Complete workflow view



workflow view

complete

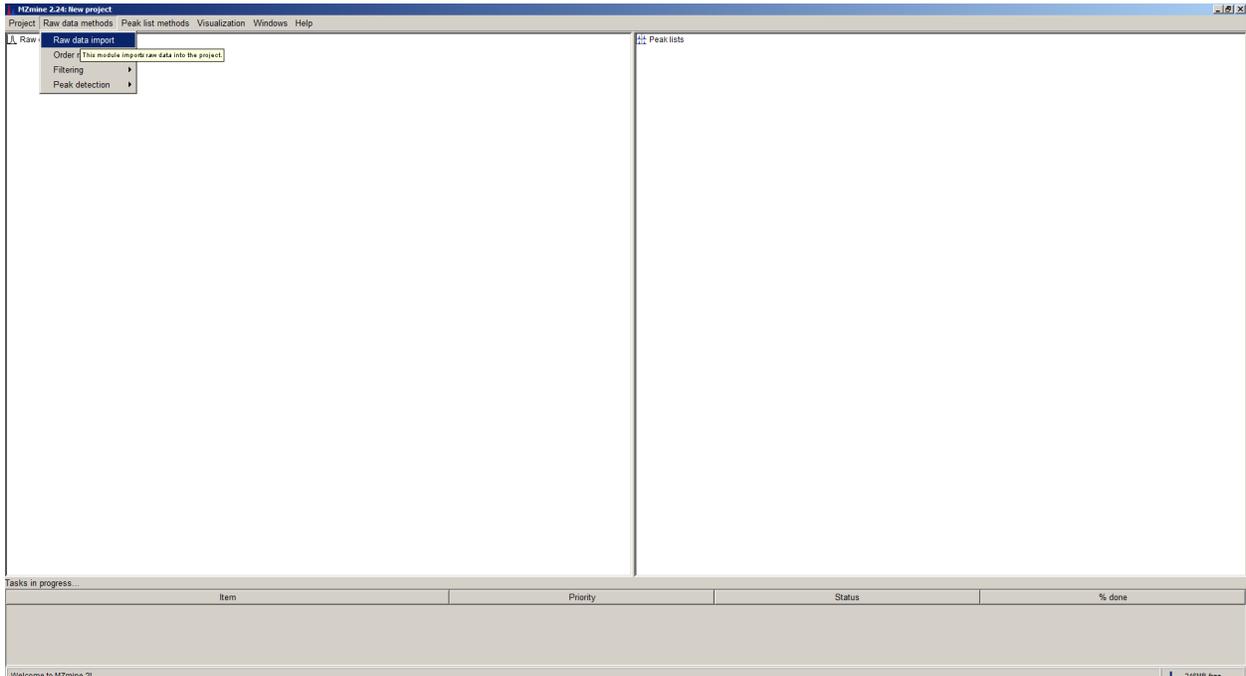
1. Start mzMine2



start

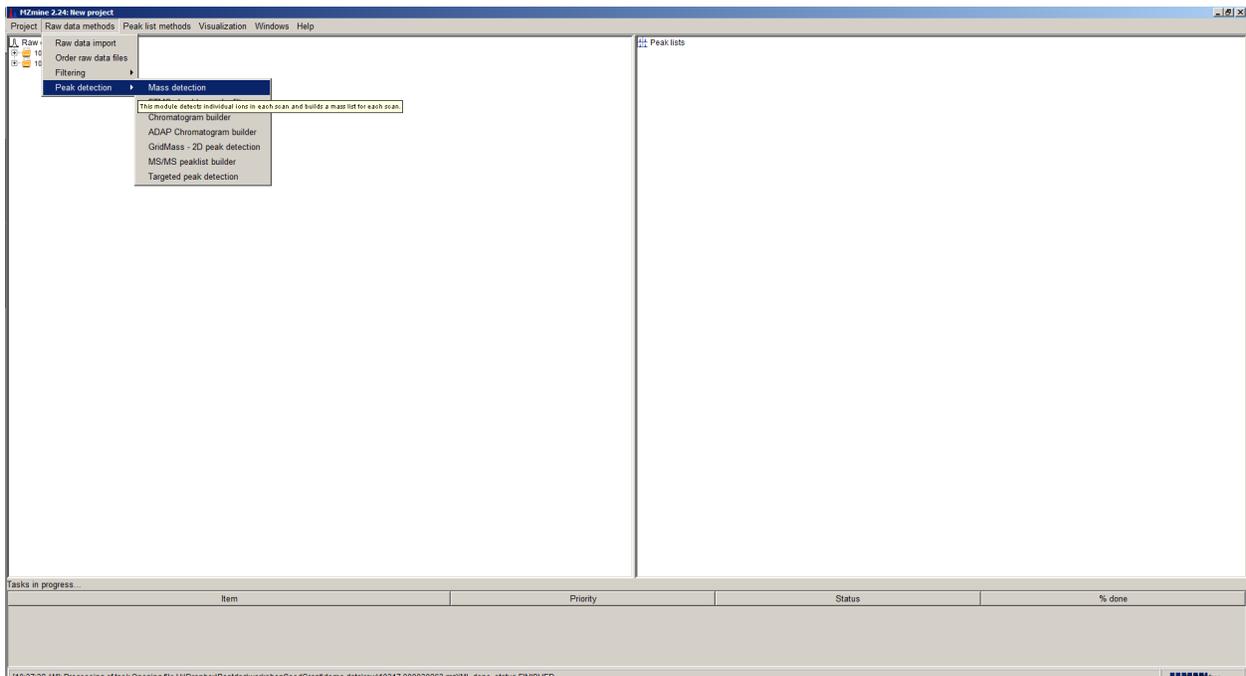
mzMine

2. Click on raw data import in drop down menu and select .mzxml files



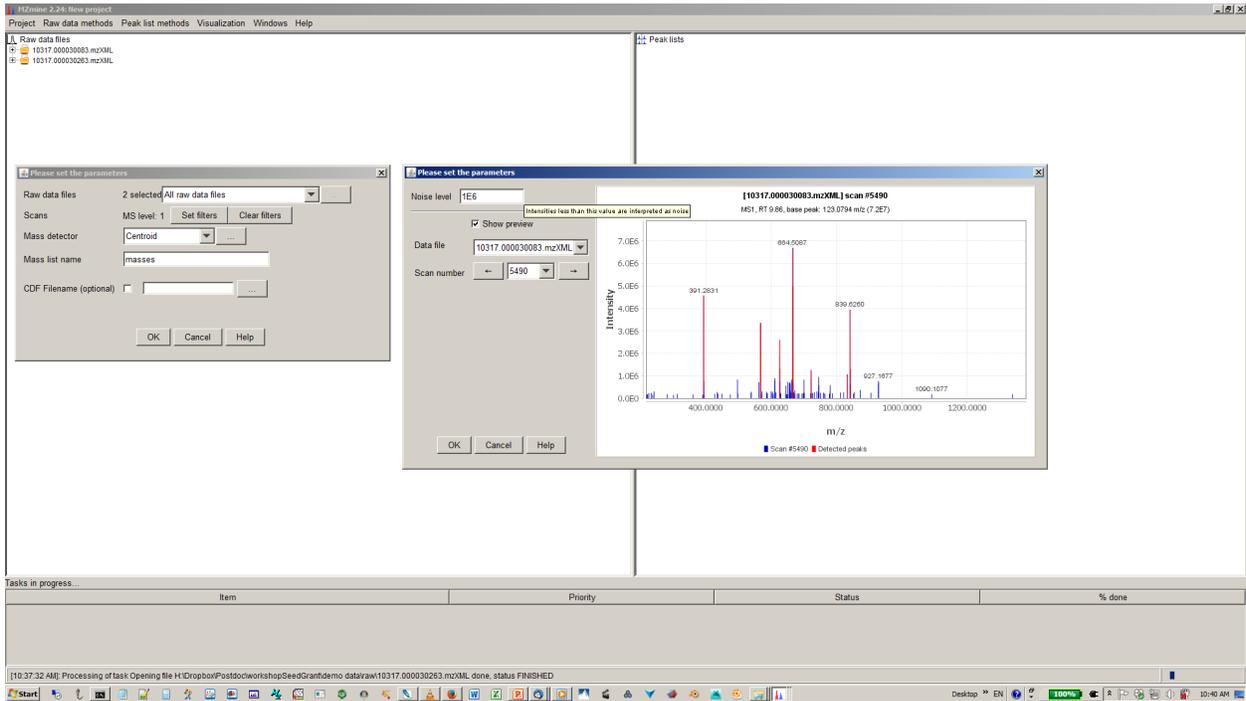
import data

3. Click on mass detection in drop down menu



mass detection

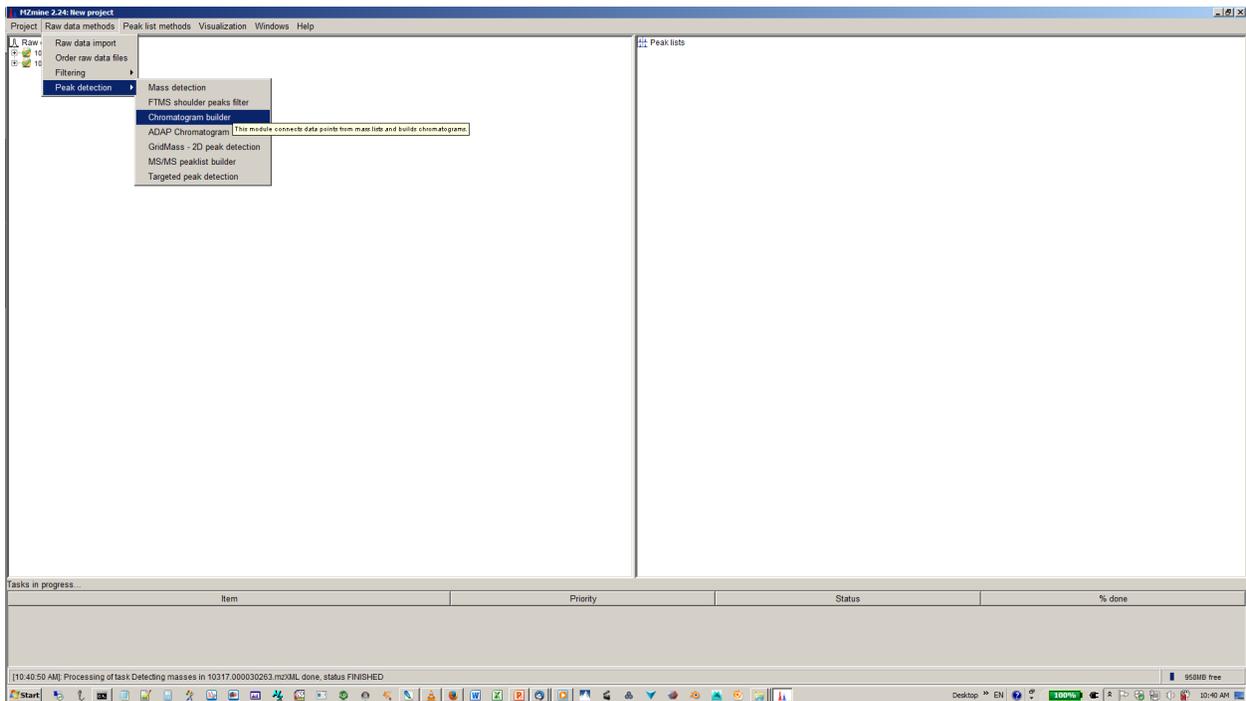
4. Specify intensity cut-off and mass list



intensity cut-off

specify

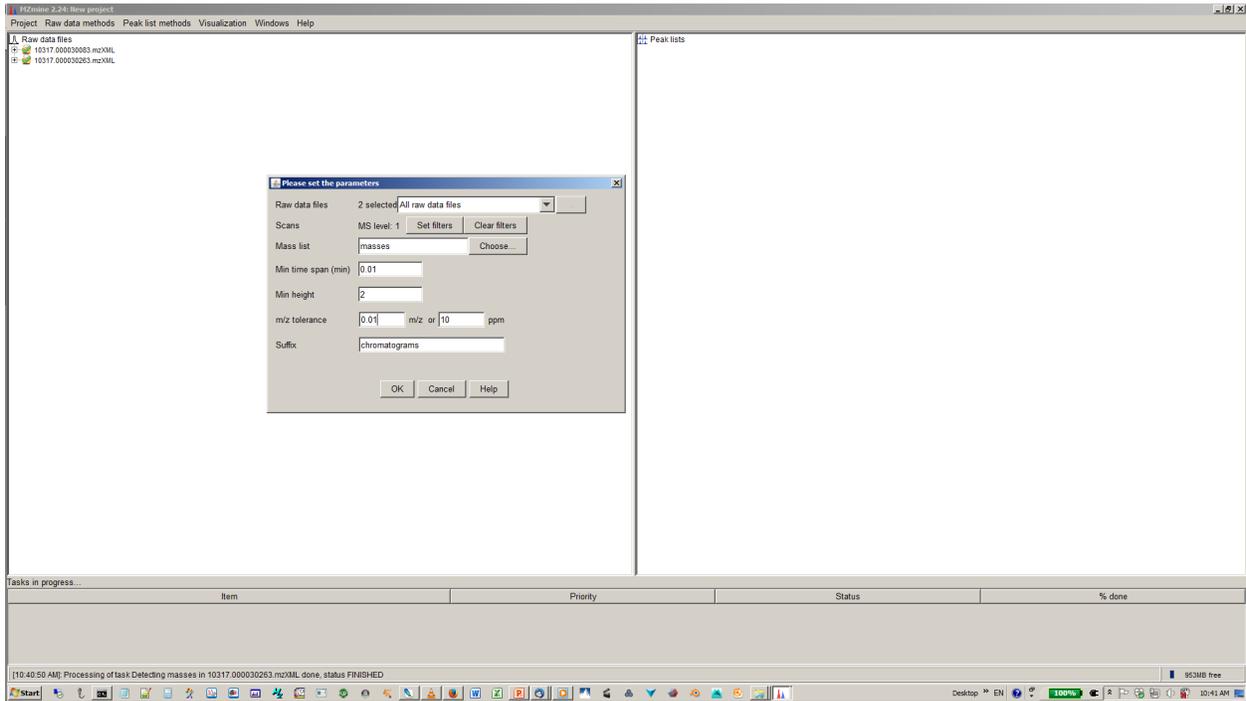
5. Build XICs with chromatogram builder



XICs with chromatogram builder

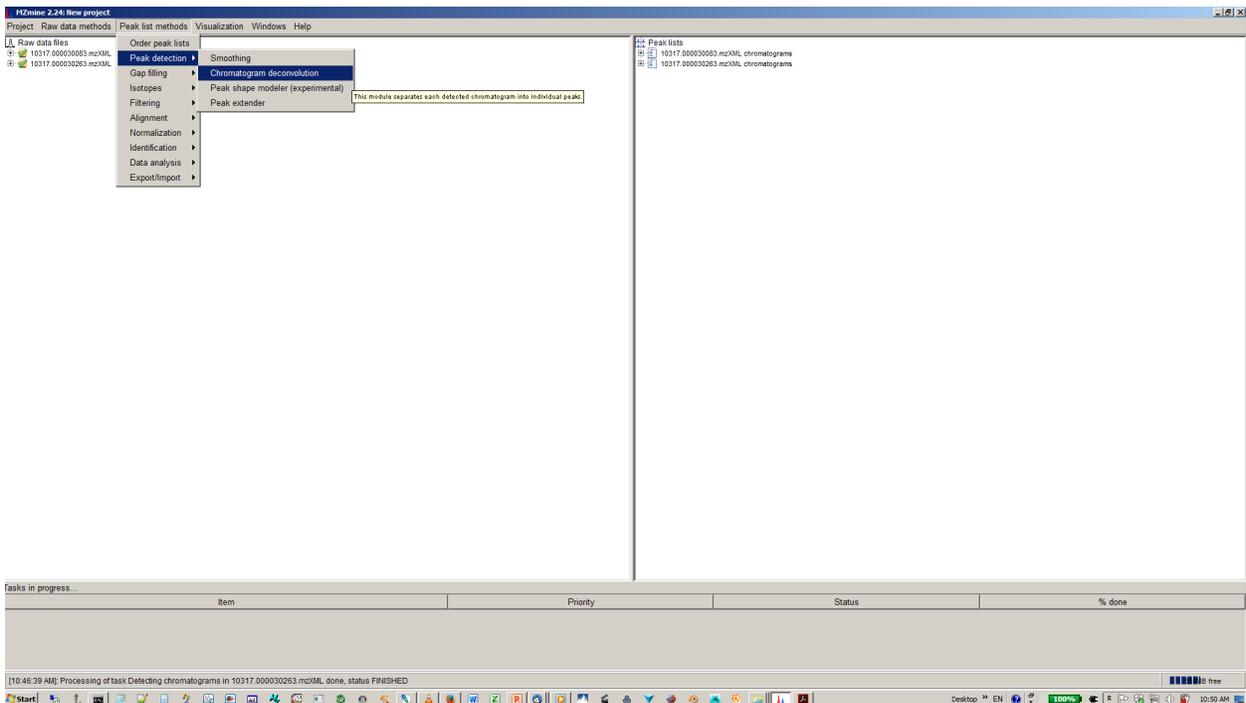
Build

6. Specify mass list, mass tolerance min. time span and min. height



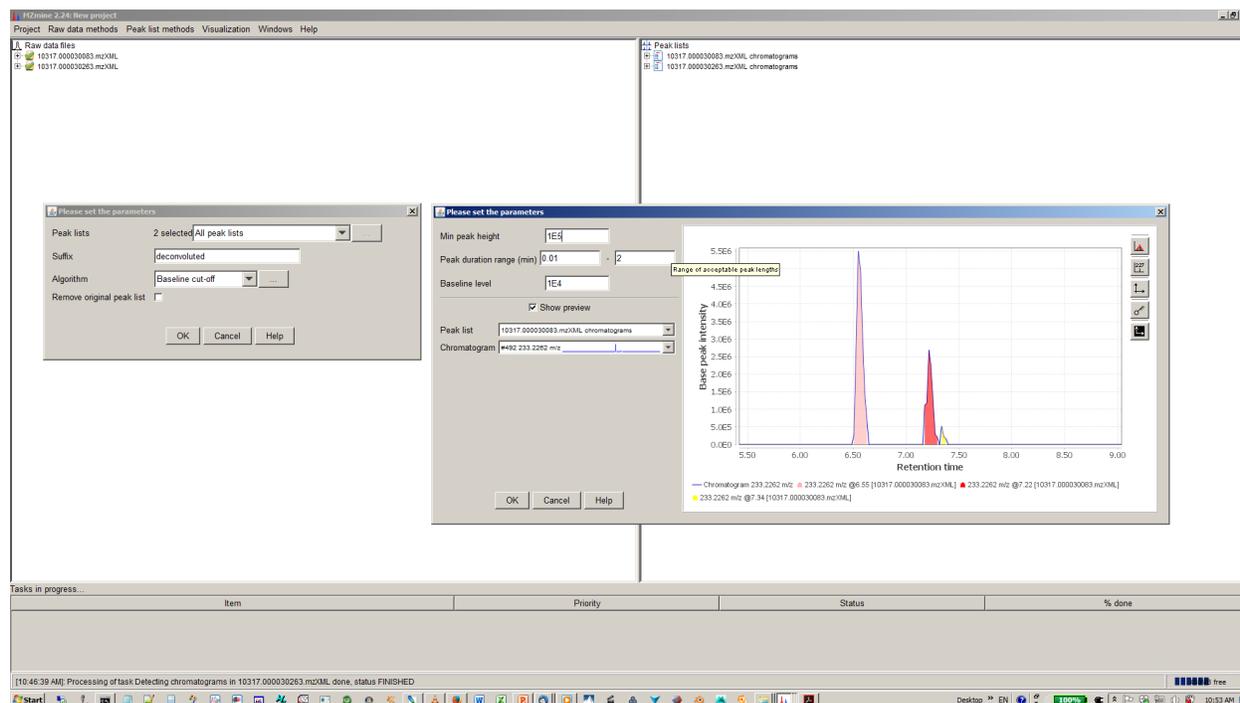
Specify mass list, mass tolerance min. time span and min. height

7. Deconvolute isobaric peaks with chromatogram deconvolution



Deconvolute isobaric peaks with chromatogram deconvolution

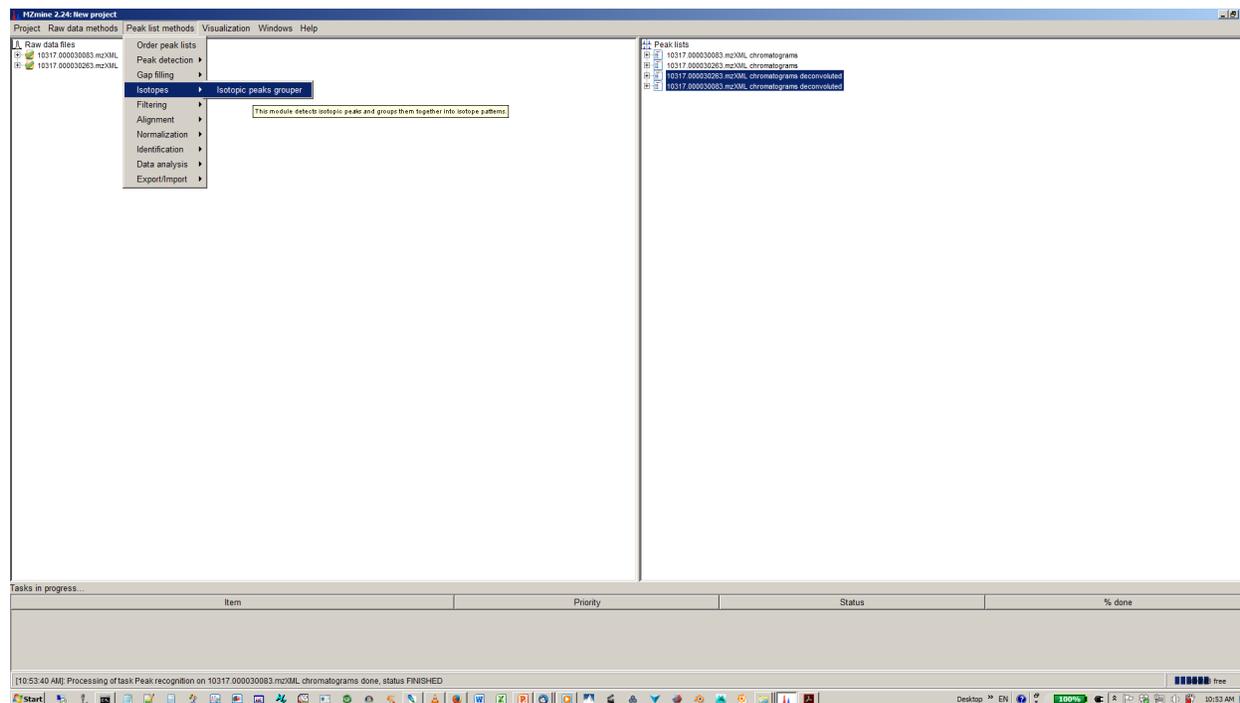
8. Specify algorithm (base line cut-off or local minimum search and parmeters)



algorithm (base line cut-off or local minimum search and parmeters

Specify

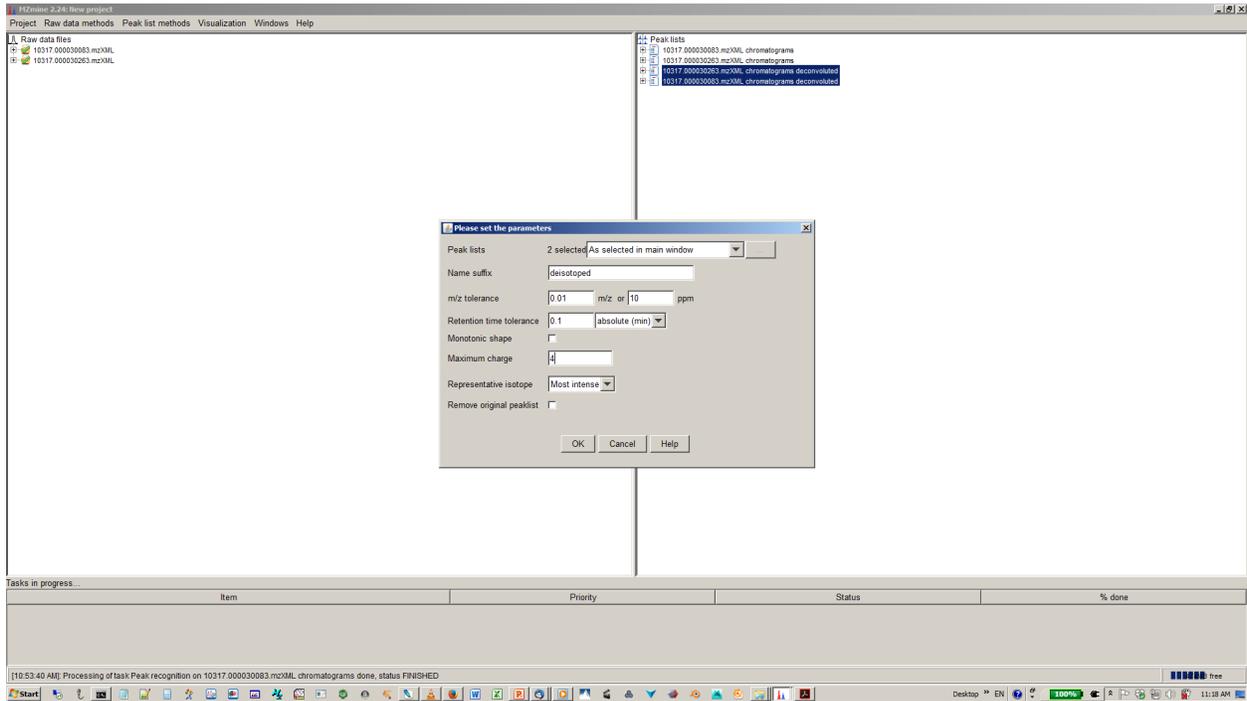
9. Perform deisotopization through isotope peak grouper



deisotopization through isotope peak grouper

Perform

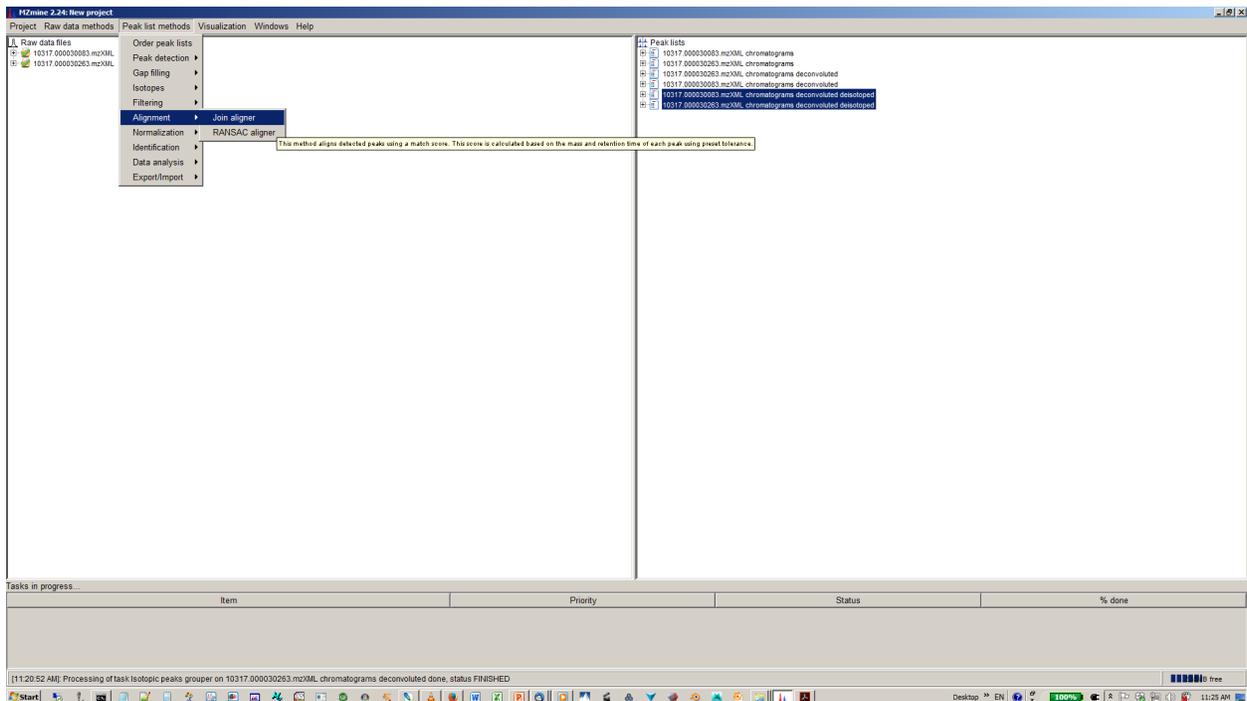
10. Specify parameters for isotope peak grouping



parameters for isotope peak grouping

Specify

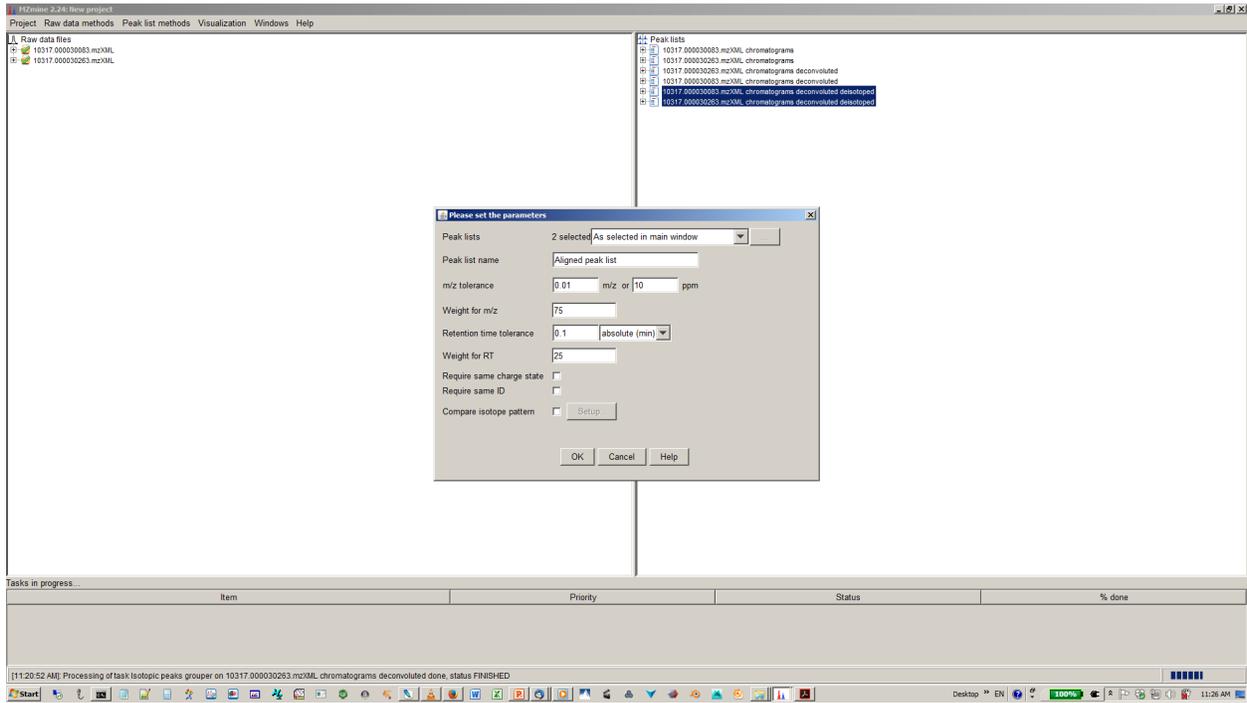
11. Align XICs from different sample to one matrix



XICs from different sample to one matrix

Align

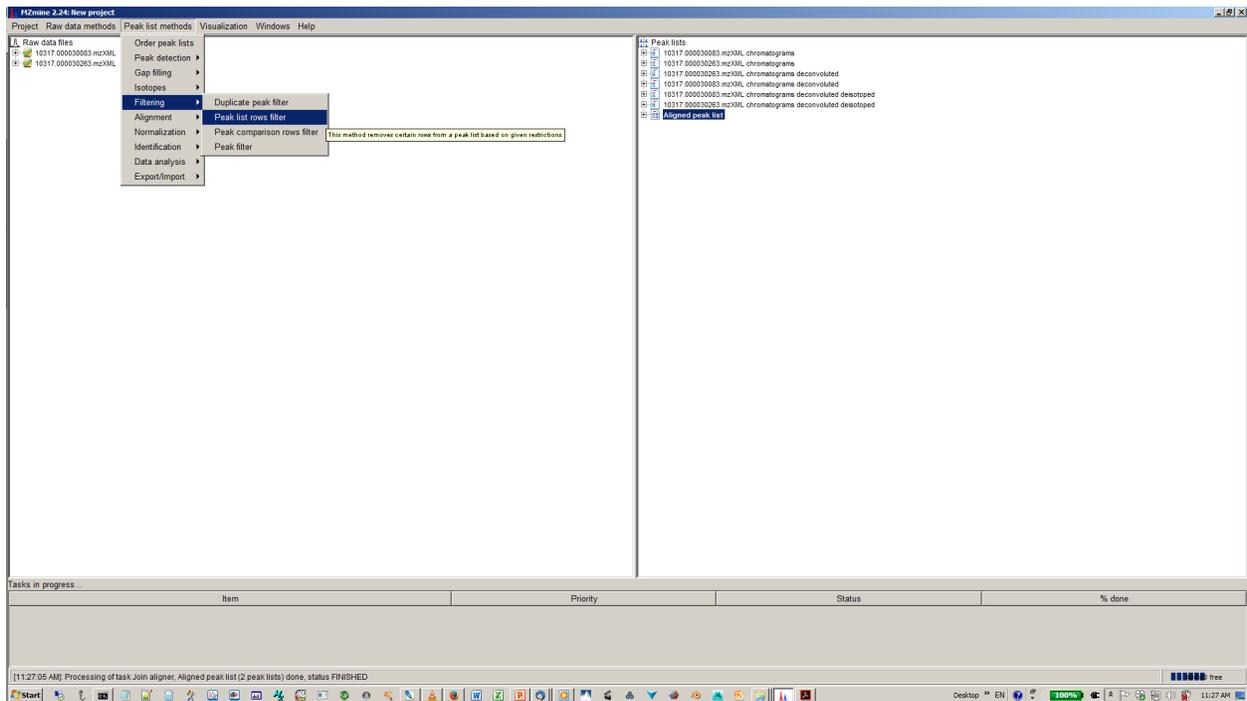
12. Specify join aligner parameters



join aligner parameters

Specify

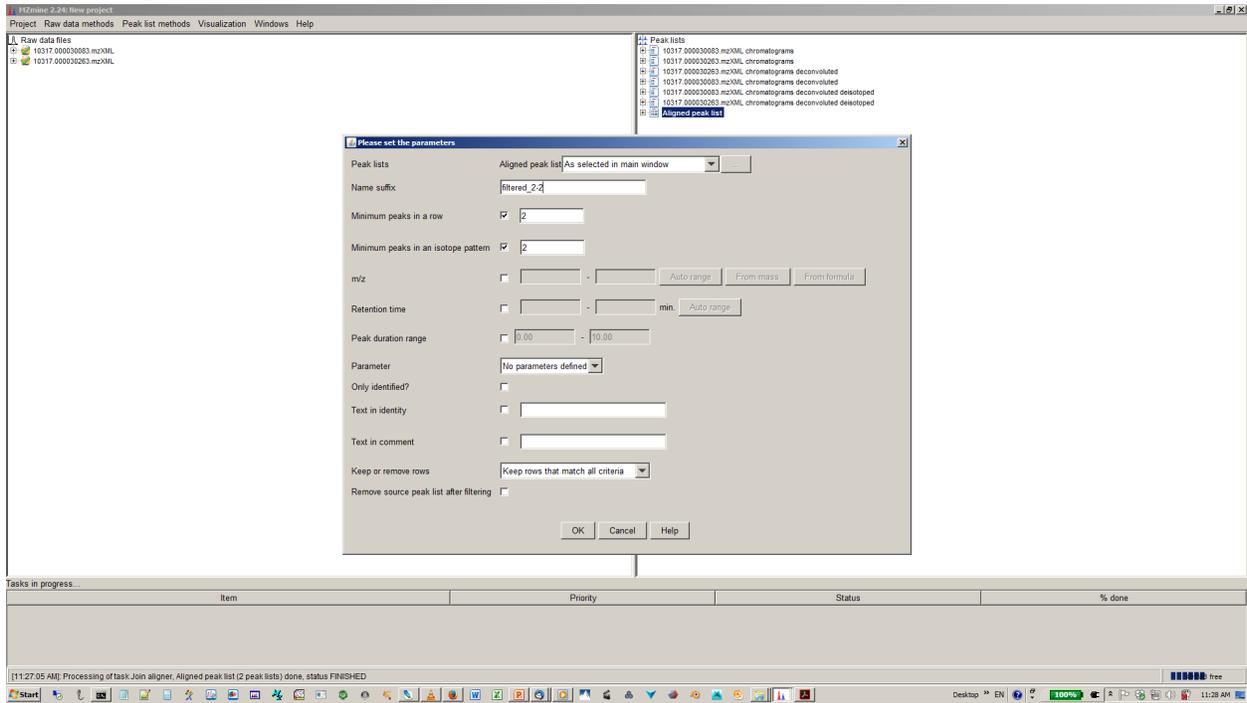
13. [optional] Filter aligned feature matrix with peak list row filter



aligned feature matrix with peak list row filter

Filter

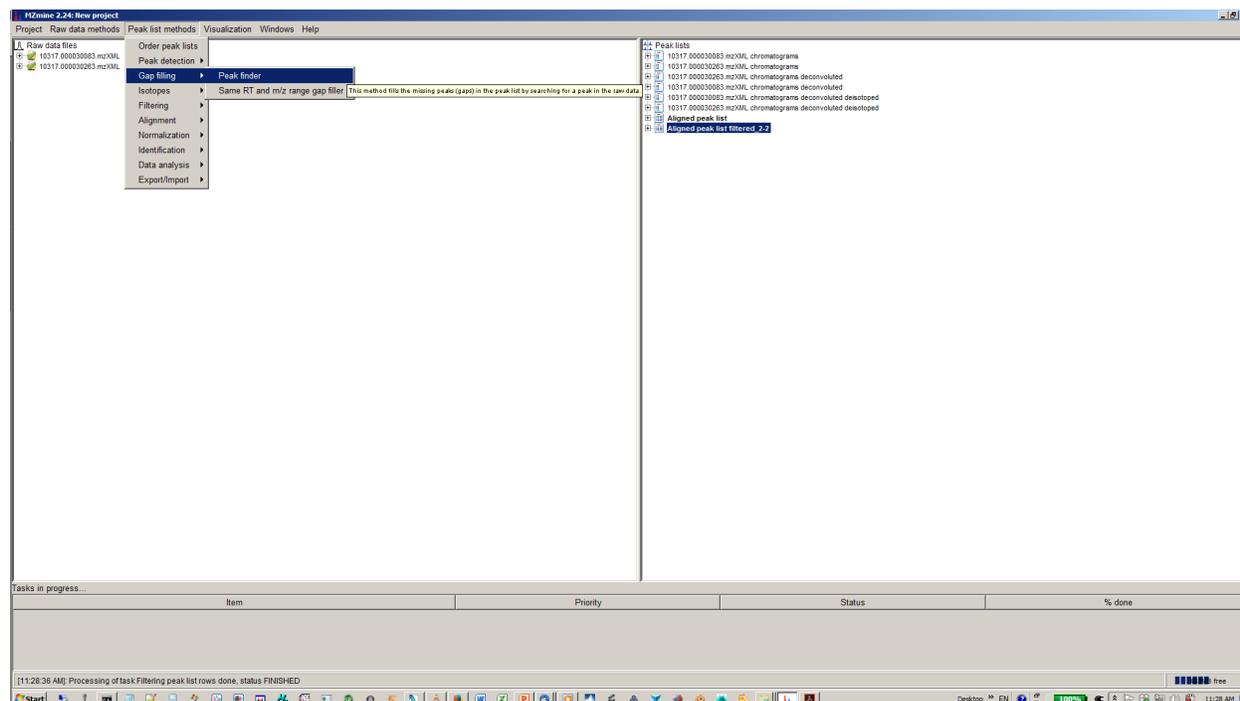
14. [optional] Depending of your experimental design use n minimum peaks in a row (n should be around the number of replicates or samples you expect to be similar) and 2-3 minimum peaks per isotope pattern



n minimum peaks in a row

use

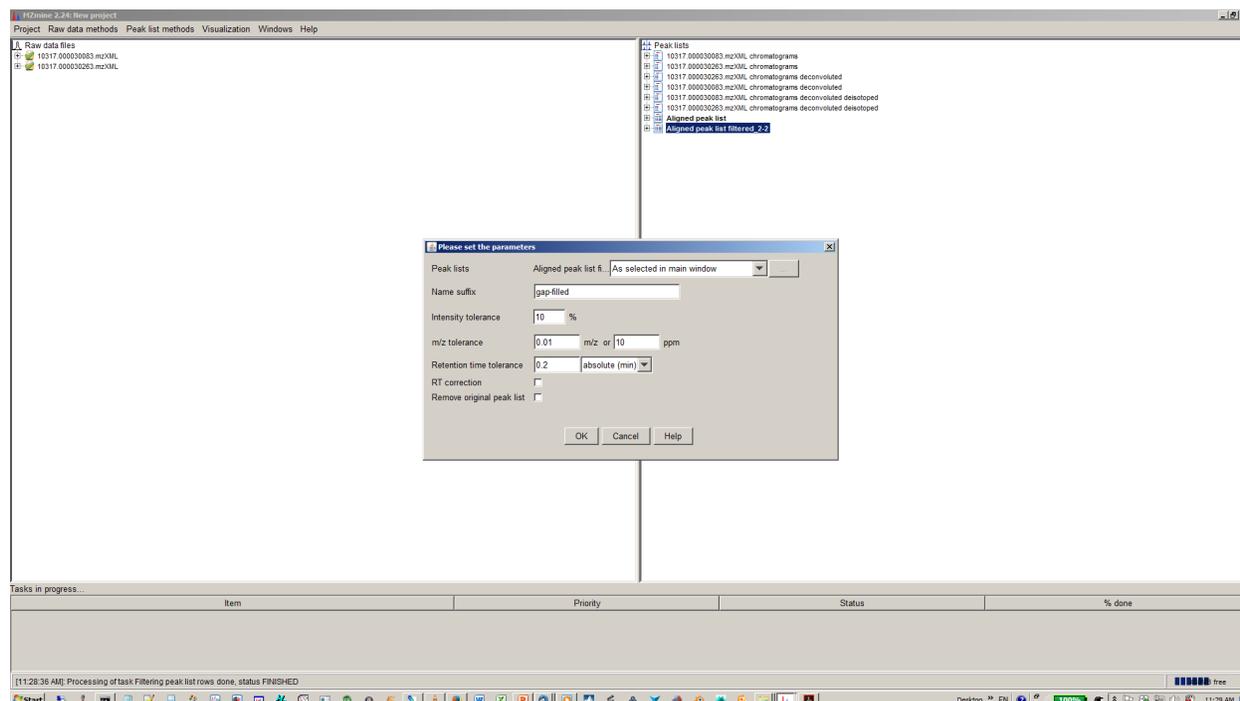
15. [optional] You gap filling the re-analyses missed peaks and fill gaps in the feature matrix



gap filling the re-analyses missed peaks and fill gaps in the feature matrix

You

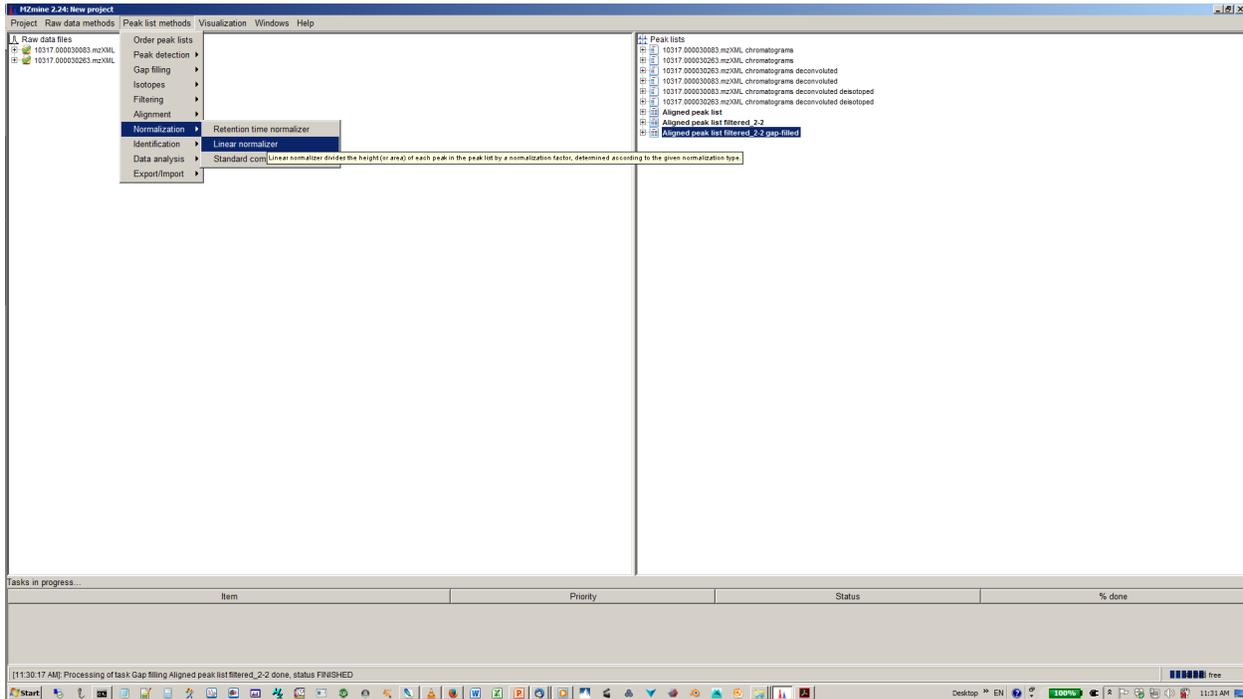
16. [optional] Depending on experimental design you can normalize your peak intensities to internal standards, TICs or total peak area.



your peak intensities to internal standards, TICs or total peak area

normalize

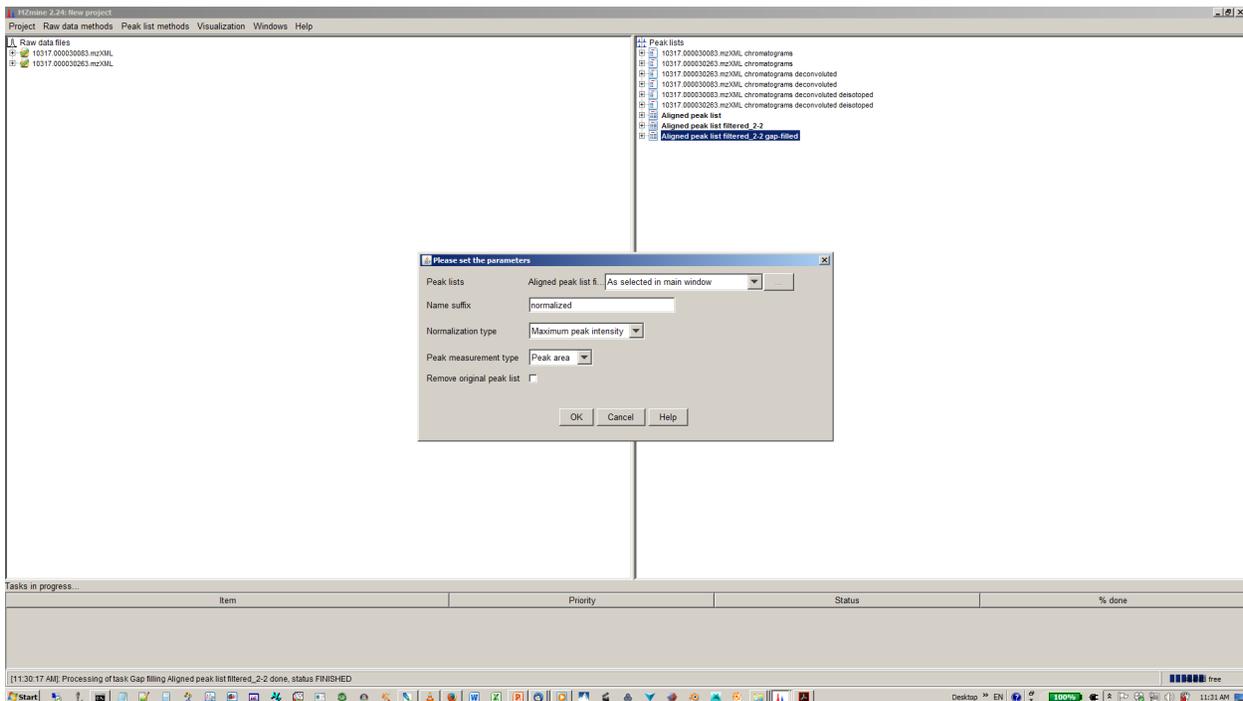
17. [optional] Specify normalization parameters



normalization parameters

Specify

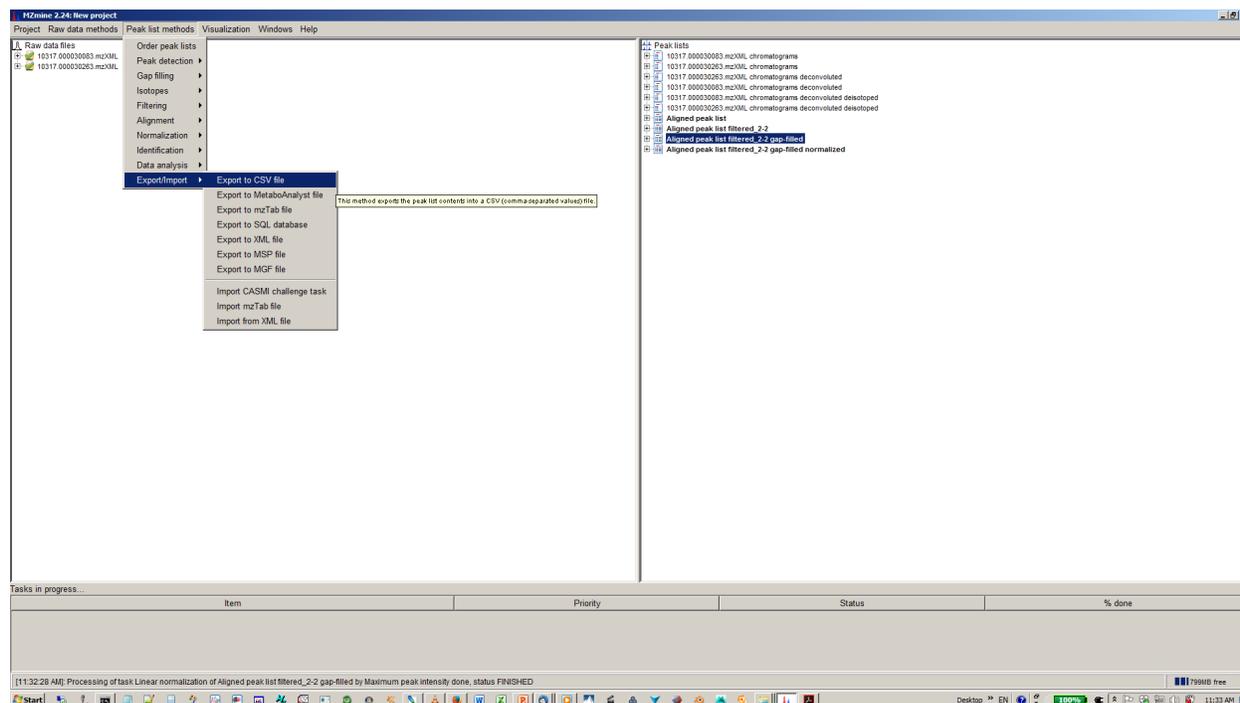
18. Export your matrix as .csv file for down stream data analysis



your matrix as .csv file for down stream data analysis

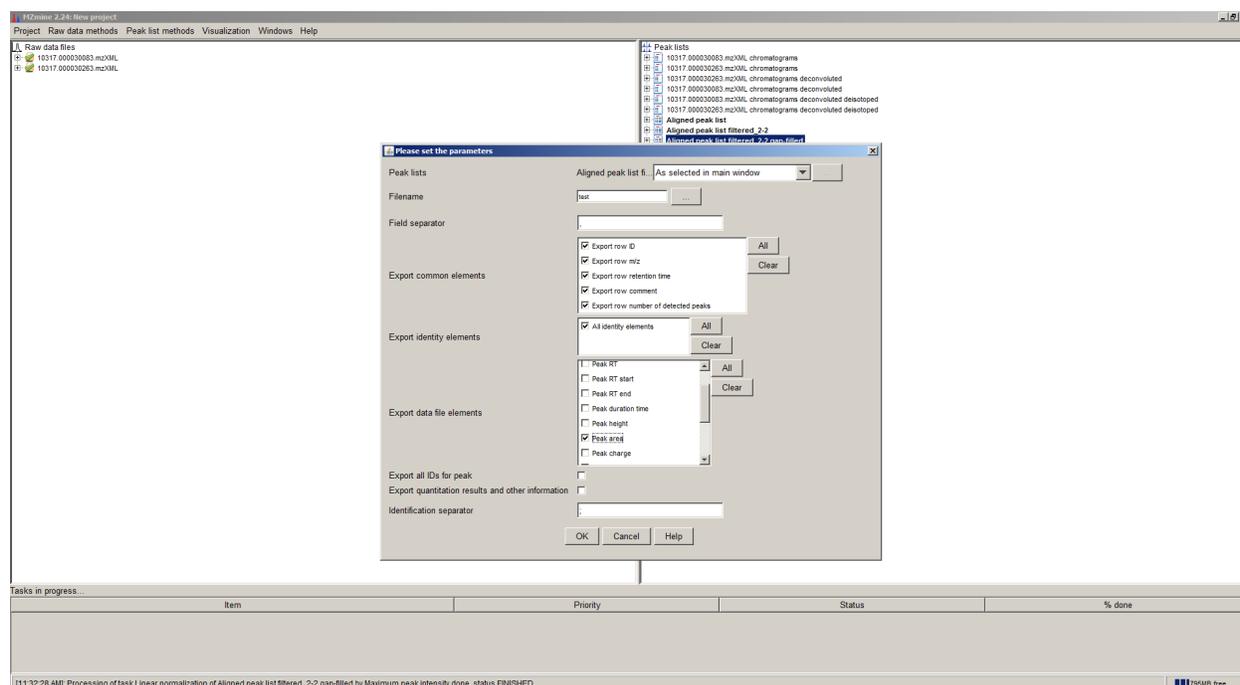
Export

19. select file name and parameters you want to export



select

file name and parameters you want to export



select

file name and parameters you want to export

Here is also a video for [MZmine 2 documentation](#):

1.20 Metabolomics demo data in Qiita

- Refer to the Qiita documentation about Principal Coordinates Analysis (PCoA) [here](#)

1.21 GNPS tutorial for MS/MS data annotation

Global Natural Products Social Molecular Networking GNPS web-platform provides public data set deposition and/or retrieval through the Mass Spectrometry Interactive Virtual Environment (MassIVE) data repository. The GNPS analysis infrastructure further enables online dereplication, automated molecular networking analysis, and crowdsourced MS/MS spectrum curation. Each data set added to the GNPS repository is automatically reanalyzed in the next monthly cycle of continuous identification. For more information, please check out the GNPS paper published in Nature Biotechnology by Ming et al 2016 [here](#) as well as the video and the resource on [Youtube](#), and well as on the online [documentation](#)

1.21.1 Tutorial: Generation of Molecular Networks in 15 minutes: Exploring MS/MS data with the GNPS Data Analysis workflow

Step 1- Go to GNPS and create an account

Go to GNPS main page in an other window <http://gnps.ucsd.edu> and create your own account first (important!)



Please Login to Use Workflows

The Future of Natural Products Research and Mass Spectrometry

Login

Step 2- Find a MS/MS dataset on MassIVE (Mass spectrometry Interactive Virtual Environment)



The Future of Natural Products Research and

A) Go to [GNPS](#) and access the MassIVE datasets repository.

B) Search for the MassIVE datasets named “GNPS Workshop” (or “GNPS_AMG_SeedGrant” for a larger example with American Gut Projects samples). Explore its content, and copy the MassIVE ID number (MSV)

Filter	Title	MassIVE ID	ProteomeXchange ID	Submission Type	Uploaded By	Principal Investigator	Upload Date	# Files	Total Size (KB)
	AMG								
1	GNPS_AMG_SeedGrant	MSV000080469		Partial	rsilva	Rob Knight	Jan, 13, 2017, 10:30 AM	24	226,716

Massive

Note: If you want to upload your own data, follow the [DorresteinLab youtube chanel](#), here is the video:

Step 3 - Access to the Data Analysis workflow

Go to back GNPS main page and open the Data Analysis workflow.

The Future of Natural Products Research and Mass Spectrometry

Cluster	Cluster Index	Filename	Scan	Precursor list	RT	Parent Mass	Gold	Silver	Bronze
10	1	1001.ms2016	4028	83991.000	2409.500	445.120	Gold	Silver	Bronze
10	2	1001.ms2016	4128	101720.000	2450.320	445.120	Gold	Silver	Bronze
10	3	1001.ms2016	4229	730279.000	2491.010	445.120	Gold	Silver	Bronze
10	4	1001.ms2016	4338	800548.000	2531.740	445.121	Gold	Silver	Bronze
10	5	1001.ms2016	4446	874482.000	2572.560	445.120	Gold	Silver	Bronze
10	6	1001.ms2016	4860		2712.330	445.120	Gold	Silver	Bronze
10	7	1001.ms2016	4981		2752.870	445.120	Gold	Silver	Bronze
10	8	1001.ms2016	5113		2793.700	445.121	Gold	Silver	Bronze
10	9	1001.ms2016	5285		2836.530	445.120	Gold	Silver	Bronze
10	10	1001.ms2016	5604	827557.000	2949.780	445.121	Gold	Silver	Bronze
10	11	1001.ms2016	5730	868837.000	2990.680	445.121	Gold	Silver	Bronze
10	12	1001.ms2016	5867	861482.000	3025.540	445.121	Gold	Silver	Bronze
10	13	1001.ms2016	6126	682002.000	3118.280	445.121	Gold	Silver	Bronze
10	14	1001.ms2016	6324	604958.000	3185.280	445.121	Gold	Silver	Bronze
10	15	1001.ms2016	6889	630093.000	3363.510	445.121	Gold	Silver	Bronze
10	16	1001.ms2016	7077	723469.000	3468.280	445.121	Gold	Silver	Bronze

[Tweet](#) [Share](#)

Data Analysis

The [Data Analysis](#) portal will allow you to organize and visualize your mass spectrometry data. Leveraging the molecular networking techniques, there are additional tools to aid in understanding the unknowns in your sample. Check out the [documentation](#) and live [demo](#). Further, a separate [dereplication workflow](#) is provided as a standalone workflow.

Create Public Massive Datasets

[Submit](#) your own data to be made public Massive datasets. These Massive datasets must be prefixed with GNPS to be visible to other GNPS users. Take advantage of [continuous identification](#) to learn more about your dataset after publication automatically. New hits to the community curated libraries and related datasets are reported. [Documentation](#)

Massive

Step 4 - Configure and launch the Data Analysis workflow

Workflow Selection

Title:

Search Protocol:

Networking Parameter Presets

Basic Options

Spectral Library: 0 files and 1 folder are selected

Spectrum files must be centroided and be in an open spectrum format (mzXML, mzML, or mgf)

Spectrum Files (Required): 0 files and 1 folder are selected

Spectrum Files G2:

Spectrum Files G3:

Spectrum Files G4:

Spectrum Files G5:

Spectrum Files G6:

Precursor Ion Mass Tolerance: Da Da Da

Advanced Network Options

Advanced Library Search Options

Advanced Filtering Options

Advanced Output Options

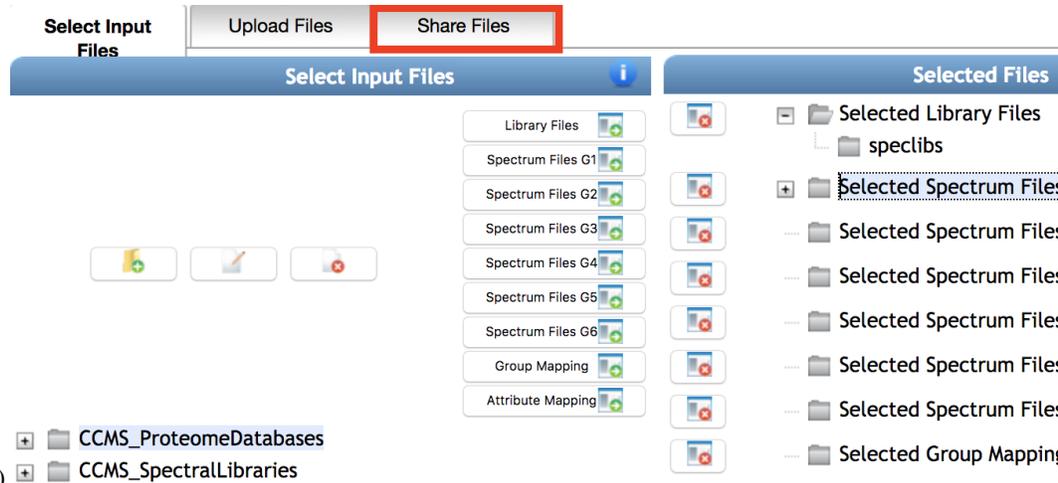
Workflow Submission

Email me at

start

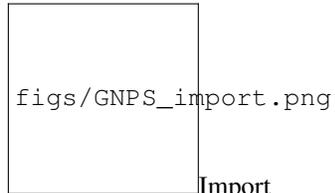
the GNPS job

A) Indicate a Title.



B) Clic on Spectrum Files (required) on Spectrum Files

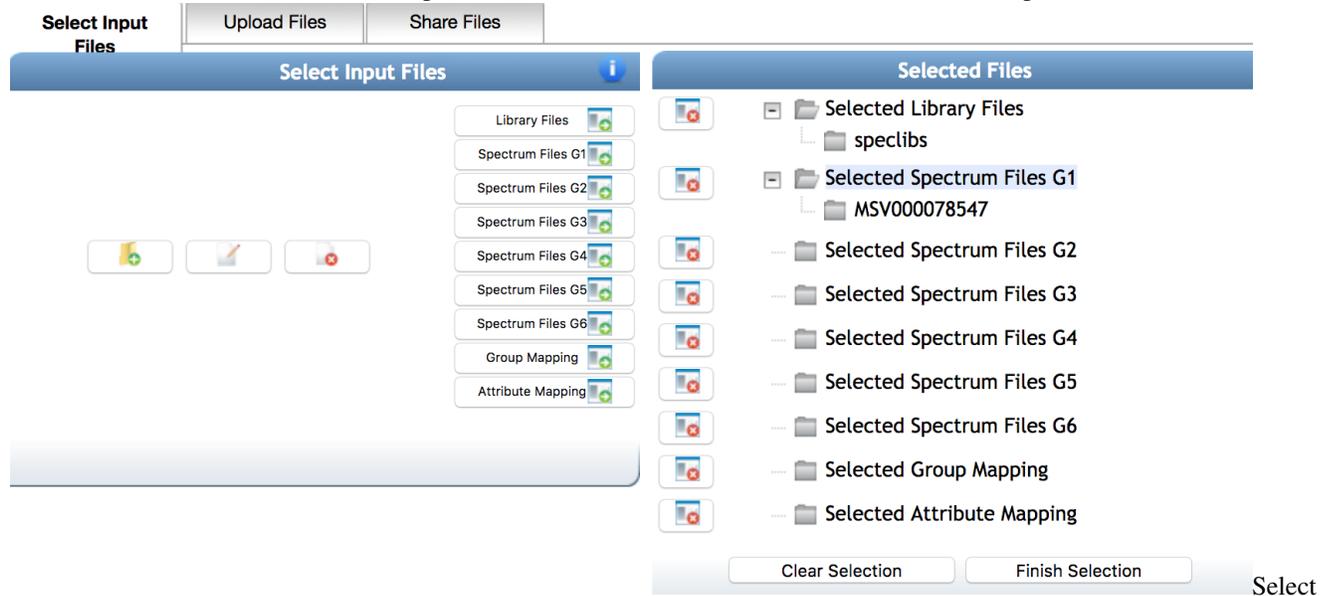
C) Go to the Share Files spreadsheet and import the Massive dataset files for the “GNPS workshop” or



“GNPS_AMG_SeedGrant” with the Import Data Share (use the MassIVE ID). Import

D) Go back to the Select Input Files spreadsheet.

E) Add the files from the imported datasets “GNPS_AMG_SeedGrant” into Spectrum Files G1.



F) Validate the selection with Finish Selection button.

G) Modify parameters to meet high-resolution mass spectrometry: Precursor Ion Mass Tolerance (0.02), Fragment Ion Mass Tolerance (0.02), Min Pairs Cos (0.6), Minimum Matched Fragment Ions (2), Minimum cluster size (use 1)

Workflow Selection

Title: Search Protocol:

Networking Parameter Presets

Basic Options

Spectral Library: 0 files and 1 folder are selected

Spectrum files must be centroided and be in an open spectrum format (mzXML, mzML, or mgf)

Spectrum Files (Required): 0 files and 1 folder are selected

Spectrum Files G2:

Spectrum Files G3:

Spectrum Files G4:

Spectrum Files G5:

Spectrum Files G6:

Precursor Ion Mass Tolerance: Da Fragment Ion Mass Tolerance: Da

Advanced Network Options

Advanced Library Search Options

Advanced Filtering Options

Advanced Output Options

Workflow Submission

Email me at

job

prepare

H) Launch the Data Analysis workflow using the Submit button.

Step 5 - Visualize the Data Analysis workflow output

A) Return to GNPS main page and go to the Jobs page. Please find here an example of GNPS data analysis output with American Gut Project.



The Future of Natural Products Research and Mass Spectrometry

view

Job Status

Workflow METABOLOMICS-SNETS

Status

DONE
[\[Clone\]](#) [\[Restart\]](#) [\[Delete\]](#)
[View All Library Hits](#) [View All Clusters With IDs](#) [View All Compounds](#)]

Methods and Citation for Manuscripts
[\[Networking Parameters and Written Network Description \]](#)

Experimental Views
[\[Reanalyze Cluster Spectra](#) | [View Raw Spectra](#) | [Topology Signatures](#) | [Topology Signatures Histogram](#)]

Auxiliary Views
[\[View Network, Node Centric](#) | [View Network Pairs](#) | [Networking Statistics](#)]

Advanced Views - Networking Graphs
[\[Nodes, MZ Histogram](#) | [Edges, MZ Delta Histogram](#) | [Edges, Score vs MZ Delta Plot](#) | [Library Search, PPM Error Histogram](#)]

Community Matches
[\[Dataset Matches \]](#)

Network Visualizations
[\[View Spectral Families \(In Browser Network Visualizer\)](#) | [Network Summarizing Graphs](#)]

Export
[\[Download Clustered Data](#) | [Download Cytoscape Data](#) | [Download Bucket Table](#) | [Make Public Dataset](#)]

User lfnothias (lfnothias@ucsd.edu), UCSD, Dorrestein Lab

Title Workshop AMG

Re-Analyze Task Outputs [Import to Re-analyze Task Data](#) [Attach Reanalysis Results to Dataset](#)

Date Created 2017-01-19 15:39:19.0

Execution Time 9 minutes 12 seconds



results

results **B)** Explore the molecule annotated using public spectral library available on GNPS. Click on [View All Library](#)

Workshop AMG Hits 1 - 30 out of 43

Filter	ViewLib	Compound Name	Library Class	Cosine	MZErrorPPM	MassDiff	LibMZ	Instrument	IonMode	PI	Ion Source
1	ViewLib	Abyssomin B	Gold	0.95	2657	1.005	378.16	Hybrid Ft	Positive	Dorrestein	DI-ESI

Query

Library

Hits.

[Back to main page](#)

[Back to status page](#)

[Collapse all](#)

[Download](#)

Go to Go

ClusterIdx	Library Class	Cosine	SharedPeaks	TIC Query	RT Query	MZErrorPPM	MassDiff	Spe
View Raw	Bronze	0.99	14	56473.80	0.00	1281	0.47	3

results **C)** Go back to the Status Page

results **D)** Click on the [View Spectral families](#) and visualize the molecular network 1

Status	Auxiliary Views [View Network , Node Centric View Network Pairs Networking Statistics]
	Advanced Views - Networking Graphs [Nodes, MZ Histogram Edges, MZ Delta Histogram Edges, Score vs MZ Delta Plot Library Search, PPM Error Histogram]
	Community Matches [Dataset Matches]
	Network Visualizations [View Spectral Families (In Browser Network Visualizer) Network Summarizing Graphs]
	Export [Download Clustered Data Download Cytoscape Data Download Bucket Table Make Public Dataset]
User	lfnothias (lnothiascaglia@ucsd.edu), UCSD, Dorrestein Lab
Title	Workshop AMG

results E) In Node Labels (bottom left), map the parent mass, or the LibraryID, in the molecular network.

results F) Visualize a first MS/MS spectrum by left-clicking on one node. Visualize a second MS/MS spectrum by right-clicking on a second node.

More on navigating into the results with the following video: