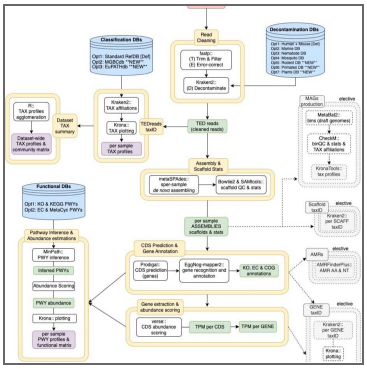

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WGSA2 workflow - a tutorial

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WGSA2

Angelina Angelova

ABSTRACT

The exploration of the microbiome has gained significant attention, leading to the development of powerful computational tools and online pipelines. These tools relieve researchers from the burdensome task of bioinformatics processing for their metagenomics data. Some existing tools (e.g. MetaPhlAn) enable the extraction of taxonomic and functional information directly from shotgun metagenomic short reads. However, more comprehensive analyses rely on tools that often require longer contiguous sequences (e.g. KEGG tools, BLASTp). Unfortunately, there is a scarcity of online tools that provide researchers with computational resources and a command line-free experience to assemble short-read metagenomic datasets for deeper exploration.

To address this computational gap, our Nephele2 team at the National Institute for Allergies and Infectious Diseases (NIAID) designed, developed, and integrated a command line-free Whole Metagenome Sequencing Assembly-based pipeline called WGSA2, into our cloud-based microbiome analysis platform, [Nephele] (https://nephele.niaid.nih.gov/). WGSA2 facilitates the processing of shotgun datasets derived from complex microbial communities and diverse habitats, including both host-associated and environmental samples. This pipeline starts by processing raw reads and proceeds to perform functional and taxonomic annotations, to bin the assemblies and to generate graphics to summarize various tabular outputs.

The pipeline offers a user-friendly experience that omits computational demands and expertise. It allows for customizations of profiling strategies and selection of databases (e.g. RefSeq, MGBC, KEGG, MetaCyc). It enables efficient acquisition of biological detail and grants users with easy access to assembly-based sequences

Protocol status: Working
We use this protocol and it's working

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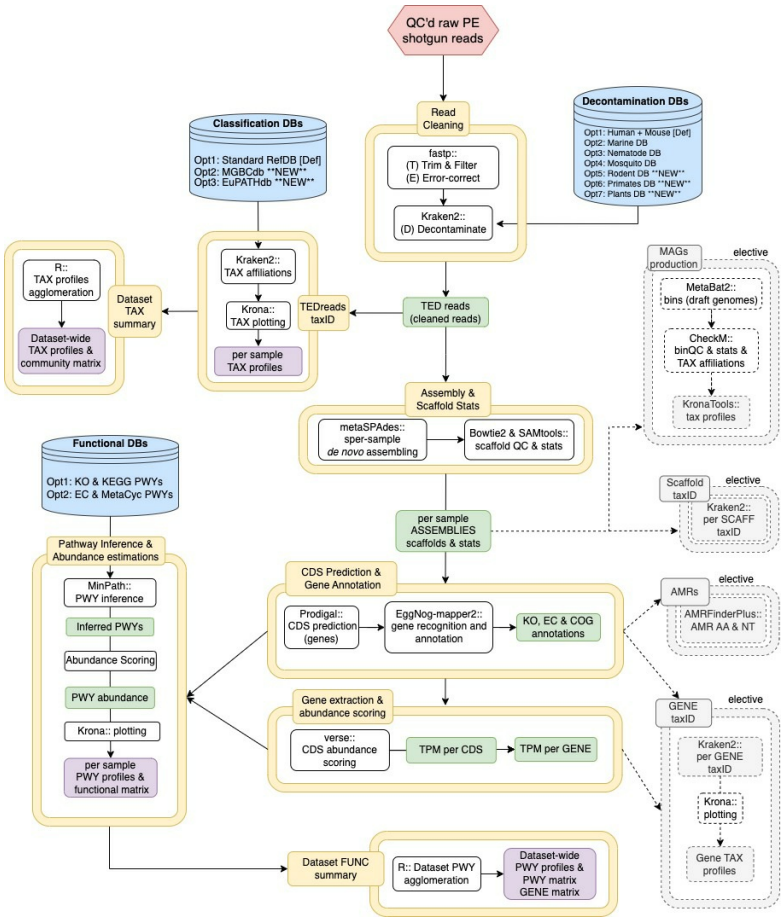
and analysis of their datasets. Overall, WGSA2 fills a computational void in metagenomics, enhances accessibility and comprehension of the data, and paves the way for deeper exploration of the microbiome.

This protocol goes through the steps of the WGSA2 workflow, explaining the tools and processing applied in each step.



WGSA2 workflow

ATTACHMENTS

WGSA2.2_WorkflowDiagram.jpg

NEPHELE_wgsa_example_mapping.xlsx

GUIDELINES

This is a tutorial on the usage, processing steps and result interpretation of WGSA2 pipeline hosted on cloud-based microbial analysis platform Nephele (https://nephele.niaid.nih.gov/).

MATERIALS

1. Visit Nephele online at https://nephele.niaid.nih.gov/
2. Globus endpoint for shotgun metagenomic dataset, shared with Nephele
3. Nephele WGSA2-formatted metadata file of your dataset

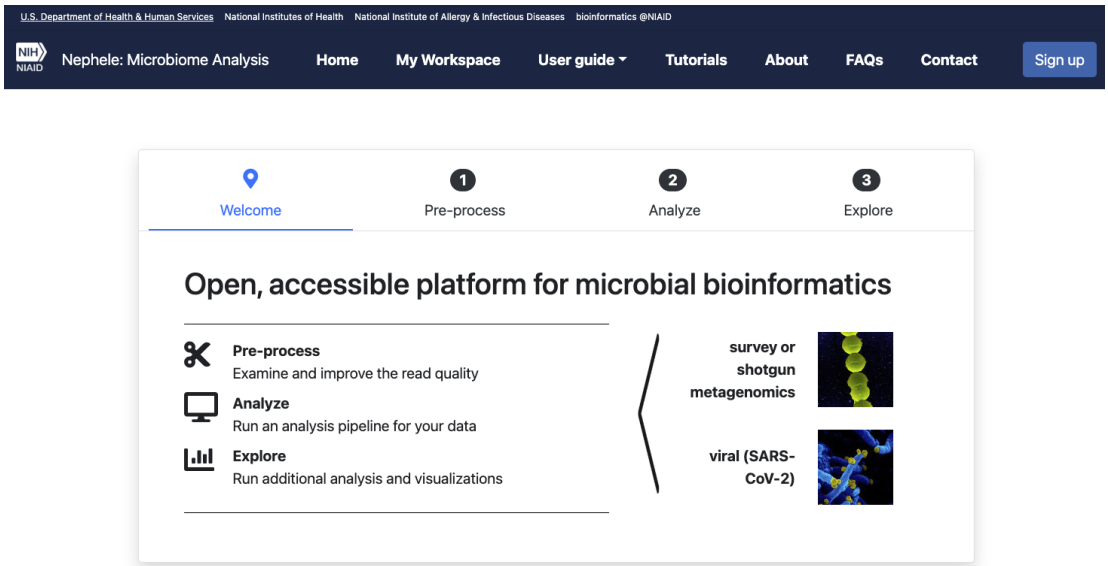
BEFORE START INSTRUCTIONS

1) Set up a Globus endpoint for your dataset (if needed). See details here: https://nephele.niaid.nih.gov/using_globus/

2) Download Nephele metadata template for WGSA2 and fill it out. The template is available in the Materials.

Start WGSA2 job

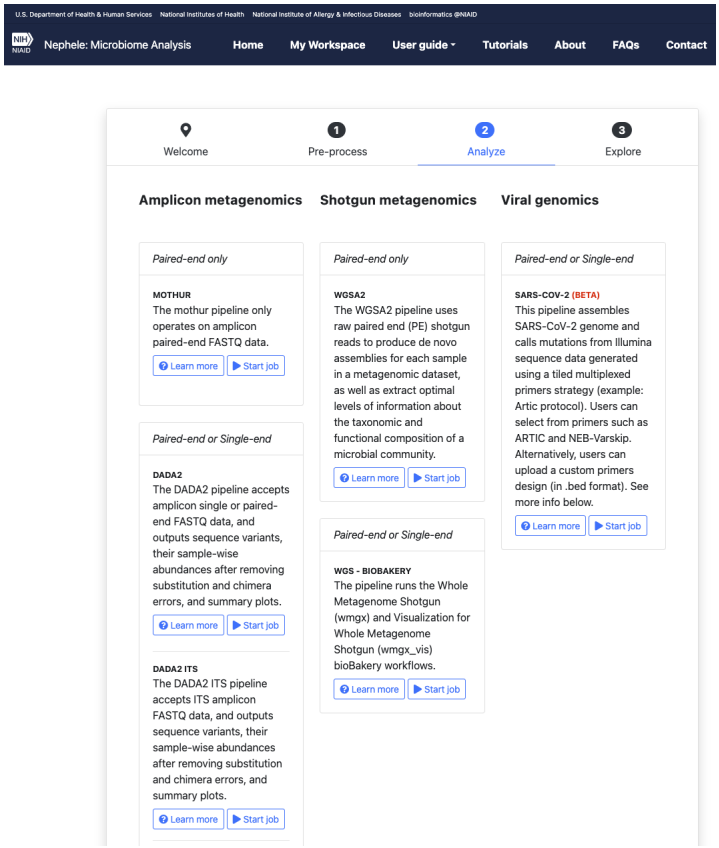
1 **Start by going to the Nephele web platform at** https://nephele.niaid.nih.gov/



Front page of Nephele Web Platform

2 **Select the "Analyze" tab and proceed to select the WGSA2.2 pipeline.**

We will start a WGSA2 job by following simple steps, described below



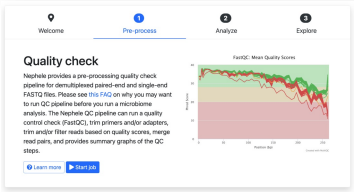
The "Analyze" tab

Note

We strongly recommend that you first run the Pre-processing (QA) pipeline, and choose to trim and filter reads. This is to ensure only the best quality reads are being used for WGSA2 processing.

You can read more about the QA pipeline from its details page (documentation). An example/template mapping file specific for this QA pipeline can be downloaded here. To download a mapping file for the QA pipeline, use this:

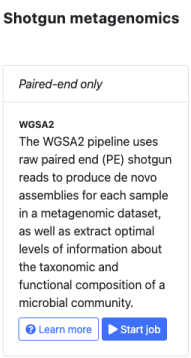
QC_pipeline_template_paired_end_N2.xlsx



Pre-processing tab

3 Run the "WGSA2" pipeline by clicking the "Start job" button

To initiate a job with WGSA2, click on the "Start job" button. This will take you through the subsequent steps of submitting a job



Selecting the WGSA2 pipeline

Note

You can learn more about this pipeline, its tools, methods and workflow by clicking the 'Learn more' button, or go to https://nephele.niaid.nih.gov/details_wgsa/

4 General submission tasks (data upload)

More detail about these steps, including how to upload data, can be found in the section "How to submit a job".

4.1 Make an account and sign in

In order to run any job in Nephele, you need to make an account using some basic information (<2 min) and log in.

Making an account with Nephele is easy! All you need is an email and a trusty password. The account is free as well as running all the available workflows.

4.2 Upload sequence data

Nephele provides multiple ways for data upload including FTP, direct upload and via Globus endpoint. More about how to upload data can be found in the section "How to submit a job".

- The input sequences **must be paired-end (PE)**, **whole metagenome shotgun** reads (**not merged**) in **FASTQ format**.
- **No special characters** in sequence file names.
- Dataset size should not exceed the **150Gb size limit (gzipped)**
- Both **zipped (.gz) and unzipped FASTQ** files are accepted (.fastq.gz & .fastq)

Note

Due to the huge resource and time requirements of WGSA2, which grows with dataset size, the entire submitted dataset must not exceed 150Gb gzipped. In the rare cases your dataset might exceed this size, please consider splitting it into separate submissions.

4.3 Upload metadata

The metadata table will be used by the WGSA2 pipeline to locate and identify your files, associate them with sample names and group them appropriately for the community exploration at the last steps of the pipeline.

This is a tab-delimited file containing information about submitted samples (sample names),

the names of their respective FASTQ files, and a simple grouping column (called "TreatmentGroup") that provides grouping information for dataset stats and visualization steps. It is advised such a file is prepared before submission. The template can be downloaded here. Mapping file can be an .xlsx file or a simple .txt file with the following format:

#SampleID	ForwardFastqFile	ReverseFastqFile	TreatmentGroup
SampName1	SampName1_R1.fastq.gz	SampName1_R2.fastq.gz	Control
SampName2	SampName2_R1.fastq.gz	SampName2_R2.fastq.gz	Control
SampName3	SampName3_R1.fastq.gz	SampName3_R2.fastq.gz	Treatment
SampName4	SampName4_R1.fastq.gz	SampName4_R2.fastq.gz	Treatment

Metadata file format

For your convenience, an example / template metadata file, specific for WGSA2, is attached below.

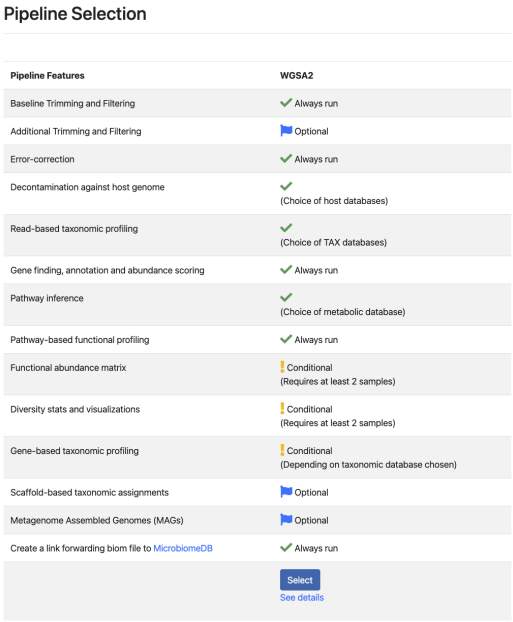
wgsa_example_mapping.xlsx

Note

It is a good rule of thumb to have your metadata file filled out and ready to upload at this step of the job submission. However, it is not a problem if you need to download and fill out the template during submission. You will **not** be logged out from our system or lose your progress within the submission process, for a few hours.

5 Main pipeline info

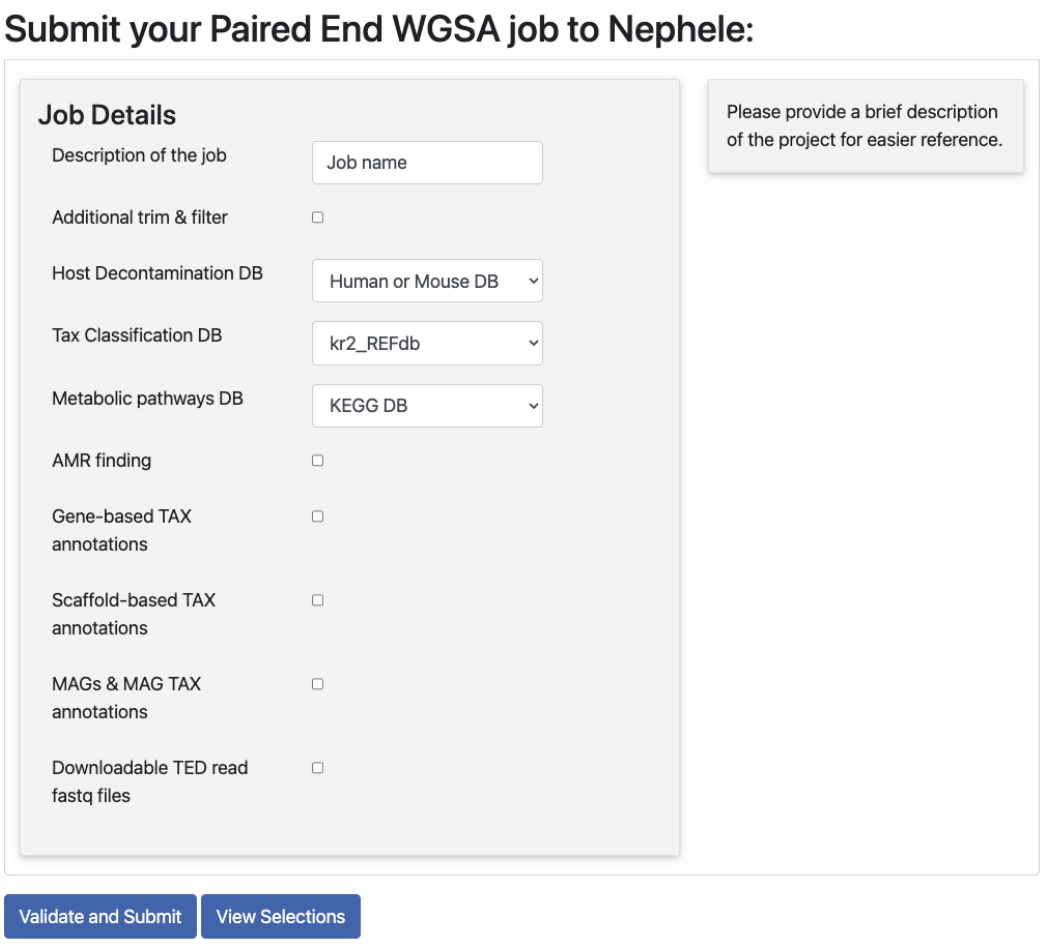
Once your data and metadata are uploaded, the Nephele job submission system will take you to a "Pipeline Selection" screen that allows to you familiarize yourself with the main steps of processing that WGSA2 will go through. This screen is primarily informational and mostly makes you aware that certain processing steps will always happen while others are conditional or optional to datasets (with 2+ samples or user elections). Read through and hit "select".



Pipeline main steps info

6 Selecting WGSA2 job settings (WGSA2 job options menu)

In this menu, you get to customize the settings of WGSA2 based on your processing needs for your dataset.



WGSA2 job customization options

Apart from the basic Job Name (a brief description of your job for your ease of recognition), these settings include additional trimming and filtering, choice of databases for decontamination, taxonomic classification and metabolic pathways hierarchy. Users may also select WGSA2 to perform additional steps of analysis such as AMR finding, gene-based taxonomic classification, MAGs formation, and more.

Note

The **default parameters of WGSA2** are a great starting point for any metagenomic dataset! You may choose to run your first job on any dataset with the pre-set default parameters, and the results will provide you with enough information to familiarize yourself with your dataset's communities and pipeline outputs.

Once you understand the workflow, parameters, and output of the pipeline, you may customize the jobs for any dataset at will.

In the next sections of this protocol, we will be describing what each step of the pipeline's workflow does, and how the customization options from this menu affect the pipeline's behavior and output.

We hope that through those instructions, you will gain the understanding needed to independently choose the best options for any of your datasets and research goals.

7 **Submit job**

For now, let us leave the dataset to the default settings (as is) and **click 'Submit'**.

Your job with WGSA2 will then start, and you will be provided with a **job ID**. This job ID is important for customer support in case of problems, as well as keeping track of your job status. An email will also be sent to you upon job start and job completion. This email will include the job ID, job details and other job information.

You can have multiple jobs submitted and running at once.

Upon completion of the pipeline, you will be able to view some results directly in your browser. All detailed results and outputs will be provided as downloadable files.

> Note
>
> WGSA2 is a completely command line free & automated pipeline. Beyond submitting a job, you will not need to do anything to produce the pipeline results.

WGSA2 processing: 1 - TEDing module (pre-assembly process... 30m

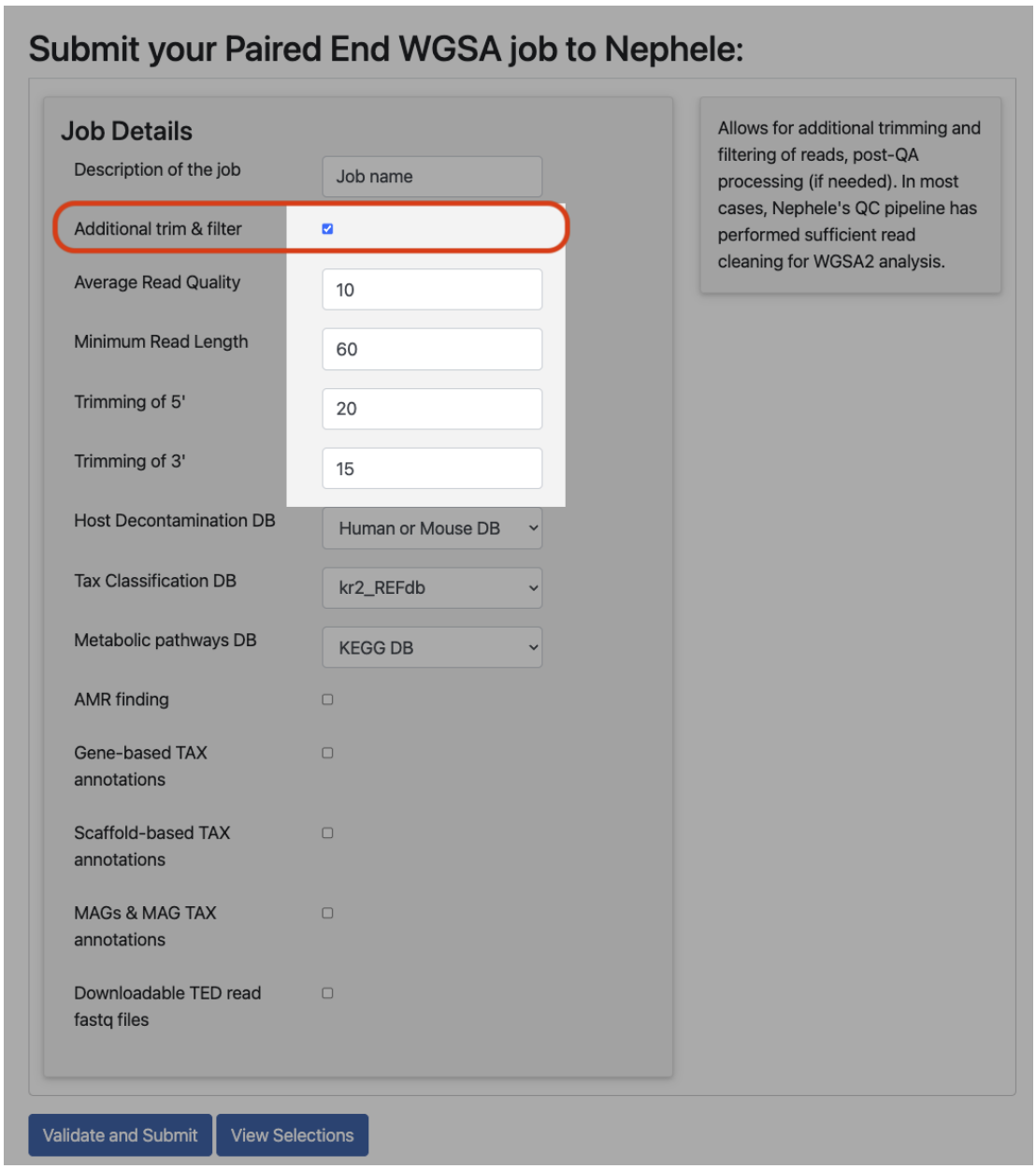
8 **(T) Trimming & filtering of raw reads** 5m

Trimming and filtering steps are performed with fastp.

> Note
>
> Note: This step is not as thorough as the recommended pre-processing performed by our QC pipeline and therefore should not be considered as its replacement. If you have run your dataset through the QC pipeline, feel free to leave this option unselected.

In this quick trim and filtering step, WGSA2 verifies that the reads from the submitted dataset have met the minimal quality and length standards required for assembly.

However, if the user has selected customizations through the **"Additional trim & filter"** option in the ***Job options menu*** (see step 6 of this protocol), then those more stringent parameters will be applied.



Additional trim & filter

Note

If you choose to select the additional trim and filter checkbox from the WGSA2 options menu (see step 6 of this protocol), a few more options will appear where you can provide more detail for the trimming & filtering parameters of your choice.

9 **(E) Error-correction** 5m

Performed automatically with fastp.

PE sequences are merged, and sequencing errors are corrected within the PE overlapping regions. This process is specifically designed to improve assembly efficiency and success.

This process is mandatory for the pipeline and undergoes no customization (see step 5, Pipeline selection section)

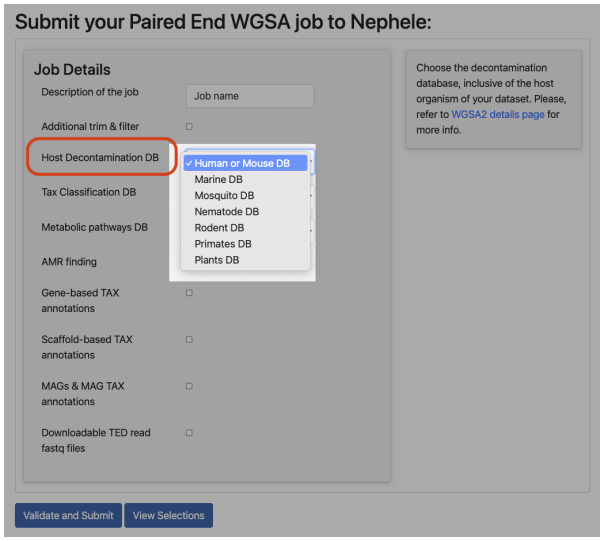
10 **(D) Decontamination** 15m

Performed with Kraken 2.

In the pre-assembly processing, the dataset undergoes a thorough cleaning process to remove host DNA and non-informative elements, such as homopolymeric or simple sequence repeats. This is achieved by utilizing a **Host Decontamination database**, containing a curated collection of thematically related host organism genomes.

The decontamination process is crucial for reducing the dataset size, by excluding biologically irrelevant data (non-informative host DNA, junk and repeat sequences). Thus the assembly quality of each sample is significantly improved.

The **Host Decontamination database** used in this processing step, is user-electable in the ***Job options menu*** (see step 6 of this protocol). By default, WGSA2 will decontaminate any dataset against the **Human & Mouse DB**, however you may elect (from the drop-down menu of the Job Options) a more appropriate decontamination database for your submitted dataset, *depending on the host organism or environment from which your dataset's microbial community is collected*.



Host Decontamination DB choices

All decontamination databases of WGSA2 are custom curated based on a theme of host organisms or environments that a microbial community might be sourced from.

The user choices for **Host Decontamination DBs** are:

- **Human & Mouse Host DB** (includes human + mouse host genomes)[**default** DB for quickest removal of most common host contaminants]
- **Marine Hosts DB** (includes human + 18 common marine host organism genomes)
- **Mosquito Hosts DB** (includes human + 2 common mosquito species genomes)
- **Nematode Hosts DB** (includes human + 15 common nematode host organism genomes)
- **Rodent Hosts DB** (includes human + 5 common rodent host organisms)
- **Primates Hosts DB** (includes human + 9 common primate host organisms)
- **Plant Hosts DB** (includes human + >110 common plant host organisms)

Please see WGSA2's documentation page for more detail on the specific genomes and assembly versions each database contains.

WGSA2 processing: 2 - TAX profiling module

30m

STEP CASE

TEDing module output From 5 to 14 steps

The TEDing module will output the same TED reads:

These are the cleaned sequences of your dataset that have undergone
1) Trimming & filtering,
2) Error-correction, and
3) Decontamination from host genomic and repeat sequences

Upon pipeline completion, you may download the resulting output folder from your WGSA2 job, and find these TED reads in subfolder named "**TEDreads**"

The **TEDreads folder** will contain per-sample logs and reports from the TEDing steps (e.g. *_fastplog.html , _decontamLOG.txt & more*), where you can find information about the trimming success & host contamination levels removed from each sample.

```
Loading database information... done.
247878 sequences (120.94 Mbp) processed in 1.526s
(9749.1 Kseq/m, 4756.69 Mbp/m).
  149797 sequences classified (60.43%)
  98081 sequences unclassified (39.57%)
```

Content of TEDreads/Samp1_decontamLOG.txt

11 Taxonomic profiling

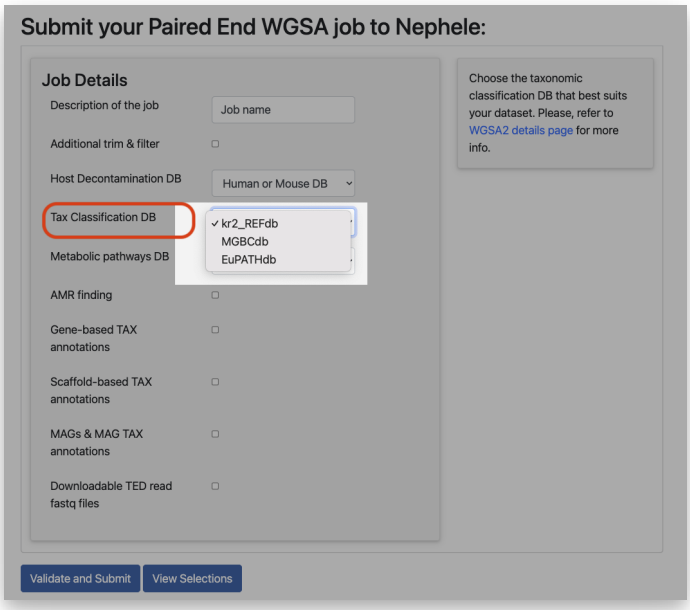
Performed with Kraken 2

By default, WGSA2 generates taxonomic profiles from the TEDreads of every sample individually, utilizing the Kraken2 tool. This highly accurate and computationally efficient method for taxonomic identification relies on a comprehensive database of k-mers derived from entire genomes of known organisms. This enables accurate taxonomic assignments for both coding and non-coding sequences, contained within the TEDreads.

For this processing step, the **taxonomic classification DB** is also *user-electable*. The applied **default** is a Kraken2 version of NCBI's RefSeq DB (Kraken2 Standard Reference Genomes DB; **kr2_REFdb**). However, depending on interest and dataset source, user may choose one of a few other options: The Mouse Gastrointestinal Gut Catalogue DB (**MGBCdb**), containing >26.6K prokaryotic organisms characteristic for mouse gut microbiomes (both reference and non-reference organisms), and the Eukaryotic Pathogens DB (**EuPATHdb**), containing genomes of known eukaryotic pathogens.

For more information about the available classification DBs, please, refer to WGSA2 documentation page.

To have WGSA2 perform TAX classification based on a different DB, other than the default, the user may elect a **taxonomic classification DB** in the ***Job options menu*** (see step 6 of this protocol) upon job submission.



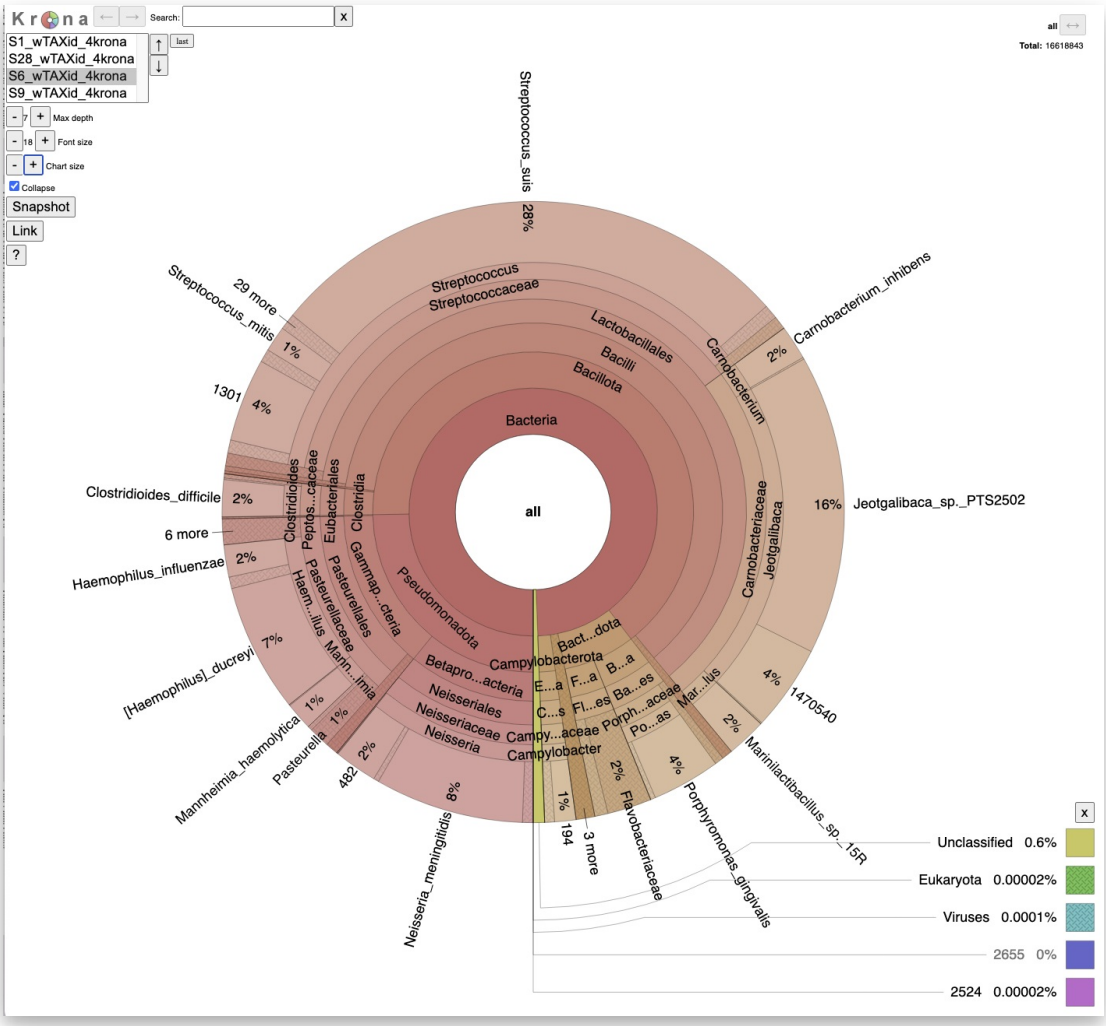
Taxonomic classification DB

12 TAX profile visualizations

2m

Performed with Krona Tools

In this processing step, the resulting taxonomic profiles (from the previous step) are re-formatted and visualized using **Krona tools** on a per-sample basis. The resulting plots are in HTML format and present the community's taxonomic composition in a circular interactive plot produced per sample. With the latest WGSA2 pipeline update, the highest taxonomic level is presented in the taxonomic ID (UUID or TAXid) for each detected organism.



Example Krona taxonomic profile plot (per sample)

Note

Since the MGBCdb include non-reference organisms and taxonomically undefined (yet) genomes, its taxonomic IDs do not correspond to NCBI's UUIDs.

WGSA2 processing: 3 - Assembly module

10h

STEP CASE

Files associated with Krona visualizations

From 3 to 12 steps

The output from this module can be found in the WGSA2 results folder: ***TAXprofiles > readsTAX_{DBname}/***, *where* the {DBname} suffix will be

naming the TAX classification DB elected by user.

The outputs of the Kraken2 step are text format samples, contained within the *> reports/ folder* . These text files are used for community profiles visualizations, as well as in the subsequently produced dataset-wide community matrix table, which is the foundation of microbial community analysis studies.

The krona re-formatted Kraken2 reports (produced from the previous step) can be found in the > *bin/* foler. Those are used by KronaTools to produce the interactive HTML report ***TAXprofiles > readsTAX_{DBname} > TAXplots_readsTAX_<DBname>.html***

13 Assembly

Performed by metaSPAdes assembler

In this step, a *de novo* assembly of the TED reads is performed for each sample independently. This produces long contiguous sequences (contigs), that are then arranged, oriented, and connected (with Ns), based on PE data of the incorporated reads until scaffolds of contigs are achieved. As metagenomic datasets are composed of numerous genomes, the produced assemblies are composed of numerous long (and short) scaffolds, each representing a genomic loci of a contained organism.

> **Note**
>
> This is a rather time and resource consuming step and tends to take even longer for large datasets containing complex communities! Please be patient with the WGSA2 processing.

14 Assembly processing - Read mapping (read alignments)

4h 30m

Performed with Bowtie2 and SAMtools

In this step the TED reads are again used to align to the corresponding sample's scaffolds (a process called 'mapping'). The alignment of the reads is also QC'd and cleaned (de-replicated, mate pairs are fixed, etc.) to ensure each pair of reads maps best and only once to the produced assembly.

> **Note**
>
> This is one of the most time and resource consuming stages of the pipeline and depending on sample size may take longer than a few hours per sample. Parallelization is used to achieve assembly of multiple samples simultaneously (however, patience is still required). Pipeline failures in this processing stage suggests problematic sample depths or read quality.

15 **Assembly processing - Abundance scoring** 10m

Performed by SAMtools and some math

The aligned reads are evaluated (GC content, plus/minus strand alignments) and enumerated. Abundance scores are produced per scaffold per sample (scaffold length, depths, coverage, reads per million (RPM), etc.), and other statistics are collected. This information is used for downstream abundance assessments, as well as every other downstream processing step of the pipeline.

WGSA2 processing: 4 - Gene discovery module 6h

STEP CASE

Output of processed assembly

From 3 to 9 steps

For each assembled sample an individual folder is produced:

asmbMetaSpades > {SampleName}_asmb/

This collects only that sample's information, including the assembly's FASTA file, assembly stats, alignments and all other files related to the processed assembly. All files produced from the assembly (step 13) and assembly processing steps (steps 14-15) carry the {final.assembly} prefix and are the main files used for all downstream processing. These include:

- ***final.assembly.fasta*** (the FASTA representation of the assembled sample)
- ***final.assembly_stats.txt*** (statistical information about the assembled sample (e.g. N50)
- ***final.assembly_scaffCoverage.txt*** (scaffold abundance, coverage, read counts, length and other per-scaffold in {SampleName} related information
- *final.assembly.bam* (processed read alignment file) [can be used if user wishes to extract specific short reads aligned to a genomic locus of choice (e.g. gene)]
- *contigs.fasta* (a contig-based representation of the assembled sample)

Note:
Sub-folders are also present within this main assembly folder. Those are produced from other, subsequent steps of the WGSA2 pipeline and may vary based on user options elected. The content of such sub-folder will be described in the corresponding sections.

16 **Gene prediction**

Performed with Prodigal

The tool scans through scaffold sequence in each sample and predicts potential partial or full-length continuous coding regions (features, genes) that commonly occur in prokaryotes, and unicellular eukaryotes.

17 **Gene abundance scoring** 15m

Performed by VERSE tool (used setting: “featureCounts”) and pipeline math

Gene abundance scores are computed by enumerating the number of reads aligned to the gene

coordinates of the scaffold.

Reads per Million (RPM) mapped reads and transcripts per million (TPM) values are calculated based on *per transcript instance* in each scaffold *in each sample* and produce the abundance scores of each gene.

In WGSA2 pipeline, the notation 'iTPM' is used to denote conventional TPM scores. This is to distinguish from another statistic of the pipeline 'geneTPM' which represents calculated average abundance score (TPM) for each *unique annotation* within sample. A geneTPM abundance matrix is also provided with WGSA2.

STEP CASE

Gene prediction & abundance scoring result

From 1 to 7 steps

Expected output is contained within each sample's assembly folder at:
asmbMetaSpades > {SampleName}_asmb > genes/ folder.

This includes files with the *PREDgenes* prefix, containing the predicted gene's NT and AA sequence information (*PREDgenes.fna and PREDgenes.faa*) as well as a gene annotation file (*PREDgene.gff*). These files can be used by the user for custom gene annotation (e.g. the KEGG's GhostKoala tool) or identifying the location coordinates of each predicted feature.

The outputs also contain summary statistics information such as gene scoring stats (PREDgenes_stats.txt) and **gene abundance information (PREDgenes_ABUN.txt),** such as gene length, aligned reads, coverage, RPK of gene and **gene's iTPK (the TPM for that particular instance of the gene)**.

18 **Gene annotation**

Performed by EggNOG-mapper2 and integrated EggNOG-v5 database

The predicted features are compared to a database of known genes (EggNOG-v5) and upon sufficient homology the gene annotation is transferred to the feature. Gene annotations obtained are based on KO, EC and COG annotation systems.

WGSA2 processing: 5 - Pathway profiling module

30m

STEP CASE

Gene annotations

6 steps

Gene annotations are outputted in the folder:
asmbMetaSpades > {SampleName}_asmb > genes > annotations/.

The content includes the annotations ({*annots}* prefix) produced by the EggNOG-mapper2, as well as the NT and AA files based on the KEGG (***annots.ko.{faa, fna, txt}***) and the EC annotations (***annots.ec.{faa, fna, txt}***).

Important summary and abundance scoring files from the annotated output are presented in the

ANNOTgenes_ABUNDtab.{ko,ec}.txt files (including the iTPM for each instance of the annotated gene).

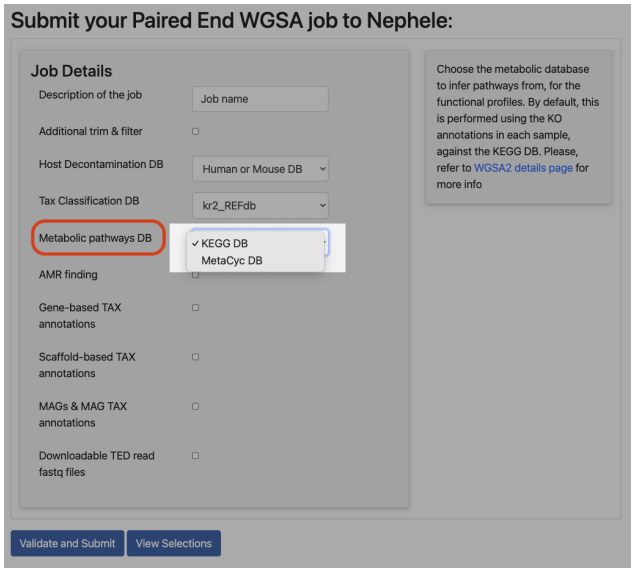
With the latest WGSA2 update, the pipeline now also outputs a **per unique gene annotation summary** files ***{SampleName}_geneTPM.{ko, ec}.txt,*** which contains the **average TPM per unique annotation (geneTPM)** as well as the gene's short and long names. These are also gathered from each sample and stored at **PWYprofiles/<PWYdb_name>/genebin/** for downstream utilization (e.g. data collation).

19 Pathway (PWY) inference

Performed with MinPath

The produced gene annotations (KO or EC) are mapped against a user-elected database of cellular pathways (KEGG or MetaCyc). A parsimony approach is then used to reconstruct the minimal set of pathways (from the selected DB) that can be described by the annotations (query of genes) within each sample (MinPath).

The default WGSA2 database for PWY inference is the KEGG db, along with the KO annotations produced in each sample. Users may opt for a different approach: to use the EC annotations of the samples to map against the MetaCyc db of pathways (instead of KEGG), which provides a different metabolic PWY classification system and will therefore produce a slightly different metabolic representation for each sample. The DB of choice is to be elected based on your convenience and familiarity. Although the results may slightly differ, there is no known incorrect option.



PWY DB election

20 PWY abundance estimation

10m

Performed by 'genes.to.kronaTable.py' & pipeline math

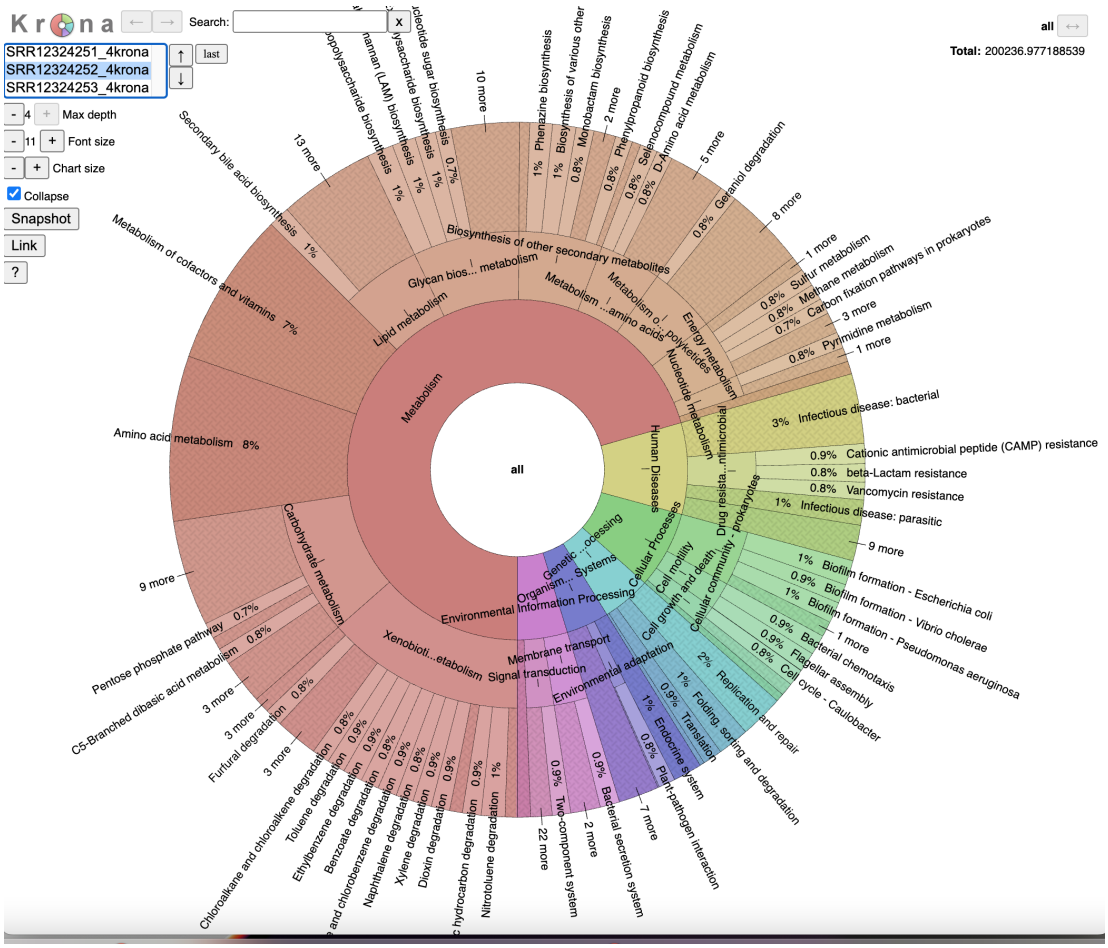
The abundance of each pathway is then calculated by averaging the TPM values from each

instance of gene, within each inferred pathway.

21 **PWY profile visualizations** 5m

Performed by Krona Tools

The metabolic characterization for each sample (representing the functional profile) are summarized for each sample in an interactive HTML report.



PWY krona plot

WGSA2 processing: 6 - Dataset summary module 20m

STEP CASE

PWY inference & profiling 3 steps

The PWY inference and abundance estimation text reports are outputted in folder **asmbMetaSpades/{SampleName}_asmb/genes/pathways/.**

Among these files is a text report of the final functional profile from each sample ({SampleName}_4krona.txt).

This file is collected from each sample and gathered into a pwy_bin/ folder at **PWYprofiles/<PWYdb_name>/pwy_bin/** for downstream utilization (e.g. KronaTools visualizations, data collations, etc)

To obtain a visual representation of these functional profiles, KronaTools is again used. The functional profile HTML interactive report is presented in **PWYprofiles/<PWYdb_name>/PWYplots_<PWYdb_prefix>.html**, where <PWYdb_prefix> is either *ko2gg* or *ec2mc*, depending on user election if functional database.

22 Data collation

Performed in R statistical language

The steps in this module are among the most important ones for the summary characterizations of any dataset. Since the pipeline has already created per-sample individualized profiles and summaries, this module is conditional to datasets with multiple samples (best if >2 samples).

In this module, the feature characterizations and abundance information from each individual sample are collected and summarized (collated) into a dataset-wide abundance matrix. This matrix is a non-redundant feature (TAX, PWY or GENE) representation of each sample's composition (abundance matrix).

Note

The abundance matrix is a table that contains the feature identity (taxonomic or functional) and abundance information for each sample within a dataset. It is the **abundance matrix** that is the fundamental part of any **downstream community-based microbial exploration**.

	Kingdom	Phylum	Class	Order	Family	Genus	Species	Sample 1	Sample 2	Sample 3	Sample 4
id816	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_sp	342851	375620	396511	4092
id821	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Phocaeicola	Phocaeicola_vulgatus	332671	455127	397689	15303
id46506	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_stercoris	319501	181507	345431	17253
id2955582	Viruses	Uroviricota	Caudoviricetes	Crassvirales	Intestiviridae	Carjivirus	Carjivirus_communis	262251	0	262251	32785
id853	Bacteria	Bacillota	Clostridia	Eubacteriales	Oscillospiraceae	Faecalibacterium	Faecalibacterium_prausnitzii	138249	190576	165474	6537
id216851	Bacteria	Bacillota	Clostridia	Eubacteriales	Oscillospiraceae	Faecalibacterium	Faecalibacterium_sp	133307	181445	159228	6013
id28116	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_ovatus	131259	71500	141473	7474
id820	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_uniformis	120766	128131	139070	917
id2714355	Bacteria	Bacillota	Clostridia	Eubacteriales	Oscillospiraceae	Vescimonas	Vescimonas_coprocola	58163	2043	58455	7019
id909656	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Phocaeicola	Phocaeicola_sp	51924	64400	61124	1556
id46503	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Tannerellaceae	Parabacteroides	Parabacteroides_merdae	47974	43007	54118	625
id2955583	Viruses	Uroviricota	Caudoviricetes	Crassvirales	Intestiviridae	Carjivirus	Carjivirus_hominis	43428	0	43428	5433
id418240	Bacteria	Bacillota	Clostridia	Eubacteriales	Lachnospiraceae	Blautia	Blautia_wexlerae	42607	4454	43243	4773
id2585118	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_communis	42012	13002	43869	3630
id214856	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_finegoldii	41735	21150	44756	2577
id186803	Bacteria	Bacillota	Clostridia	Eubacteriales	Lachnospiraceae		Lachnospiraceae_sp	38232	42352	44282	511
id815	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae		Bacteroidaceae_sp	36359	70135	46378	4218
id357276	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Phocaeicola	Phocaeicola_dorei	34903	15585	37129	2419
id2929495	Bacteria	Bacillota	Clostridia	Eubacteriales	Oscillospiraceae	Faecalibacterium	Faecalibacterium_l4-3-84	30756	7302	31799	2936
id818	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_thetaiotaomicron	25452	57238	33629	3969
id28118	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Odoribacteraceae	Odoribacter	Odoribacter_splanchnicus	24049	23490	27405	74

Example feature abundance table

23 Convert to biom format

5m

Performed by biom-format

The taxonomic and functional abundance matrices are further formatted into **biom** files that include the user metadata. These biom files can be conveniently uploaded into [MicrobiomeDB] or customized as needed for other user-specific purposes.

STEP CASE

Abundance matrixes 1 step

All the most important taxonomic and functional analysis compositional abundance matrices created during the pipeline run, can be found within the **merged_tables/** folders within the corresponding **TAXprofiles or PWYprofiles/** folders.

These compositional tables include:

- **Taxonomic compositional matrix for dataset** - the taxonomic information and abundance from each sample, collated into one non-redundant matrix. This is used for any taxonomic microbiome analysis and statistical comparison between communities. Stored in **TAXprofiles/readsTAX_<DBname>/merged_tables/**merged_Counts+TAX.txt

- **Genetic compositional matrix for dataset** - the gene information and abundance from each sample collated into one non-redundant matrix. This is used for any gene-focused microbiome explorations. Stored in **PWYprofiles/<PWYdb_name>/merged_tables/**merged_geneTPMtable.txt

- **Pathway compositional matrix for dataset** - the functional (inferred pathway) information and abundance from each sample, collated into 1 non-redundant matrix. This is used for any functional analyses of communities and statistical comparisons between them. Stored in **PWYprofiles/<DBname>PWYs.MP/merged_tables/**merged_Counts+PWY.txt

All files in these folders have a generally similar structure. They represent an abundance matrix for the dataset, inclusive of all the samples and various features (TAX or PWYs or GENEs). This information however is presented in different ways:
1) the per-sample counts alone (merged_Counts.txt)
2) the features identities alone (merged_{TAX, PWY}.txt)
3) the per-sample counts with the features table adjoined, in various combinations: each rank/level as separate columns (**merged_Counts+{TAX, PWY}.txt**), or concatenated together as lineage within 1 column (e.g. merged_Counts+Lineage.txt), or both (merged_Counts+TAX+Lineage.txt).
4) the per-sample abundance table (merged_Counts+Lineage.txt), presented as a *json biom* file (**merged_Counts+Llineage_json.biom**).

These files present the same information but separated by format for convenient utilization in downstream analytical tools.

24 Exploratory visualizations and statistics

Performed with R statistical language

The steps in this module are conditional to datasets containing multiple samples (best if >3 samples), as they require statistical calculations and summary visualizations.

The obtained **TAX and PWY abundance matrices**, are used to produce community characterizing visualization plots (e.g. alpha and beta diversity plots, abundance profile heat-maps etc.). These are especially useful for quick familiarization with the composition of the samples in your dataset and understanding some of the community relations.