NMDC Workflows Documentation

Release 0.2a

National Microbiome Data Collaborative

Dec 14, 2021

Contents:

CHAPTER 1

Overview

1.1 NMDC

The National Microbiome Data Collaborative (NMDC) is a new initiative, launched in July 2019 and funded by the Department of Energy's (DOE) Office of Science, Biological and Environmental Research program, that aims to empower the research community to more effectively harness microbiome data. The NMDC is building an open-source, integrated data science ecosystem aimed at leveraging existing efforts in the microbiome research space, including data standards, quality, integration, and access, to create a linked data discovery portal. Read the Nature Reviews Microbiology Comment on the NMDC or visit the NMDC website.

Four national laboratories are working together to produce the NMDC:

- Lawrence Berkeley National Laboratory
- Los Alamos National Laboratory
- Oak Ridge National Laboratory
- Pacific Northwest National Laboratory

1.2 NMDC Workflows

1.3 General Guidelines

NMDC aims to integrate existing open-source bioinformatics tools into standardized workflows for processing raw multi-omics data to produce interoperable and reusable annotated data products. Any commercial software are optional alternatives and not required.

1.4 Execution Evironment

Two common ways to install and run the NMDC workflows:

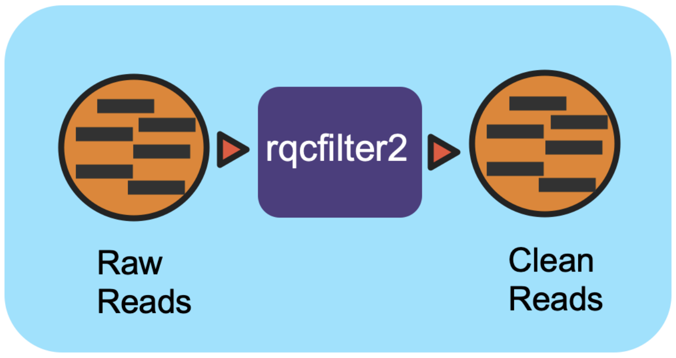
- Native installation
- Containers

The NMDC workflows have been written in WDL and require a WDL-capable Workflow Execution Tool (i.e., Cromwell). To ease the native installation, Docker images have been created for the third-party tools for all of the workflows as well. The workflows use the corresponding Docker images to run the required third-party tools. Databases must be downloaded and installed for most of the workflows.

The NMDC workflows are also available as a web application called NMDC EDGE . The application has only the NMDC workflows integrated into an updated framework for EDGE Bioinformatics ; this provides the workflows, third-party software, and requisite databases within a platform with a user-friendly interface. NMDC EDGE is provided as a web application especially for users who are not comfortable with running command line tools or without the computational resources to run the command line/ Docker versions.

CHAPTER 2

Reads QC Workflow (v1.0.1)



2.1 Workflow Overview

This workflow utilizes the program “rqcfilter2” from BBTools to perform quality control on raw Illumina reads. The workflow performs quality trimming, artifact removal, linker trimming, adapter trimming, and spike-in removal (using BBDuk), and performs human/cat/dog/mouse/microbe removal (using BBMap).

2.2 Workflow Availability

The workflow from GitHub uses all the listed docker images to run all third-party tools. The workflow is available in GitHub: https://github.com/microbiomedata/ReadsQC; the corresponding Docker image is available in DockerHub: https://hub.docker.com/r/microbiomedata/bbtools.

2.3 Requirements for Execution

(recommendations are in **bold**)

- WDL-capable Workflow Execution Tool (**Cromwell**)
- Container Runtime that can load Docker images (**Docker v2.1.0.3 or higher**)

2.4 Hardware Requirements

- Disk space: 106 GB for the RQCFilterData database
- Memory: >40 GB RAM

2.5 Workflow Dependencies

2.5.1 Third party software (This is included in the Docker image.)

- BBTools v38.90 (License: BSD-3-Clause-LBNL)

2.5.2 Requisite database

The RQCFilterData Database must be downloaded and installed. This is a 106 GB tar file which includes reference datasets of artifacts, adapters, contaminants, the phiX genome, and some host genomes.

The following commands will download the database:

```
mkdir refdata
wget http://portal.nersc.gov/dna/microbial/assembly/bushnell/RQCFilterData.tar
tar -xvf RQCFilterData.tar -C refdata
rm RQCFilterData.tar
```

2.6 Sample dataset(s)

Zymobiomics mock-community DNA control (SRR7877884); the original gzipped dataset is ~4 GB.

Note: If the input data is paired-end data, it must be in interleaved format. The following command will interleave the files, using the above dataset as an example:

```
paste <(zcat SRR7877884_1.fastq.gz | paste - - - -) <(zcat SRR7877884_2.fastq.gz |␣
↪paste - - - -) | tr '\t' '\n' | gzip -c > SRR7877884-int.fastq.gz
```

For testing purposes and for the following examples, we used a 10% sub-sampling of the above dataset: SRR7877884-int-0.1.fastq.gz. This dataset is already interleaved.

2.7 Inputs

A JSON file containing the following information:

1. the path to the database
2. the path to the interleaved fastq file (input data)
3. the path to the output directory
4. (optional) parameters for memory
5. (optional) number of threads requested

An example input JSON file is shown below:

```
{
    "jgi_rqcfilter.database": "/path/to/refdata",
    "jgi_rqcfilter.input_files": [
        "/path/to/SRR7877884-int-0.1.fastq.gz "
    ],
    "jgi_rqcfilter.outdir": "/path/to/rqcfiltered",
    "jgi_rqcfilter.memory": "35G",
    "jgi_rqcfilter.threads": "16"
}
```

Note: In an HPC environment, parallel processing allows for processing multiple samples. The “jgi_rqcfilter.input_files” parameter is an array data structure. It can be used for multiple samples as input separated by a comma (,). Ex: “jgi_rqcfilter.input_files”:[“first-int.fastq”,”second-int.fastq”]

2.8 Output

A directory named with the prefix of the FASTQ input file will be created and multiple output files are generated; the main QC FASTQ output is named prefix.anqdpht.fastq.gz. Using the dataset above as an example, the main output would be named SRR7877884-int-0.1.anqdpht.fastq.gz. Other files include statistics on the quality of the data; what was trimmed, detected, and filtered in the data; a status log, and a shell script documenting the steps implemented so the workflow can be reproduced.

Part of an example output JSON file is shown below:

```
SRR7877884-int-0.1
|-- SRR7877884-int-0.1.anqdpht.fastq.gz
|-- filterStats.txt
|-- filterStats.json
|-- filterStats2.txt
|-- adaptersDetected.fa
|-- reproduce.sh
|-- spikein.fq.gz
|-- status.log
|-- ...
```

Below is an example of all the output directory files with descriptions to the right.

FileName	Description
SRR7877884-int-0.1.anqdpht.fastq.gz	main output (clean data)
adaptersDetected.fa	adapters detected and removed
bhist.txt	base composition histogram by position
cardinality.txt	estimation of the number of unique kmers
commonMicrobes.txt	detected common microbes
file-list.txt	output file list for rqcfilter2.sh
filterStats.txt	summary statistics
filterStats.json	summary statistics in JSON format
filterStats2.txt	more detailed summary statistics
gchist.txt	GC content histogram
human.fq.gz	detected human sequence reads
ihist_merge.txt	insert size histogram
khist.txt	kmer-frequency histogram
kmerStats1.txt	synthetic molecule (phix, linker, lamda, pJET) filter run log
kmerStats2.txt	synthetic molecule (short contamination) filter run log
ktrim_kmerStats1.txt	detected adapters filter run log
ktrim_scaffoldStats1.txt	detected adapters filter statistics
microbes.fq.gz	detected common microbes sequence reads
microbesUsed.txt	common microbes list for detection
peaks.txt	number of unique kmers in each peak on the histogram
phist.txt	polymer length histogram
refStats.txt	human reads filter statistics
reproduce.sh	the shell script to reproduce the run
scaffoldStats1.txt	detected synthetic molecule (phix, linker, lamda, pJET) statistics
scaffoldStats2.txt	detected synthetic molecule (short contamination) statistics
scaffoldStatsSpikein.txt	detected skipe-in kapa tag statistics
sketch.txt	mash type sketch scanned result against nt, refseq, silva database sketches.
spikein.fq.gz	detected skipe-in kapa tag sequence reads
status.log	rqcfilter2.sh running log
synth1.fq.gz	detected synthetic molecule (phix, linker, lamda, pJET) sequence reads
synth2.fq.gz	detected synthetic molecule (short contamination) sequence reads

2.9 Version History

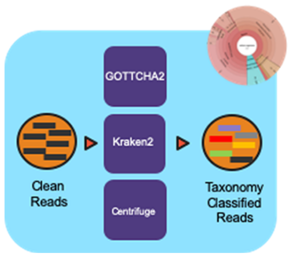
- 1.0.1 (release date **02/16/2021**; previous versions: 1.0.0)

2.10 Point of contact

- Original author: Brian Bushnell <bbushnell@lbl.gov>
- Package maintainer: Chienchi Lo <chienchi@lanl.gov>

CHAPTER 3

The Read-based Taxonomy Classification (v1.0.1)



3.1 Workflow Overview

The pipeline takes in sequencing files (single- or paired-end) and profiles them using multiple taxonomic classification tools with the Cromwell as the workflow manager.

3.2 Workflow Availability

The workflow is available in GitHub: https://github.com/microbiomedata/ReadbasedAnalysis; the corresponding Docker image is available in DockerHub: https://hub.docker.com/r/microbiomedata/nmdc_taxa_profilers

3.2.1 Requirements for Execution:

(recommendations are in **bold**)

- WDL-capable Workflow Execution Tool (**Cromwell**)
- Container Runtime that can load Docker images (**Docker v2.1.0.3 or higher**)

3.2.2 Hardware Requirements:

- Disk space: 152 GB for databases (55 GB, 89 GB, and 8 GB for GOTTCHA2, Kraken2 and Centrifuge databases, respectively)
- 60 GB RAM

3.3 Workflow Dependencies

3.3.1 Third party software:

(These are included in the Docker image.)

- GOTTCHA2 v2.1.6 (License: BSD-3-Clause-LANL)
- Kraken2 v2.0.8 (License: MIT)
- Centrifuge v1.0.4 (License: GPL-3)

3.3.2 Requisite databases:

The database for each tool must be downloaded and installed. These databases total 152 GB.

- GOTTCHA2 database (gottcha2/):

The database RefSeqr90.cg.BacteriaArchaeaViruses.species.fna contains complete genomes of bacteria, archaea and viruses from RefSeq Release 90. The following commands will download the database:

```
wget https://edge-dl.lanl.gov/GOTTCHA2/RefSeq-r90.cg.BacteriaArchaeaViruses.species.
↪tar
tar -xvf RefSeq-r90.cg.BacteriaArchaeaViruses.species.tar
rm RefSeq-r90.cg.BacteriaArchaeaViruses.species.tar
```

- Kraken2 database (kraken2/):

This is a standard Kraken 2 database, built from NCBI RefSeq genomes. The following commands will download the database:

```
mkdir kraken2
wget https://genome-idx.s3.amazonaws.com/kraken/k2_standard_20201202.tar.gz
tar -xzvf k2_standard_20201202.tar.gz -C kraken2
rm k2_standard_20201202.tar.gz
```

- Centrifuge database (centrifuge/):

This is a compressed database built from RefSeq genomes of Bacteria and Archaea. The following commands will download the database:

```
mkdir centrifuge
wget https://genome-idx.s3.amazonaws.com/centrifuge/p_compressed_2018_4_15.tar.gz
tar -xzvf p_compressed_2018_4_15.tar.gz -C centrifuge
rm p_compressed_2018_4_15.tar.gz
```

3.3.3 Sample dataset(s):

Zymobiomics mock-community DNA control (SRR7877884); this dataset is ~7 GB.

Input: A JSON file containing the following information: 1. selection of profiling tools (set as true if selected) 2. the paths to the required database(s) for the tools selected 3. the paths to the input fastq file(s) (paired-end data is shown; this can be the output of the Reads QC workflow in interleaved format which will be treated as single-end data.) 4. the prefix for the output file names 5. the path of the output directory 6. CPU number requested for the run.

```
{
    "ReadbasedAnalysis.enabled_tools": {
        "gottcha2": true,
        "kraken2": true,
        "centrifuge": true
    },
    "ReadbasedAnalysis.db": {
        "gottcha2": "/path/to/database/RefSeq-r90.cg.BacteriaArchaeaViruses.species.
↪fna",
        "kraken2": " /path/to/kraken2",
        "centrifuge": "/path/to/centrifuge/p_compressed"
    },
    "ReadbasedAnalysis.reads": [
        "/path/to/SRR7877884.1.fastq.gz",
        "/path/to/SRR7877884.2.fastq.gz"
    ],
    "ReadbasedAnalysis.paired": true,
    "ReadbasedAnalysis.prefix": "SRR7877884",
    "ReadbasedAnalysis.outdir": "/path/to/ReadbasedAnalysis",
    "ReadbasedAnalysis.cpu": 4
}
```

3.3.4 Output:

The workflow creates an output JSON file and individual output sub-directories for each tool which include tabular classification results, a tabular report, and a Krona plot (html).:

```
ReadbasedAnalysis/
|-- SRR7877884.json
|-- centrifuge
|   |-- SRR7877884.classification.tsv
|   |-- SRR7877884.report.tsv
|   `-- SRR7877884.krona.html
|
|-- gottcha2
|   |-- SRR7877884.full.tsv
|   |-- SRR7877884.krona.html
|   `-- SRR7877884.tsv
|
`-- kraken2
    |-- SRR7877884.classification.tsv
    |-- SRR7877884.krona.html
    `-- SRR7877884.report.tsv
```

Below is an example of the output directory files with descriptions to the right.

FileName	Description
SRR7877884.json	ReadbasedAnalysis result JSON file
centrifuge/SRR7877884.classification.tsv	Centrifuge output read classification TSV file
centrifuge/SRR7877884.report.tsv	Centrifuge output report TSV file
centrifuge/SRR7877884.krona.html	Centrifuge krona plot HTML file
gottcha2/SRR7877884.full.tsv	GOTTCHA2 detail output TSV file
gottcha2/SRR7877884.tsv	GOTTCHA2 output report TSV file
gottcha2/SRR7877884.krona.html	GOTTCHA2 krona plot HTML file
kraken2/SRR7877884.classification.tsv	Kraken2 output read classification TSV file
kraken2/SRR7877884.report.tsv	Kraken2 output report TSV file
kraken2/SRR7877884.krona.html	Kraken2 krona plot HTML file

3.4 Version History

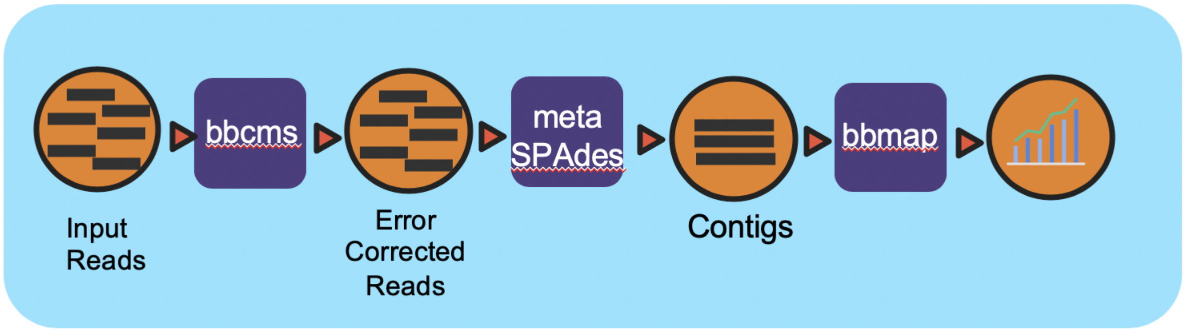
1.0.1 (release date 01/14/2021; previous versions: 1.0.0)

3.5 Point of contact

Package maintainer: Po-E Li <po-e@lanl.gov>

CHAPTER 4

Metagenome Assembly Workflow (v1.0.1)



4.1 Workflow Overview

This workflow takes in paired-end Illumina reads in interleaved format and performs error correction, then reformats the interleaved file into two FASTQ files for downstream tasks using bbcms (BBTools). The corrected reads are assembled using metaSPAdes. After assembly, the reads are mapped back to contigs by bbmap (BBTools) for coverage information. The .wdl (Workflow Description Language) file includes five tasks, *bbcms*, *assy*, *create_agp*, *read_mapping_pairs*, and *make_output*.

1. The *bbcms* task takes in interleaved FASTQ inputs and performs error correction and reformats the interleaved fastq into two output FASTQ files for paired-end reads for the next tasks.
2. The *assy* task performs metaSPAdes assembly
3. Contigs and Scaffolds (output of metaSPAdes) are consumed by the *create_agp* task to rename the FASTA header and generate an AGP format which describes the assembly
4. The *read_mapping_pairs* task maps reads back to the final assembly to generate coverage information.
5. The final *make_output* task adds all output files into the specified directory.

4.2 Workflow Availability

The workflow from GitHub uses all the listed docker images to run all third-party tools. The workflow is available in GitHub: https://github.com/microbiomedata/metaAssembly; the corresponding Docker images are available in DockerHub: https://hub.docker.com/r/microbiomedata/spades and https://hub.docker.com/r/microbiomedata/bbtools

4.3 Requirements for Execution

(recommendations are in **bold**)

- WDL-capable Workflow Execution Tool (**Cromwell**)
- Container Runtime that can load Docker images (**Docker v2.1.0.3 or higher**)

4.4 Hardware Requirements

- Memory: >40 GB RAM

The memory requirement depends on the input complexity. Here is a simple estimation equation for the memory required based on kmers in the input file:

```
predicted_mem = (kmers * 2.962e-08 + 1.630e+01) * 1.1 (in GB)
```

Note: The kmers variable for the equation above can be obtained using the kmercountmulti.sh script from BBTools.

kmercountmulti.sh -k=31 in=your.read.fq.gz

4.5 Workflow Dependencies

4.5.1 Third party software: (This is included in the Docker image.)

- metaSPades v3.15.0 (License: GPLv2)
- BBTools:38.90 (License: BSD-3-Clause-LBNL)

4.6 Sample dataset(s)

Zymobiomics mock-community DNA control (SRR7877884); this dataset is ~4 GB.

Note: If the input data is paired-end data, it must be in interleaved format. The following command will interleave the files, using the above dataset as an example:

```
paste <(zcat SRR7877884_1.fastq.gz | paste - - - -) <(zcat SRR7877884_2.fastq.gz |␣
↪paste - - - -) | tr '\t' '\n' | gzip -c > SRR7877884-int.fastq.gz
```

For testing purposes and for the following examples, we used a 10% sub-sampling of the above dataset: (SRR7877884-int-0.1.fastq.gz). This dataset is already interleaved.

4.7 Input

A JSON file containing the following information:

1. the path to the input FASTQ file (Illumina paired-end interleaved FASTQ) (recommended the output of the Reads QC workflow.)
2. the contig prefix for the FASTA header
3. the output path
4. memory (optional) ex: "jgi_metaASM.memory": "105G"
5. threads (optional) ex: "jgi_metaASM.threads": "16"

An example input JSON file is shown below:

```
{
    "jgi_metaASM.input_file":["/path/to/SRR7877884-int-0.1.fastq.gz "],
    "jgi_metaASM.rename_contig_prefix":"projectID",
    "jgi_metaASM.outdir":"/path/to/ SRR7877884-int-0.1_assembly",
    "jgi_metaASM.memory": "105G",
    "jgi_metaASM.threads": "16"
}
```

4.8 Output

The output directory will contain four output sub-directories: bbcms, final_assembly, mapping and spades3. The main output, the assembled contigs, are in final_assembly/assembly.contigs.fasta.

Part of an example output JSON file is shown below:

```
├── bbcms
│   ├── berkeleylab-jgi-meta-60ade422cd4e
│   ├── counts.metadata.json
│   ├── input.corr.fastq.gz
│   ├── input.corr.left.fastq.gz
│   ├── input.corr.right.fastq.gz
│   ├── readlen.txt
│   └── unique31mer.txt
├── final_assembly
│   ├── assembly.agp
│   ├── assembly_contigs.fasta
│   ├── assembly_scaffolds.fasta
│   └── assembly_scaffolds.legend
├── mapping
│   ├── covstats.txt (mapping_stats.txt)
│   ├── pairedMapped.bam
│   ├── pairedMapped.sam.gz
│   ├── pairedMapped_sorted.bam
│   └── pairedMapped_sorted.bam.bai
└── spades3
```

(continues on next page)

(continued from previous page)

```
        ├── assembly_graph.fastg
        ├── assembly_graph_with_scaffolds.gfa
        ├── contigs.fasta
        ├── contigs.paths
        ├── scaffolds.fasta
        └── scaffolds.paths
```

The table provides all of the output directories, files, and their descriptions.

Directory	File Name	Description
bbcms		Error correction result directory
bbcms/berkeleylab-jgi-meta-60ade422cd4e		directory containing checking resource s
bbcms/	counts.metadata.json	bbcms commands and summary statistic
bbcms/	input.corr.fastq.gz	error corrected reads in interleaved form
bbcms/	input.corr.left.fastq.gz	error corrected forward reads
bbcms/	input.corr.right.fastq.gz	error corrected reverse reads
bbcms/	rc	cromwell script sbumit return code
bbcms/	readlen.txt	error corrected reads statistics
bbcms/	resources.log	resource checking log
bbcms/	script	Task run commands
bbcms/	script.background	Bash script to run script.submit
bbcms/	script.submit	cromwell submit commands
bbcms/	stderr	standard error where task writes error m
bbcms/	stderr.background	standard error where bash script writes e
bbcms/	stderr.log	standard error from bbcms command
bbcms/	stdout	standard output where task writes error r
bbcms/	stdout.background	standard output where bash script writes
bbcms/	stdout.log	standard output from bbcms command
bbcms/	unique31mer.txt	the count of unique kmer, K=31
spades3		metaSPAdes assembly result directory
spades3/K33		directory containing intermediate files fr
spades3/K55		directory containing intermediate files fr
spades3/K77		directory containing intermediate files fr
spades3/K99		directory containing intermediate files fr
spades3/K127		directory containing intermediate files fr
spades3/misc		directory containing miscellaneous files
spades3/tmp		directory for temp files
spades3/	assembly_graph.fastg	metaSPAdes assembly graph in FASTG
spades3/	assembly_graph_with_scaffolds.gfa	metaSPAdes assembly graph and scaffol
spades3/	before_rr.fasta	contigs before repeat resolution
spades3/	contigs.fasta	metaSPAdes resulting contigs
spades3/	contigs.paths	paths in the assembly graph correspondi
spades3/	dataset.info	internal configuration file
spades3/	first_pe_contigs.fasta	preliminary contigs of iterative kmers as
spades3/	input_dataset.yaml	internal YAML data set file
spades3/	params.txt	information about SPAdes parameters in
spades3/	scaffolds.fasta	metaSPAdes resulting scaffolds
spades3/	scaffolds.paths	paths in the assembly graph correspondi
spades3/	spades.log	metaSPAdes log
final_assembly		create_agp task result directory

Table 1 – continued from previous page

Directory	File Name	Description
final_assembly/berkeleylab-jgi-meta-60ade422cd4e		directory containing checking resource s
final_assembly/	assembly.agp	an AGP format file describes the assemb
final_assembly/	assembly_contigs.fna	Final assembly contig fasta
final_assembly/	assembly_scaffolds.fna	Final assembly scaffolds fasta
final_assembly/	assembly_scaffolds.legend	name mapping file from spades node na
final_assembly/	rc	cromwell script sbumit return code
final_assembly/	resources.log	resource checking log
final_assembly/	script	Task run commands
final_assembly/	script.background	Bash script to run script.submit
final_assembly/	script.submit	cromwell submit commands
final_assembly/	stats.json	assembly statistics in json format
final_assembly/	stderr	standard error where task writes error m
final_assembly/	stderr.background	standard error where bash script writes e
final_assembly/	stdout	standard output where task writes error r
final_assembly/	stdout.background	standard output where bash script writes
mapping		maps reads back to the final assembly re
mapping/	covstats.txt	contigs coverage informaiton
mapping/	mapping_stats.txt	contigs coverage informaiton (same as c
mapping/	pairedMapped.bam	reads mapping back to the final assembl
mapping/	pairedMapped.sam.gz	reads mapping back to the final assembl
mapping/	pairedMapped_sorted.bam	reads mapping back to the final assembl
mapping/	pairedMapped_sorted.bam.bai	reads mapping back to the final assembl
mapping/	rc	cromwell script sbumit return code
mapping/	resources.log	resource checking log
mapping/	script	Task run commands
mapping/	script.background	Bash script to run script.submit
mapping/	script.submit	cromwell submit commands
mapping/	stderr	standard error where task writes error m
mapping/	stderr.background	standard error where bash script writes e
mapping/	stdout	standard output where task writes error r
mapping/	stdout.background	standard output where bash script writes

4.9 Version History

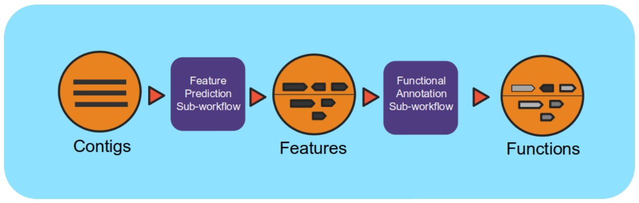
- 1.0.1 (release date **02/16/2021**; previous versions: 1.0.0)

4.10 Point of contact

- Original author: Brian Foster <bfoster@lbl.gov>
- Package maintainer: Chienchi Lo <chienchi@lanl.gov>

CHAPTER 5

Metagenome Annotation Workflow (v1.0.0)


Feature Prediction Sub-workflow
Functional Annotation Sub-workflow
Contigs
Features
Functions

5.1 Workflow Overview

This workflow takes assembled metagenomes and generates structural and functional annotations. The workflow uses a number of open-source tools and databases to generate the structural and functional annotations.

The input assembly is first split into 10MB splits to be processed in parallel. Depending on the workflow engine configuration, the split can be processed in parallel. Each split is first structurally annotated, then those results are used for the functional annotation. The structural annotation uses tRNAscan_se, RFAM, CRT, Prodigal and GeneMarkS. These results are merged to create a consensus structural annotation. The resulting GFF is the input for functional annotation which uses multiple protein family databases (SMART, COG, TIGRFAM, SUPERFAMILY, Pfam and Cath-FunFam) along with custom HMM models. The functional predictions are created using Last and HMM. These annotations are also merged into a consensus GFF file. Finally, the respective split annotations are merged together to generate a single structural annotation file and single functional annotation file. In addition, several summary files are generated in TSV format.

5.2 Workflow Availability

The workflow is available in GitHub: https://github.com/microbiomedata/mg_annotation/ and the corresponding Docker image is available in DockerHub: https://hub.docker.com/r/microbiomedata/mg-annotation.

5.3 Requirements for Execution (recommendations are in bold):

- WDL-capable Workflow Execution Tool **(Cromwell)**
- Container Runtime that can load Docker images **(Docker v2.1.0.3 or higher)**

5.4 Hardware Requirements:

- Disk space: 106 GB for the reference databases
- Memory: >100 GB RAM

5.5 Workflow Dependencies

- **Third party software (This is included in the Docker image.)**
  - Conda (3-clause BSD)
  - tRNAscan-SE >= 2.0 (GNU GPL v3)
  - Infernal 1.1.2 (BSD)
  - CRT-CLI 1.8 (Public domain software, last official version is 1.2)
  - Prodigal 2.6.3 (GNU GPL v3)
  - GeneMarkS-2 >= 1.07 (Academic license for GeneMark family software)
  - Last >= 983 (GNU GPL v3)
  - HMMER 3.1b2 (3-clause BSD)
  - TMHMM 2.0 (Academic)
- Requisite databases: The databases are available by request. Please contact NMDC (support@microbiomedata.org) for access.

5.6 Sample datasets

https://raw.githubusercontent.com/microbiomedata/mg_annotation/master/example.fasta

Input: A JSON file containing the following:

1. The path to the assembled contigs fasta file
2. The ID to associate with the result products (e.g. sample ID)

An example JSON file is shown below:

```
{
 "annotation.imgap_input_fasta": "/path/to/fasta.fna",
 "annotation.imgap_project_id": "samp_xyz123"}
}
```

Output: The final structural and functional annotation files are in GFF format and the summary files are in TSV format. The key outputs are listed below but additional files are available.

- GFF: Structural annotation
- GFF: Functional annotation
- TSV: KO Summary
- TSV: EC Summary
- TSV: Gene Phylogeny Summar

The output paths can be obtained from the output metadata file from the Cromwell Exectuion. Here is a snippet from the outputs section of the full metadata JSON file.

```
{
  "annotation.cath_funfam_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-
↪4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_cath_funfam.gff",
  "annotation.cog_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_cog.gff",
  "annotation.ko_ec_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_ko_ec.gff",
  "annotation.product_names_tsv": "/output/cromwell-executions/annotation/a67a5a0f-
↪1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_product_names.
↪tsv",
  "annotation.gene_phylogeny_tsv": "/output/cromwell-executions/annotation/a67a5a0f-
↪1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_gene_phylogeny.
↪tsv",
  "annotation.pfam_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_pfam.gff",
  "annotation.proteins_tigrfam_domtblout": "/output/cromwell-executions/annotation/
↪a67a5a0f-1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_
↪proteins.tigrfam.domtblout",
  "annotation.structural_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-
↪4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_structural_
↪annotation.gff",
  "annotation.ec_tsv": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_ec.tsv",
  "annotation.supfam_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_supfam.gff",
  "annotation.proteins_supfam_domtblout": "/output/cromwell-executions/annotation/
↪a67a5a0f-1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_
↪proteins.supfam.domtblout",
  "annotation.tigrfam_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-
↪4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_tigrfam.gff",
  "annotation.stats_tsv": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-final_stats/execution/samp_xyz123_structural_annotation_
↪stats.tsv",
  "annotation.proteins_cog_domtblout": "/output/cromwell-executions/annotation/
↪a67a5a0f-1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_
↪proteins.cog.domtblout",
  "annotation.ko_tsv": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_ko.tsv",
  "annotation.proteins_pfam_domtblout": "/output/cromwell-executions/annotation/
↪a67a5a0f-1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_
↪proteins.pfam.domtblout",
  "annotation.proteins_smart_domtblout": "/output/cromwell-executions/annotation/
↪a67a5a0f-1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_
↪proteins.smart.domtblout",
  "annotation.crt_crisprs": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-
↪4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_crt.crisprs",
  "annotation.functional_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-
↪4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_functional_
↪annotation.gff",
```

(continues on next page)

(continued from previous page)

```
  "annotation.proteins_faa": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-
↪4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123.faa",
  "annotation.smart_gff": "/output/cromwell-executions/annotation/a67a5a0f-1ad7-4469-
↪bb0c-780f4ef20307/call-merge_outputs/execution/samp_xyz123_smart.gff",
  "annotation.proteins_cath_funfam_domtblout": "/output/cromwell-executions/
↪annotation/a67a5a0f-1ad7-4469-bb0c-780f4ef20307/call-merge_outputs/execution/samp_
↪xyz123_proteins.cath_funfam.domtblout"
}
```

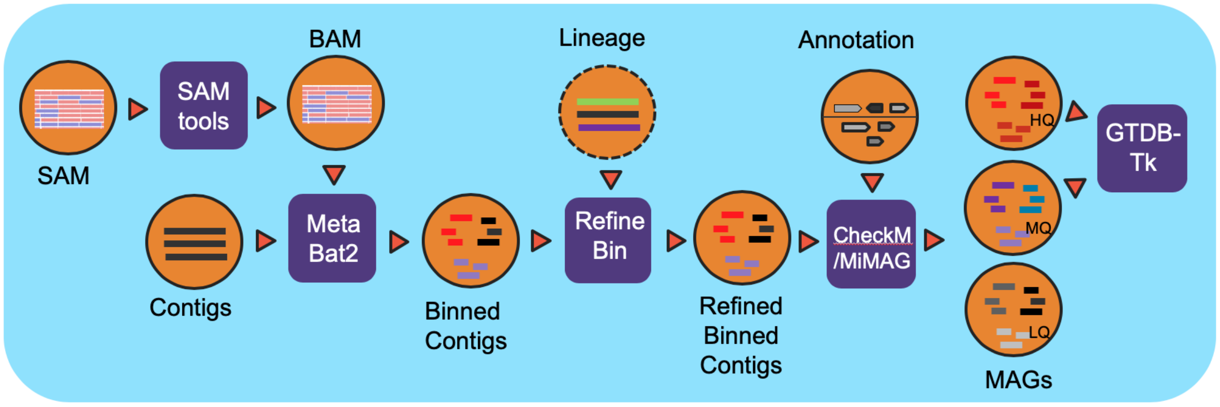
Version History: 1.0.0 (release data)

5.7 Point of contact

- Package maintainer: Shane Canon <scanon@lbl.gov>

CHAPTER 6

Metagenome Assembled Genomes Workflow (v1.0.2)



6.1 Workflow Overview

The workflow is based on IMG metagenome binning pipeline and has been modified specifically for the NMDC project. For all processed metagenomes, it classifies contigs into bins using MetaBat2. Next, the bins are refined using the functional Annotation file (GFF) from the Metagenome Annotation workflow and optional contig lineage information. The completeness of and the contamination present in the bins are evaluated by CheckM and bins are assigned a quality level (High Quality (HQ), Medium Quality (MQ), Low Quality (LQ)) based on MiMAG standards. In the end, GTDB-Tk is used to assign lineage for HQ and MQ bins.

6.2 Workflow Availability

The workflow from GitHub uses all the listed docker images to run all third-party tools. The workflow is available in GitHub: https://github.com/microbiomedata/metaMAGs The corresponding Docker image is available in DockerHub: https://hub.docker.com/r/microbiomedata/nmdc_mbin

6.3 Requirements for Execution

(recommendations are in **bold**):

- WDL-capable Workflow Execution Tool (**Cromwell**)
- Container Runtime that can load Docker images (**Docker v2.1.0.3 or higher**)

6.4 Hardware Requirements

- Disk space: > 27 GB for the CheckM and GTDB-Tk databases
- Memory: ~120GB memory for GTDB-tk.

6.5 Workflow Dependencies

6.5.1 Third party software (These are included in the Docker image.)

- Biopython v1.74 (License: BSD-3-Clause)
- Sqlite (License: Public Domain)
- Pymysql (License: MIT License)
- requests (License: Apache 2.0)
- samtools > v1.9 (License: MIT License)
- Metabat2 v2.15 (License: BSD-3-Clause)
- CheckM v1.1.2 (License: GPLv3)
- GTDB-TK v1.2.0 (License: GPLv3)
- FastANI v1.3 (License: Apache 2.0)
- FastTree v2.1.10 (License: GPLv2)

6.5.2 Requisite databases

The GTDB-Tk database must be downloaded and installed. The CheckM database included in the Docker image is a 275MB file contains the databases used for the Metagenome Binned contig quality assessment. The GTDB-Tk (27GB) database is used to assign lineages to the binned contigs.

- The following commands will download and unarchive the GTDB-Tk database:

```
wget https://data.ace.uq.edu.au/public/gtdb/data/releases/release89/89.0/gtdbtk_
↪r89_data.tar.gz
tar -xvzf gtdbtk_r89_data.tar.gz
mv release89 GTDBTK_DB
rm gtdbtk_r89_data.tar.gz
```

6.6 Sample dataset(s)

The following test dataset include an assembled contigs file, a BAM file, and a functional annotation file: metaMAGs_test_dataset.tgz

6.7 Input

A JSON file containing the following:

1. the number of CPUs requested
2. The number of threads used by pplacer (Use lower number to reduce the memory usage)
3. the path to the output directory
4. the project name
5. the path to the Metagenome Assembled Contig fasta file (FNA)
6. the path to the Sam/Bam file from read mapping back to contigs (SAM.gz or BAM)
7. the path to contigs functional annotation result (GFF)
8. the path to the text file which contains mapping of headers between SAM or BAM and GFF (ID in SAM/FNA<tab>ID in GFF)
9. the path to the database directory which includes *checkM_DB* and *GTDBTK_DB* subdirectories.
10. (optional) scratch_dir: use –scratch_dir for gtdbtk disk swap to reduce memory usage but longer runtime

An example JSON file is shown below:

```
{
    "nmdc_mags.cpu":32,
    "nmdc_mags.pplacer_cpu":1,
    "nmdc_mags.outdir":"/path/to/output",
    "nmdc_mags.proj_name":" Ga0482263",
    "nmdc_mags.contig_file":"/path/to/Ga0482263_contigs.fna ",
    "nmdc_mags.sam_file":"/path/to/pairedMapped_sorted.bam ",
    "nmdc_mags.gff_file":"/path/to/Ga0482263_functional_annotation.gff",
    "nmdc_mags.map_file":"/path/to/Ga0482263_contig_names_mapping.tsv",
    "nmdc_mags.gtdbtk_database":"/path/to/GTDBTK_DB"
    "nmdc_mags.scratch_dir":"/path/to/scratch_dir"
}
```

6.8 Output

The workflow creates several output directories with many files. The main output files, the binned contig files from HQ and MQ bins, are in the *hqmq-metabat-bins* directory; the corresponding lineage results for the HQ and MQ bins are in the *gtdbtk_output* directory.

A partial JSON output file is shown below:

```
|-- MAGs_stats.json
|-- 3300037552.bam.sorted
|-- 3300037552.depth
```

(continues on next page)

(continued from previous page)

```
|-- 3300037552.depth.mapped
|-- bins.lowDepth.fa
|-- bins.tooShort.fa
|-- bins.unbinned.fa
|-- checkm-out
|   |-- bins/
|   |-- checkm.log
|   |-- lineage.ms
|   `-- storage
|-- checkm_qa.out
|-- gtdbtk_output
|   |-- align/
|   |-- classify/
|   |-- identify/
|   |-- gtdbtk.ar122.classify.tree -> classify/gtdbtk.ar122.classify.tree
|   |-- gtdbtk.ar122.markers_summary.tsv -> identify/gtdbtk.ar122.markers_summary.tsv
|   |-- gtdbtk.ar122.summary.tsv -> classify/gtdbtk.ar122.summary.tsv
|   |-- gtdbtk.bac120.classify.tree -> classify/gtdbtk.bac120.classify.tree
|   |-- gtdbtk.bac120.markers_summary.tsv -> identify/gtdbtk.bac120.markers_summary.
↪tsv
|   |-- gtdbtk.bac120.summary.tsv -> classify/gtdbtk.bac120.summary.tsv
|   `-- ..etc
|-- hqmq-metabat-bins
|   |-- bins.11.fa
|   |-- bins.13.fa
|   `-- ... etc
|-- mbin-2020-05-24.sqlite
|-- mbin-nmdc.20200524.log
|-- metabat-bins
|   |-- bins.1.fa
|   |-- bins.10.fa
|   `-- ... etc
```

Below is an example of all the output directory files with descriptions to the right.

FileName/DirectoryName	Description
1781_86104.bam.sorted	sorted input bam file
1781_86104.depth	the contig depth coverage
1781_86104.depth.mapped	the name mapped contig depth coverage
MAGs_stats.json	MAGs statistics in json format
bins.lowDepth.fa	lowDepth (mean cov <1) filtered contigs fasta file by metaBat2
bins.tooShort.fa	tooShort (< 3kb) filtered contigs fasta file by metaBat2
bins.unbinned.fa	unbinned fasta file
metabat-bins/	initial metabat2 binning result fasta output directory
checkm-out/bins/	hmm and marker genes analysis result directory for each bin
checkm-out/checkm.log	checkm run log file
checkm-out/lineage.ms	lists the markers used to assign taxonomy and the taxonomic level to which the
checkm-out/storage/	intermediate file directory
checkm_qa.out	checkm statistics report
hqmq-metabat-bins/	HQ and MQ bins contigs fasta files directory
gtdbtk_output/identify/	gtdbtk marker genes identify result directory
gtdbtk_output/align/	gtdbtk genomes alignment result directory
gtdbtk_output/classify/	gtdbtk genomes classification result directory

Continued on next

Table 1 – continued from previous page

FileName/DirectoryName	Description
gtdbtk_output/gtdbtk.ar122.classify.tree	archaeal reference tree in Newick format containing analyzed genomes (bins)
gtdbtk_output/gtdbtk.ar122.markers_summary.tsv	summary tsv file for gtdbtk marker genes identify from the archaeal 122 marke
gtdbtk_output/gtdbtk.ar122.summary.tsv	summary tsv file for gtdbtk archaeal genomes (bins) classification
gtdbtk_output/gtdbtk.bac120.classify.tree	bacterial reference tree in Newick format containing analyzed genomes (bins)
gtdbtk_output/gtdbtk.bac120.markers_summary.tsv	summary tsv file for gtdbtk marker genes identify from the bacterial 120 mark
gtdbtk_output/gtdbtk.bac120.summary.tsv	summary tsv file for gtdbtk bacterial genomes (bins) classification
gtdbtk_output/gtdbtk.bac120.filtered.tsv	a list of genomes with an insufficient number of amino acids in MSA
gtdbtk_output/gtdbtk.bac120.msa.fasta	the MSA of the user genomes (bins) and the GTDB genomes
gtdbtk_output/gtdbtk.bac120.user_msa.fasta	the MSA of the user genomes (bins) only
gtdbtk_output/gtdbtk.translation_table_summary.tsv	the translation table determined for each sgenome (bins)
gtdbtk_output/gtdbtk.warnings.log	gtdbtk warning message log
mbin-2021-01-31.sqlite	sqlite db file stores MAGs metadata and statistics
mbin-nmdc.20210131.log	the mbin-nmdc pipeline run log file
rc	cromwell script sbumit return code
script	Task run commands
script.background	Bash script to run script.submit
script.submit	cromwell submit commands
stderr	standard error where task writes error message to
stderr.background	standard error where bash script writes error message to
stdout	standard output where task writes error message to
stdout.background	standard output where bash script writes error message to
complete.mbin	the dummy file to indicate the finish of the pipeline

6.9 Version History

- 1.0.2 (release date **02/24/2021**; previous versions: 1.0.1)

6.10 Point of contact

- Original author: Neha Varghese <njvarghese@lbl.gov>
- Package maintainer: Chienchi Lo <chienchi@lanl.gov>

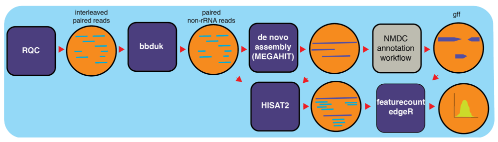
CHAPTER 7

Metatranscriptome Workflow (v0.0.2)

7.1 Summary

MetaT is a workflow designed to analyze metatranscriptomes, building on top of already existing NMDC workflows for processing input. The metatranscriptoimics workflow takes in raw data and starts by quality filtering the reads using the RQC worfklow. With filtered reads, the workflow filters out rRNA reads (and separates the interleaved file into separate files for the pairs) using bbduk (BBTools). After the filtering steps, reads are assembled into transcripts and using MEGAHIT and annotated using the Metagenome Anotation Workflow; producing GFF funtional annotation files. Features are counted with Subread's featureCounts which assigns mapped reads to genomic features and generating RPKMs for each feature in a GFF file for sense and antisense reads.

7.2 Workflow Diagram



7.3 Workflow Availability

The workflow uses the listed docker images to run all third-party tools. The workflow is available in GitHub: https://github.com/microbiomedata/metaT; and the corresponding Docker images that have all the required dependencies are available in following DockerHub (https://hub.docker.com/r/microbiomedata/bbtools, https://hub.docker.com/r/microbiomedata/meta_t, and https://hub.docker.com/r/intelliseqngs/hisat2)

7.4 Requirements for Execution (recommendations are in bold):

1. WDL-capable Workflow Execution Tool (**Cromwell**)
2. Container Runtime that can load Docker images (**Docker v2.1.0.3 or higher**)

7.5 Workflow Dependencies

7.5.1 Third-party software (These are included in the Docker images.)

1. BBTools v38.94. (License: BSD-3-Clause-LBNL.)
2. BBMap v38.94. (License: BSD-3-Clause-LBNL.)
3. Python v3.7.6. (License: Python Software Foundation License)
4. featureCounts v2.0.2. (License: GNU-GPL)
5. R v3.6.0. (License: GPL-2/GPL-3)
6. edgeR v3.28.1. (R package) (License: GPL (>=2))
7. pandas v1.0.5. (python package) (License: BSD-3-Clause)
8. gffutils v0.10.1. (python package) (License: MIT)

7.5.2 Requisite database

The RQCFilterData Database must be downloaded and installed. This is a 106 GB tar file which includes reference datasets of artifacts, adapters, contaminants, the phiX genome, rRNA kmers, and some host genomes. The following commands will download the database:

```
wget http://portal.nersc.gov/dna/microbial/assembly/bushnell/RQCFilterData.tar
tar -xvf RQCFilterData.tar
rm RQCFilterData.tar
```

7.6 Sample dataset(s)

The following files are provided with the GitHub download in the test_data folder:

1. Raw reads: test_data/test_interleave.fastq.gz (output from ReadsQC workflow)
2. Annotation file: test_functional_annotation.gff (output from mg_annotation workflow)

7.6.1 Input: A JSON file containing the following

1. a name for the analysis
2. the number of cpus requested
3. the path to the clean input interleaved fastq file (recommended: the output from the Reads QC workflow)
4. the path to the rRNA_kmer database provided as part of RQCFilterData
5. the path to the assembled transcripts (output of part 1)

6. the paths to the reads with rRNA removed (paired-end files) (output of part 1)
7. the path to the annotation file (from the Metagenome Annotation workflow)

An example JSON file is shown below:

```
{
  "metat_omics.project_name": "test",
  "metat_omics.no_of_cpus": 1,
  "metat_omics.rqc_clean_reads": "test_data/test_interleave.fastq",
  "metat_omics.ribo_kmer_file": "/path/to/riboKmers20fused.fa.gz",
  "metat_omics.metat_contig_fn": "/path/to/megahit_assem.contigs.fa",
  "metat_omics.non_ribo_reads": [
    "/path/to/filtered_R1.fastq",
    "/path/to/filtered_R2.fastq"
  ],
  "metat_omics.ann_gff_fn": "test_data/test_functional_annotation.gff"
}
```

7.6.2 Output

Output is split up between steps of the workflow. The first half of the workflow will output rRNA-filtered reads and the assembled transcripts. After annotations and featureCount steps include a JSON file that contain RPKMs for both sense and antisense, reads, and information from annotation for each feature. An example of JSON outpus:

```
{
        "featuretype": "transcript",
        "seqid": "k123_15",
        "id": "STRG.2.1",
        "source": "StringTie",
        "start": 1,
        "end": 491,
        "length": 491,
        "strand": ".",
        "frame": ".",
        "extra": [],
        "cov": "5.928717",
        "FPKM": "76638.023438",
        "TPM": "146003.046875"
   }
```

Below is an example of the output directory files with descriptions to the right.

Table 1: Title

Directory/File Name	Description
metat_output/sense_out.json	RPKM for each feature on + strand
metat_output/antisense_out.json	RPKM for each feature on - strand
assembly/megahit_assem.contigs.fa	assembled transcripts
mapback/mapped_sorted.bam	alignment of reads and transcripts
qa/_interleaved.fastq	non-ribosomal reads
qa/filterStats.txt	summary statistics in JSON format
qa/filterStats2.txt	more detailed summary statistics
annotation/annotations.json	annotation information
annotation/features.json	feature information
annotation/_cath_funfam.gff	features from cath database
annotation/_cog.gff	features from cog databse
annotation/_ko_ec.gff	features from ko database
annotation/_pfam.gff	features from pfam database
annotation/_smart.gff	features from smart database
annotation/_structural_annotation.gff	structural features
annotation/_supfam.gff	features from supfam databse
annotation/_tigrfam.gff	features from trigfam database
annotation/_functional_annotation.gff	functional features
annotation/_ec.tsv	ec terms tsv
annotation/_ko.tsv	ko terms tsv
annotation/proteins.faa	fasta containing protiens

7.7 Version History

- 0.0.2 (release date 01/14/2021; previous versions: 0.0.1)
- 0.0.3 (release date 07/28/2021; previous versions: 0.0.2)

7.8 Points of contact

- Author: Migun Shakya <migun@lanl.gov>

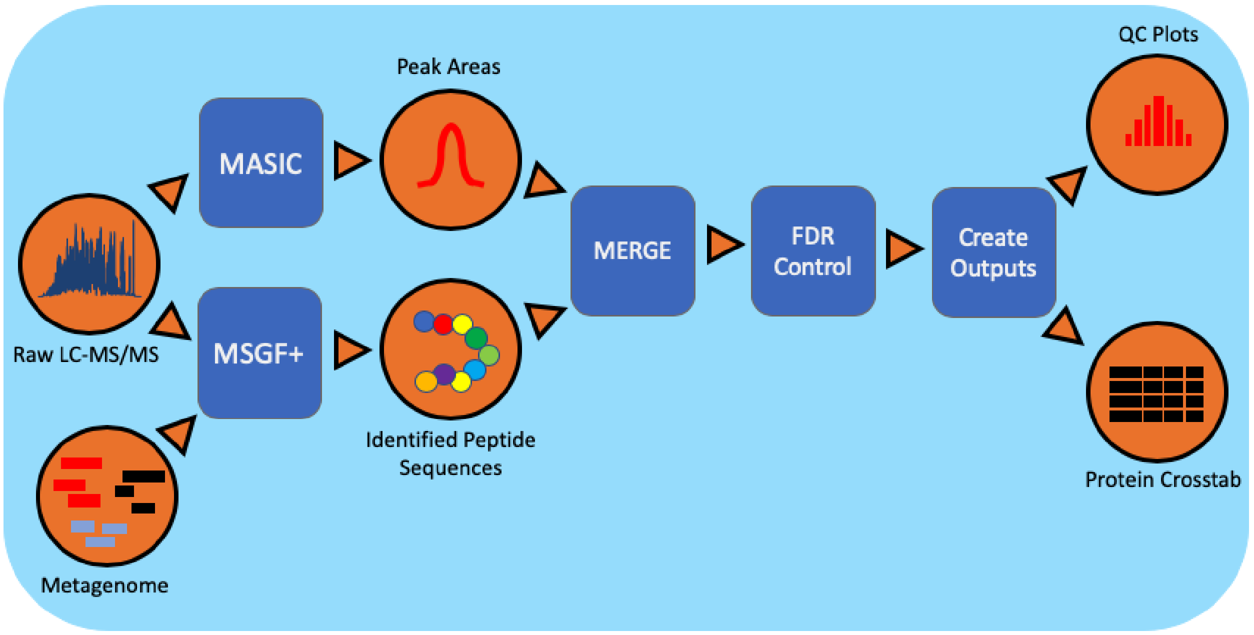
CHAPTER 8

Metaproteomic Workflow (v1.0.0)

8.1 Summary

The metaproteomics workflow/pipeline is an end-to-end data processing workflow for protein identification and characterization using MS/MS data. Briefly, mass spectrometry instrument generated data files(.RAW) are converted to mzML, an open data format, using MSConvert. Peptide identification is achieved using MSGF+ and the associated metagenomic information in the FASTA (protein sequences) file format. Intensity information for identified species is extracted using MASIC and combined with protein information.

8.2 Workflow Diagram



8.3 Workflow Dependencies

8.3.1 Third party software

```
|----------------------------|------------------------------------------|
| MSGFPlus                   | v20190628                                |
| Mzid-To-Tsv-Converter      | v1.3.3                                   |
| PeptideHitResultsProcessor | v1.5.7130                                |
| pwiz-bin-windows           | x86_64-vc141-release-3_0_20149_b73158966 |
| MASIC                      | v3.0.7235                                |
| sqlite-netFx-full-source   | 1.0.111.0                                |
| Conda                      | (3-clause BSD)                           |
|                            |                                          |
```

8.4 Workflow Availability

The workflow is available in GitHub: https://github.com/microbiomedata/metaPro

The container is available at Docker Hub (microbiomedata/mepro): https://hub.docker.com/r/microbiomedata/mepro

8.4.1 Inputs

- *.raw, metagenome, parameter files : MSGFplus & MASIC, contaminant_file*

8.4.2 Outputs

1. Processing multiple datasets.

```
.
├── Data/
├── FDR_table.csv
├── Plots/
├── dataset_job_map.csv
├── peak_area_crosstab_by_dataset_id.csv
├── protein_peptide_map.csv
├── specID_table.csv
└── spectra_count_crosstab_by_dataset_id.csv
```

2. Processing single FICUS dataset.

- metadatafile, [Example](https://jsonblob.com/400362ef-c70c-11ea-bf3d-05dfba40675b)

```
| Keys                 | Values                                                            ␣
↪          |
|--------------------|-----------------------------------------------------------------
↪----------|
| id                 | str: "md5 hash of $github_url+$started_at_time+$ended_at_time"␣
↪          |
| name               | str: "Metagenome:$proposal_extid_$sample_extid:$sequencing_
↪project_extid |
| was_informed_by    | str: "GOLD_Project_ID"                                            ␣
↪          |
| started_at_time    | str: "metaPro start-time"                                         ␣
↪          |
| ended_at_time      | str: "metaPro end-time"                                           ␣
↪          |
| type               | str: tag: "nmdc:metaPro"                                          ␣
↪          |
| execution_resource | str: infrastructure name to run metaPro                           ␣
↪          |
| git_url            | str: "url to a release"                                           ␣
↪          |
| dataset_id         | str: "dataset's unique-id at EMSL"                                ␣
↪          |
| dataset_name       | str: "dataset's name at EMSL"                                     ␣
↪          |
| has_inputs         | json_obj                                                          ␣
↪          |
| has_outputs        | json_obj                                                          ␣
↪          |
| stats              | json_obj                                                          ␣
↪          |

has_inputs :
| MSMS_out          | str: file_name \|file_size \|checksum                            ␣
↪                                                   |
| metagenome_file   | str: file_name \|file_size \|checksum \|
                      int: entry_count(#of gene sequences) \|
                      int: duplicate_count(#of duplicate gene sequences) |
| parameter_files   | str: for_masic/for_msgfplus : file_name \|file_size \|checksum
                      parameter file used for peptide identification search
| Contaminant_file | str: file_name \|file_size \|checksum
```

(continues on next page)

(continued from previous page)

```
                     (FASTA containing common contaminants in proteomics)

has_outputs:
| collapsed_fasta_file | str: file_name \|file_size \|checksum                    ␣
↪                     |
| resultant_file       | str: file_name \|file_size \|checksum                    ␣
↪                     |
| data_out_table       | str: file_name \|file_size \|checksum                    ␣
↪                     |

stats:
| from_collapsed_fasta | int: entry_count(#of unique gene sequences)               ␣
↪                                                                                ␣
↪                                                                                ␣
↪                                                                                ␣
↪                                                                                ␣
↪                                                                                ␣
↪              |
| from_resultant_file  | int: total_protein_count                                  ␣
↪                                                                                ␣
↪                                                                                ␣
↪                                                                                ␣
↪                                                                                ␣
↪                                                                                ␣
↪                   |
| from_data_out_table  | int: PSM(# of MS/MS spectra matched to a peptide sequence at␣
↪5% false discovery rate (FDR)
                         float: PSM_identification_rate(# of peptide matching MS/MS␣
↪spectra divided by total spectra searched (5% FDR)
                         int: unique_peptide_seq_count(# of unique peptide sequences␣
↪observed in pipeline analysis 5% FDR)
                         int: first_hit_protein_count(# of proteins observed assuming␣
↪single peptide-to-protein relationships)
                         int: mean_peptide_count(Unique peptide sequences matching to␣
↪each identified protein.)
```

- data_out_table

```
| DatasetName | PeptideSequence | FirstHitProtein | SpectralCount |␣
↪sum(MasicAbundance) | GeneCount | FullGeneList | FirstHitDescription |␣
↪DescriptionList | min(Qvalue) |
```

- collapsed_fasta_file
- resultant_file

8.5 Requirements for Execution

- Docker or other Container Runtime

8.6 Version History

- 1.0.0

8.7 Point of contact

Package maintainer: Anubhav <anubhav@pnnl.gov>

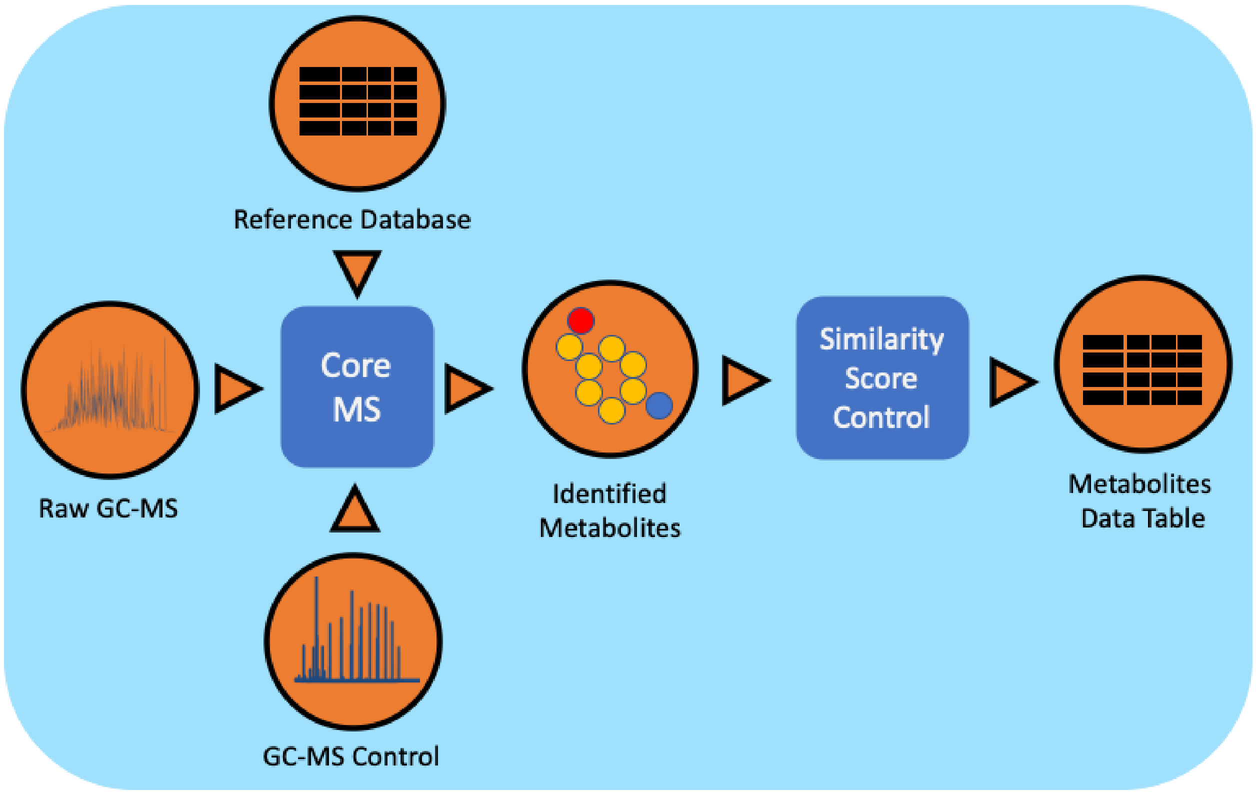
CHAPTER 9

Metabolomics Workflow (v2.1.0)

9.1 Summary

The gas chromatography-mass spectrometry (GC-MS) based metabolomics workflow (metaMS) has been developed by leveraging PNNL's CoreMS software framework. The current software design allows for the orchestration of the metabolite characterization pipeline, i.e., signal noise reduction, m/z based Chromatogram Peak Deconvolution, abundance threshold calculation, peak picking, spectral similarity calculation and molecular search, similarity score calculation, and confidence filtering, all in a single step.

9.2 Workflow Diagram



9.3 Workflow Dependencies

9.3.1 Third party software

- CoreMS (2-clause BSD)
- Click (BSD 3-Clause “New” or “Revised” License)

9.3.2 Database

- PNNL GC-MS Spectral Database

9.4 Workflow Availability

The workflow is available in GitHub: https://github.com/microbiomedata/metaMS

The container is available at Docker Hub (microbiomedata/metaMS): https://hub.docker.com/r/microbiomedata/metams

The python package is available on PyPi: https://pypi.org/project/metaMS/

The databases are available by request. Please contact NMDC (support@microbiomedata.org) for access.

9.5 Test datasets

https://github.com/microbiomedata/metaMS/blob/master/data/GCMS_FAMES_01_GCMS-01_20191023.cdf

9.6 Execution Details

Please refer to:

https://github.com/microbiomedata/metaMS/blob/master/README.md#usage

9.6.1 Inputs

- ANDI NetCDF for GC-MS (.cdf)
- CoreMS Hierarchical Data Format (.hdf5)
- CoreMS Parameter File (.JSON)
- MetaMS Parameter File (.JSON)

9.6.2 Outputs

- **Metabolites data-table**
    - CSV, TAB-SEPARATED TXT
    - HDF: CoreMS HDF5 format
    - XLSX : Microsoft Excel
- **Workflow Metadata:**
    - JSON

9.7 Requirements for Execution

- Docker Container Runtime
- Python Environment >= 3.6
- Python Dependencies are listed on requirements.txt

9.8 Version History

- 2.1.0

9.9 Point of contact

Package maintainer: Yuri E. Corilo <corilo@pnnl.gov>

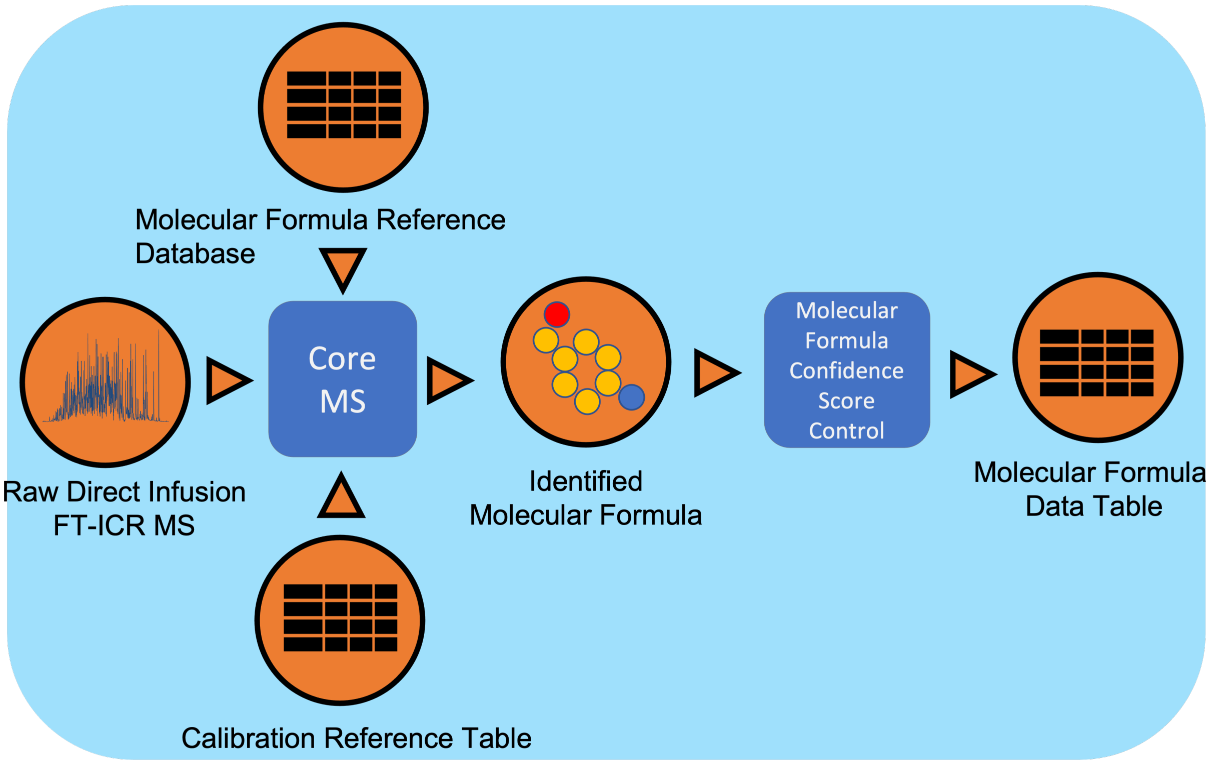
CHAPTER 10

Natural Organic Matter Workflow

10.1 Summary

Direct Infusion Fourier Transform mass spectrometry (DI FT-MS) data undergoes signal processing and molecular formula assignment leveraging EMSL's CoreMS framework. Raw time domain data is transformed into the m/z domain using Fourier Transform and Ledford equation. Data is denoised followed by peak picking, recalibration using an external reference list of known compounds, and searched against a dynamically generated molecular formula library with a defined molecular search space. The confidence scores for all the molecular formula candidates are calculated based on the mass accuracy and fine isotopic structure, and the best candidate assigned as the highest score.

10.2 Workflow Diagram


Molecular Formula Reference Database
Raw Direct Infusion FT-ICR MS
Core MS
Calibration Reference Table
Identified Molecular Formula
Molecular Formula Confidence Score Control
Molecular Formula Data Table

10.3 Workflow Dependencies

10.3.1 Third party software

- CoreMS (2-clause BSD)
- Click (BSD 3-Clause "New" or "Revised" License)

10.3.2 Database

- CoreMS dynamic molecular database search and generator

10.4 Workflow Availability

The workflow is available in GitHub: https://github.com/microbiomedata/enviroMS

The container is available at Docker Hub (microbiomedata/metaMS): https://hub.docker.com/r/microbiomedata/enviroms

The python package is available on PyPi: https://pypi.org/project/enviroMS/

The databases are available by request. Please contact NMDC (support@microbiomedata.org) for access.

10.5 Test datasets

https://github.com/microbiomedata/enviroMS/tree/master/data

10.6 Execution Details

Please refer to:

https://github.com/microbiomedata/enviroMS#enviroms-installation

10.6.1 Inputs

- **Supported format for Direct Infusion FT-MS data:**
  - Thermo raw file (.raw)
  - Bruker raw file (.d)
  - Generic mass list in profile and/or centroid mode (inclusive of all delimiters types and Excel formats)
- **Calibration File:**
  - Molecular Formula Reference (.ref)
- **Parameters:**
  - CoreMS Parameter File (.json)
  - EnviroMS Parameter File (.json)

10.6.2 Outputs

- **Molecular Formula Data-Table, containing m/z measuments, Peak height, Peak Area, Molecular Formula Identification, I**
  - CSV, TAB-SEPARATED TXT
  - HDF: CoreMS HDF5 format
  - XLSX : Microsoft Excel
- **Workflow Metadata:**
  - JSON

10.7 Requirements for Execution

- Docker Container Runtime or
- Python Environment >= 3.8 and
- Python Dependencies are listed on requirements.txt

10.8 Version History

- 4.1.5

10.9 Point of contact

Package maintainer: Yuri E. Corilo <corilo@pnnl.gov>