**MetaWRAP module descriptions**

**MetaWRAP-Read\_qc**

The Read\_qc module is meant to pre-process raw Illumina sequencing reads in preparation for assembly and alignment. The raw reads are trimmed with Trim-galore v0.4.3 at default settings, and then the human-derived reads (contamination) are removed with bmtagger v3.101. Read pairs with a single suspected human read are also removed. FASTQC is then used to generate quality reports of the raw and final read sets in order to assess read quality improvement. The user has control over which of the above features he wishes to use.

**MetaWRAP-Assembly**

The Assembly module allows the user to assemble a set of metagenomic reads with either metaSPAdes or MegaHit, both set to default assembly settings. While metaSPAdes results in a superior assembly in most samples, MegaHit scales well with large datasets, and is therefore set as the defualt. The assemblies are then formatted to include the scaffold length and kmer depth, sorted by length, and contigs shorter than 1000bp are removed. An assembly report is then generated with QUAST v4.5 with default settings.

**MetaWRAP-Kraken**

The Kraken module takes in any number of FastQ or FastA files, classifies the contained sequences with KRAKEN v0.10.6, and reports the taxonomy distribution in an interactive html kronagram using KronaTools v2.7. If a passed FastA file is an assembly file from the Assembly module, the taxonomy of each contig is weighted based on its length and coverage [weight=coverage\*length], which are encoded in the scaffold naming.

**MetaWRAP-Binning**

The Binning module is meant to be a convenient wrapper around three metagenomic binning software: metaBAT v2.12.1, Maxbin v2.2.4, and CONCOCT v0.4.0. First the metagenomic assembly is indexed and paired end reads from any number of samples are aligned to it with bwa v0.7.15. The alignments are sorted and compressed with Samtools v1.6, and library insert size statistics are also gathered at the same time (insert size average and standard deviation). MetaBAT2’s jgi\_summarize\_bam\_contig\_depths function is used to generate contig abundance table, and it is then converted into the correct format for each of the three binning softwares. The assembly is binned with software(s) of the user’s choice, and the resulting bins are optionally evaluated with CheckM v1.0.7.

**MetaWRAP-Bin\_refinement**

The Bin\_refinement module utilizes a hybrid approach to take in two or three bin sets that were obtained with different binning approaches and produces a consolidated, improved bin set. First, binning\_refiner v1.2 is used to hybridize the bin sets in every possible combination. If there are three original bin sets A, B, and C, they will be hybridized to produce bin sets AB, BC, AC, and ABC. CheckM is then run to evaluate the completion and contamination of the bins in each of the 7 bin sets (3 originals, 4 hybridized). The bins sets are then iteratively compared to each other, and each pair is consolidated into an improved bin set. To do this, the same bin is identified within the two bin sets based on a minimum of 80% overlap in genome length, and the better bin is selected based on the scoring function S=Completion-5\*Contamination. Only bins that meet the minimum completion (-c) and maximum contamination (-x) criteria are considered. After all bin sets are incorporated into a consolidated set, duplicate contigs are removed. By default, duplicate kept only in the superior bin (based on scoring function). CheckM is then re-run on the final bin set and a final report file is generated with a custom script (.stats files). Completion and contamination rank plots are also made to compare the quality of the original bins and the Bin\_refinement module output.

**MetaWRAP-Reassemble\_bins**

The Reassemble\_bins module aims to improve a set of bins by extracting reads that belong to each bin and re-assembling them. First, entire original metagenomic assembly is indexed and FastQ reads are aligned back to it with bwa v0.7.15. Reads pairs mapping back to contigs belonging to the provided bins are stored in separate FastQ files, even if only one read mate aligned. Two sets of reads are stored for each bin – reads mapping perfectly (strict), and reads mapping with <3 mismatches (permissive). Each set of reads is then reassembled with SPAdes v3.11 with the --carefull setting, and short contigs (<1000bp) are removed. CheckM is used to evaluate the completion and contamination of each of the three versions of each bin – the original bin, the “strict” re-assembled bin, and “permissive” reassembled bin. The best version is chosen based on a scoring funciton S=Completion-5\*Contamination. The final bins set it then re-evaluated with CheckM, and summary statistics are generated. Additionally, a N50, completion and contamination rank plots is generated to evaluate the improvements in the bin sets following reassembly.

**MetaWRAP-Quant\_bins**

The Quant\_bins module rapidly estimates the abundance of bins across a number of samples. Salmon v0.9.1 is used to index the entire metagenomic assembly and align reads from each sample back to the assembly. Coverage tables are generated estimating the abundance of each contig in each sample. The average abundance of each bin in each sample is calculated by taking the length-weighted average of the bins’s contig abundances. A final bin abundance table is made, and a clustered heatmap is generated with Seaborn v0.8.1 to visualize bin abundance variation across samples. The bin abundances are standardized to 100 million reads in each sample library before plotting.

**MetaWRAP-Blobology**

The Blobology module uses a modified version of the original Blobology software to create blobplots (a GC vs abundance plot of all the contigs) of a metagenomic assembly, and annotates it with phylogenetic information or bin information. The taxonomy of each contig is estimated with Mega-BLAST v2.7.1 with NCBI\_nt as the database. The assembly is then indexed and the reads from any number of samples are aligned against it with bowtie2. Blobology’s gc\_cov\_annotate.pl function is used to generate a blobplot file with the GC, coverage (in all samples), and taxonomy of each contig. If the user provided a set of bins to annotate, the contigs are also annotated with the bins they belong to. Finally, Blobology’s makeblobplot.R function is used to make the blobplots of the contigs across all the provided samples, with taxonomic and bin membership annotations.

**MetaWRAP-Classify\_bins**

The Classify\_bins module is a conservative way to assign taxonomy to a set of metagenomic bins. First, the contigs in all bins are combined into one file, and MegaBLAST v2.7.1 is used to align the contigs to the NCBI\_nt database. The alignment results are then used by taxator-kt to estimate the taxonomy of each contig. The most likely taxonomy of each bin is then estimated from individual contig predictions. Taxonomy of each contig are added to a phylogenetic tree, adding weight to each branch based on the length of that contig. The tree is then traversed from the root down the heaviest branches until the next likely branch is <50% of the current branch weight. Once no further taxonomic rank can be estimated, the final taxonomy of that bin is reported.

**MetaWRAP-Annotate\_bins**

The Annotate\_bins module takes in a set of bins and quickly functionally annotates them with PROKKA v1.12. The annotation process is parallelized for any number of bins and threads. For each bin, the module returns the annotation file in GFF format, and two FastA files with untranslated and translated genes.