Step	Analysis	Purpose	Software	Time
1	Reads preprocessing	Quality check	Trimmomatic and FastQC	15 min
2	Metagenomic assembly	Assemble RNA and DNA	Megahit	6 h
3	Assembly evaluation	See if assembly is succesful	Quast	45 min
4	Binning	Assign genomes to correct species	Metabat	>30 min
5	Quality check of assembly and bins	Quality evaluation	CheckM	2 h
6	Basic phylogenetic placement of bins	taxonomic ID	CheckM	2 h
7	Phylogenetic placement	Reconstruct phylogenetic tree	Phylophlan	6 h
8	Functional annotation	Metabolic reconstruction	Prokka	1 h
9a	Mapping	align the RNA against ref genome	BWA	4-6 h
9b	Read counting	count the aligned reads	HTSeq package in Python	n/a
9c	Expression analysis	which genes are more expressed	DeSeq2 package in R	n/a
Step	Extra Analysis	Purpose	Software	Time
9a	Abundance of different organisms/bins	n/a	BWA	n/a
7	Refine taxonomic ID	n/a	FastTree2	n/a
4 (after binning)	Metabolic pathway reconstructions	n/a	Submit to IMG (Integrated Microbial Genomes)	n/a
after 9	Analysis of expression data	n/a	n/a	n/a
after 9	Comparisons across bins	n/a	n/a	n/a
after 9	Comparative genomics of bins	n/a	n/a	n/a
9c	Ortholog gene clustering of bins	n/a	DeSeq2 package in R	n/a

* Yellow means that the time step is extra time-consuming