

# Metagenomic Binning Pipelines - the State of the Art

## Abstract

New generations of sequencing platforms coupled with numerous bioinformatics tools have led to rapid technological progress in metagenomics and metatranscriptomics to investigate complex microorganism communities. Nevertheless, a combination of different bioinformatic tools remains necessary to draw conclusions out of microbiota studies. As sequencing costs have dropped at a rate above 'Moore's law', a greater number of large data sets are being produced than ever before. Newer algorithms that take advantage of the size of these datasets are continually being developed. Binning algorithms are defined as the grouping of assembled metagenomic contigs by their genome of origin. Selecting the most appropriate binning algorithm can be a daunting task and is influenced by many factors. This review serves as a guide to direct the researcher to the binning algorithm that best suits their needs.

## Background

The explosion in popularity and success in the field of metagenomics over the last 25 years can be largely attributed to the advances in computing technologies. An example of the outcomes of this can be found in the Human Microbiome Project; a project that has been greatly improved the understanding of the microbial flora involved in human health and disease. These advances have brought with them greater demands for storage, CPU time, and consequently more efficient algorithms. The main function of binning tools is to reconstruct species/biological entities from metagenomic samples. Compared to amplicon, shotgun metagenome can provide functional gene profiles directly and reach a much higher resolution of taxonomic annotation. However, due to the high demands on computational resources, cost, and expertise necessary to perform this analysis, researchers have historically been limited in their capacity to collect and analyse sequencing data. As the cost of sequencing is rapidly falling, this burden has been significantly lessened. At the time

26 of writing, shotgun metagenomic sequencing costs on average three times as much as 16S sequencing  
27 comparatively. The objectives of this review is for the reader to be better informed about the  
28 latest algorithms (since 2017) for binning metagenomic samples. The second part of this review  
29 is for the reader to be informed about distinguishing factors between the methods. The last part  
30 is for the reader to make an informed decision based on those factors for their needs. This review  
31 will be broken down into the following sections:

- 32 • *List of the binning algorithms*
- 33 • *Classify binning algorithms based on their objectives, guideline for algorithm choice, subsection msp mag*
- 34
- 35 • *Current limitations and Future directions*

## 36 Recent methods for metagenomic binning

37 A metagenomic sample is composed of many organisms and the standard procedure is to retrieve  
38 the sequences from the mixture of organisms. The final goal of binning is to reconstruct the se-  
39 quences from each organism present in the original sample. Among the binning tools developed  
40 in recent years we can distinguish a subset dedicated to cluster reads (read-binning) (MetaBBC-  
41 LR, BioBloom Tools, CLAME, LVQ-KKN, Meta VW, HirBin, MEGAN-LR). The main purpose  
42 of read-binning tools is to preprocess reads into clusters for a posterior targeted assembly, here  
43 we find reference-free and non-reference-free tools, and tools designed for short-read or long-read  
44 sequencing technologies. The majority of binning tools we can find are oriented toward cluster-  
45 ing contigs (contig-binning) into bins, which may represent the genome from a single biological  
46 entity/organism. Contig-binning tools normally rely on coverage information and sequence compo-  
47 sition. Progress in contig-binning algorithms can be seen in the proposals to integrate new sources  
48 of information (for example, from scaffold-graphs(Binnacle), paired-end reads(COCACOLA), or  
49 3D contact information(MetaTOR)) and state of the art algorithms in machine learning (CoCoNet,  
50 Variational Autoencoders for Metagenomic Binning (VAMB)).

## 51 Metagenome Assembled Genomes

52 A Metagenome-Assembled Genome (MAG) is a single-taxon assembly based on one or more binned  
53 metagenomes that has been asserted to be a close representation to an actual individual genome  
54 (that could match an already existing isolate or represent a novel isolate).

## 55 **Binning microbial genomes with deep learning**

56 The integration of deep learning techniques into the field of metagenomics has revolutionised the  
57 field of metagenomics. The VAMB pipeline was developed to take advantage of variational au-  
58 toencoders; a generative machine learning model that uses a combination. Improved metagenome  
59 binning and assembly using deep variational autoencoders. Nature biotechnology the VAMB  
60 pipeline (Nissen et al., n.d.).

## 61 **Binning for viral genomes**

62 New insights from uncultivated genomes of the global human gut microbiome (Nayfach, Shi,  
63 Seshadri, Pollard, & Kyrpides, 2019).

## 64 **Chosing the most appropriate binning algorithm (Classifica-** 65 **tion by output)**

66 A review on the benchmarking binning algorithms was done by Yue et al., 2020. Resource man-  
67 agement is an important factor in the choice of binning algorithm. The tradeoff between number  
68 of Central Processing Units (CPUs), memory, and time are important considerations. Newer ad-  
69 vances in pipeline technologies have ameliorated these costs. Alignment based or alignment free.  
70 An analysis pipeline is defined as a program that combines several programs in a defined order to  
71 complete a complex analysis. Improperly developed, validated, and/or monitored pipelines may  
72 generate inaccurate results.

## 73 **MSPs, binning co-abundant genes**

74 Binning of co-abundant genes represents an alternative proposal to reconstruct species/biological  
75 entities from a set of metagenomic samples. Co-abundant gene binning methods assume each  
76 gene coming from a shared chromosome will display proportional abundances across samples, if  
77 you have enough samples from a common environment you can identify the sets of genes from  
78 a common organism of origin (MLGs Chameleon-clust 2012, CAGs and MGSs Canopy 2014,  
79 Markovclust-MGCs Karlsson 2013, MSPs MSPminner 2018). To the extent of our knowledge, in  
80 the past few years MSPminner is the only available Software exploiting this approach. MSPminner  
81 introduced a robust proportionality measure detecting co abundant but no necessarily co occurring.

Table 1: Comparison of binning algorithms

Software/Algorithm	Year	Description/purpose	Comment/Highlight	Doi	PubmedID
CoNet	2021	Deep learning tool for Viral Metagenome Binning	Reconstructs viral genomes	10.1093/bioinformatics/btab213	33822891
Biatic	2021	Using scaffolds to improve Metagenomic bin quality	Incorporates scaffold information	10.3389/fmicb.2021.685614	33717083
VAMB	2021	Metagenomic binning and MAG assembly	Autocoder algorithm, fast processing	10.1186/s13057-020-00777-4	33398153
phyloFlash	2020	mRNA profiling and MAG assembly	Incorporates asRNA profiling info into MAG as...	10.1093/bioinformatics/btaa441	33109753
hyBRCC-LR	2020	Metagenomic binning for Long-Reads	Suitable for Long Reads sequencing technology	10.1093/bioinformatics/btaa441	32657364
BinBam Tools	2020	Refined binning of metagenomic contigs using as...	Data preparation for targeted assembly, using s...	10.1093/bioinformatics/btaa180	32641514
ChibiBin	2020	Simulating metagenomic stable isotope probing d...	Incorporates assembly graphs	10.1093/bioinformatics/btaa180	32167328
MetaSPSim	2020	Unsupervised binning k-mers and coverage, focus...	Augment binning resolution with extra experimen...	10.1186/s12859-020-3372-6	32000876
MetaCon	2019	Binning viral haplotypes from assembled contigs	Enriches different levels of contigs	10.1186/s12859-019-2904-4	31757198
ViBin	2019	Framework for Proton and analysis of MAGs	Viral haplotypes MAGs	10.1186/s12859-019-3138-1	31634576
MAGO (*only tool pipeline)	2019	Genome separation of Endosymbionts from mixed s...	Identification of endosymbiont	10.1093/mbe/mbz237	31633780
SeqDox	2019	High quality MAGs from mammalian gits using met...	Incorporates 2D contact information	10.3389/fgene.2019.00853	31608107
MetaTOR	2019	Adaptive binning algorithm for genome recons...	Eliminates misassembly patterns	10.7717/peerj.7359	31481973
MetaBAT 2	2019	Scalable binning algorithm for large-scale meta...	Employs sample X mappings of mapped read counts	10.1093/bioinformatics/btz677	31388474
PolyCRACKER	2019	Method for partitioning polyploid bacterial genomes b...	Haplotypes for polyploid genomes	10.1186/s12864-019-5828-5	31299888
SolidBin	2019	Improving metagenomic binning with individual extraction of metagenomic bins	NaN	10.1093/bioinformatics/btz253	30977806
Autmeta	2019	Signal processing method for aligning free met...	Handles eukaryotic contamination	10.1093/bioinformatics/btz253	30838416
MLBP MrGBP (Algorithm)	2019	Aligning metagenomic reads from individual	Alternative description of sequences designed f...	10.1038/s41586-018-38197-9	30770850
CLAME	2018	Signal processing method for aligning free met...	Alignment based for reads	10.1186/s12864-018-5191-y	30537931
3D BH SNE (Algorithm)	2018	Fuzzy binning of metagenomic sequence fragments	Horizontal gene transfer and regions of uncerta...	10.1109/EMBC.2018.8512529	30440833
LVQ-KNN	2018	Classification based RNA or DNA binning of short s...	Classify into DNA or RNA sequence	10.1016/j.virus.2018.10.002	30291874
MSPinner	2018	Abundance based reconstruction of microbial pan...	Pan genome reconstruction	10.1093/bioinformatics/btz830	30252023
MetaWRAP*	2018	Flexible pipeline for genome resolved metagenom...	Hybrid bin extraction algorithm	10.1186/s40168-018-0541-1	30219103
MetaVW	2018	Large scale Machine Learning Sequence classific...	Machine learning for reads based on Kmer profile	10.1007/978-1-4939-8561-6_2	30030800
BM3C	2018	Metagenomic binning through low density binning	Improvement at higher taxonomic levels, discove...	10.1093/bioinformatics/btz611	30010790
BM3C	2018	Binning contigs using codon usage sequence comp...	Add codon usage information	10.1093/bioinformatics/btz611	29947757
AMBER tool	2018	Assessment of Metagenome Binners	NaN	10.1093/bioinformatics/btz611	29893851
DAS Tool	2018	Derreplication aggregation and scoring strategy	Combines several binning algorithm results	10.1038/s41564-018-0171-1	29807988
MEGAN-LR	2018	Long Read/ contigs taxonomic binning	Alignment of long reads against reference sequences	10.1186/s13062-018-0208-7	29678199
CoMet	2018	Binning workflow using contain coverage and com...	Single sample, include gc content and 4mer fre...	10.1186/s12859-017-1967-3	29297295
?	2017	Metagenomic binning and association of plasmids...	Plasmid binning at strain level using methylati...	10.1038/nbt.4037	29227468
MetaGen	2017	Reference-free learning with multiple metagenom...	Requires multiple samples	10.1186/s13059-017-1323-y	28974263
d2sBin add onn	2017	Improved formula for calculate oligonucleotide...	Math formula to calculate oligo sequence dissim...	10.1186/s12859-017-1835-1	28931373
BusiBee Web	2017	Bootstrapped supervised binning and annotation	2d interactive scatterplots supervised binning	10.1093/nar/nkx348	28472498
ICoVer	2017	Interactive visualisation tool for verification...	Interactive visualisation tool	10.1186/s12859-017-1653-5"	28464793
HiBin*	2017	High resolution identification of differential...	Supervised annotation, unsupervised clustering ...	10.1186/s12864-017-3686-6	28431529
BinSanity	2017	Unsupervised clustering using coverage and affi...	Reduce bias for high/low abundance	10.7717/peerj.3035	28289564
Binning-refiner	2017	Improve genome bins through the combination of ...	Combination of different binning algorithms	10.1093/bioinformatics/btz086	28186226
IFCM add on	2016	Improved binning using Fuzzy C-Means Method	Add estimated distribution of real genome lengths	10.1109/TCBB.2016.2576452	27295684
COCACOLA	2016	Binning contigs using composition, read coverage...	Adds paired end read and coalignment information	10.1093/bioinformatics/btz290	27256312
GroupM (2)	2014	Tool for automatic recovery of population genom...	Adds differential coverage to complement compos...	10.7717/peerj.603	25289188

82 This tools groups co-abundant genes into Metagenomic Species Pan-genomes or Metagenomic  
 83 Species Pan-genomess (MSPs) and classify genes within an MSP as core, accesory and shared.  
 84 The factors that impact directly on MSP quality include the sample composition, the sequencing  
 85 depth, the previos bioinforamtic steps to build the reference gene dataset and to map the reads.  
 86 A high number of samples with varying phenotypes improve the quality of MSPs. MSPs can  
 87 be employed for taxonomic profiles of new samples from similar ecosystems, to compare strains  
 88 between samples building a presence/absence table of accesory genes and for biomarker discovery.  
 89 By binning contigs carrying genes from the same MSP it is also possible to build a MAG.

## 90 Metagenomic Species Pan-genomes

91 Microbial pan-genomes are gene repertoires composed of core genes present in all strains and  
 92 accessory genes present in only some of them (Tettelin et al., 2005). In a shotgun metagenomic  
 93 sequencing context, we define as shared the genes detected in some samples where the species is  
 94 not present. A strain found in a sample is an instance of the species pan-genome: it is made of all  
 95 the species (shared) core genes and of a subset of (shared) accessory genes. Core genes are suitable  
 96 for taxonomic profiling at species-level while accessory genes can be used to compare strains across  
 97 samples. Genes tagged as shared should be used carefully as they contain false positives counts  
 98 or are subject to horizontal transfer.

## 99 Conclusion

100 New and open areas of research in which the application of metagenomic pipelines are relevant  
 101 The increased impact of machine learning in analysis Short section - just for past-present-future  
 102 completeness Future developments for metagenomic analysis

## 103 References

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