*Hey look! MetaWRAP is a neat program that might be useful to biologists!*

**MetaWRAP is a modular, comprehensive platform** for analysis, interpretation, and visualization of metagenomic data, with special emphasis on extracting and analyzing high-quality draft genomes (bins). With the help of Conda, metaWRAP is easy to download and install for biologists without significant computation experience. The metaWRAP installation includes a comprehensive bioinformatics environment of over 150 commonly used software and libraries, saving the user from installing and configuring them independently. Each of metaWRAP’s modules is a standalone program, allowing the user to choose only the functions they are interested in. Alternatively, the user may follow the intuitive workflow starting from raw metagenomic sequencing reads.

**The metaWRAP workflow** starts with the Read\_qc module, which trims the raw sequence reads and removes human contamination from each of the sequenced samples. Quality control reports are also generated to evaluate the sequencing quality. The reads from all given samples are then assembled with the Assembly module, also producing an assembly report. Both the reads from each sample and the assembly can be taxonomically profiled with the Kraken module, producing interactive kronagrams of the sample taxonomy. The Binning module is then used to bin the scaffolds of the joint assembly using three metagenomic binners – MaxBin2, metaBAT2, and CONCOCT. These bin sets are then passed to the Bin\_refinement module, which hybridizes the bin sets with Binning\_refiner, and then finds the best version of each bin based on completion and contamination metrics estimated with CheckM. The scaffolds in the final bin set is then de-replicated, and a final report of their completion, contamination, and other metrics is produced. These bins can then be visualized by using the Blobology module, which plots the contigs of the joint assembly on a GC vs abundance plot, and annotating them with their taxonomy and bin. The Quant\_bins module can be used to quickly estimate the abundance of each bin in each of the metagenomic samples, and Classify\_bins can be used to conservatively, but accurately estimate their taxonomy. Finally, Reassemble\_bins can be used to reassemble the reads belonging to each bin, improving their N50, completion, and contamination.

*Oh, and it does binning really well!*

In order to **test the efficacy of the Bin\_refinement** module in improving bins based on three different bin sets, we tested applied the module to the data set from the CAMI challenge, which included synthetically generated metagenomes with high, medium, and low diversity. The “golden standard” assembly from each CAMI challenge was binned with metaBAT2 v2.12.1, Maxbin2 v2.2.4, and CONCOCT v0.4.0 using the Binning module of metaWRAP. The resulting three bin sets were then passed to DAS\_Tool, binning\_refiner, and metaWRAP:Bin\_refinement to attempt to improve the bin sets. To simulate a realistic metagenomic pipeline, the completion and contamination of the bins in all six bin sets was first evaluated with CheckM, and bins with a completion less than 50% or a contamination greater than 10% were discarded. The true recall and precision of the bins within the six resulting bin sets was then determined with Amber, which compared the bins against the true original genomes (Figure S4). Bin recall and precision were converted to completion and contamination percentages for easier viewing (Figure 2).

Between the **original binning software**, metaBAT2 consistently outperformed the others, producing 385 high quality bins (completion greater than 90% and contamination less than 5%), and 271 near-perfect bins (completion greater than 95% and contamination less than 1%). MaxBin2 came in second with 275 high quality bins and 164 near-perfect bins. Finally, CONCOCT performed rather poorly in all but the smallest CAMI challenge data sets, producing 58 high quality bins and 40 near-perfect bins.

**DAS\_Tool** consistently produced high-completion bins, with an average bin completion of 96.14 +/- 5.8% between the three CAMI challenges. However DAS\_Tool bins were had relatively high contamination when compared to binning\_refiner and metaWRAP, with an average contamination of 1.57% +/- 3.05%. Between all three challenges, DAS\_Tool was able to produce 426 high quality bins and 263 near-perfect bins.

**Binning\_refiner** produced very pure bins, which an average contamination of 0.2% +/- 1.61%, but did so at the expense of significantly reduced completion, with an average of 92.5% +/- 12.2% between the three CAMI challenge samples. In total, Binning\_refiner was able to produce 289 high quality bins and 210 near-perfect bins.

**MetaWRAP**’s Bin\_refinent module was able to consistently produce high completion and low contamination bins, coming close to DAS\_Tool in completion, with an average of 94.7% +/- 10.2%, and coming close to Binning\_refiner in contamination with an average of 0.8% +/- 2.6%. In total, metaWRAP produced 457 high quality bins and 339 near-perfect bins. (Figures 2, S4)

* Bin\_refinement module also produces the best bins on real data sets from a range of microbiome types (Figures 3, 5)
* Bin\_refinement module dynamically adapts its output to prioritize completion and contamination thresholds that the user wants (Figures 5, S5, S6)
* Reasemble\_bins module consistently and significantly improves bins (except in soil) (Figures 4, 5)
* Combining the Bin\_refinement and Reassemble\_bins modules, metaWRAP as a whole produces the best binning results out of all current competitors (Figure 5)
* The Kraken module estimates taxonomy distributions of communities (Figure S7)
* The Quant\_bins estimates bin abundances in different samples (Figure S8)
* Classify\_bins is a conservative first-pass way to estimate bin taxonomy (Figures S9, S10)
* Blobology is a useful way to visualize binning success (Figures 6, S11).