
CONCOCT Documentation

Release 0.4.0

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CONCOCT “bins” metagenomic contigs. Metagenomic binning is the process of clustering sequences into clusters corresponding to operational taxonomic units of some level.

For any known issues with CONCOCT check the issue tracker: <https://github.com/BinPro/CONCOCT/issues>

Features

CONCOCT does unsupervised binning of metagenomic contigs by using nucleotide composition - kmer frequencies - and coverage data for multiple samples. CONCOCT can accurately (up to species level) bin metagenomic contigs. For optimal performance:

- Map several samples against your assembled contigs.
- Cut longer contigs into 10 - 20 kb pieces prior to mapping.
- Evaluate your bins using single copy genes.

Installation

For a comprehensive guide on how to install CONCOCT and all its dependencies, see [Installation](#).

Contribute

- Issue Tracker: [github](#)
- Source Code: [github](#)

Support

If you are having issues, please let us know. We have a mailing list located at: concoct-support@lists.sourceforge.net which you can subscribe to [here](#).

Licence

FreeBSD

Contents:

6.1 Installation

6.1.1 Dependencies

Fundamental dependencies

```
python v2.7.*  
gcc  
gsl
```

These items are prerequisites for the installation of concoct as described below. The installation procedure varies on different systems, and described in this README is only how to proceed with a linux (ubuntu) distribution.

The first item, `python v2.7.*`, should be installed on a modern Ubuntu distribution. A c-compiler, e.g. `gcc`, is needed to compile the c parts of concoct that uses the GNU Scientific Library `gsl`. For linux (ubuntu) this is installed through:

```
apt-get install build-essential libgsl0-dev
```

Python packages

```
cython>=0.19.2  
numpy>=1.7.1  
scipy>=0.12.0  
pandas>=0.11.0  
biopython>=1.62b  
scikit-learn>=0.13.1
```

These are the python packages that need to be installed in order to run concoct. If you follow the installation instructions below, these will be installed automatically, but are listed here for transparency.

Optional dependencies

- For assembly, use your favorite, here is one
 - Velvet
 - * In velvet installation directory Makefile, set 'MAXKMERLENGTH=128', if this value is smaller in the default installation.

- To create the input table (containing average coverage per sample and contig)
 - [BEDTools](#) version $\geq 2.15.0$ (only `genomeCoverageBed`)
 - [Picard](#) tools version ≥ 1.110
 - [samtools](#) version $\geq 0.1.18$
 - [bowtie2](#) version $\geq 2.1.0$
 - [GNU parallel](#) version ≥ 20130422
 - Python packages: `pysam` ≥ 0.6
- For validation of clustering using single-copy core genes
 - [Prodigal](#) ≥ 2.60
 - Python packages: `bcbio-gff` ≥ 0.4
 - R packages: `gplots`, `reshape`, `ggplot2`, `ellipse`, `getopt` and `grid`

If you want to install these dependencies on your own server, you can take a look at `doc/Dockerfile.all_dep` for ideas on how to install them.

6.1.2 Installation

Here we describe two recommended ways of getting `concoct` to run on your computer/server. The first option, using `Anaconda`, should work for any *nix (e.g. Mac OS X or Linux) system even where you do not have ‘sudo’ rights (e.g. on a common computer cluster). The second option is suitable for a linux computer where you have root privileges and you prefer to use a virtual machine where all dependencies to run `concoct` are included. `Docker` does also run on Mac OS X through a virtual machine. For more information check out the [Docker documentation](<http://docs.docker.com/installation/>).

Using Anaconda

This instruction shows how to install all dependencies (except the ‘Fundamental dependencies’ and the ‘Optional dependencies’ listed above) using an `Anaconda` environment. `Anaconda` is a tool to isolate your python installation, which allows you to have multiple parallel installations using different versions of different packages, and gives you a very convenient and fast way to install the most common scientific python packages. `Anaconda` is free but not open source, you can download `Anaconda` [here](#). Installation instructions can be found [here](#).

After installing `Anaconda`, create a new environment that will contain the `concoct` installation:

```
conda create -n concoct_env python=2.7.6
```

After choosing to proceed, run the suggested command:

```
source activate concoct_env
```

then install the `concoct` dependencies into this environment:

```
conda install cython numpy scipy biopython pandas pip scikit-learn
```

Finally, download the `CONCOCT` distribution from <https://github.com/BinPro/CONCOCT/releases> (stable) and extract the files, or clone the repository with github (potentially unstable). Resolve all dependencies, see above and then execute within the `CONCOCT` directory:

```
python setup.py install
```

Using Docker

If you have root access to a machine where you want to install concoct and you have storage for roughly 2G “virtual machine” then Docker provides a very nice way to get a Docker image with concoct and its dependencies installed. This way the only thing you install on your host system is Docker, the rest is contained in an Docker image. This allows you to install and run programs in that image without it affecting your host system. You should [get to know Docker here](#). You need to [get Docker installed](#) and specially if you have [Ubuntu](#). When Docker is installed you need to download and log into the concoct image.

We provide a Docker image:

binpro/concoct_latest contains CONCOCT and all its dependencies for the complete workflow with the exception of the SCG evaluation.

The following command will then download the image from the Docker image index, map the Data folder to the image and log you into the docker image.

```
sudo docker run -v /home/USER/Data:/opt/Data -i -t binpro/concoct_latest bash
```

To test concoct you can then do:

```
$ cd /opt/CONCOCT_latest
$ nosetests
```

Which should execute all tests without errors.

6.2 Usage

CONCOCT uses several command line options to control the clustering, here is a complete documentation of these. These can also be viewed by typing `concoct -h` on the command line.:

```
usage: concoct [-h] [--coverage_file COVERAGE_FILE]
               [--composition_file COMPOSITION_FILE] [-c CLUSTERS]
               [-k KMER_LENGTH] [-l LENGTH_THRESHOLD] [-r READ_LENGTH]
               [--total_percentage_pca TOTAL_PERCENTAGE_PCA] [-b BASENAME]
               [-s SEED] [-i ITERATIONS] [-e EPSILON] [--no_cov_normalization]
               [--no_total_coverage] [-o] [-d] [-v]
```

optional arguments:

```
-h, --help                show this help message and exit
--coverage_file COVERAGE_FILE
                           specify the coverage file, containing a table where
                           each row correspond to a contig, and each column
                           correspond to a sample. The values are the average
                           coverage for this contig in that sample. All values
                           are separated with tabs.
--composition_file COMPOSITION_FILE
                           specify the composition file, containing sequences in
                           fasta format. It is named the composition file since
                           it is used to calculate the kmer composition (the
                           genomic signature) of each contig.
-c CLUSTERS, --clusters CLUSTERS
                           specify maximal number of clusters for VGMM, default
                           400.
-k KMER_LENGTH, --kmer_length KMER_LENGTH
                           specify kmer length, default 4.
-l LENGTH_THRESHOLD, --length_threshold LENGTH_THRESHOLD
```

```
        specify the sequence length threshold, contigs shorter
        than this value will not be included. Defaults to
        1000.
-r READ_LENGTH, --read_length READ_LENGTH
        specify read length for coverage, default 100
--total_percentage_pca TOTAL_PERCENTAGE_PCA
        The percentage of variance explained by the principal
        components for the combined data.
-b BASENAME, --basename BASENAME
        Specify the basename for files or directory where
        output will be placed. Path to existing directory or
        basenamewith a trailing '/' will be interpreted as a
        directory. If not provided, current directory will be
        used.
-s SEED, --seed SEED
        Specify an integer to use as seed for clustering. 0
        gives a random seed, 1 is the default seed and any
        other positive integer can be used. Other values give
        ArgumentError.
-i ITERATIONS, --iterations ITERATIONS
        Specify maximum number of iterations for the VBGMM.
        Default value is 500
-e EPSILON, --epsilon EPSILON
        Specify the epsilon for VBGMM. Default value is 1.0e-6
--no_cov_normalization
        By default the coverage is normalized with regards to
        samples, then normalized with regards of contigs and
        finally log transformed. By setting this flag you skip
        the normalization and only do log transform of the
        coverage.
--no_total_coverage
        By default, the total coverage is added as a new
        column in the coverage data matrix, independently of
        coverage normalization but previous to log
        transformation. Use this tag to escape this behaviour.
-o, --converge_out
        Write convergence info to files.
-d, --debug
        Debug parameters.
-v, --version
        show program's version number and exit
```

6.3 Complete Example V0.4

This documentation page aims to be a complete example walk through for the usage of the CONCOCT package version 0.3. It assumes you have successfully gone through the installation description found in the README.

6.3.1 Required software

To run the entire example you need to install all dependencies as stated in the README dependencies. This includes all the optional dependencies. You can also look at doc/Dockerfile to help you install these packages on your server.

Another way to get everything set up is to use our full Docker image (binpro/concoct_latest) as suggested in the README docker.

It is not required to run all steps. The output files for each step are in the test data repository. At the end of this example the results should be the same as the results in the corresponding test data repository: <https://github.com/BinPro/CONCOCT-test-data/releases>. The version numbers listed above are the ones used to generate the results in that repository. Using newer versions will probably not be a problem, but your results may be different in that case.

6.3.2 Downloading test data

First download the test data repository of CONCOCT corresponding to the version of CONCOCT, that you have installed. The test data repository can be downloaded [here](#). Then extract it in a suitable location.

If you are running the current unstable master branch of concocct, you need to clone the latest version of the test-data-repository as well.

6.3.3 Setting up the test environment

Using Docker

On your HOST machine create the following folder structure below (Data, Data/CONCOCT-complete-example, Data/CONCOCT-test-data):

```
mkdir -p /HOST/path/to/Data
mkdir /HOST/path/to/Data/CONCOCT-complete-example
```

Move the test data that was downloaded and extracted (CONCOCT-test-data) to the Data folder

```
# Move the test data you extracted in the download part into the Data folder
mv /HOST/extracted/test/data/CONCOCT-test-data /HOST/path/to/Data/CONCOCT-test-data
```

Now you want to execute the following command to log into our Docker image and to map the /HOST/path/to/Data to your image and the Data folder will be accessible in /opt/Data:

```
sudo docker run -v /HOST/path/to/Data:/opt/Data/ -i -t binpro/concocct_latest bash
```

This will download the 2G image to your machine and then leaves you in a BASH shell. In the Docker image, the following environmental variables have been set. So if you have your folders set up differently in the steps above you need to alter these variables accordingly:

```
CONCOCT=/opt/CONCOCT_latest
CONCOCT_TEST=/opt/Data/CONCOCT-test-data
CONCOCT_EXAMPLE=/opt/Data/CONCOCT-complete-example
```

Your own setup

After obtaining the test data, create a folder where you want all the output from this example to go:

```
mkdir CONCOCT-complete-example
cd CONCOCT-complete-example
```

Set three variables with full paths. One pointing to the root directory of the CONCOCT software, one pointing to the test data repository, named CONCOCT_TEST and one to the directory we just created. If you now have these in the folder /home/username/src/, for instance, then use:

```
CONCOCT=/home/username/src/CONCOCT
CONCOCT_TEST=/home/username/src/CONCOCT-test-data
CONCOCT_EXAMPLE=/home/username/CONCOCT-complete-example
```

You can see the full path of a directory you are located in by running the command `pwd`.

6.3.4 Assembling Metagenomic Reads

The first step in the analysis is to assemble all reads into contigs, here we use the software [Velvet](#) for this. This step can be computationally intensive but for this small data set comprising a synthetic community of four species and 16 samples (100,000 reads per sample) it can be performed in a few minutes. If you do not wish to execute this step, the resulting contigs are already in the test data repository, and you can copy them from there instead. The commands for running Velvet are:

```
cd $CONCOCT_EXAMPLE
cat $CONCOCT_TEST/reads/Sample*_R1.fa > All_R1.fa
cat $CONCOCT_TEST/reads/Sample*_R2.fa > All_R2.fa
velveth velveth_k71 71 -fasta -shortPaired -separate All_R1.fa All_R2.fa
velvetg velveth_k71 -ins_length 400 -exp_cov auto -cov_cutoff auto
```

After the assembly is finished create a directory with the resulting contigs and copy the result of Velvet there (this output is also in \$CONCOCT_TEST/contigs):

```
mkdir contigs
cp velveth_k71/contigs.fa contigs/velvet_71.fa
rm All_R1.fa
rm All_R2.fa
```

6.3.5 Cutting up contigs

In order to give more weight to larger contigs and mitigate the effect of assembly errors we cut up the contigs into chunks of 10 Kb. The final chunk is appended to the one before it if it is < 10 Kb to prevent generating small contigs. This means that no contig < 20 Kb is cut up. We use the script `cut_up_fasta.py` for this:

```
cd $CONCOCT_EXAMPLE
python $CONCOCT/scripts/cut_up_fasta.py -c 10000 -o 0 -m contigs/velvet_71.fa > contigs/velvet_71_c10K.fa
```

6.3.6 Map the Reads onto the Contigs

After assembly we map the reads of each sample back to the assembly using [bowtie2](#) and remove PCR duplicates with [MarkDuplicates](#). The coverage histogram for each bam file is computed with [BEDTools](#) `genomeCoverageBed`. The script that calls these programs is provided with CONCOCT.

If you are not using the Docker image, then one does need to set an environment variable with the full path to the MarkDuplicates jar file. `$MRK DUP` which should point to the MarkDuplicates jar file e.g.

```
#NOTE not necessary if using the Docker image
export MRK DUP=/home/username/src/picard-tools-1.77/MarkDuplicates.jar
```

It is typically located within your picard-tools installation.

The following command is to be executed in the `$CONCOCT_EXAMPLE` dir you created in the previous part. First create the index on the assembly for bowtie2:

```
cd $CONCOCT_EXAMPLE
bowtie2-build contigs/velvet_71_c10K.fa contigs/velvet_71_c10K.fa
```

Then run this for loop, which for each sample creates a folder and runs `map-bowtie2-markduplicates.sh`:

```
for f in $CONCOCT_TEST/reads/*_R1.fa; do
  mkdir -p map/$(basename $f);
  cd map/$(basename $f);
```

```

    bash $CONCOCT/scripts/map-bowtie2-markduplicates.sh -ct 1 -p '-f' $f $(echo $f | sed s/R1/R2/) pa
    cd ../../.;
done

```

The parameters used for `map-bowtie2-markduplicates.sh` are:

- `-c` option to compute coverage histogram with `genomeCoverageBed`
- `-t` option is number of threads
- `-p` option is the extra parameters given to `bowtie2`. In this case `-f`.

The five arguments are:

- `pair1`, the fasta/fastq file with the #1 mates
- `pair2`, the fasta/fastq file with the #2 mates
- `pair_name`, a name for the pair used to prefix output files
- `assembly`, a fasta file of the assembly to map the pairs to
- `assembly_name`, a name for the assembly, used to postfix outputfiles
- `outputfolder`, the output files will end up in this folder

6.3.7 Generate coverage table

Use the bam files of each sample to create a table with the coverage of each contig per sample.

```

cd $CONCOCT_EXAMPLE/map
python $CONCOCT/scripts/gen_input_table.py --isbedfiles \
    --samplenames <(for s in Sample*; do echo $s | cut -d'_' -f1; done) \
    ../contigs/velvet_71_c10K.fa */bowtie2/asm_pair-smds.coverage \
> concoct_inputtable.tsv
mkdir $CONCOCT_EXAMPLE/concoct-input
mv concoct_inputtable.tsv $CONCOCT_EXAMPLE/concoct-input/

```

6.3.8 Generate linkage table

The same bam files can be used to give linkage per sample between contigs:

```

cd $CONCOCT_EXAMPLE/map
python $CONCOCT/scripts/bam_to_linkage.py -m 8 \
    --regionlength 500 --fullsearch \
    --samplenames <(for s in Sample*; do echo $s | cut -d'_' -f1; done) \
    ../contigs/velvet_71_c10K.fa Sample*/bowtie2/asm_pair-smds.bam \
> concoct_linkage.tsv
mv concoct_linkage.tsv $CONCOCT_EXAMPLE/concoct-input/

```

6.3.9 Run concoct

To see possible parameter settings with a description run

```
$CONCOCT/bin/concoct --help
```

We will only run `concoct` for some standard settings here. First we need to parse the input table to just contain the mean coverage for each contig in each sample:

```
cd $CONCOCT_EXAMPLE
cut -f1,3-26 concoct-input/concoct_inputtable.tsv > concoct-input/concoct_inputtableR.tsv
```

Then run concoct with 40 as the maximum number of cluster `-c 40`, that we guess is appropriate for this data set:

```
cd $CONCOCT_EXAMPLE
concoct -c 40 --coverage_file concoct-input/concoct_inputtableR.tsv --composition_file contigs/velvet
```

When concoct has finished the message “CONCOCT Finished, the log shows how it went.” is piped to stdout. The program generates a number of files in the output directory that can be set with the `-b` parameter and will be the present working directory by default.

6.3.10 Evaluate output

This will require that you have Rscript with the R packages `gplots`, `reshape`, `ggplot2`, `ellipse`, `getopt` and `grid` installed. The package `grid` does not have to be installed for R version > 1.8.0

First we can visualise the clusters in the first two PCA dimensions:

```
cd $CONCOCT_EXAMPLE
mkdir evaluation-output
Rscript $CONCOCT/scripts/ClusterPlot.R -c concoct-output/clustering_gt1000.csv -p concoct-output/PCA
```

<https://github.com/BinPro/CONCOCT-test-data/tree/master/evaluation-output/ClusterPlot.pdf>

We can also compare the clustering to species labels. For this test data set we know these labels, they are given in the file `clustering_gt1000_s.csv`. For real data labels may be obtained through taxonomic classification, e.g. using:

<https://github.com/umerijaz/TAXAassign>

In either case we provide a script `Validate.pl` for computing basic metrics on the cluster quality:

```
cd $CONCOCT_EXAMPLE
cp $CONCOCT_TEST/evaluation-output/clustering_gt1000_s.csv evaluation-output/
$CONCOCT/scripts/Validate.pl --cfile=concoct-output/clustering_gt1000.csv --sfile=evaluation-output/c
```

This script requires the clustering output by concoct `concoct-output/clustering_gt1000.csv` these have a simple format of a comma separated file listing each contig id followed by the cluster index and the species labels that have the same format but with a text label rather than a cluster index. The script should output:

N	M	TL	S	K	Rec.	Prec.	NMI	Rand	AdjRand
684	684	6.8023e+06	5	4	0.897224	0.999604	0.841911	0.911563	0.823200

This gives the no. of contigs `N` clustered, the number with labels `M`, the number of unique labels `S`, the number of clusters `K`, the recall, the precision, the normalised mutual information (NMI), the Rand index, and the adjusted Rand index. It also generates a file called a `confusion matrix` with the frequencies of each species in each cluster. We provide a further script for visualising this as a heatmap:

```
$CONCOCT/scripts/ConfPlot.R -c evaluation-output/clustering_gt1000_conf.csv -o evaluation-output/c
```

This generates a file with normalised frequencies of contigs from each cluster across species:

https://github.com/BinPro/CONCOCT-test-data/tree/master/evaluation-output/clustering_gt1000_conf.pdf

6.3.11 Validation using single-copy core genes

We can also evaluate the clustering based on single-copy core genes. You first need to find genes on the contigs and functionally annotate these. Here we used prodigal (<https://github.com/hyatt/Prodigal>) for gene prediction and

annotation, but you can use anything you want:

```
cd $CONCOCT_EXAMPLE
mkdir -p $CONCOCT_EXAMPLE/annotations/proteins
prodigal -a annotations/proteins/velvet_71_c10K.faa \
-i contigs/velvet_71_c10K.fa \
-f gff -p meta > annotations/proteins/velvet_71_c10K.gff
```

We used RPS-Blast to COG annotate the protein sequences using the script `RSBLAST.sh`. You need to set the environmental variable `COGSDB_DIR`:

```
export COGSDB_DIR=/proj/b2010008/nobackup/database/cog_le/
```

The script furthermore requires GNU parallel and rpsblast. Here we run it on eight cores:

```
$CONCOCT/scripts/RPSBLAST.sh -f annotations/proteins/velvet_71_c10K.faa -p -c 8 -r 1
mkdir $CONCOCT_EXAMPLE/annotations/cog-annotations
mv velvet_71_c10K.out annotations/cog-annotations/
```

The blast output has been placed in:

```
$CONCOCT_TEST/annotations/cog-annotations/velvet_71_c10K.out
```

Finally, we filtered for COGs representing a majority of the subject to ensure fragmented genes are not over-counted and generated a table of counts of single-copy core genes in each cluster generated by CONCOCT. Remember to use a real email address, this is supplied since information is fetched from ncbi using their service eutils, and the email is required to let them know who you are.

```
cd $CONCOCT_EXAMPLE
$CONCOCT/scripts/COG_table.py -b annotations/cog-annotations/velvet_71_c10K.out \
-m $CONCOCT/scgs/scg_cogs_min0.97_max1.03_unique_genera.txt \
-c concoct-output/clustering_gt1000.csv \
--cdd_cog_file $CONCOCT/scgs/cdd_to_cog.tsv > evaluation-output/clustering_gt1000_scg.tab
```

The script requires the clustering output by `concoct concoct-output/clustering_gt1000.csv`, a file listing a set of SCGs (e.g. a set of COG ids) to use `scgs/scg_cogs_min0.97_max1.03_unique_genera.txt` and a mapping of Conserved Domain Database ids (<https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) to COG ids `$CONCOCT/scgs/cdd_to_cog.tsv`. If these protein sequences were generated by Prokka, the names of the contig ids needed to be recovered from the gff file. Since prodigal has been used, the contig ids instead are recovered from the protein ids using a separator character, in which case only the string before (the last instance of) the separator will be used as contig id in the annotation file. In the case of prodigal the separator that should be used is `_` and this is the default value, but other characters can be given through the `'-separator'` argument.

The output file is a tab-separated file with basic information about the clusters (cluster id, ids of contigs in cluster and number of contigs in cluster) in the first three columns, and counts of the different SCGs in the following columns.

This can also be visualised graphically using the R script:

```
cd $CONCOCT_EXAMPLE
$CONCOCT/scripts/COGPlot.R -s evaluation-output/clustering_gt1000_scg.tab -o evaluation-output/clust
```

The plot is downloadable here:

https://github.com/BinPro/CONCOCT-test-data/tree/master/evaluation-output/clustering_gt1000_scg.pdf

6.3.12 Incorporating linkage information

To perform a hierarchical clustering of the clusters based on linkage we simply run:

```
$CONCOCT/scripts/ClusterLinkNOverlap.pl --cfile=concoct-output/clustering_gt1000.csv --lfile=concoct-
```

The output indicates that the clusters have been reduced from four to three. The new clustering is given by `concoct-output/clustering_gt1000_1.csv`. This is a significant improvement in recall:

```
$CONCOCT/scripts/Validate.pl --cfile=concoct-output/clustering_gt1000_1.csv --sfile=evaluation-output
```

N	M	TL	S	K	Rec.	Prec.	NMI	Rand	AdjRand
684	684	6.8400e+02	5	3	1.000000	0.997076	0.995805	0.999979	0.999957

The algorithm is explained in more depth in the paper on [arXiv](#)