# The GroopM manual

Version 0.2.10.14 Last updated 21st December 2013

---

**GroopM was developed to be used in conjunction with a specific experimental design pattern. Before you try GroopM please ensure:**

- You are using a MODERN sequencing platform. Preferably Illumina based
- You have sampled your metagenomic community at at least 3 time points / spatial positions

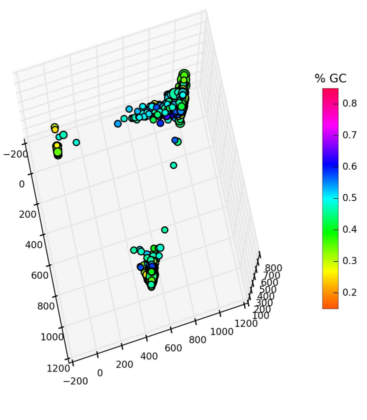
**Please be aware. GroopM will not work well (or at all) in the following situations:**

- For sets of unrelated metagenomes
- For fewer than 3 samples
- For low (very) coverage data sets
- For (very) bad assemblies

**How do you know if one of these (unfortunate) descriptions applies to your data?**

GroopM needs at least 3 samples at reasonable depth and a pretty decent collection of long contigs to bootstrap itself into action. Not all your contigs need to be super long, just a decent chunk. If your longest contig is only 3 Kbp long then GroopM may not do very well. Perhaps you need to review your sampling / filtering / assembly / post assembly work flows. GroopM requires that your samples come from *related metagenomes*. By this we mean that you have sampled a collection of similar habitats, for example 20 human gut microbiomes OR a set of replicated reactors OR the same habitat multiple times. GroopM assumes that a substantial number of reads from each sample will map to most organisms in all samples. If your environments differ too greatly GroopM will probably choke. To see how you'll go you can parse in the data and then run:

```
$ groopm explore -m allcontigs database.gm
```



If you see only clusters of contigs at the extremities of the resulting plot (as shown here) then GroopM probably won't work for your data.

**GroopM has been designed to be user friendly. So far we've used it to get good results from a wide variety of habitats with highly varying sequencing approaches.**

**There's no need to be scared. Chances are, GroopM will work with your data.**

## For impatient people

1. Make a co-assembly of ALL of your data using Velvet or similar
2. Map each of your read sets to these contigs using BWA or similar*
3. `$ groopm parse db.gm contigs.fa *.bam`
4. `$ groopm core db.gm`
5. `$ groopm refine db.gm`
6. `$ groopm recruit db.gm`
7. `$ groopm extract db.gm contigs.fa`

*BAM files must be indexed and sorted. GroopM has been known to have difficulty parsing Picard-generated BAM files. If this occurs then re-sorting the BAM file with samtools usually fixes the problem.

## Table of Contents

**Data storage** 4

**GroopM command line overview** 4

**Typical work flow overview and corresponding commands** 5

Loading your data into GroopM 6
*GroopM parse* ........ *6*

Creating “core” bins 6
*GroopM core* ........ *6*

Refining bins 7
*GroopM refine* ........ *7*

Recruiting unbinned contigs 7
*GroopM recruit* ........ *7*

Extracting your bins 7
*GroopM extract* ........ *7*

**Manual bin editing utilities** 8

*GroopM merge* ........ *8*
*GroopM split* ........ *8*
*GroopM delete* ........ *9*

**Printing, visualisation and data extraction** 9

Extracting data from the database 9
*groopm print* ........ *9*
*GroopM dump* ........ *10*

Visualising your bins 10
*groopm plot* ........ *10*
*groopm highlight* ........ *11*
*GroopM explore* ........ *11*

**Appendix 1 .gm (.sm) database layout** 13

## Data storage

GroopM stores all of it's data in one database. A single database provides two awesome advantages. 1)  One project, one file. Each data type used by GroopM is stored as a table or group of tables within the database. Thus users do not need to worry about the format and location of several csv files. 2) Other programs can be made aware of this database structure and can add to or modify its contents. ALL GroopM commands expect to be given the database name as a parameter.

We currently use HDF5 for this purpose but we are shortly going to move over to using plain old sqllite3. We recommend that databases generated using groopm should have the suffix ".gm" or ".sm". Technically you can make it whatever you like, but this is the convention we have adopted. The current implementation uses a standard hdf5 layout, so you can view and modify the contents of a GroopM database with third party tools such as hdfview. We are working on the standardization of this data structure, as well as developing programming APIs to access and modify its contents. If this is something you think would interest you, please contact me at mike@mikeimelfort.com.

## GroopM command line overview

GroopM is executed from the command line and can be run in several different "modes". Some modes are used to build or modify the .gm database, some produce bins and others print or visualise data. This is a brief overview of GroopM commands. Detailed information for each command is given in the sections below.

### Typical work flow commands:

| | | |
|---|---|---|
| parse | → | Load raw data (contigs /mappings) and create database |
| core | → | Create core bins |
| refine | → | Interactively refine core bins (merge / split etc.) |
| recruit | → | Add unbinned contigs to the cores / enlarge cores |
| extract | → | Extract binned contigs or read identifiers |

### Manual bin editing utilities:

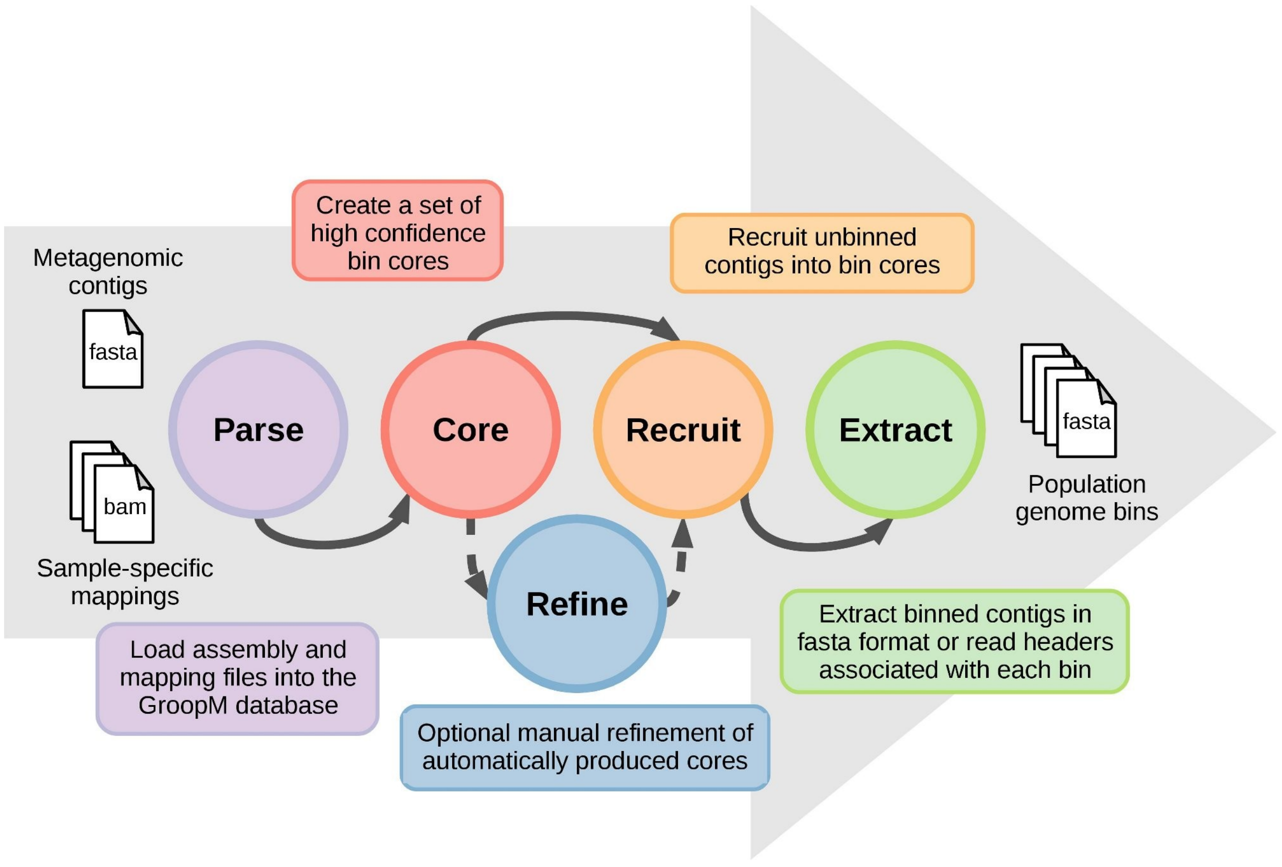
| | | |
|---|---|---|
| merge | → | Merge two or more bins |
| split | → | Split a bin into N parts |
| delete | → | Delete a bin |

### Printing, visualisation and data extraction:

| | | |
|---|---|---|
| explore | → | Methods for visualising bins and contigs |
| plot | → | Plot bins |
| highlight | → | Highlight individual bins and apply labels |
| print | → | Print summary statistics about bins |
| dump | → | Write database fields to csv |

## Typical work flow overview and corresponding commands

Before you can use GroopM you'll need to assemble and map your reads. The general recipe is to make a co-assembly of ALL of your data using Velvet or similar. Take these contigs and map each of your read sets to them using BWA or similar. If you have N sampling points then your aim is to produce N sorted-indexed BAM files. samtools can help with this. The typical workflow for GroopM is as follows:


Metagenomic contigs
fasta
bam
Sample-specific mappings
Create a set of high confidence bin cores
Recruit unbinned contigs into bin cores
Parse
Core
Recruit
Extract
Refine
fasta
Population genome bins
Load assembly and mapping files into the GroopM database
Optional manual refinement of automatically produced cores
Extract binned contigs in fasta format or read headers associated with each bin

Please note that this is the core work flow. GroopM also contains a number of visual exploratory tools and some bin editing tools including the optional 'refine' step. This step can be run after 'core' or after 'recruit' or after both. GroopM was designed to be as parameter-free as possible. For more information on any of these steps simply type:

```
$ groopm OPTION -h
```

The rest of this manual provides detailed information on and useful examples of all of the GroopM command modes.

## Loading your data into GroopM

*parse*

Description:

Load the raw data (contigs /mappings) and create database

Required arguments:

| | |
|---|---|
| dbname | name of the database being created |
| reference | FASTA file containing BAM reference sequences (your contigs) |
| bamfiles | BAM files to parse |

Optional arguments:

| | | |
|---|---|---|
| -f --force | [False] | overwrite existing DB file without prompting |

Example usage:

```
$ groopm parse database.gm contigs.fa sample1.bam sample2.bam sample3.bam
```

This example will parse your contigs (contigs.fa) and the three corresponding BAM files (Sample1.bam, … ) and store the information in database.gm. You will use this database in all remaining GroopM steps.

## Creating "core" bins

*core*

Description:

Create a set of high quality "trusted" bins

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |

Optional arguments:

| | | |
|---|---|---|
| -c --cutoff | [1500] | cutoff contig size for core creation |
| -s --size | [10] | minimum number of contigs which define a core |
| -b --bp | [1000000] | cumulative size of contigs which define a core regardless of number of contigs |
| -f --force | [False] | overwrite existing DB file without prompting |
| -g –graphfile | | output graph of micro bin mergers |
| -p --plot | [False] | create plots of bins after basic refinement |
| -m --multiplot | [0] | create plots during core creation – (0-3) MAKES MANY IMAGES! |

Example usage:

```
$ groopm core database.gm
$ groopm core -c 500 database.gm
```

The first example will attempt to bin all contigs at least as long as the default cut off length (1500 bp). You can modify this using the -c option as demonstrated in the second example which tries to bin all contigs that are at least 500 bp long. All bins created using this command are stored in the database.gm.

## Refining bins

***refine***

Description:

Examine your bins and try fix any errors you find

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |

Optional arguments:

| | | |
|---|---|---|
| -a --auto | [False] | automatically refine bins |
| -r --no_transform | [False] | skip data transformation (3 stoits only) |
| -p --plot | [False] | create plots of bins after refinement |

Example usage:

```
$ groopm refine database.gm
```

This will start GroopM's  interactive bin editing work flow. MORE about this soon!

## Recruiting unbinned contigs

***recruit***

Description:

Recruit unbinned contigs using the core bins as a 'template'

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |

Optional arguments:

| | | |
|---|---|---|
| -c --cutoff | [500] | cutoff contig size |
| -f --force | [False] | overwrite existing db file without prompting |
| -s --step | [200] | step size for iterative recruitment |
| -i --inclusivity | [2.5] | make recruitment more or less inclusive |

Example usage:

**$ groopm recruit database.gm**

This will recruit unbinned contigs into your core bins. By default it tries to recruit all unbinned contigs. You can specify a lower length cutoff using the '-c' option. Increasing the '-i' option makes your bins bigger, but not necessarily better.

### Extracting your bins

***GroopM extract***

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |
| data | data file(s) to extract from bam or fasta |

Optional arguments:

| | | |
|---|---|---|
| -b --bids | [] | bin ids to use (None for all) |
| -c --cutoff | [0] | cutoff size (ignored for reads, 0 = no cutoff) |
| -m --mode | [contigs] | what to extract [reads contigs] |
| -o –outfolder | [] | write to this folder (None for current dir) |
| -B --no_separate_bams | [False] | use one file for all stoits |

Example usage:

```
$ groopm extract database.gm contigs.fa
$ groopm extract database.gm -m reads Sample1.bam Sample2.bam
```

The first usage will create a collection of multiple FASTA files, one for each bin, that contain binned contigs. The second usage will extract read headers from the bam files provided and create one file of headers for each bin. If the user specifies the '-B' option, then GroopM will produce B * M output files where B is the number of bins and M is the number of BAM files provided. Each file will contain read headers from one bin and from one sample.

## Manual bin editing utilities

***GroopM merge***

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |
| bids | bin ids to merge. |

Optional arguments:

| | | |
|---|---|---|
| -f --force | [False] | merge without prompting |

Example usage:

```
$ groopm merge database.gm 5 10
$ groopm merge database.gm 5 10 11
```

The first usage  will merge bins 5 and 10. You will be given the chance to review the merge before confirming (GroopM will produce some plots). If you don't want this then use the '-f' option. The contigs from bin 10 will be placed into bin 5 and bin 10 will be deleted. The second usage will merge bin 10 with bin 5 and then bin 11 with bin 5.

### *GroopM split*

Required arguments:

| | | |
|---|---|---|
| dbname | name of the database to open | |
| bid | bin id to split | |
| parts | number of parts to split the bin into | |

Optional arguments:

| | | |
|---|---|---|
| -m --mode | [kmer] | profile to split on [kmer, cov, length] |
| -f --force | [False] | split without prompting |

Example usage:

```
$ groopm split database.gm 7 3
$ groopm split database.gm -m cov 7 3
```

The first usage will split bin 7 into three parts using k-means clustering based on tetranucleotide signatures. You will be given the chance to review the split before confirming (GroopM will produce some plots). If you don't want this then use the '-f' option. The second usage will do the same type of split but use coverage profiles instead.

### *GroopM delete*

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |
| bids | bin ids to delete |

Optional arguments:

| | | |
|---|---|---|
| -f –force | [False] | delete without prompting |

Example usage:

```
$ groopm delete database.gm 87
$ groopm delete database.gm 87 88 89
```

The first usage will delete bin 87. The second usage will delete bins 87, 88 and 89.

## Printing, visualisation and data extraction

### Extracting data from the database

#### *groopm print*

Description:

Print summary statistics about bins.

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |

Optional arguments:

| | | |
|---|---|---|
| -b --bids | [] | bin ids to print (None for all) |
| -o --outfile | [] | print to file not STDOUT |
| -f --format | [bins] | output format [bins contigs] |
| -u --unbinned | [False] | print unbinned contig IDs too |

Example usage:

```
$ groopm print database.gm
$ groopm print database.gm -f contigs -b 7 4
```

The first usage will print some summary statistics about all the bins including the number of contigs, average GC content etc. The second usage will print detailed information about bins 7 and 4 on a per contig basis.

#### *GroopM dump*

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |

Optional arguments:

| | | |
|---|---|---|
| -f --fields | [names,bins] | fields to extract* |
| -o --outfile | [GMdump.csv] | write data to this file |
| -s --separator | [,] | data separator |
| --no_headers | [False] | don't add headers |

*Build a comma separated list from [names mers gc coverage tcoverage ncoverage lengths bins] or just use all

Example usage:

```
$ groopm dump database.gm
$ groopm dump database.gm -f names,coverage,bins,gc -o summary.csv
```

More soon

## Visualising your bins

### *groopm plot*

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |

Optional arguments:

| | | |
|---|---|---|
| -b --bids | [] | bin ids to plot (None for all) |
| -t --tag | [BIN] | tag to add to output filename |
| -f --folder | [] | save plots in folder |
| -p --points | [False] | ignore contig lengths when plotting |
| -C --cm | [HSV] | set colormap* |

*[HSV Accent Blues Spectral Grayscale Discrete DiscretePaired]

Example usage:

```
$ groopm plot database.gm
```

More soon

### *groopm highlight*

Required arguments:

| | |
|---|---|
| dbname | name of the database to open |

Optional arguments:

| | | |
|---|---|---|
| -L --binlabels | [] | replace bin IDs with user specified labels (use 'none' to force no labels) |
| -C --contigcolors | [] | specify contig colors (use 'none' to force no colors) |
| -r --radius | [False] | draw placement radius to help with label moving |
| -c --cutoff | [1000] | cutoff contig size |
| -e --elevation | [25.0] | elevation in printed image |
| -a --azimuth | [-45.0] | azimuth in printed image |
| -f --file | [gmview] | name of image file to produce |
| -t --filetype | [jpg] | type of file to produce |
| -d --dpi | [300] | image resolution |
| -s --show | [False] | load image in viewer only |
| -p --points | [False] | ignore contig lengths when plotting |
| -b --bids | [] | bin ids to plot (None for all) |

Example usage:

```
$ groopm highlight database.gm
$ groopm highlight database.gm -s -L bin_labels.csv -t png -f myBins
$ groopm highlight database.gm -L bin_labels.csv -f myBins -d 600 -a 20 -e 95
```

More soon

### *GroopM explore*

Required arguments:

| | | |
|---|---|---|
| dbname | name of the database to open | |

Optional arguments:

| | | |
|---|---|---|
| -b --bids | [] | bin ids to plot (None for all) |
| -c --cutoff | [1000] | cutoff contig size |
| -m --mode | [binids] | Exploration mode* |
| -r --no_transform | [False] | skip data transformation (3 stoits only) |
| -k --kmers | [False] | include kmers in figure (only used when mode == together) |
| -p --points | [False] | ignore contig lengths when plotting |
| -C --cm | [HSV] | set colormap** |

*[binpoints binids allcontigs unbinnedcontigs binnedcontigs binassignments compare sidebyside together]

**[HSV Accent Blues Spectral Grayscale Discrete DiscretePaired]

Example usage:

```
$ groopm explore database.gm
$ groopm explore database.gm -m allcontigs
```

More soon

# Appendix 1 .gm (.sm) database layout

This is the structure of the PyTables implementation of the data storage used in GroopM.

## METADATA

| | |
|---|---|
| Group: | 'meta' |
| Description: | Information about the database, bins and contigs |

### Metadata

| | |
|---|---|
| Table name: | 'meta' |
| Description: | Information about the data set and DB |
| Fields: | |

| | | |
|---|---|---|
| 'stoitColNames' | : String (512) : | Names of the BAM files supplied to parse |
| 'numStoits' | : Int : | Number of samples |
| 'merColNames' | : String (4096) : | Concatenated Nucl strings of kmers used |
| 'merSize' | : Int : | Length of the kmer used in the signature |
| 'numMers' | : Int : | Number of kmers used to make signatures |
| 'numCons' | : Int : | Number of contigs stored in DB |
| 'numBins' | : Int : | Number of bins stored in DB |
| 'clustered' | : Bool : | Set to true after clustering is complete |
| 'complete' | : Bool : | Unused |
| 'formatVersion' | : Int : | GroopM file version |

### PC variance

| | |
|---|---|
| Table name: | 'kpca_variance' |
| Description: | Variance of kmer signature PCAs |
| Fields: | |

| | | |
|---|---|---|
| 'pc1_var' | : Float : | Variance of 1st kmer principal component |
| 'pc2_var' | : Float : | Variance of 2nd kmer principal component |
| 'pc3_var' | : Float : | |
| ... | | |

### Contigs

| | |
|---|---|
| Table name: | 'contigs' |
| Description: | Information about the contigs being worked with |
| Fields: | |

| | | |
|---|---|---|
| 'cid' | : String (512) : | Fasta header of contig |

| | | |
|---|---|---|
| 'bid' | : Int : | Bin ID for this contig |
| 'length' | : Int : | Contig length |
| 'gc' | : Float : | GC percentage of this contig |

### Bins

Table name: 'bins'

Description: Information about the bins

Fields:

| | | |
|---|---|---|
| 'bid' | : Int : | ID of the bin |
| 'numMembers' | : Int : | Number of contigs in thebin |
| 'isLikelyChimeric' | : Bool : | Has the bin been flagged as chimeric |

### Transformed coverage corners

Table name: 'transCoverageCorners'

Description: Coordinates of the 'corners' in transformed coverage space

Fields:

| | | |
|---|---|---|
| 'x' | : Float : | X-coordinate of the corner point |
| 'y' | : Float : | Y-coordinate of the corner point |
| 'z' | : Float : | Z-coordinate of the corner point |

## PROFILES

Group: 'profile'

Description: The profile group stores all the information about contigs. All tables in the profile group have the same number of rows. Identical rows in different tables describe the same contig.

### Kmer Signature

Table name: 'kms'

Description: Tetranucleotide signatures

Fields:

| | | |
|---|---|---|
| 'mer1' | : Float : | Frequency of first kmer |
| 'mer2' | : Float : | Frequency of second kmer |
| 'mer3' | : Float : | |
| ... | | |

### Kmer Vals

Table name: 'kpca'

Description: Principal components of tetranucleotide signatures

Fields:

|  |  |  |
|---|---|---|
| 'pc1' | : Float : | First principal component |
| 'pc2' | : Float : | Second Principal component |
| 'pc3' | : Float : |  |
| ... |  |  |

### Coverage profile

|  |  |
|---|---|
| Table name: | 'coverage' |
| Description: | Raw coverage profiles (coverage in samples) |
| Fields: |  |

|  |  |  |
|---|---|---|
| 'stoit1' | : Float : | Coverage in first sample |
| 'stoit2' | : Float : | Coverage in second sample |
| 'stoit3' | : Float : |  |
| ... |  |  |

### Transformed coverage profile

|  |  |
|---|---|
| Table name: | 'transCoverage' |
| Description: | Coverage profiles in GroopM transformed coverage space |
| Fields: |  |

|  |  |  |
|---|---|---|
| 'x' | : Float : | X-coordinate in transformed space |
| 'y' | : Float : | Y-coordinate in transformed space |
| 'z' | : Float : | Z coordinate in transformed space |

### Normalised coverage profile

|  |  |
|---|---|
| Table name: | 'normCoverage' |
| Description: | Normalised coverage (Euclidean distance from origin to raw coverage profile point) |
| Fields: |  |

|  |  |  |
|---|---|---|
| 'normCov' | : Float : | Value |

## LINKS

|  |  |
|---|---|
| Group: | 'links' |
| Description: | Storage of paired read links between contigs (unused in this version) |

### Links

|  |  |
|---|---|
| Table name: | 'links' |
| Description: | Storage of paired read links between contigs |
| Fields: |  |

|  |  |  |
|---|---|---|
| 'contig1' | : Int : | Reference to index in meta/contigs |
| 'contig2' | : Int : | Rreference to index in meta/contigs |

| | | |
|---|---|---|
| 'numReads' | : Int : | Number of reads supporting this link |
| 'linkType' | : Int : | The type of the link (SS, SE, ES, EE) |
| 'gap' | : Int : | The estimated gap between the contigs |