**Supplementary Information**

**Title:** Accurate binning of metagenomic contigs via automated clustering sequences using information of genomic signatures and marker genes

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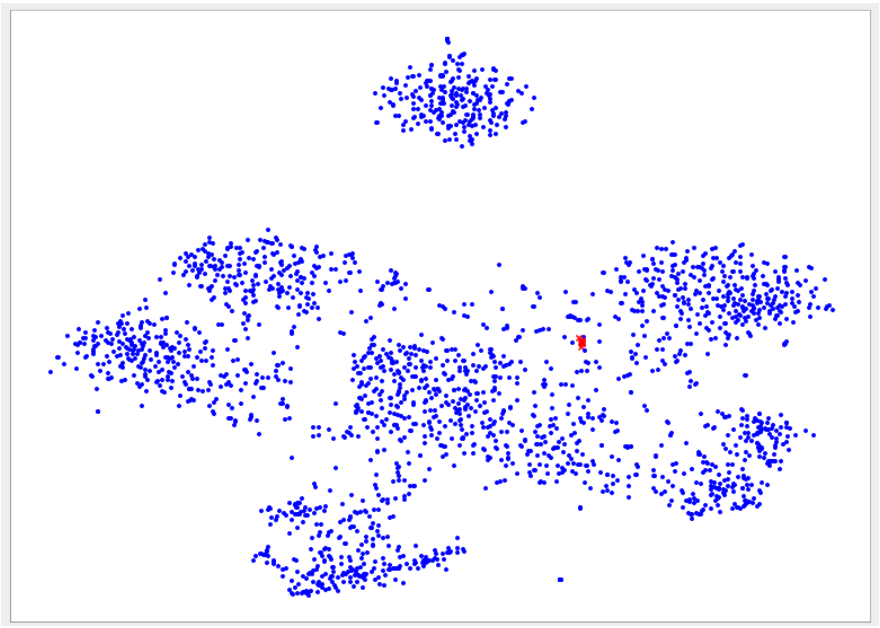
Index

Figure S1 ..... 2
Figure S2 ..... 3
Figure S3 ..... 4
Figure S4 ..... 5
Figure S5 ..... 6
Figure S6 ..... 7
Figure S7 ..... 8
Figure S8 ..... 9
Figure S9 ..... 10
Table S1 ..... 11
Supplementary Note ..... 13
Install MyCC ..... 13
Run MyCC ..... 16
10 Genomes ..... 16
100 Genomes ..... 23
25 Genomes ..... 25
64 Genomes ..... 28
Sharon's Dataset ..... 30
Drosophila Dataset ..... 36
Docker of MyCC ..... 44
Supplementary Methods ..... 46

**(a)**



**(b)**

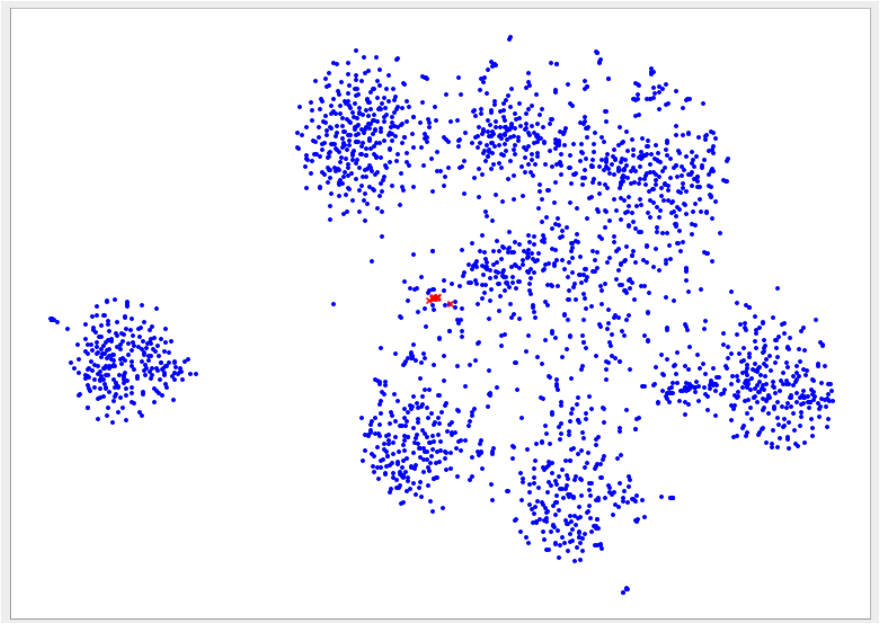


Figure S1 Scatter plot visualization in VizBin for Sharon's dataset employing: (a) 5mer signatures and (b) 4mer signatures. Red crosses highlight contigs of *Enterococcus faecalis*.

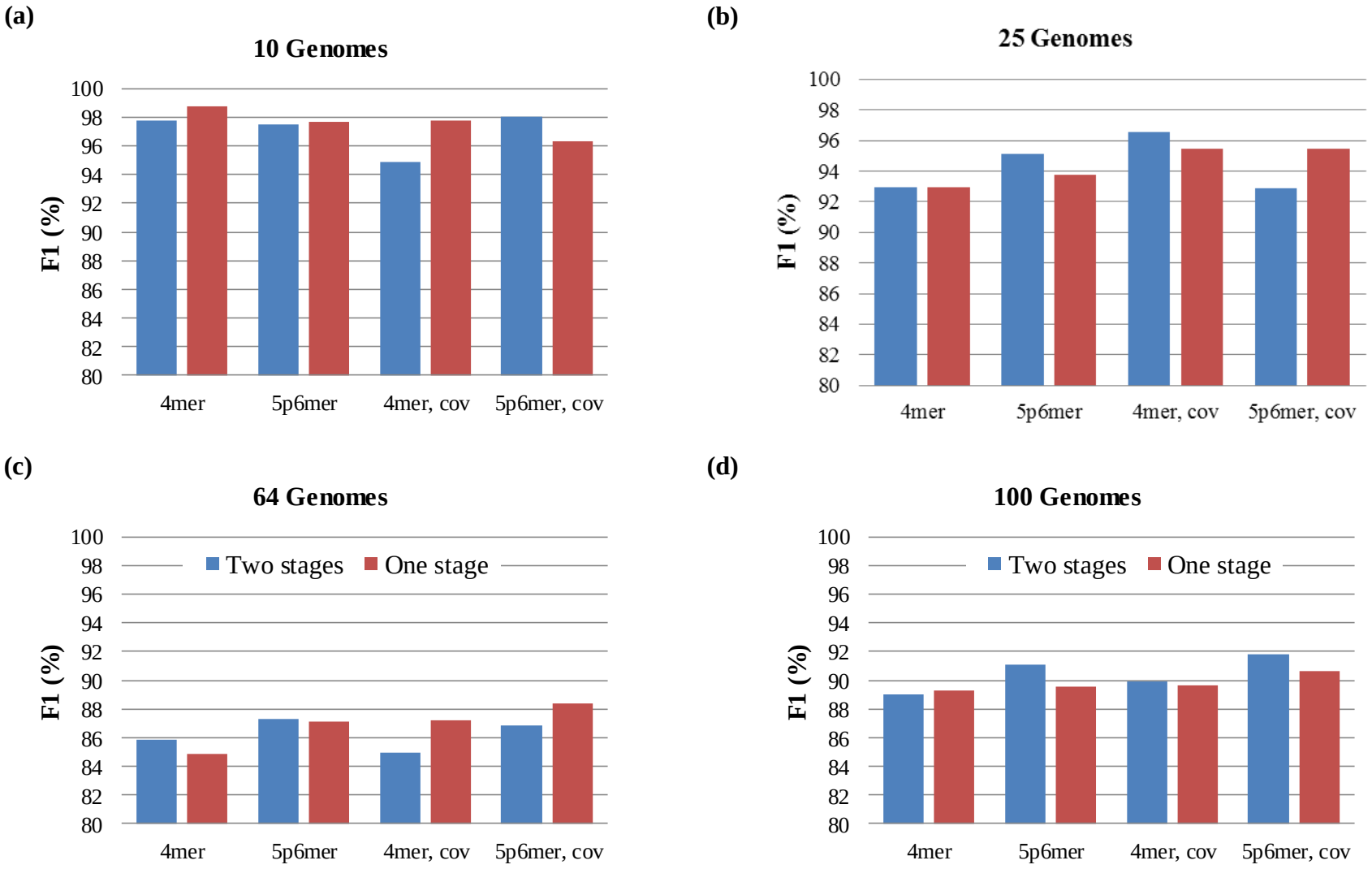



Figure S2 Effects of parameter settings in MyCC.

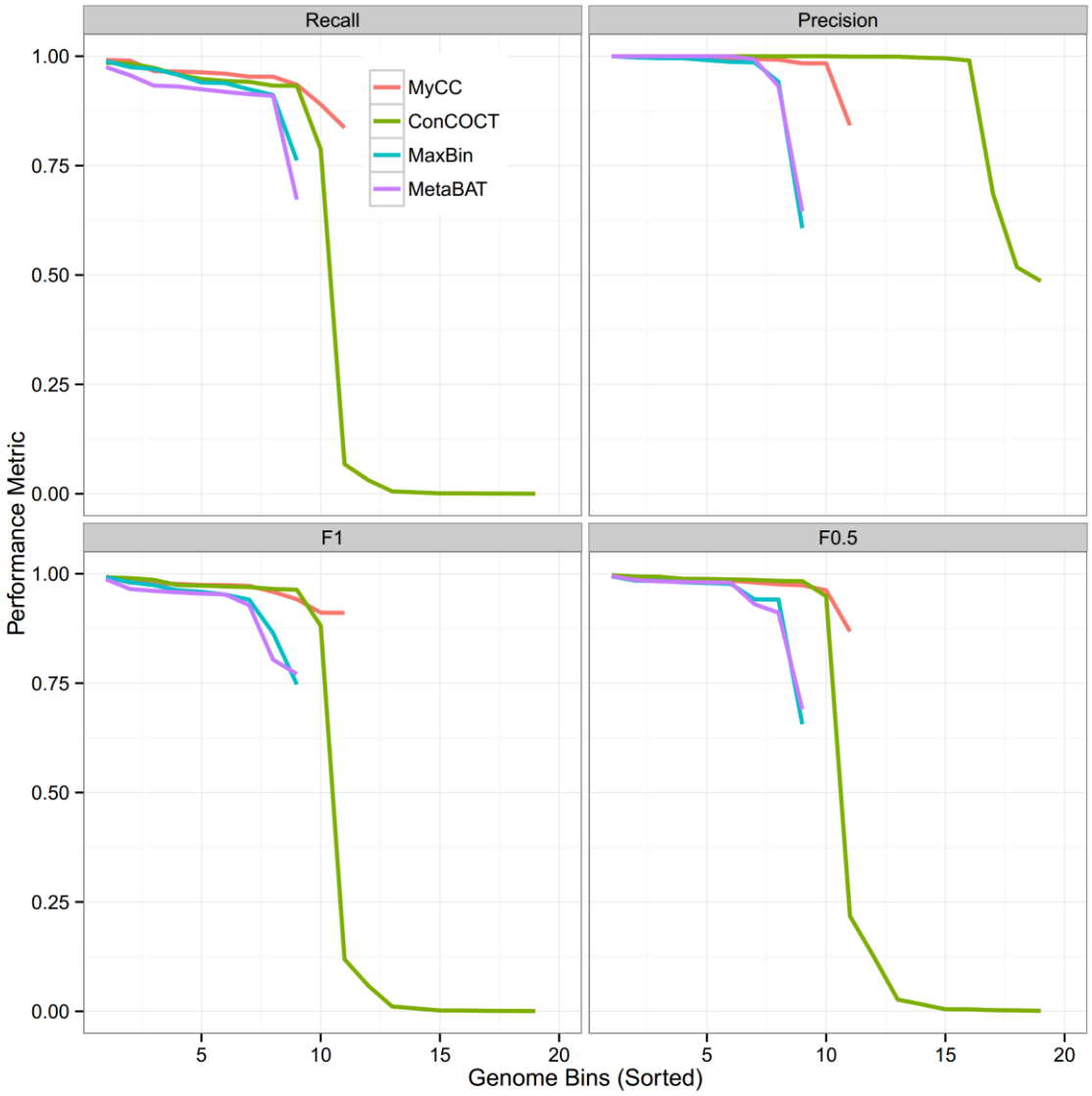



Figure S3 Relative performance of various binning tools evaluated by benchmark.R on the dataset of 10 genomes. Binning results are available at http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/benchmark/10s.zip.

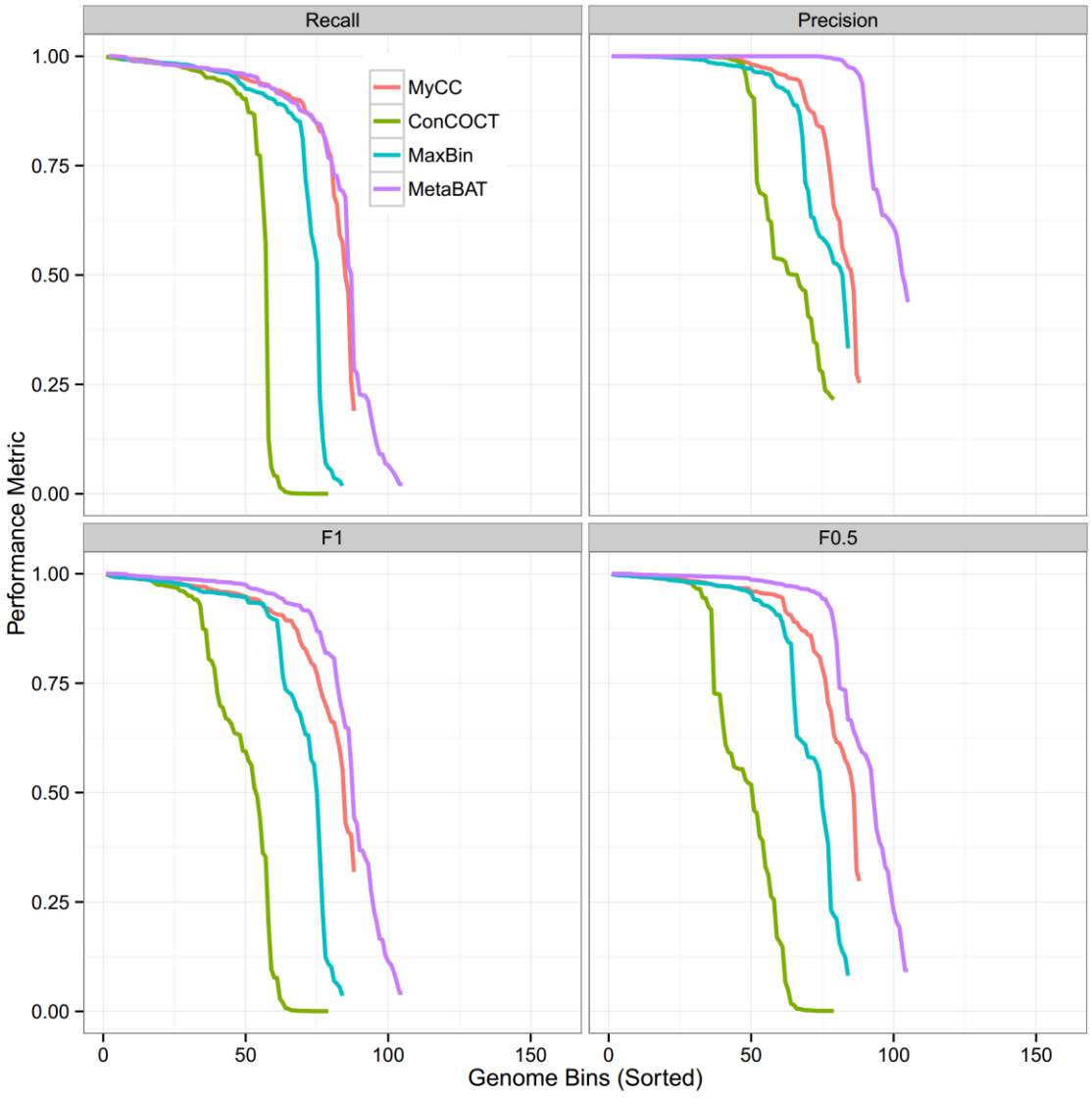



Figure S4 Relative performance of various binning tools evaluated by benchmark.R on the dataset of 100 genomes. Binning results are available at http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/benchmark/100s.zip

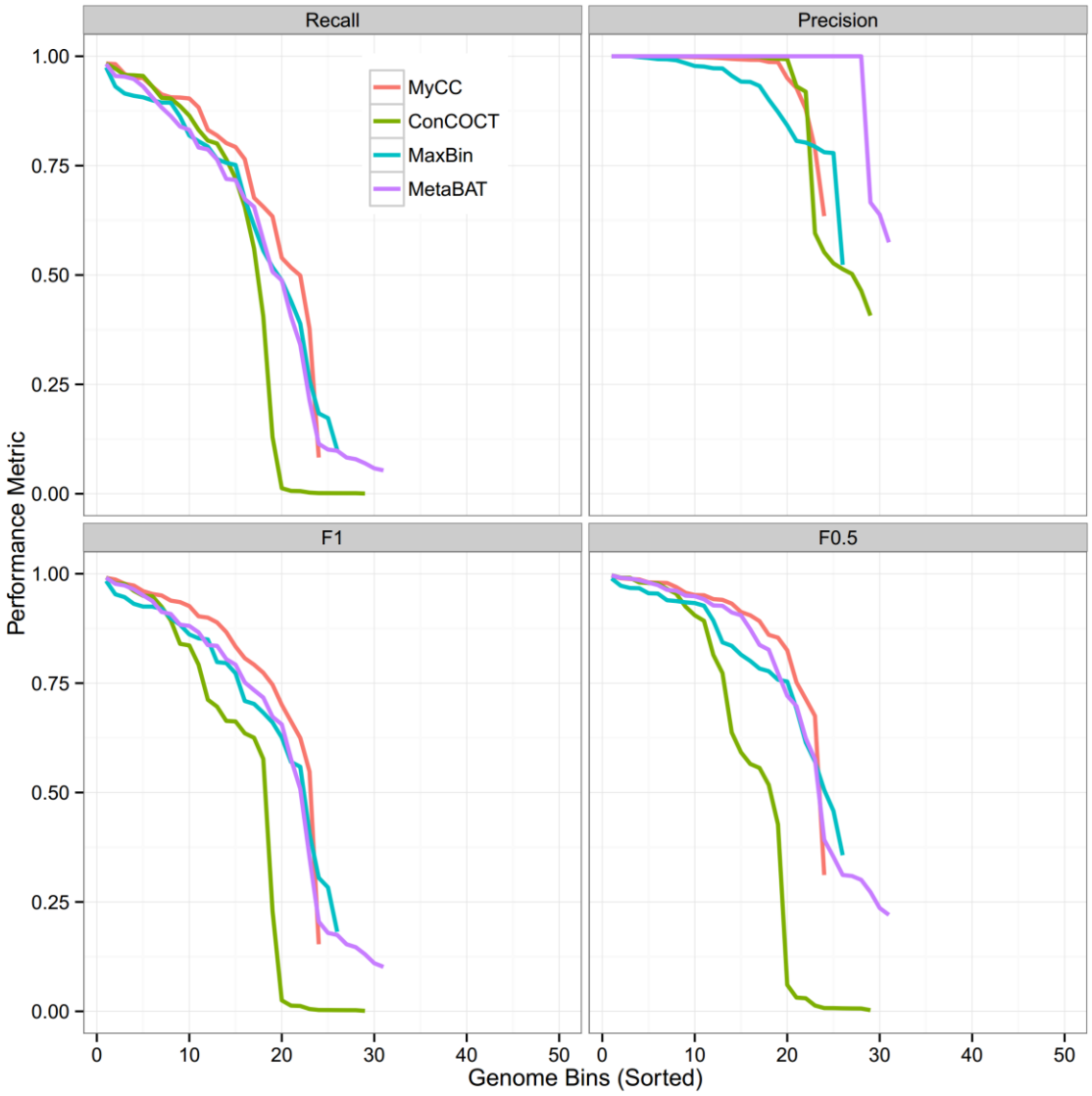



Figure S5 Relative performance of various binning tools evaluated by benchmark.R on the dataset of 25 genomes. Binning results are available at http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/benchmark/25s.zip.

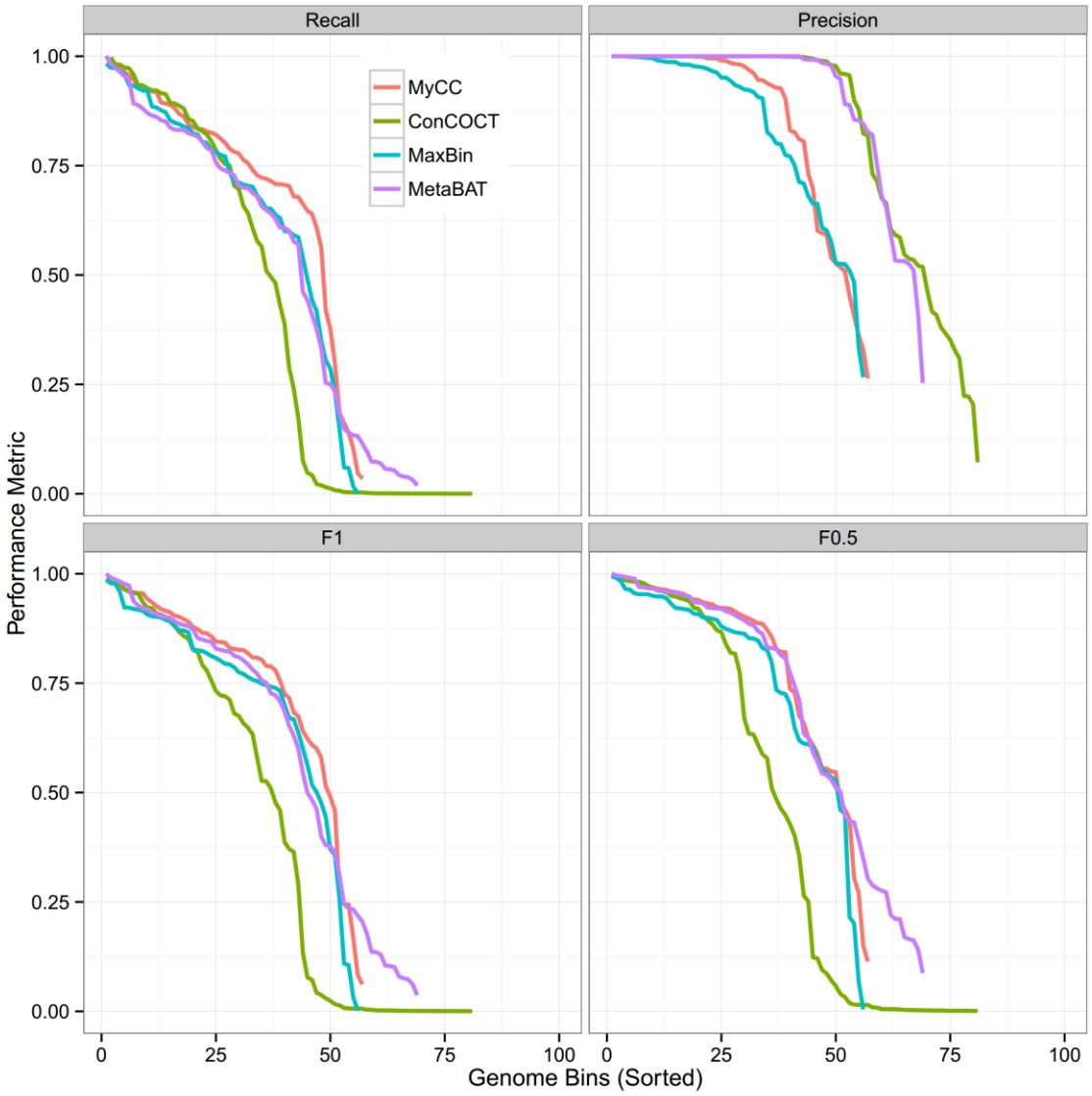



Figure S6 Relative performance of various binning tools evaluated by benchmark.R on the dataset of 64 genomes. Binning results are available at http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/benchmark/64s.zip.

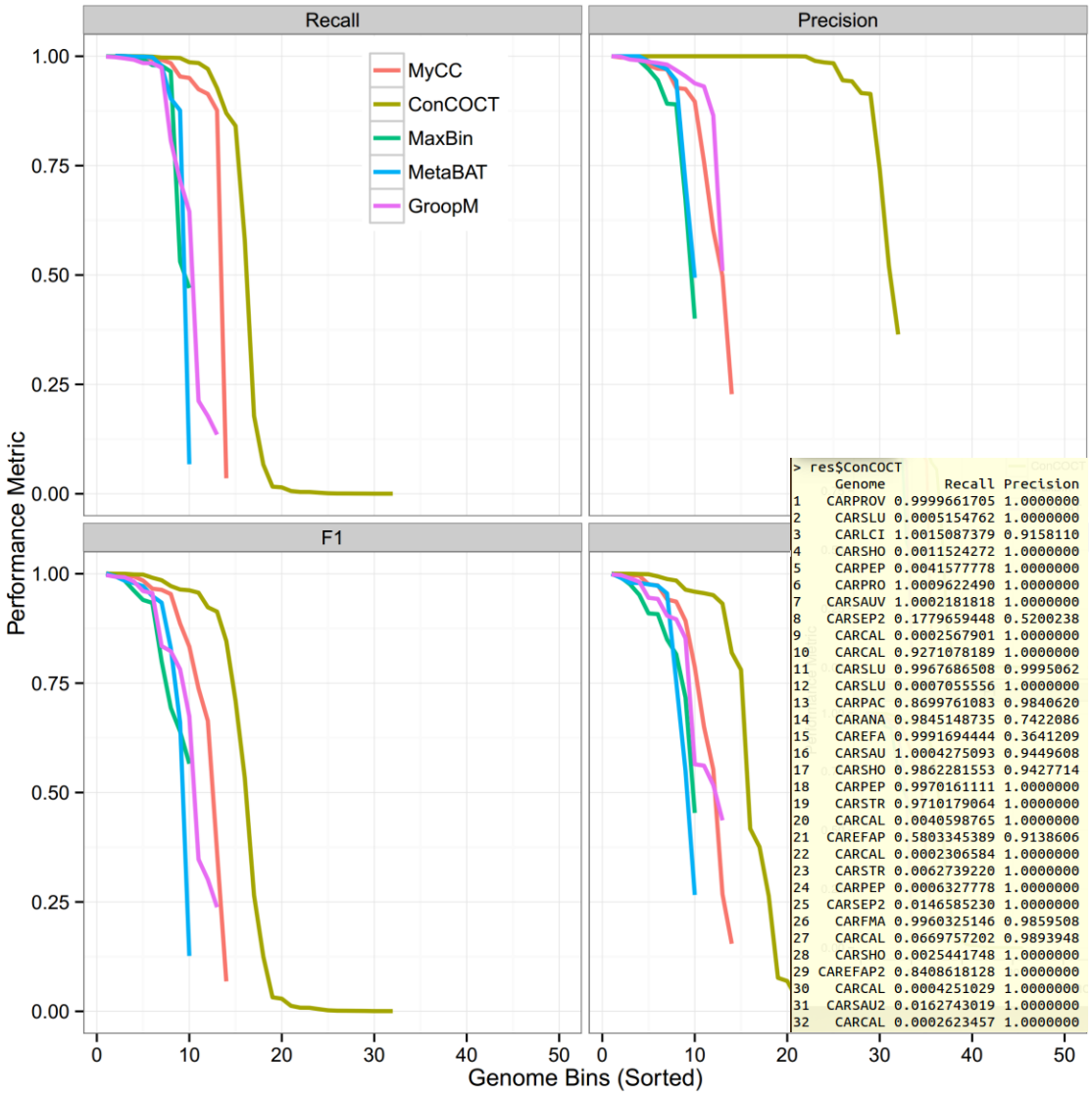



Figure S7 Relative performance of various binning tools evaluated by benchmark.R on the Sharon's dataset. Binning results are available at http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/benchmark/Sharon.zip. Please note that CONCOCT produced many small bins containing only several contigs, which result in high-precision but low-recall binning performance.

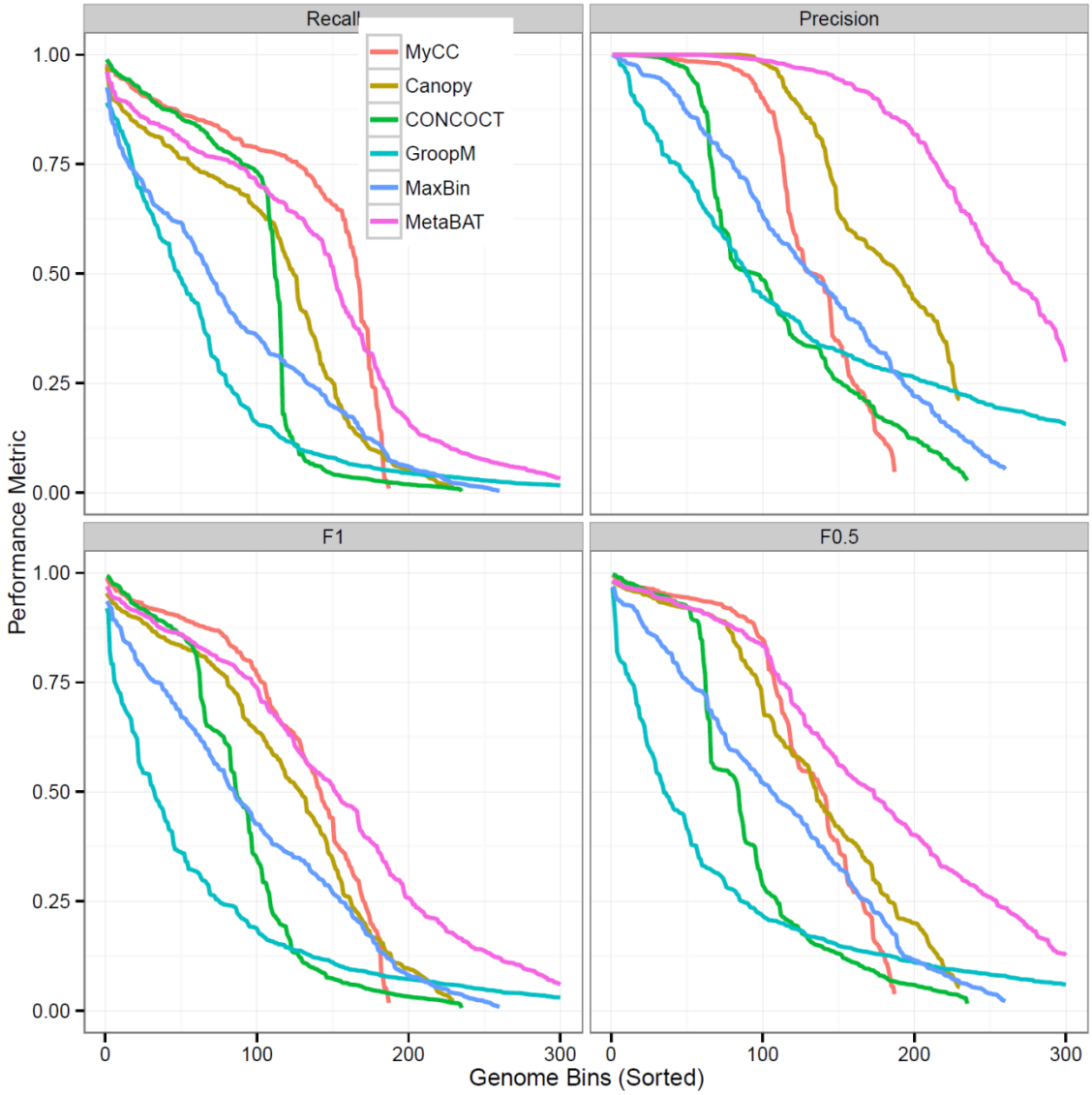



Figure S8 Relative performance of various binning tools evaluated by benchmark.R on the MetaHIT dataset.

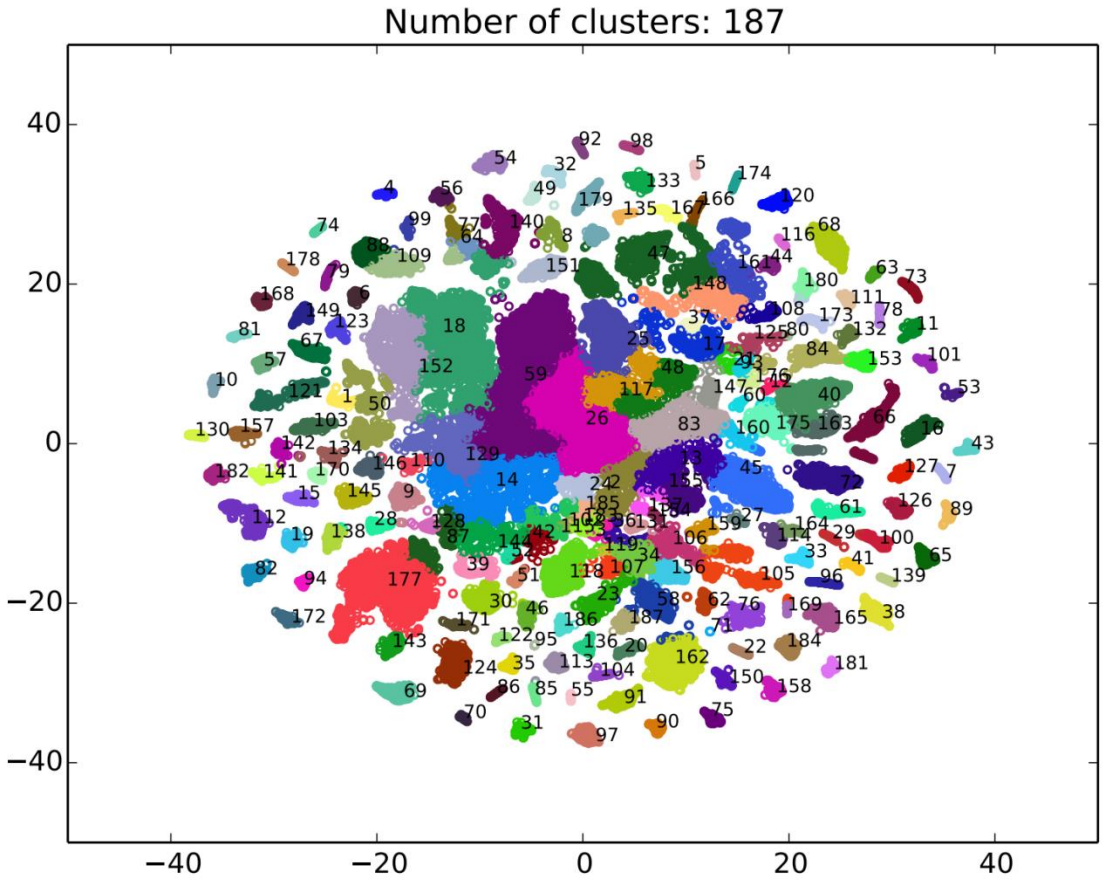



Figure S9 Visualization of metagenomes derived from the 264 MetaHIT human gut metagenome data.

Table S1 Estimation of completeness, contamination and strain heterogeneity provided by CheckM

| CONCOCT | | | | |
|---|---|---|---|---|
| Bin ID | Markder lineage | Completeness | Contamination | Heterogeneity |
| Cluster_22 | root (UID1) | 100 | 204.17 | 33.92 |
| Cluster_23 | g__Staphylococcus (UID301) | 99.51 | 2.91 | 60 |
| **Cluster_25** | **o__Clostridiales (UID1120)** | **98.95** | **0** | **0** |
| **Cluster_14** | **o__Actinomycetales (UID1530)** | **97.86** | **0** | **0** |
| **Cluster_24** | **g__Staphylococcus (UID298)** | **95.39** | **0.57** | **0** |
| Cluster_19 | g__Staphylococcus (UID298) | 84.07 | 0.02 | 0 |
| Cluster_11 | f__Leuconostocaceae (UID486) | 45.08 | 0.23 | 0 |
| Cluster_18 | k__Archaea (UID2) | 31.63 | 8.54 | 0 |
| Cluster_3 | o__Clostridiales (UID1125) | 28.73 | 0 | 0 |
| Cluster_26 | g__Streptococcus (UID576) | 16.31 | 0.33 | 0 |
| Cluster_20 | k__Bacteria (UID203) | 5.49 | 0 | 0 |
| Cluster_21 | k__Bacteria (UID203) | 4.73 | 0 | 0 |
| Cluster_4 | k__Archaea (UID2) | 3.74 | 0 | 0 |
| 19 Clusters | root (UID1) | 0 | 0 | 0 |
| GroopM | | | | |
| Bin ID | Markder lineage | Completeness | Contamination | Heterogeneity |
| myDB_bin_10 | root (UID1) | 100 | 104.17 | 96.55 |
| **myDB_bin_11** | **g__Staphylococcus (UID301)** | **99.51** | **0.08** | **0** |
| **myDB_bin_16** | **o__Lactobacillales (UID544)** | **99.25** | **0** | **0** |
| **myDB_bin_9** | **o__Actinomycetales (UID1530)** | **97.86** | **0** | **0** |
| myDB_bin_6 | g__Staphylococcus (UID298) | 95.81 | 1.75 | 16.67 |
| myDB_bin_4 | g__Staphylococcus (UID298) | 82.52 | 3.03 | 0 |
| myDB_bin_2 | k__Archaea (UID2) | 34.43 | 9.48 | 5.88 |
| myDB_bin_7 | f__Leuconostocaceae (UID486) | 24.66 | 0 | 0 |
| myDB_bin_1 | o__Clostridiales (UID1125) | 24.37 | 0.15 | 0 |
| myDB_bin_13 | g__Streptococcus (UID576) | 13.81 | 0.53 | 0 |
| myDB_bin_17 | k__Bacteria (UID203) | 13.79 | 0 | 0 |
| myDB_bin_19 | k__Bacteria (UID203) | 3.45 | 0 | 0 |
| myDB_bin_18 | root (UID1) | 0 | 0 | 0 |
| MaxBin2 | | | | |
| Bin ID | Markder lineage | Completeness | Contamination | Heterogeneity |
| maxbin.008 | k__Bacteria (UID203) | 100 | 24.37 | 0 |
| **maxbin.005** | **g__Staphylococcus (UID301)** | **99.51** | **0.08** | **0** |

| | | | | |
|---|---|---|---|---|
| **maxbin.001** | **o__Lactobacillales (UID544)** | **99.25** | **0** | **0** |
| **maxbin.002** | **o__Clostridiales (UID1120)** | **98.95** | **0** | **0** |
| maxbin.007 | g__Staphylococcus (UID294) | 97.91 | 3.37 | 81.82 |
| maxbin.006 | g__Staphylococcus (UID294) | 97 | 1.12 | 100 |
| maxbin.009 | g__Staphylococcus (UID298) | 84.9 | 2.53 | 0 |
| maxbin.010 | k__Bacteria (UID203) | 72.81 | 37.95 | 0 |
| maxbin.004 | o__Actinomycetales (UID1530) | 66.94 | 0 | 0 |
| maxbin.003 | k__Bacteria (UID203) | 22.41 | 0 | 0 |

MetaBAT

| Bin ID | Markder lineage | Completeness | Contamination | Heterogeneity |
|---|---|---|---|---|
| myOutput.4 | k__Bacteria (UID203) | 100 | 37.96 | 0 |
| myOutput.2 | root (UID1) | 100 | 108.33 | 93.44 |
| myOutput.5 | g__Staphylococcus (UID301) | 99.51 | 2.91 | 60 |
| **myOutput.1** | **o__Lactobacillales (UID544)** | **99.25** | **0** | **0** |
| **myOutput.3** | **o__Clostridiales (UID1120)** | **98.95** | **0** | **0** |
| **myOutput.6** | **g__Staphylococcus (UID298)** | **95.39** | **0.57** | **0** |
| myOutput.9 | g__Staphylococcus (UID298) | 84.1 | 0.02 | 0 |
| myOutput.10 | f__Leuconostocaceae (UID486) | 37.7 | 0 | 0 |
| myOutput.7 | k__Archaea (UID2) | 31.55 | 8.54 | 0 |
| myOutput.8 | root (UID1) | 0 | 0 | 0 |

MyCC

| Bin ID | Markder lineage | Completeness | Contamination | Heterogeneity |
|---|---|---|---|---|
| Cluster.8 | root (UID1) | 100 | 104.17 | 96.55 |
| **Cluster.10** | **g__Staphylococcus (UID301)** | **99.51** | **0.08** | **0** |
| **Cluster.12** | **o__Lactobacillales (UID544)** | **99.25** | **0** | **0** |
| **Cluster.1** | **o__Clostridiales (UID1120)** | **98.95** | **0** | **0** |
| **Cluster.7** | **o__Actinomycetales (UID1530)** | **97.86** | **0** | **0** |
| **Cluster.13** | **g__Staphylococcus (UID298)** | **95.39** | **0.57** | **0** |
| Cluster.6 | g__Staphylococcus (UID298) | 78.73 | 0 | 0 |
| Cluster.2 | f__Leuconostocaceae (UID486) | 45.08 | 0.23 | 0 |
| Cluster.14 | o__Clostridiales (UID1125) | 38.34 | 3.48 | 0 |
| Cluster.4 | k__Archaea (UID2) | 34.43 | 9.48 | 5.88 |
| Cluster.11 | k__Bacteria (UID203) | 7.21 | 0 | 0 |
| Cluster.9 | g__Staphylococcus (UID298) | 5.36 | 0.02 | 0 |
| Cluster.3 | root (UID1) | 4.17 | 0 | 0 |
| Cluster.5 | k__Bacteria (UID203) | 2.66 | 0 | 0 |

## Supplementary Note

### Install MyCC (Virtual Box for Windows or Mac)

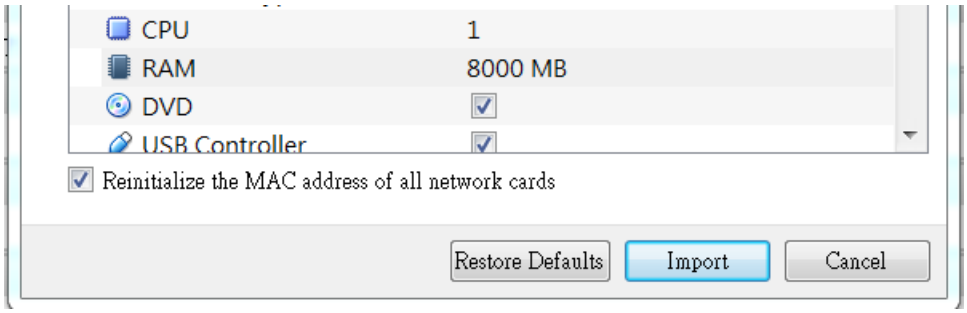
Download Virtual Box
https://www.virtualbox.org/wiki/Downloads

Download the image file of MyCC (MyCC.ova) via
http://sourceforge.net/projects/sb2nhri/files/MyCC/

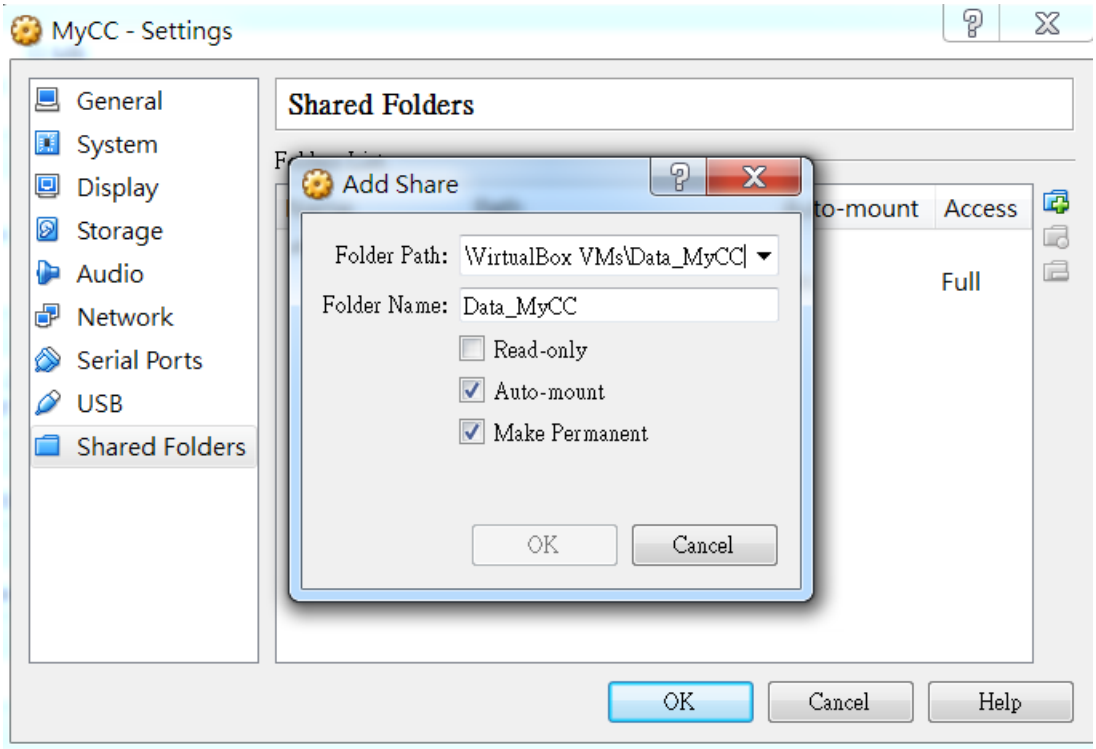
Open VirtualBox
File -> Import Appliance...
Select the file (MyCC.ova) to import

Or, directly double click on MyCC.ova

Please check the box of "Reinitialize the MAC address of all network cards"




Import
Click on Shared folders to add share (e.g. Data_MyCC in your local computer)



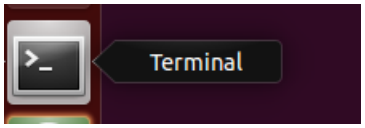

Check Auto-mount and Make-Permanent

OK -> OK

Start

Devices -> Shared Clipboard --> Bidirectional

Open Terminal




Mount to the shared folder

sudo mount -t vboxsf Data_MyCC MyData

(Password for manager: manager)

## Install MyCC (Virtual Box for Linux)

In Ubuntu 15.10:

sudo apt-get install virtualbox

wget http://sourceforge.net/projects/sb2nhri/files/MyCC/MyCC.ova

vboxmanage import MyCC.ova

vboxmanage startvm MyCC

Power off MyCC:

vboxmanage controlvm MyCC poweroff

See https://www.virtualbox.org/manual/ for further information.

## Run MyCC

**MyCC.py -h**

Usage:

MyCC.py [inputfile] [4mer/5mer/56mer default:4mer] [Options]

Options:

-a A file having coverage information.

-t Minimum contig length. [defaults: 1000 bp]

-lt A fraction of contigs for the first-stage clustering. [default: 0.7]

-ct Minimum contig lengh for first stage clustering. [bp]

-meta Change to meta mode of Prodigal. [default: single]

-p Perplexity for BH-SNE. [default: 20, range between 5 and 50]

-pm To set preferences all equal to the median of the other similarities for affinity propagation.

-st Maximum distance for sparse format of affinity propagation. [default: 500]

-mask To mask repetitive sequences.

-keep To keep temporary files.

The 10-Genome, 25-Genome, 64-Genomes, 100-Genome and Sharon's datasets were able to complete within one hour using Intel Xeon CPU E31245 3.30GHz with 4GB RAM. Please note that the following results were produced on the Ubuntu Virtual Machine System, they are slightly different from the results present in Table 1, which were produced on a Centos server with Intel Xeon E7-4820 processors 8-core 2.00 GHz and 256 GB of RAM.

mkdir Run

cd Run

Produce coverage profiles:

Please note that we used Bowtie 2 to produce BAM files. Then we run MetaBAT to produce a depth file: jgi_summarize_bam_contig_depths --outputDepth depth.txt *.bam. We took the columns of contigName and *.bam in the file of depth.txt to produce our depth file.

**Dataset: 10 Genomes**

A RayMeta assembly: raymeta_10.fasta

A filtered assembly: 10s.fasta (contigs>=1000 bp, Header without space)

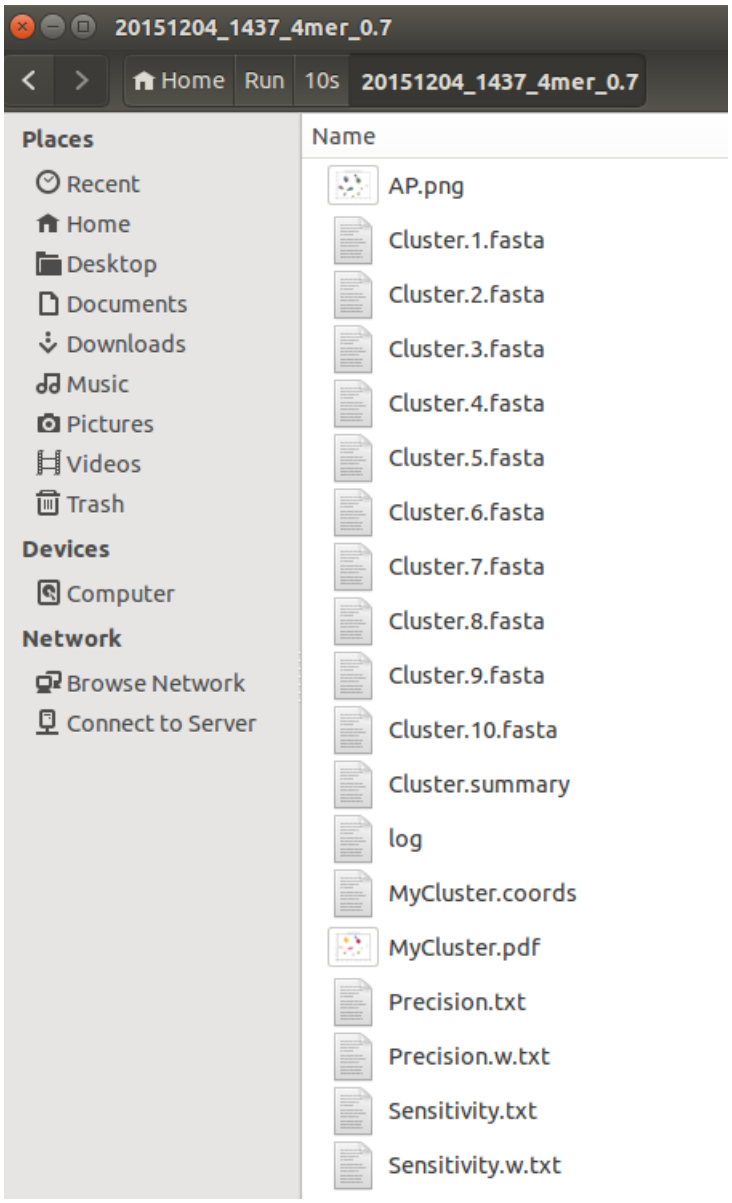
Binning gold standard: 10s.spe.txt
A coverage file: 10s.depth.txt

wget http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/10s.zip
unzip 10s.zip
cd 10s

MyCC.py 10s.fasta

```
manager@sb:~/Run/10s$ MyCC.py 10s.fasta
20151204_1437
4mer
1_rename.py /home/manager/Run/10s/10s.fasta 1000
Seqs >= 1000 : 2185
Minimum contig lengh for first stage clustering: 3786
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potentia
l_genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_4mer.py for fisrt stage clustering
2_GetFeatures_4mer.py for second stage clustering
3_GetMatrix.py 3786 for fisrt stage clustering
 1538 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151204_1443
```

MyCC outputs a folder named yyyymmdd_hhmm_kmer_lt (*e.g.*, 20151204_1437_4mer_0.7). The folder contains binning sequences for each cluster in FASTA format (**Cluster.N.fasta**), a summary file (**Cluster.summary**), a visualization plot (**MyCluster.pdf**) and a coordinate file (**MyCluster.coords**).




Cluster.summary ×

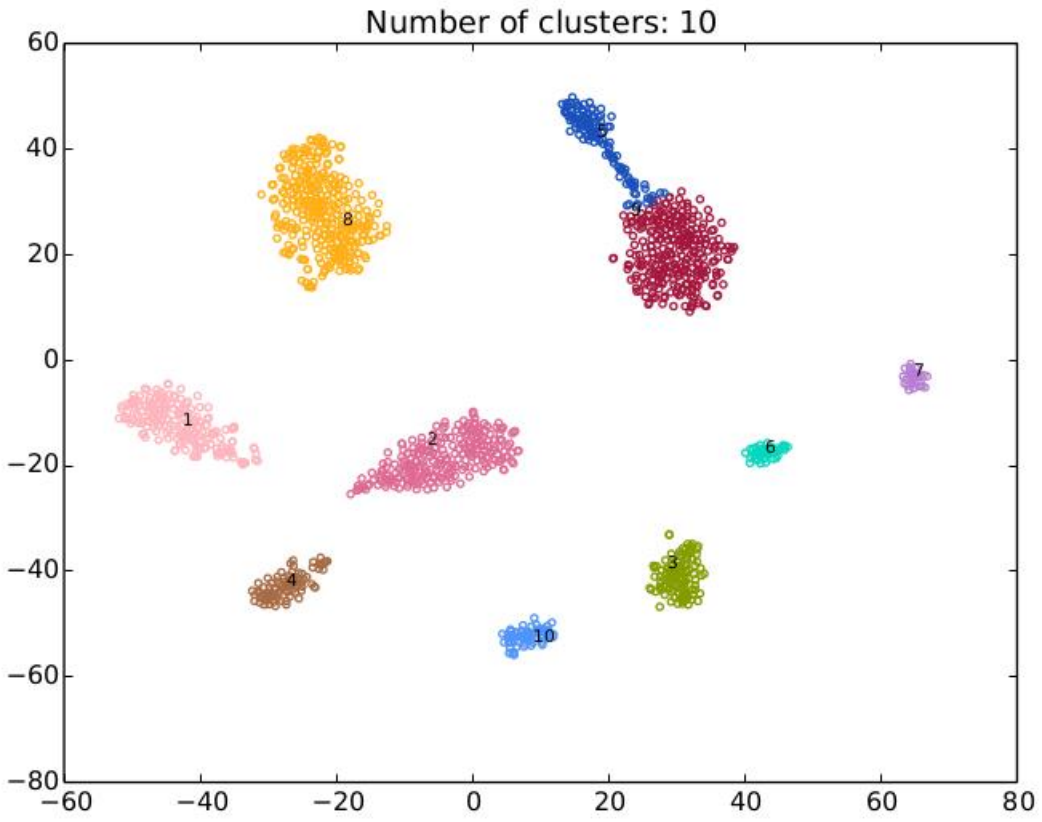
| Cluster | WholeGenome | N50 | NoOfCtg | LongestCtgLen | AvgLenOfCtg | Cogs |
|---|---|---|---|---|---|---|
| Cluster.1.fasta | 1901145 | 7154 | 359 | 22997 | 2657 | 38 |
| Cluster.2.fasta | 4516525 | 24042 | 327 | 103378 | 6907 | 37 |
| Cluster.3.fasta | 2736452 | 34074 | 164 | 91793 | 8424 | 37 |
| Cluster.4.fasta | 4220229 | 83088 | 109 | 294361 | 19374 | 41 |
| Cluster.5.fasta | 3540046 | 43992 | 155 | 145092 | 11579 | 12 |
| Cluster.6.fasta | 1696113 | 56131 | 70 | 203033 | 12900 | 37 |
| Cluster.7.fasta | 1707337 | 74948 | 52 | 202223 | 17699 | 38 |
| Cluster.8.fasta | 5316241 | 19489 | 465 | 114410 | 5716 | 39 |
| Cluster.9.fasta | 4753388 | 20166 | 405 | 120224 | 5885 | 11 |
| Cluster.10.fasta | 2760436 | 61646 | 79 | 206967 | 18251 | 37 |

Description of Cluster.summary

Cluster: cluster name

WholeGenome: the total length of contigs in a cluster
N50
NoOfCtg: the number of contigs in a cluster
LongestCtgLen: the length of the longest contig
AvgLenOfCtg: the average length of contigs in a cluster
Cogs: the number of marker genes in a cluster

MyCluster.pdf:




Evaluate:

**Evaluate.py** -h

Please input two files containing reference assignment and assembly, followed by target name (e.g., fasta).

Usage:

Evaluate.py [reference assignment] [assembly] [target name] [options]

option:

-split To split header by space.

-plot To plot references based on the MyCluster.coords.
-h Help.

cd 20151204_1437_4mer_0.7/
Evaluate.py ../10s.spe.txt ../10s.fasta fasta
(Please note that "-split" is required if the sequence headers contain space. For example, Evaluate.py ../10s.spe.txt ../raymeta_10.fasta fasta -split)

```
manager@sb:~/Run/10s$ cd 20151204_1437_4mer_0.7/
manager@sb:~/Run/10s/20151204_1437_4mer_0.7$ Evaluate.py ../10s.spe.txt ../10s.fas
ta fasta
No. of reference genomes: 10
No. of bins in evaluation: 10
No. of sequences assigned reference: 2172
No. of binned sequences: 2185
Precision: 0.944291, 0.974709
Sensitivity: 0.944291, 0.974709
```

Please note that the first value for precision/sensitivity only takes the number of contigs into account. However, we emphasize on the precision and sensitivity based on the total length of contigs in a cluster (the second value), as described in the manuscript.
Assume there are N genomes in the dataset, which were binned into M clusters. The overall precision and recall (sensitivity) are calculated as

$$\text{Precision (\%)} = \frac{\sum_{i=1}^{M} \max_{j} S_{ij}}{\sum_{i=1}^{M} \sum_{j=1}^{N} S_{ij}} \times 100$$

$$\text{Recall (\%)} = \frac{\sum_{j=1}^{N} \max_{i} S_{ij}}{\sum_{i=1}^{M} \sum_{j=1}^{N} S_{ij} + \sum length\ of\ unbinned\ sequences} \times 100$$

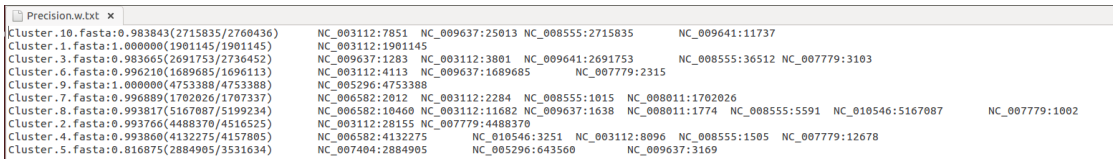
in which $S_{ij}$ indicates the total length of contigs in a cluster *i* corresponding to a reference genome *j*.
Precision based on the number of contigs: (listed in **Precision.txt**)

Precision.txt ×

```
Cluster.10.fasta:0.860759(68/79)        NC_003112:4     NC_009637:3     NC_008555:68    NC_009641:4
Cluster.1.fasta:1.000000(359/359)       NC_003112:359
Cluster.3.fasta:0.902439(148/164)       NC_009637:1     NC_003112:2     NC_009641:148   NC_008555:12    NC_007779:1
Cluster.6.fasta:0.928571(65/70) NC_003112:3     NC_009637:65    NC_007779:2
Cluster.9.fasta:1.000000(405/405)       NC_005296:405
Cluster.7.fasta:0.923077(48/52) NC_006582:2     NC_003112:1     NC_008555:1     NC_008011:48
Cluster.8.fasta:0.955947(434/454)       NC_006582:7     NC_003112:7     NC_009637:1     NC_008011:1     NC_008555:3     NC_010546:434   NC_007779:1
Cluster.2.fasta:0.969419(317/327)       NC_003112:10    NC_007779:317
Cluster.4.fasta:0.898148(97/108)        NC_006582:97    NC_010546:1     NC_003112:6     NC_008555:1     NC_007779:3
Cluster.5.fasta:0.714286(110/154)       NC_007404:110   NC_005296:42    NC_009637:2
```

Overall precision:
(68+359+148+65+405+48+434+317+97+110)/(79+359+164+70+405+52+454+327+108+154)=2051/2172=0.944291

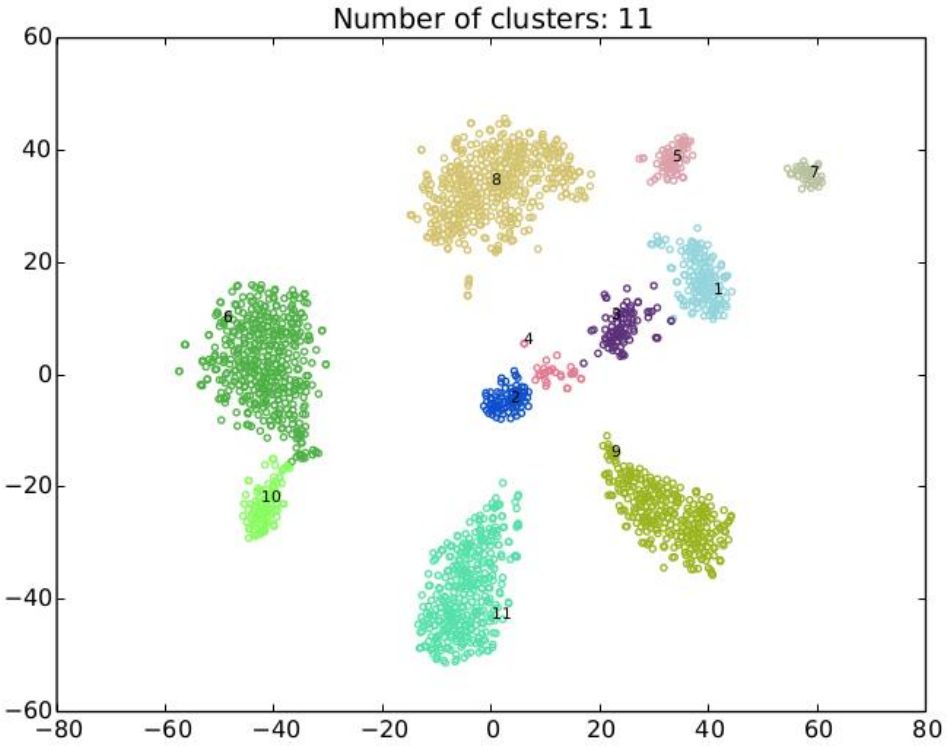
Precision based on the total length of contigs: (listed in **Precision.w.txt)**




Overall precision:
(2715835+1901145+2691753+1689685+4753388+1702026+5167087+4488370+4132275+2884905)/(2760436+1901145+2736452+1696113+4753388+1707337+5199234+4516525+4157805+3531634)=32126469/32960069=0.974709

Similarly, we set one stage by specifying "-lt 1"
MyCC.py 10s.fasta -lt 1

```
manager@sb:~/Run/10s$ MyCC.py 10s.fasta -lt 1
20151204_1500
4mer
1_rename.py /home/manager/Run/10s/10s.fasta 1000
Seqs >= 1000 : 2185
Minimum contig lengh for first stage clustering: 1001
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potential_
genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_4mer.py for fisrt stage clustering
2_GetFeatures_4mer.py for second stage clustering
3_GetMatrix.py 1001 for fisrt stage clustering
 2185 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151204_1506
```



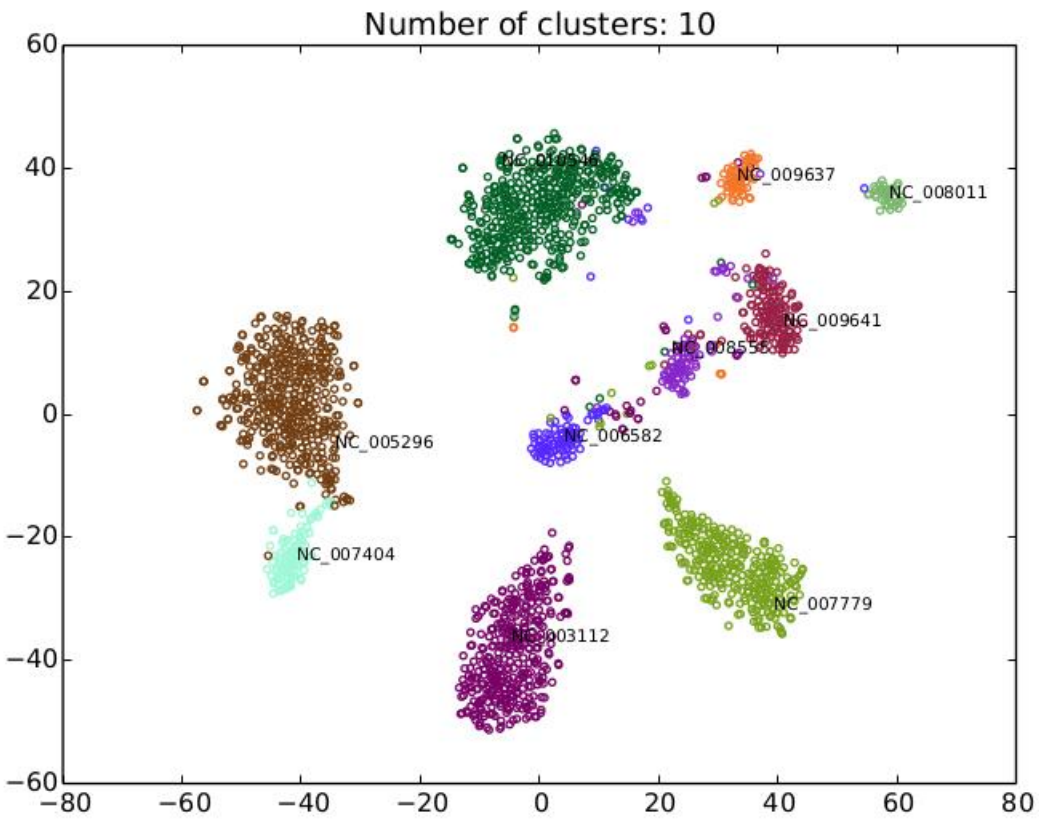

cd 20151204_1500_4mer_1.0/

Evaluate.py ../10s.spe.txt ../10s.fasta fasta -plot

```
manager@sb:~/Run/10s/20151204_1500_4mer_1.0$ Evaluate.py ../10s.spe.txt ../10s.fas
ta fasta -plot
No. of reference genomes: 10
No. of bins in evaluation: 11
No. of sequences assigned reference: 2172
No. of binned sequences: 2185
Precision: 0.954880, 0.987968
Sensitivity: 0.948435, 0.980754
```

By adding the option of "-plot", you can obtain a visualization plot (**MyReference.pdf**) labeled with the names of reference genomes.

MyReference.pdf:




**Dataset: 100 Genomes**

A RayMeta assembly: Contigs.fasta

A filtered assembly: 100s.fasta (contigs>=1000 bp, Header without space)

A coverage file: 100s.depth.txt

Binning gold standard: 100s.spe.txt

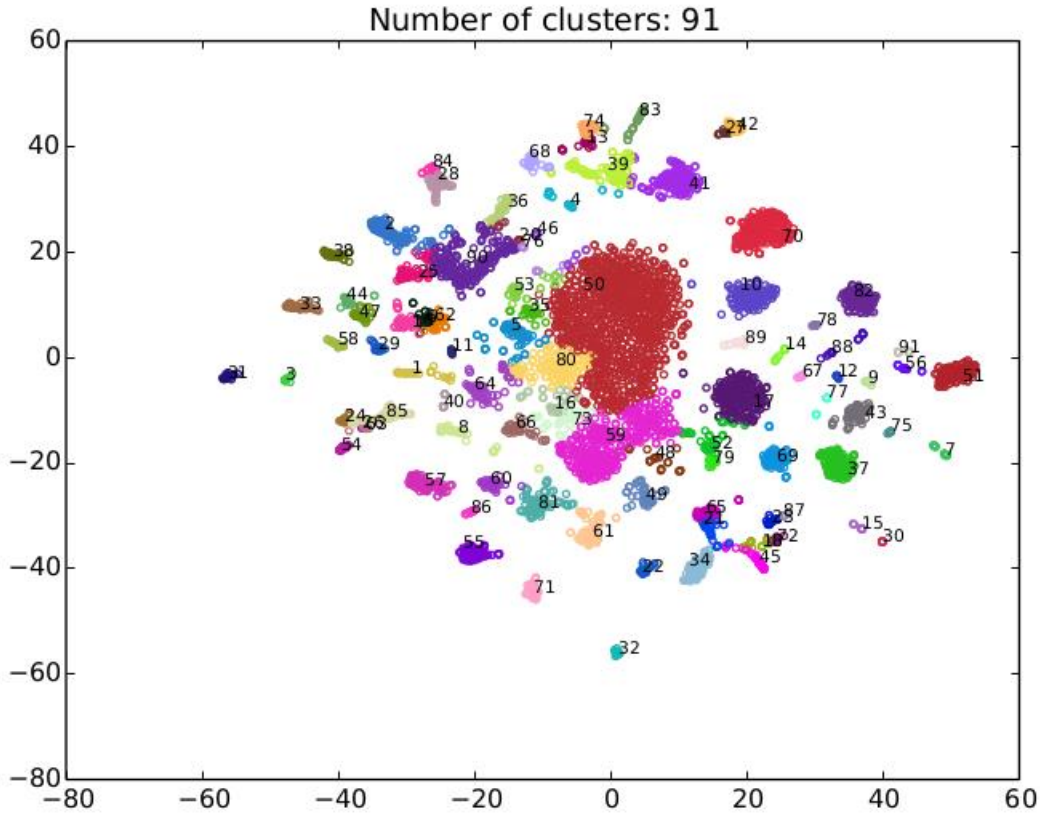
wget http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/100s.zip

unzip 100s.zip

cd 100s

MyCC.py 100s.fasta 56mer -a 100s.depth.txt

```
manager@sb:~/Run/100s$ MyCC.py 100s.fasta 56mer -a 100s.depth.txt
20151207_0925
56mer
1_rename.py /home/manager/Run/100s/100s.fasta 1000
Seqs >= 1000 : 8978
Minimum contig lengh for first stage clustering: 2475
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potentia
l_genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_5mer_p6mer.py for fisrt stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/100s/100s.depth.txt
2_GetFeatures_4mer.py for second stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/100s/100s.depth.txt
3_GetMatrix.py 2475 for fisrt stage clustering
 6297 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151207_1017
```




cd 20151207_0925_56mer_0.7_cov/

Evaluate.py ../100s.spe.txt ../100s.fasta fasta -plot

```
manager@sb:~/Run/100s/20151207_0925_56mer_0.7_cov$ Evaluate.py ../100s.spe.txt .
./100s.fasta fasta -plot
No. of reference genomes: 100
No. of bins in evaluation: 91
No. of sequences assigned reference: 8942
No. of binned sequences: 8978
Precision: 0.611832, 0.895118
Sensitivity: 0.782487, 0.940128
```

**Dataset: 25 Genomes**

Original download site:

http://portal.nersc.gov/dna/RD/Metagenome_RD/MetaBAT/Software/Mockup/

An assembly: assembly.fasta

Binning gold standard: membership.txt

Bam files: library1.bam* and library2.bam*

To generate a depth file from BAM files:

jgi_summarize_bam_contig_depths --outputDepth depth.txt *.bam

We modified the files of depth.txt and membership.txt to My.depth.txt and member.txt, respectively, for MyCC:

A coverage file: My.depth.txt

Binning gold standard: member.txt

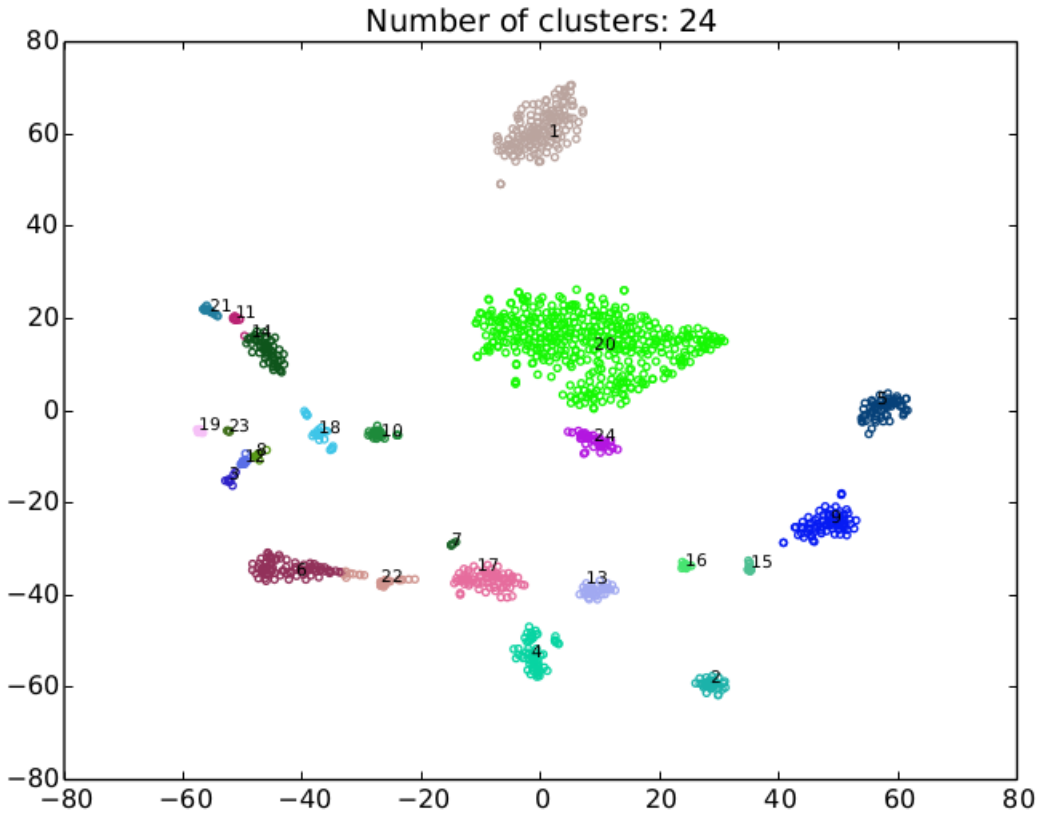
wget http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/25s.zip

unzip 25s.zip

cd 25s

MyCC.py assembly.fa -a My.depth.txt

```
manager@sb:~/Run/25s$ MyCC.py assembly.fa -a My.depth.txt
20151207_1023
4mer
1_rename.py /home/manager/Run/25s/assembly.fa 1000
Seqs >= 1000 : 1893
Minimum contig lengh for first stage clustering: 4645
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potentia
l_genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_4mer.py for fisrt stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/25s/My.depth.txt
2_GetFeatures_4mer.py for second stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/25s/My.depth.txt
3_GetMatrix.py 4645 for fisrt stage clustering
 1328 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151207_1034
```

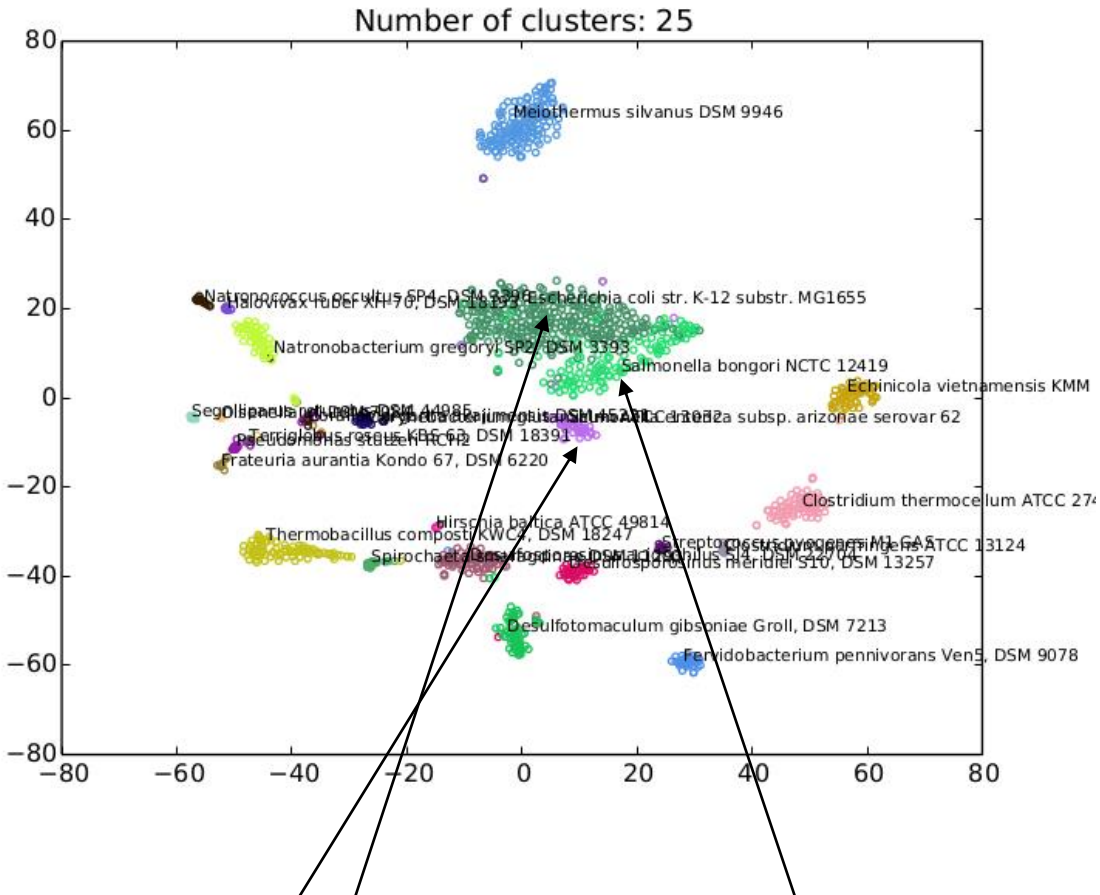



cd 20151207_1023_4mer_0.7_cov

Evaluate.py ../member.txt ../assembly.fa fasta -plot

```
manager@sb:~/Run/25s/20151207_1023_4mer_0.7_cov$ Evaluate.py ../member.txt ../as
sembly.fa fasta -plot
No. of reference genomes: 25
No. of bins in evaluation: 24
No. of sequences assigned reference: 1893
No. of binned sequences: 1893
Precision: 0.797147, 0.958671
Sensitivity: 0.853143, 0.972830
```

MyReference.pdf:




In comparing MyCluster.pdf with MyReference.pdf, we found MyCC binned three species of *Escherichia coli* str. K-12 substr. MG1655, *Salmonella bongori* NCTC 12419 and *Salmonella enterica* subsp. arizonae serovar 62 into two clusters. It binned the contigs of *E. coli* MG1655 (99.33% recall) and *S. bongori* NCTC 12419 (66.73% recall) into the Cluster.20 (65.26% precision), while the contigs of *S. enterica* subsp. serovar 62 (85.51% recall) and the rest of *S. bongori* NCTC 12419 into the Cluster.24 (86.06% precision). It is worth noting that based on the low numbers of marker genes recorded in Cluster.summay for these two clusters (7 and 12 for Cluster.20 and Cluster.24,

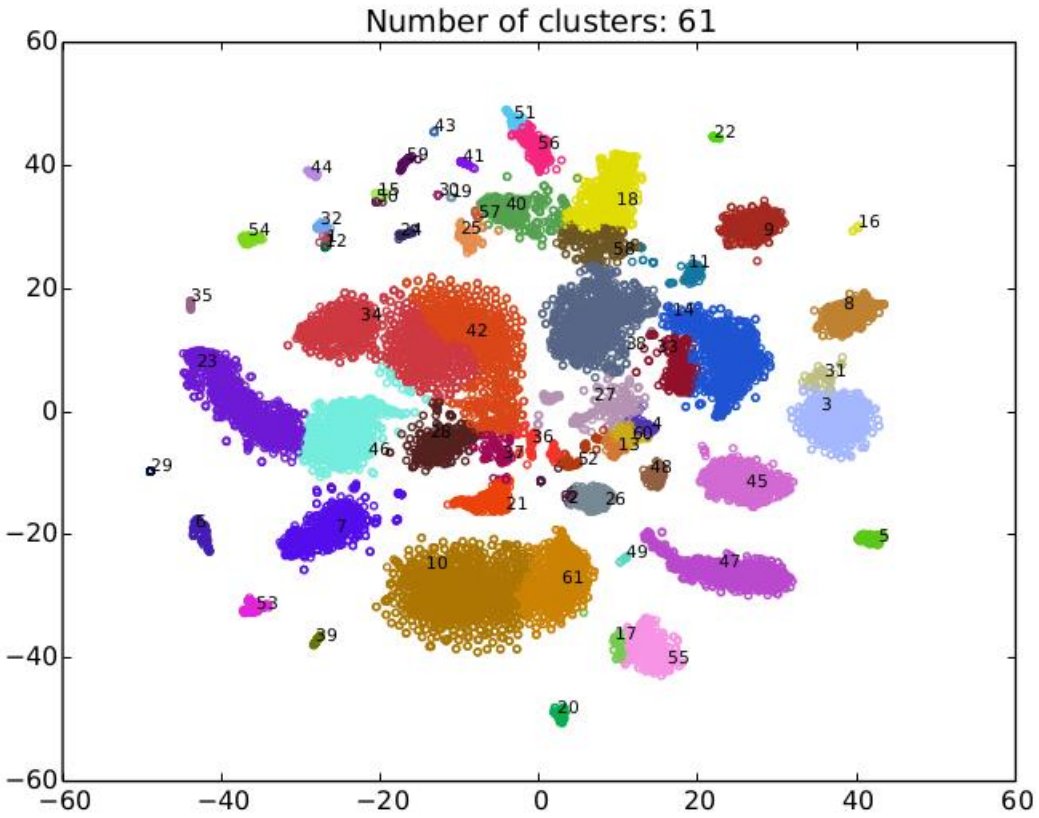
respectively) and their positions located in the center of the plot, MyCC indeed provided the evidence of confidence in binning.

**Dataset: 64 Genomes**

A RayMeta assembly: Ray.contigs.fasta
A filtered assembly: 64s.fasta (contigs>=1000 bp, Header without space)
Binning gold standard: 64s.spe.txt
A coverage file: 64s.depth.txt

wget http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/64s.zip
unzip 64s.zip
cd 64s
MyCC.py 64s.fasta 56mer -a 64s.depth.txt

```
manager@sb:~/Run/64s$ MyCC.py 64s.fasta 56mer -a 64s.depth.txt
20151207_1044
56mer
1_rename.py /home/manager/Run/64s/64s.fasta 1000
Seqs >= 1000 : 23602
Minimum contig lengh for first stage clustering: 1493
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potentia
l_genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_5mer_p6mer.py for fisrt stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/64s/64s.depth.txt
2_GetFeatures_4mer.py for second stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/64s/64s.depth.txt
3_GetMatrix.py 1493 for fisrt stage clustering
 16574 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151207_1136
```

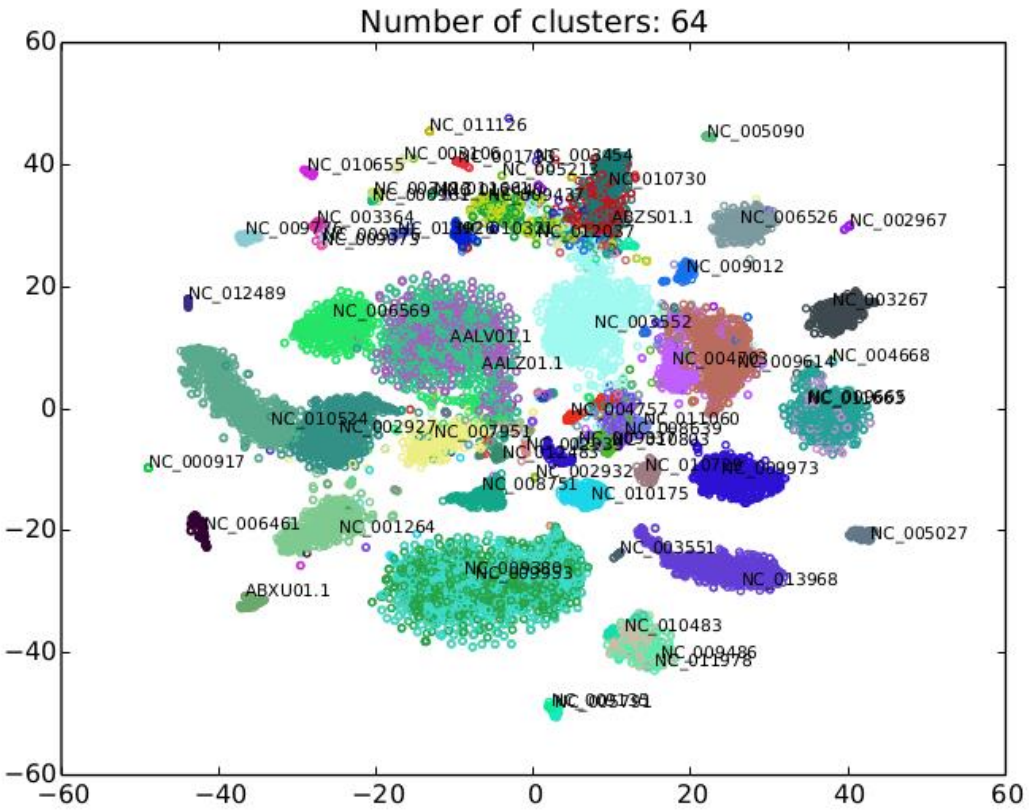



cd 20151207_1044_56mer_0.7_cov

Evaluate.py ../64s.spe.txt ../64s.fasta fasta -plot

```
manager@sb:~/Run/64s/20151207_1044_56mer_0.7_cov$ Evaluate.py ../64s.spe.txt ../
64s.fasta fasta -plot
No. of reference genomes: 64
No. of bins in evaluation: 61
No. of sequences assigned reference: 22606
No. of binned sequences: 23602
Precision: 0.731620, 0.863816
Sensitivity: 0.786207, 0.916936
```

MyReference.pdf:




**Dataset: Sharon's Dataset**

Original download site: http://ggkbase.berkeley.edu/carrol/

An assembly: carrol.contigs.fa

Binning gold standard: carrol.scaffolds_to_bin.tsv

We modify the files of carrol.contigs.fa and carrol.scaffolds_to_bin.tsv to carrol.fasta (contigs>=1000 bp, Header without space) and carrol.scaffolds_to_bin.txt, respectively, for MyCC.

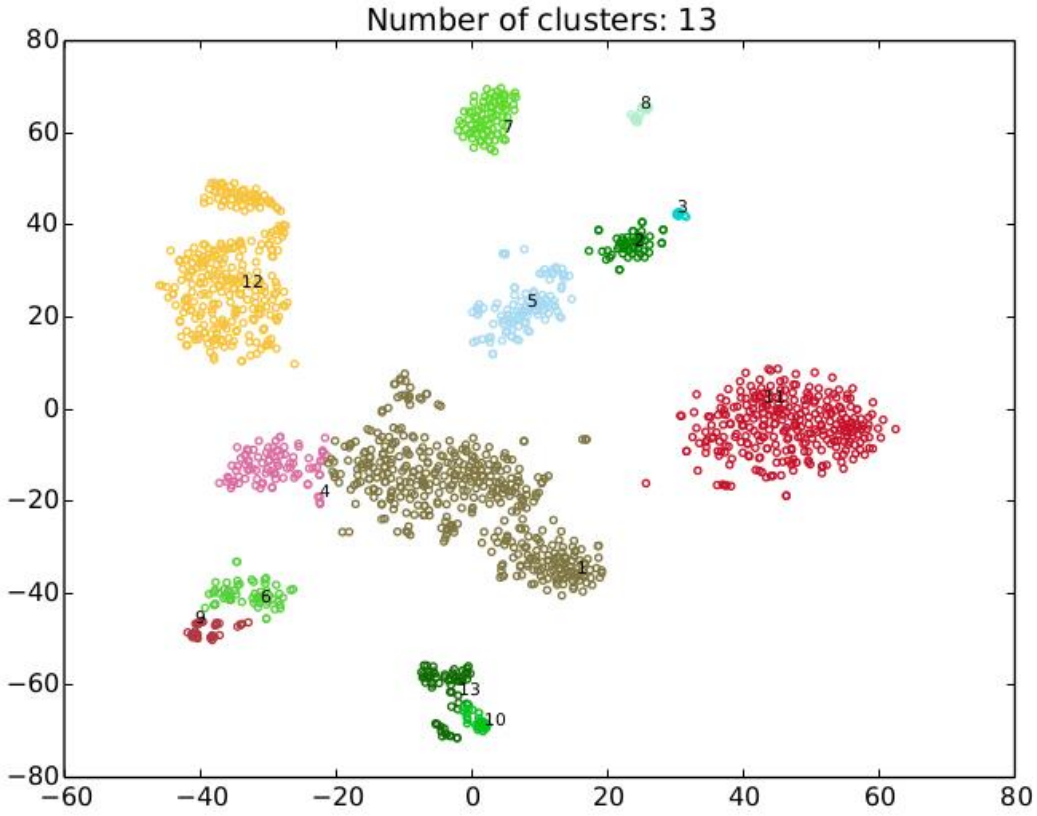
wget http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/Sharon.zip

unzip Sharon.zip

cd Sharon

MyCC.py carrol.fasta -a My.depth.txt

```
manager@sb:~/Run/Sharon$ MyCC.py carrol.fasta -a My.depth.txt
20151207_1149
4mer
1_rename.py /home/manager/Run/Sharon/carrol.fasta 1000
Seqs >= 1000 : 2294
Minimum contig lengh for first stage clustering: 1250
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potentia
l_genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_4mer.py for fisrt stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/Sharon/My.depth.txt
2_GetFeatures_4mer.py for second stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/Sharon/My.depth.txt
3_GetMatrix.py 1250 for fisrt stage clustering
 1607 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151207_1154
```

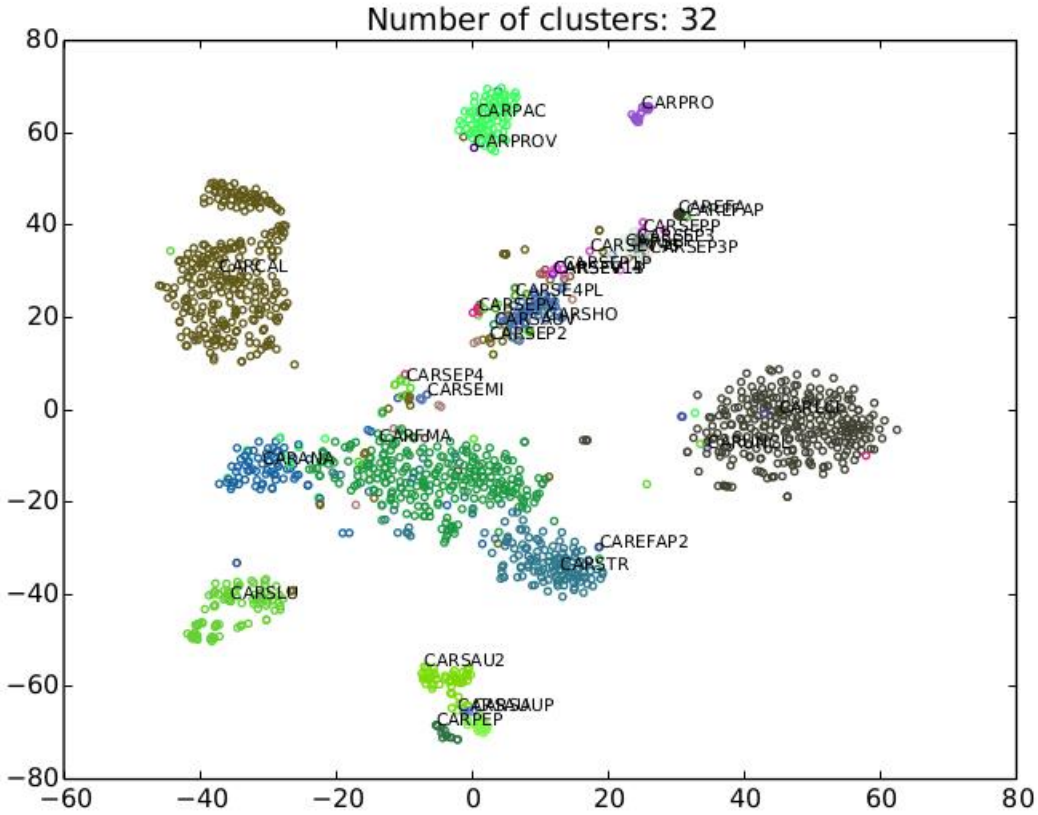



cd 20151207_1149_4mer_0.7_cov

Evaluate.py ../carrol.scaffolds_to_bin.txt ../carrol.fasta fasta -plot

```
manager@sb:~/Run/Sharon/20151207_1149_4mer_0.7_cov$ Evaluate.py ../carrol.scaffo
lds_to_bin.txt ../carrol.fasta fasta -plot
No. of reference genomes: 32
No. of bins in evaluation: 13
No. of sequences assigned reference: 2294
No. of binned sequences: 2294
Precision: 0.767219, 0.865632
Sensitivity: 0.908021, 0.965512
```

MyReference.pdf:



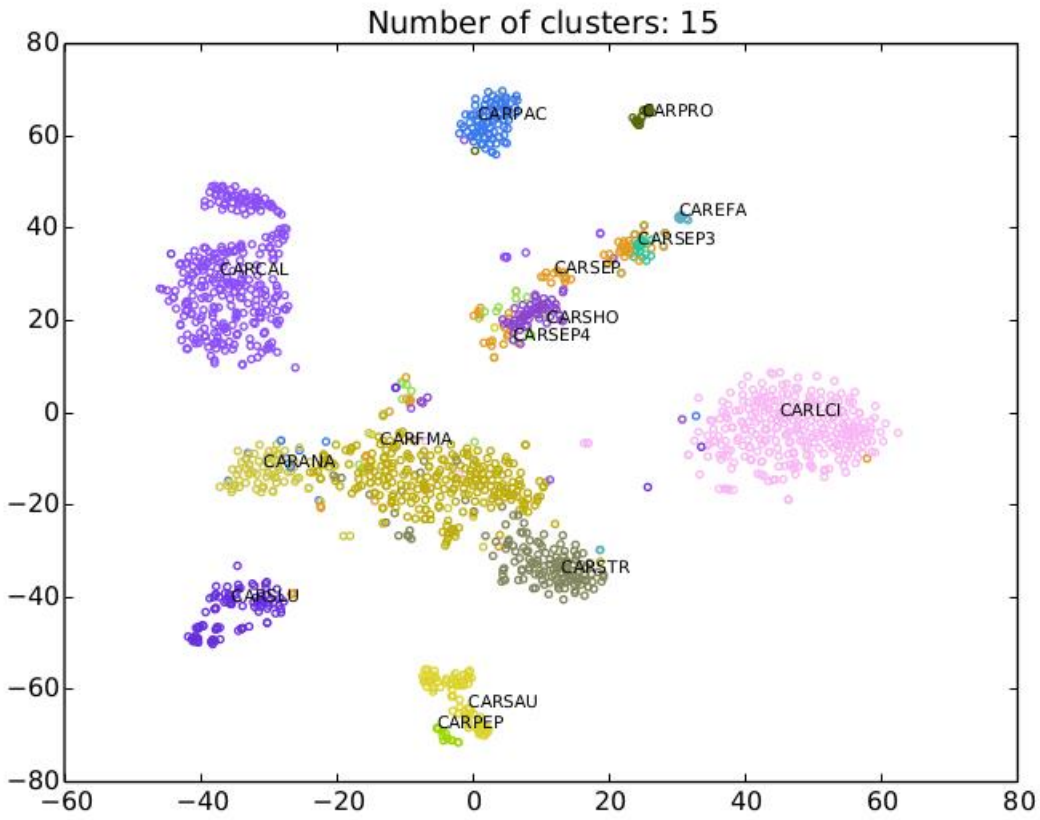

According to the description in carrol.organism_info.tsv, we found that the authors divided plasmids and viruses from a strain. Here, we group plasmids and virus into the strain and ignore some viruses.

As shown in GroupName.xls:

| Name | GroupName | name | co |
|---|---|---|---|
| CARSLU | CARSLU | Staphylococcus lugdunensis | ca |
| CARPEP | CARPEP | Peptoniphilus sp. Carrol | ca |
| CARSHO | CARSHO | Staphylococcus hominis | ca |
| CARSAUV | CARSAU | Staphylococcus aureus virus | ca |
| CARSAU2 | CARSAU | Staphylococcus aureus strain 2 | ca |
| CARSAUP | CARSAU | Staphylococcus aureus plasmid | ca |
| CARSAU | CARSAU | Staphylococcus aureus | ca |
| CARPRO | CARPRO | Propionibacterium sp. | ca |
| CARPROV | CARPRO | Propionibacterium sp. virus | ca |
| CARSEP | CARSEP | Staphylococcus epidermidis strain 1 | ca |
| CARSEPP | CARSEP | Staphylococcus epidermidis plasmid | ca |
| CARSEPV | CARSEP | Staphylococcus epidermidis viruses | ca |
| CARSEP2 | CARSEP | Staphylococcus epidermidis misc. | ca |
| CARSEP3 | CARSEP3 | Staphylococcus epidermidis strain 3 | ca |
| CARSEP4 | CARSEP4 | Staphylococcus epidermidis strain 4 | ca |
| CAREFA | CAREFA | Enterococcus faecalis | ca |
| CAREFAP | CAREFA | Enterococcus faecalis plasmid | ca |
| CAREFAP2 | CAREFA | Enterococcus faecalis plasmid 2 | ca |
| CARUNCL | | Carrol unclustered | ca |
| CARSEV46 | | Staphylococcus epidermidis virus 4-6 | ca |
| CARSEV13 | | Staphylococcus epidermidis virus 013 | ca |
| CARSEV14 | | Staphylococcus epidermidis virus 014 | ca |
| CARSEMI | | Staphylococcus epidermidis misc | ca |
| CARSEP1P | CARSEP | Staphylococcus epidermidis strain 1 plasmids | ca |
| CARSEP3P | CARSEP3 | Staphylococcus epidermidis strain 3 plasmids | ca |
| CARSE4PL | CARSEP4 | Staphylococcus epidermidis strain 4 plasmids | ca |
| ACDRDN | | Redundant | ca |
| CARCAL | CARCAL | Candida albicans | ca |
| CARFMA | CARFMA | Finegoldia magna | ca |
| CARLCI | CARLCI | Leuconostoc citreum | ca |
| CARANA | CARANA | Anaerococcus sp. | ca |
| CARPAC | CARPAC | Propionibacterium acnes | ca |
| CARSTR | CARSTR | Streptococcus sp. | ca |

Evaluate.py ../carrol.scaffolds_to_bin.groupname.txt ../carrol.fasta fasta -plot

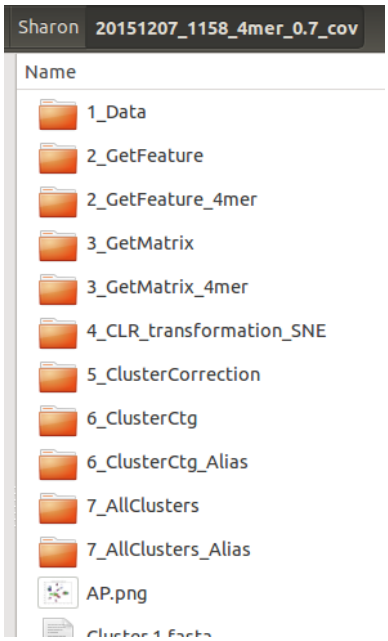
```
manager@sb:~/Run/Sharon/20151207_1149_4mer_0.7_cov$ Evaluate.py ../carrol.scaffo
lds_to_bin.groupname.txt ../carrol.fasta fasta -plot
No. of reference genomes: 15
No. of bins in evaluation: 13
No. of sequences assigned reference: 2270
No. of binned sequences: 2294
Precision: 0.782819, 0.877007
Sensitivity: 0.889427, 0.955113
```



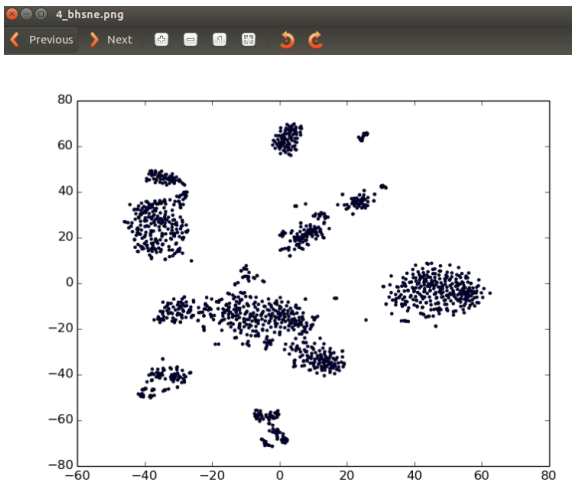

MyCC.py carrol.fasta -a My.depth.txt -keep

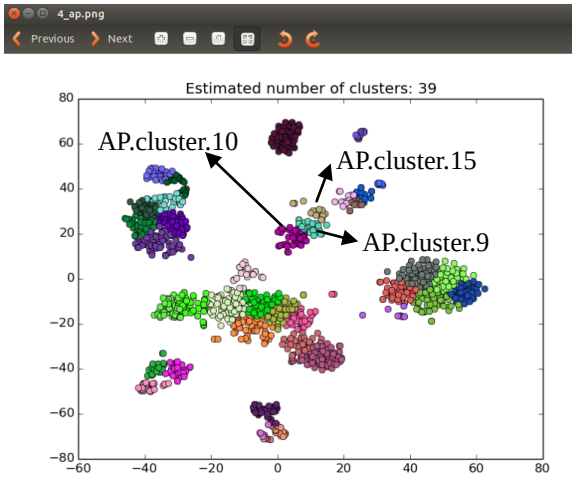
```
manager@sb:~/Run/Sharon$ MyCC.py carrol.fasta -a My.depth.txt -keep
20151207_1158
4mer
1_rename.py /home/manager/Run/Sharon/carrol.fasta 1000
Seqs >= 1000 : 2294
Minimum contig lengh for first stage clustering: 1250
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potentia
l_genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_4mer.py for fisrt stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/Sharon/My.depth.txt
2_GetFeatures_4mer.py for second stage clustering
2_GetFeatures_TimeSeries.py /home/manager/Run/Sharon/My.depth.txt
3_GetMatrix.py 1250 for fisrt stage clustering
 1607 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151207_1203
```

By adding the option of "-keep", MyCC keeps temporary files in the output folder:




So, you can find the visualization plots including Barnes-Hut-SNT and Affinity Propagation clustering (as shown below) in the fold of 4_CLR_transformation_SNE.




According to 20151207_1158_4mer_0.7_cov/5_ClusterCorrection/0_Cog_AP.txt,

9 COGs: COG0012, COG0016, COG18, COG0081, COG0087, COG0172, COG0185, COG0201 and COG0215 in AP.cluster.9

8 COGs: COG0052, COG0102, COG0124, COG0495, COG0525, COG0533, COG0541 and COG0552 in AP.cluster.10

0 COG in AP.cluster.15

After iterative splitting and merging processes, these three clusters were finally merged to form a corrected cluster (*e.g.*, Cluster.5 in page 31).

cd 20151207_1158_4mer_0.7_cov/

Evaluate.py ../carrol.scaffolds_to_bin.txt ../carrol.fasta fasta -plot

```
manager@sb:~/Run/Sharon/20151207_1158_4mer_0.7_cov$ Evaluate.py ../carrol.scaffo
lds_to_bin.txt ../carrol.fasta fasta -plot
No. of reference genomes: 32
No. of bins in evaluation: 13
No. of sequences assigned reference: 2294
No. of binned sequences: 2294
Precision: 0.767219, 0.865632
Sensitivity: 0.908021, 0.965512
```

You are able to evaluate the binning results in the first stage: (contig length >= the length of the 1606$^{th}$ contig (2294*0.7) entering the first stage)

cd 6_ClusterCtg_Alias

Evaluate.py ../../carrol.scaffolds_to_bin.txt ../../carrol.fasta fasta

```
manager@sb:~/Run/Sharon/20151207_1158_4mer_0.7_cov/6_ClusterCtg_Alias$ Evaluate.
py ../../carrol.scaffolds_to_bin.txt ../../carrol.fasta fasta
No. of reference genomes: 32
No. of bins in evaluation: 13
No. of sequences assigned reference: 2294
No. of binned sequences: 1607
Precision: 0.792782, 0.870529
Sensitivity: 0.644289, 0.941130
```

By comparing the binning result with the binning gold standard, we found that the sequences in Cluster.3 represent the genome of *Enterococcus faecalis*, which was reconstructed from infant gut microbiome by Sharon *et al*., not from the so-called "reference genomes".

The precision of Cluster.3: 96.98%

The recall of *Enterococcus faecalis*: 100%

```
Cluster.3.fasta:0.969841(2877608/2967093)      CAREFA:2877608  CAREFAP:88476   CARSEP2:1009
CAREFA:1.000000(2877608/2877608)        Cluster.3.fasta:2877608
```

**Dataset: Drosophila dataset**

A RayMeta assembly: my.ctg.fa

A coverage file: My.depth.txt

Cluster.1.COGs.fasta

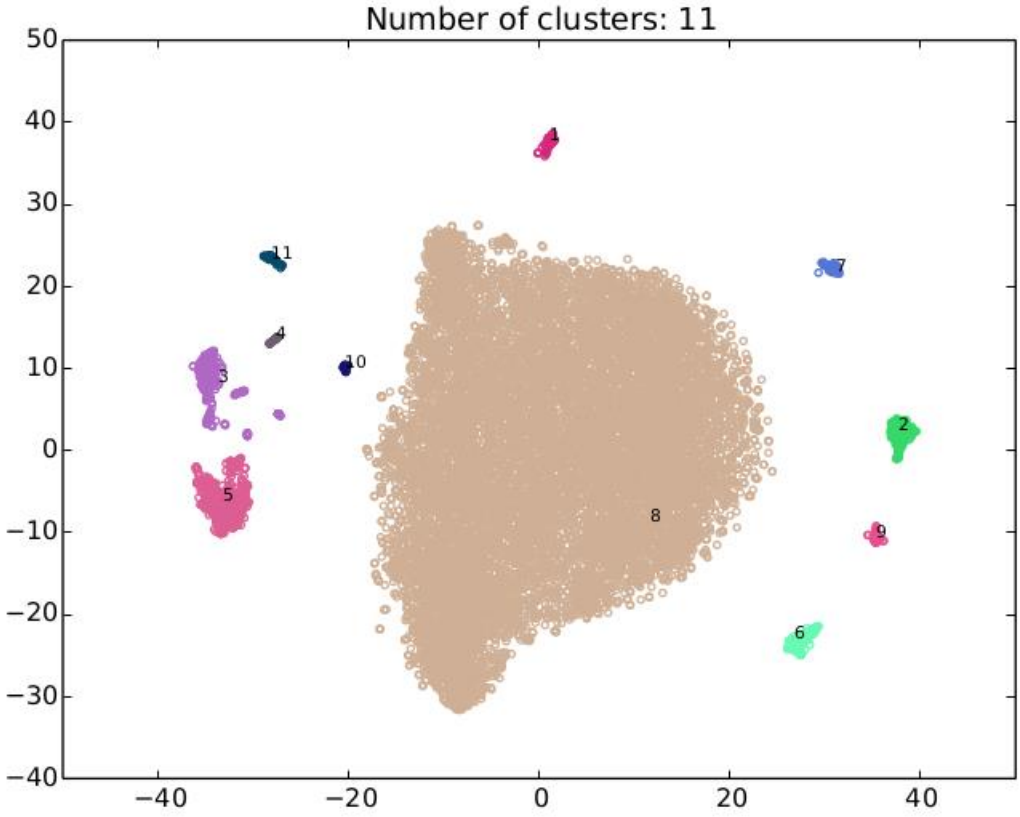
Cluster.5.COGs.fasta

Cluster.6.COGs.fasta

Cluster.5.faa

Cluster.5.tbl
Cluster.6.faa
Cluster.6.tbl

wget http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/fly.zip
unzip fly.zip
cd fly
MyCC.py my.ctg.fa -a My.depth.txt -keep




Cluster.summary

| Cluster | WholeGenome | N50 | NoOfCtg | LongestCtgLen | AvgLenOfCtg | Cogs |
|---|---|---|---|---|---|---|
| Cluster.1.fasta | 1412423 | 22190 | 110 | 54381 | 6542 | 39 |
| Cluster.2.fasta | 1418844 | 6729 | 274 | 24492 | 2595 | 10 |
| Cluster.3.fasta | 3996074 | 15023 | 465 | 55244 | 4297 | 27 |
| Cluster.4.fasta | 224814 | 5214 | 50 | 12319 | 2309 | 4 |
| Cluster.5.fasta | 2554723 | 3661 | 828 | 20009 | 1545 | 31 |
| Cluster.6.fasta | 1203054 | 5915 | 291 | 26593 | 2075 | 33 |
| Cluster.7.fasta | 452006 | 2227 | 220 | 8247 | 1032 | 7 |
| Cluster.8.fasta | 108511839 | | 9586 | 19446 | 100844 | 2790 14 |
| Cluster.9.fasta | 324829 | 3159 | 119 | 7328 | 1366 | 5 |
| Cluster.10.fasta | | 130137 | 3779 | 48 | 15046 | 1381 0 |
| Cluster.11.fasta | | 397064 | 3684 | 134 | 10352 | 1499 9 |

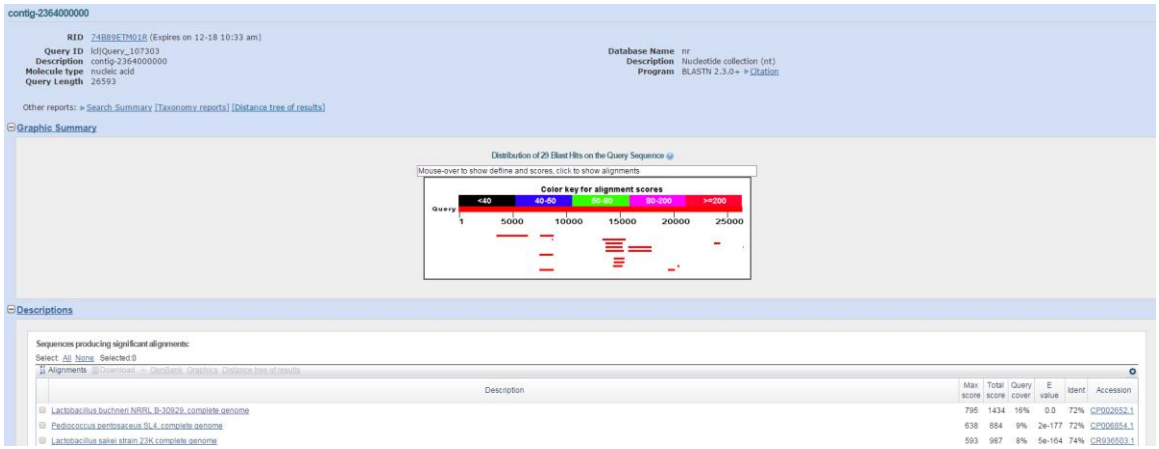
The binning results were assessed by CheckM to produce a summary table:

| Bin ID | Marker lineage | Completeness | Contamination | Strain heterogeneity |
|---|---|---|---|---|
| Cluster.1 | c__Gammaproteobacteria (UID4387) | 93.22 | 0 | 0 |
| Cluster.5 | o__Rhodospirillales (UID3754) | 90.63 | 4.79 | 53.85 |
| Cluster.3 | o__Rhodospirillales (UID3754) | 90.24 | 2.9 | 25 |
| Cluster.6 | o__Lactobacillales (UID463) | 89.56 | 1.47 | 75 |
| Cluster.8 | k__Archaea (UID2) | 75.63 | 44.89 | 2.42 |
| Cluster.2 | o__Lactobacillales (UID463) | 37.57 | 0.16 | 0 |
| Cluster.7 | k__Bacteria (UID203) | 28.92 | 0 | 0 |
| Cluster.9 | k__Bacteria (UID203) | 21.87 | 1.15 | 100 |
| Cluster.11 | o__Lactobacillales (UID463) | 11.08 | 0 | 0 |
| Cluster.4 | o__Rhodospirillales (UID3754) | 7.47 | 0 | 0 |
| Cluster.10 | root (UID1) | 0 | 0 | 0 |

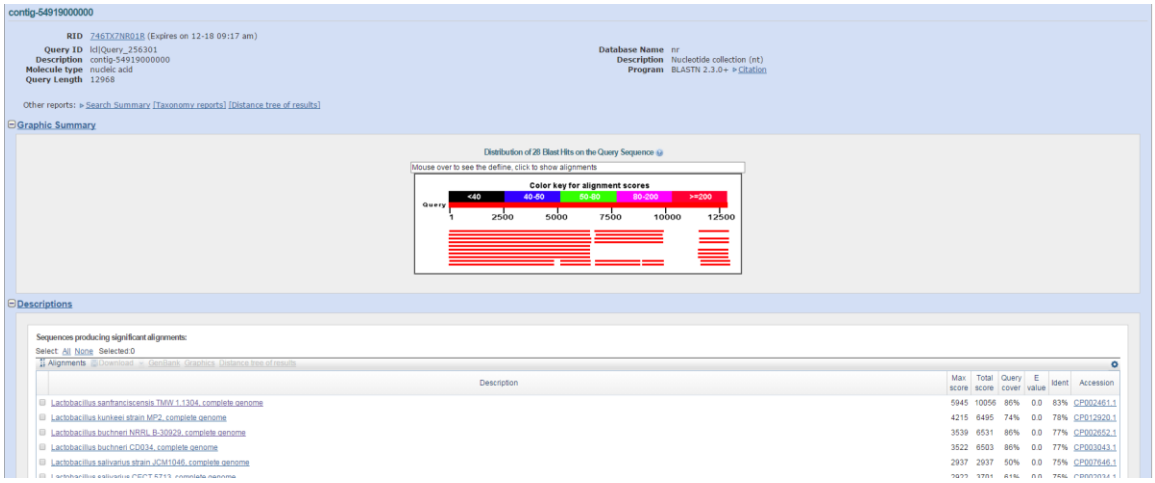
This table shows that the upper four clusters (Cluster.1, Cluster.3, Cluster. 5 and Cluster.6) are of good quality: near complete and with low contamination.

Among the 11 clusters, three clusters (Cluster.1, Cluster. 5 and Cluster.6) have large marker gene counts (39, 31 and 33, respectively) in MyCC's summary. We further examined these three clusters.

For example, looking into Cluster.6.fasta
Blast the longest contig (contig-2364000000) against Nucleotide collection:



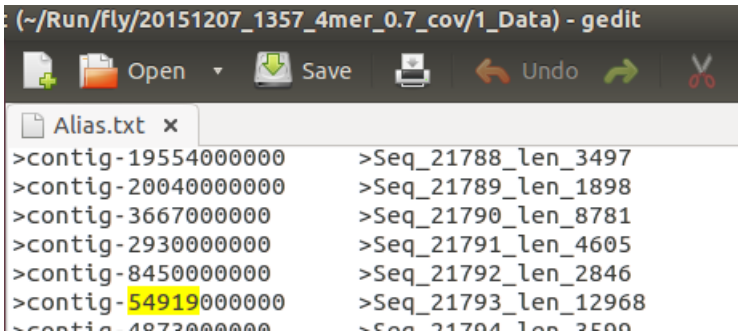

Blast contig-54919000000 against Nucleotide collection:



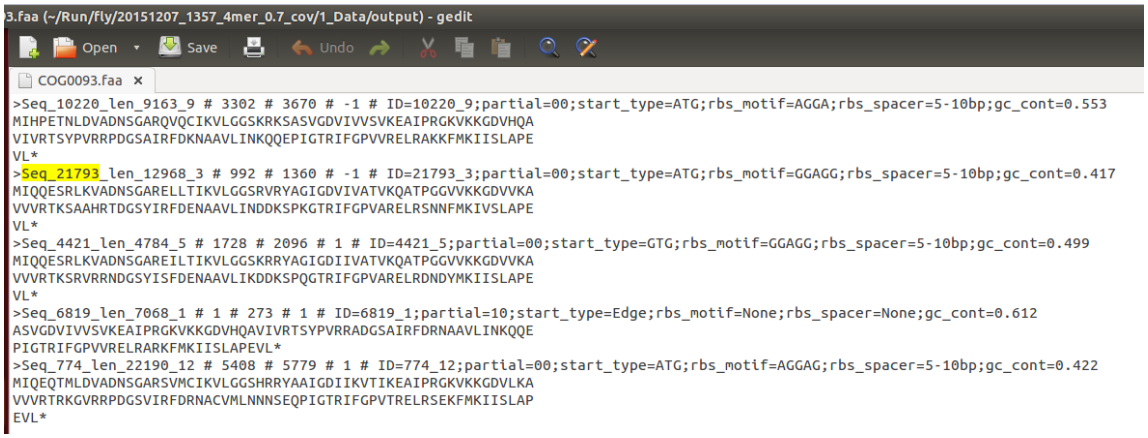

……..

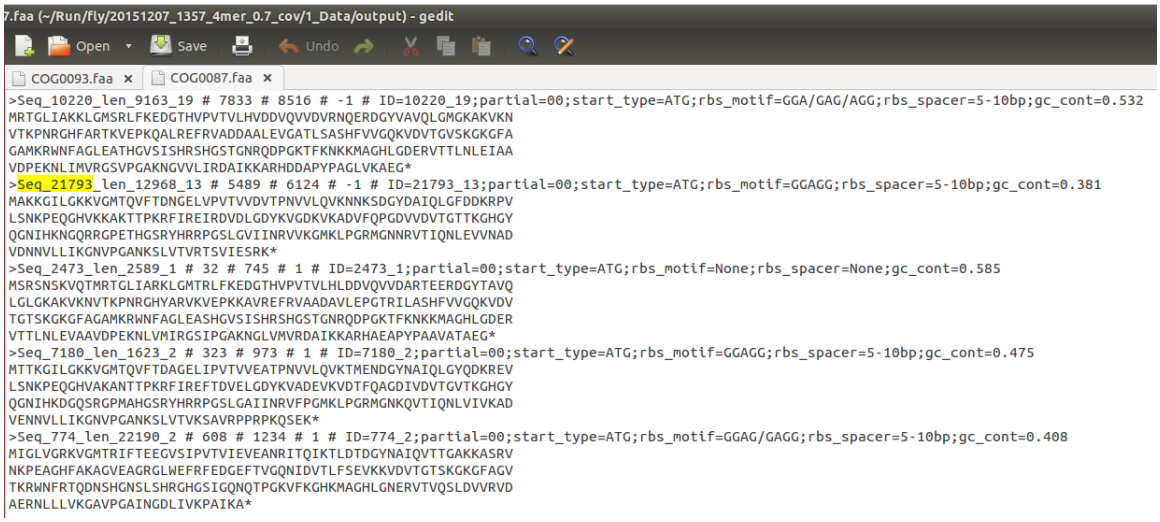
The above results suggst that sequences in this cluster are differenct from the nucleotide collection.

With Alias.txt (in the folder of 1_Data), we know the contig "contig-5491900000" is Seq_21793_len_12968.




Looking into files of marker gene (*e.g.*, COG0093.faa in 1_Data/output), protein sequences of marker genes can be retrieved.

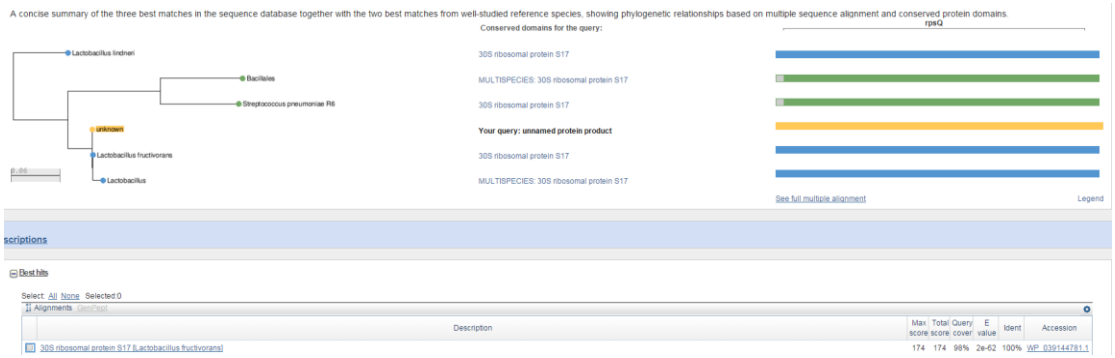




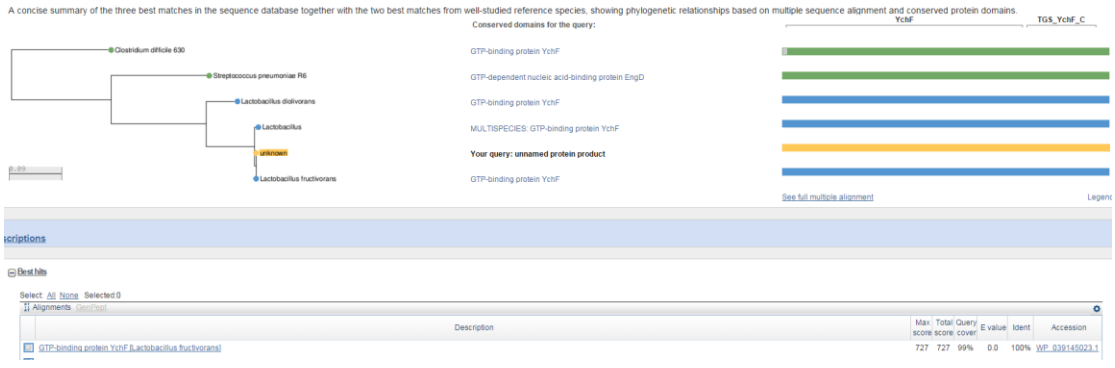

Accordingly, a file containng the protein sequences of marker genes for Cluster.6 was obtained. (**Cluster.6.COGs.fasta**)

Use SmartBLAST (http://blast.ncbi.nlm.nih.gov/smartblast/):

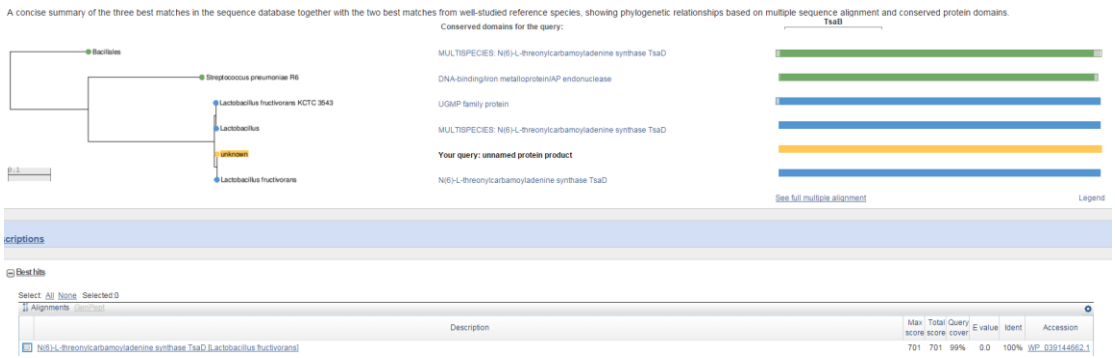
>contig-54919000000_COG0186.faa




>contig-1355000000_COG0012.faa



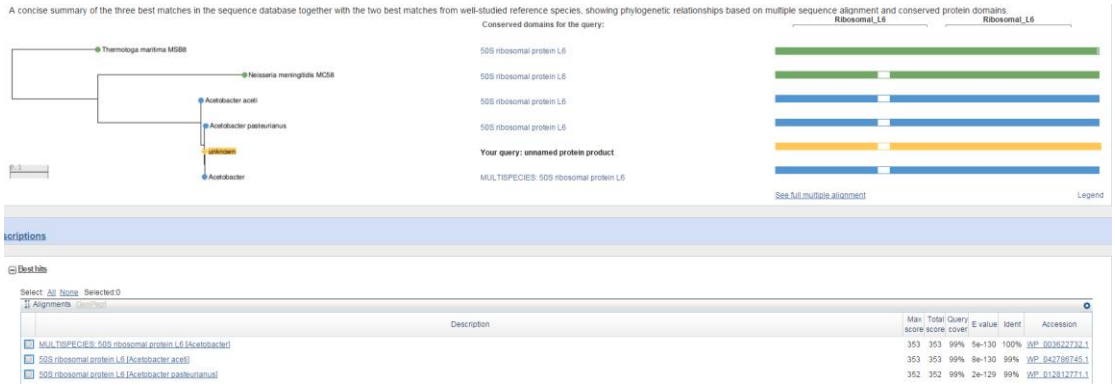

>contig-1324000000_COG0533.faa




…….

The above results (28 out of the 33 COGs) suggst that the closest species to this cluster is *Lactobacillus fructivorans*.

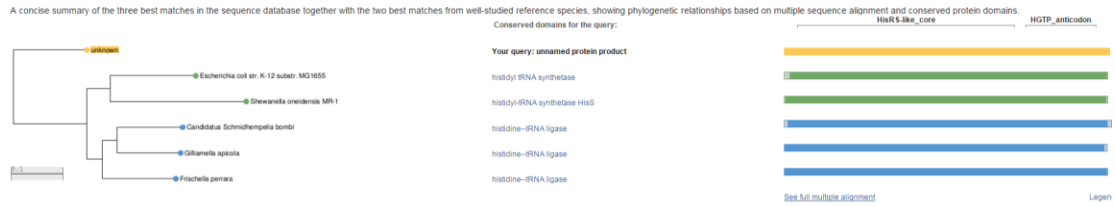
Similarly, according to the protein sequences of marker genes for Cluster.5 (**Cluster.5.COGs.fasta**), we found the closest species to Cluster.5 is *Acetobacter pasteurianus*.

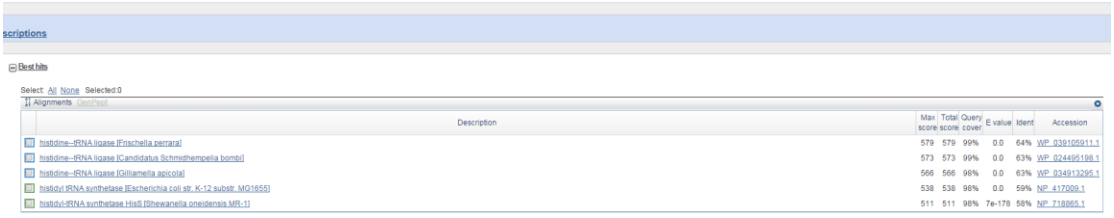



…….

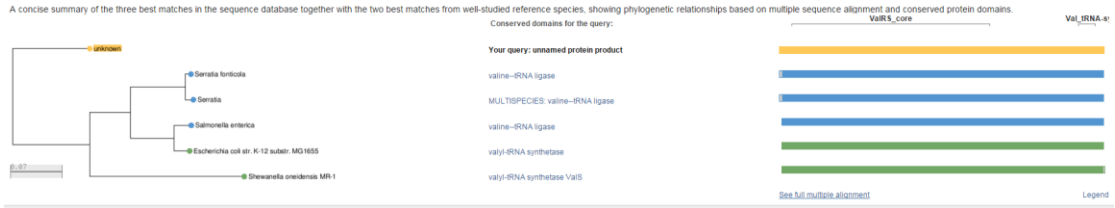
However, the protein sequences of marker genes for Cluster.1 (**Cluster.1.COGs.fasta**) suggest that the sequences in this cluster may be from a new species.

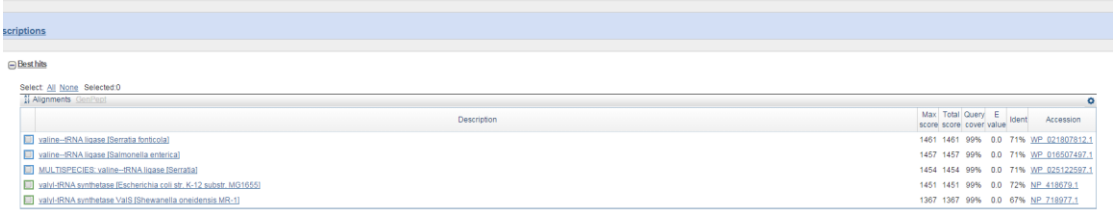
>contig-55588000000_COG0124.faa



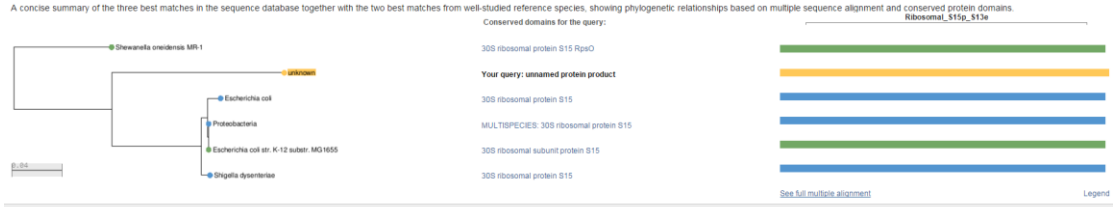



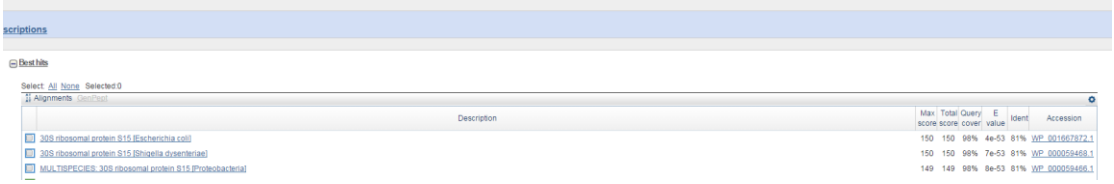

>contig-55668000000_COG0525.faa




>contig-22000000_COG0184.faa




…….

The sequences of Cluster.5.fasta and Cluster.6.fasta were annotated by Prokka with high-quality reference genomes (http://www.ncbi.nlm.nih.gov/genome/browse/reference/) as genus db.

Prokka --genus Acetobacter --usegenus Cluster.5.fasta

organism: Acetobacter species strain

contigs: 828

bases: 2554723
tRNA: 22
tmRNA: 1
CDS: 2447

Prokka --genus Lactobacillus--usegenus Cluster.6.fasta
organism: Lactobacillus species strain
contigs: 291
bases: 1203054
tRNA: 12
CDS: 1055

FASTA files of translated coding genes (**Cluster.5.faa and Cluster.6.faa**) and feature tables (**Cluster.5.tbl, and Cluster.6.tbl**) are provided in the fly.zip.

**Docker of MyCC**

Docker in Ubuntu (*This test was performed in VirtualBox of MyCC*)

curl -sSL https://get.docker.com/ | sudo sh

(Password for manager: manager)

Start docker:

```
manager@sb:~$ sudo service docker start
start: Job is already running: docker
```

Check status:

sudo service docker status

```
manager@sb:~$ sudo service docker status
docker start/running, process 6798
```

Pull the docker image of MyCC:

sudo docker run -t -i -v /home/manager/Run:/Run 990210oliver/mycc.docker:v1 /bin/bash

```
manager@sb:~$ sudo docker run -t -i -v /home/manager/Run:/Run 990210oliver/mycc.
docker:v1 /bin/bash
Unable to find image '990210oliver/mycc.docker:v1' locally
v1: Pulling from 990210oliver/mycc.docker
2880a3395ede: Pull complete
515565c29c94: Pull complete
98b15185dba7: Pull complete
2ce633e3e9c9: Pull complete
9d4ef3367e93: Pull complete
d242658d2723: Pull complete
a2cbf632e138: Pull complete
b2b78f236713: Pull complete
ecc0cd64d372: Pull complete
130b2aedb075: Pull complete
7fb33e8f384f: Pull complete
8dd940730acf: Pull complete
6b596cda3368: Pull complete
66158a4b5885: Pull complete
c54a1a4bc7d7: Pull complete
b7c3f11e4e9a: Pull complete
0fd69001d6b6: Pull complete
Digest: sha256:3832de4d8f16c0fa097a9c01dc7a51b21acab8da56d2ef453ab822ad415ebac6
Status: Downloaded newer image for 990210oliver/mycc.docker:v1
```

cd Run/10s

MyCC.py 10s.fasta

```
root@f8fc39cf5ad3:/Run/10s# MyCC.py 10s.fasta
20151207_0424
4mer
1_rename.py /Run/10s/10s.fasta 1000
Seqs >= 1000 : 2185
Minimum contig lengh for first stage clustering: 3786
run Prodigal.
/opt/prodigal.linux -i My.fa -a gene.aa -d gene.nuc -f gbk -o output -s potentia
l_genes.txt
run fetchMG.
run UCLUST.
Get Feature.
2_GetFeatures_4mer.py for fisrt stage clustering
2_GetFeatures_4mer.py for second stage clustering
3_GetMatrix.py 3786 for fisrt stage clustering
 1538 contigs entering first stage clustering
Clustering...
1_bhsne.py 20
2_ap.py /opt/ap 500 0
Cluster Correction.
to Split and Merge.
1_ClusterCorrection_Split.py 40 2
2_ClusterCorrection_Merge.py 40
Get contig by cluster.
20151207_0429
```

cd 20151207_0424_4mer_0.7/

Evaluate.py ../10s.spe.txt ../10s.fasta fasta -plot -split

```
root@f8fc39cf5ad3:/Run/10s/20151207_0424_4mer_0.7# Evaluate.py ../10s.spe.txt ..
/10s.fasta fasta -plot -split
No. of reference genomes: 10
No. of bins in evaluation: 10
No. of sequences assigned reference: 2172
No. of binned sequences: 2185
Precision: 0.944291, 0.974709
Sensitivity: 0.944291, 0.974709
```

exit

Run MyCC:

sudo docker run -t -i -v /home/manager/Run:/Run 990210oliver/mycc.docker:v1 /bin/bash

## Supplementary Methods

Evaluating binning performance by benchmark.R (http://portal.nersc.gov/dna/RD/Metagenome_RD/MetaBAT/Files/):

```
wget http://sourceforge.net/projects/sb2nhri/files/MyCC/Data/benchmark/Sharon.zip
unzip Sharon.zip -d Sharon
R
---In R console---
source('http://portal.nersc.gov/dna/RD/Metagenome_RD/MetaBAT/Files/benchmark.R')
setwd('yourpath/Sharon/')
res <- list(MyCC=calcPerf('MaxBin','MyCC'),ConCOCT=calcPerf('MaxBin','ConCOCT'),
MaxBin=calcPerf('MaxBin','maxbin'), MetaBAT=calcPerf('MaxBin','MetaBat'),
GroopM=calcPerf('MaxBin','GroopM'))
plotPerf(res,xlim=50)
pdf('infant.pdf',width=8,height=8)
plotPerf(res,xlim=50)
dev.off()
```