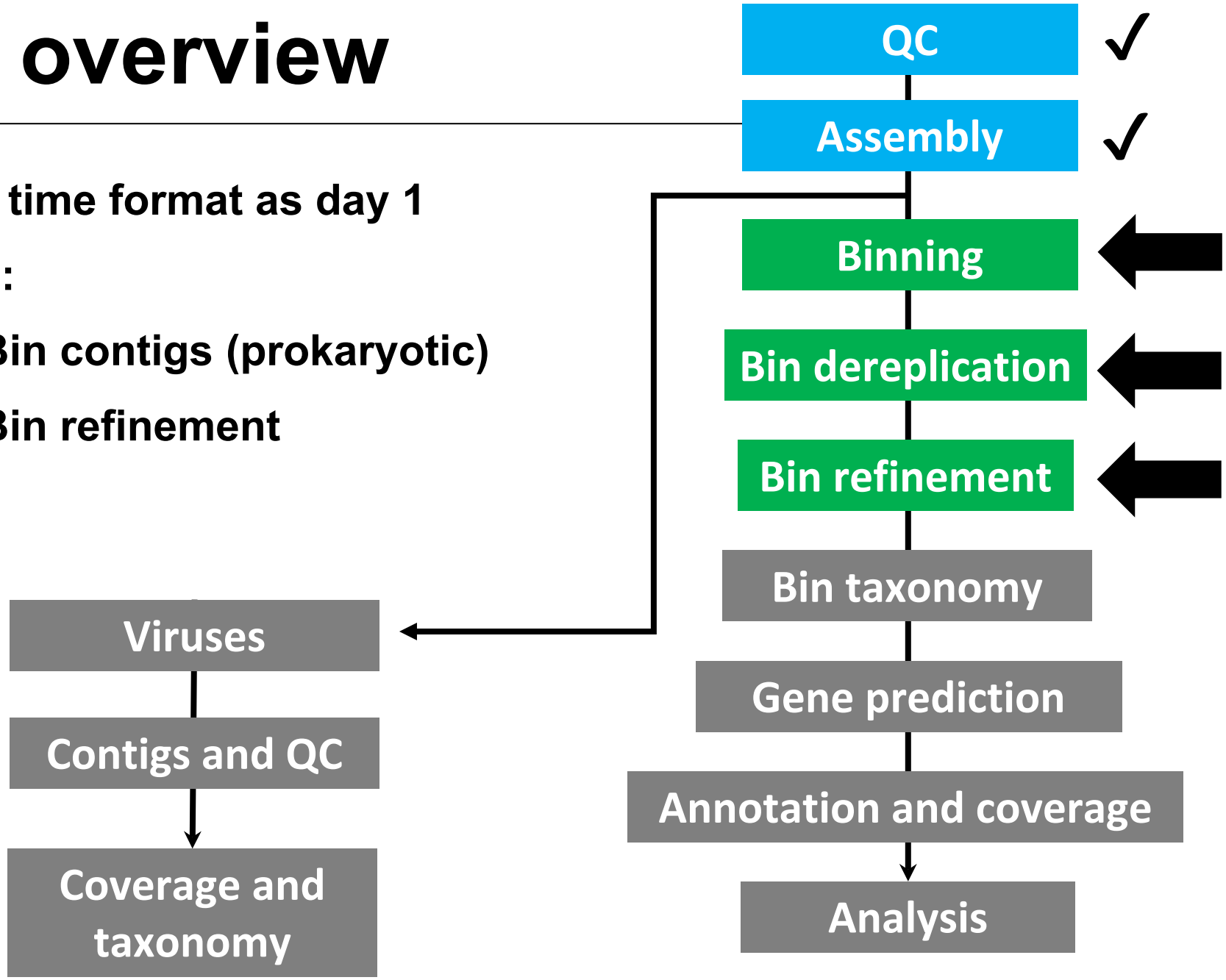
Day 2

Binning, binning, and more binning!

Day overview

- Same time format as day 1
- Goals:
  - Bin contigs (prokaryotic)
  - Bin refinement



Samples/$$$

Many samples/Limited $$$

Few samples/Ample $$$

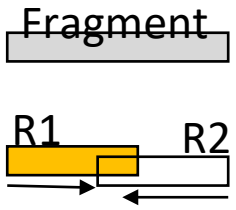
Aim:
Read analysis

Aim:
Genome recovery

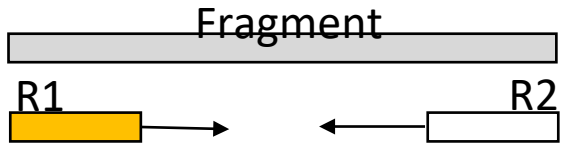
Libraries:
Short overlapping PE

Libraries:
Longer gapped PE inserts

(e.g. 200 bp DNA fragments
for 2x125 bp reads)



(e.g. >=550 bp DNA fragments)



Binning (part 1)

Overview of binning

- **Key parameters**
- **History**
- **Strategies for binning**

Overview of binning

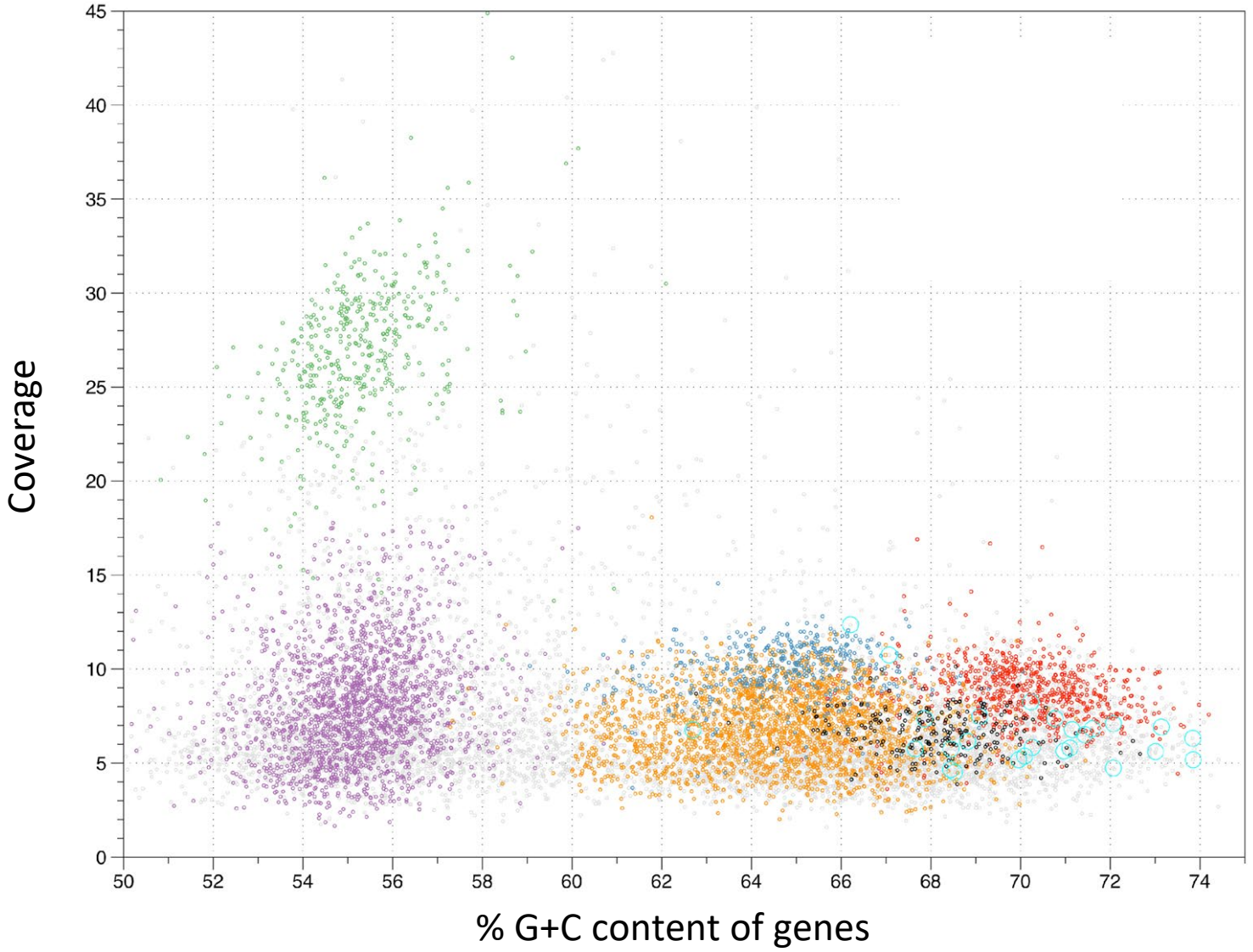
Key parameters:

- **G+C content (compositional signature)**
- **Tetranucleotide frequencies (composition signature)**

  ATCG TCGG CGGC

- **Genome coverage**
- **Differential coverage**
- **Phylogenetic affiliation of:**
  - **predicted proteins in a contig**
  - **core genes within a bin**

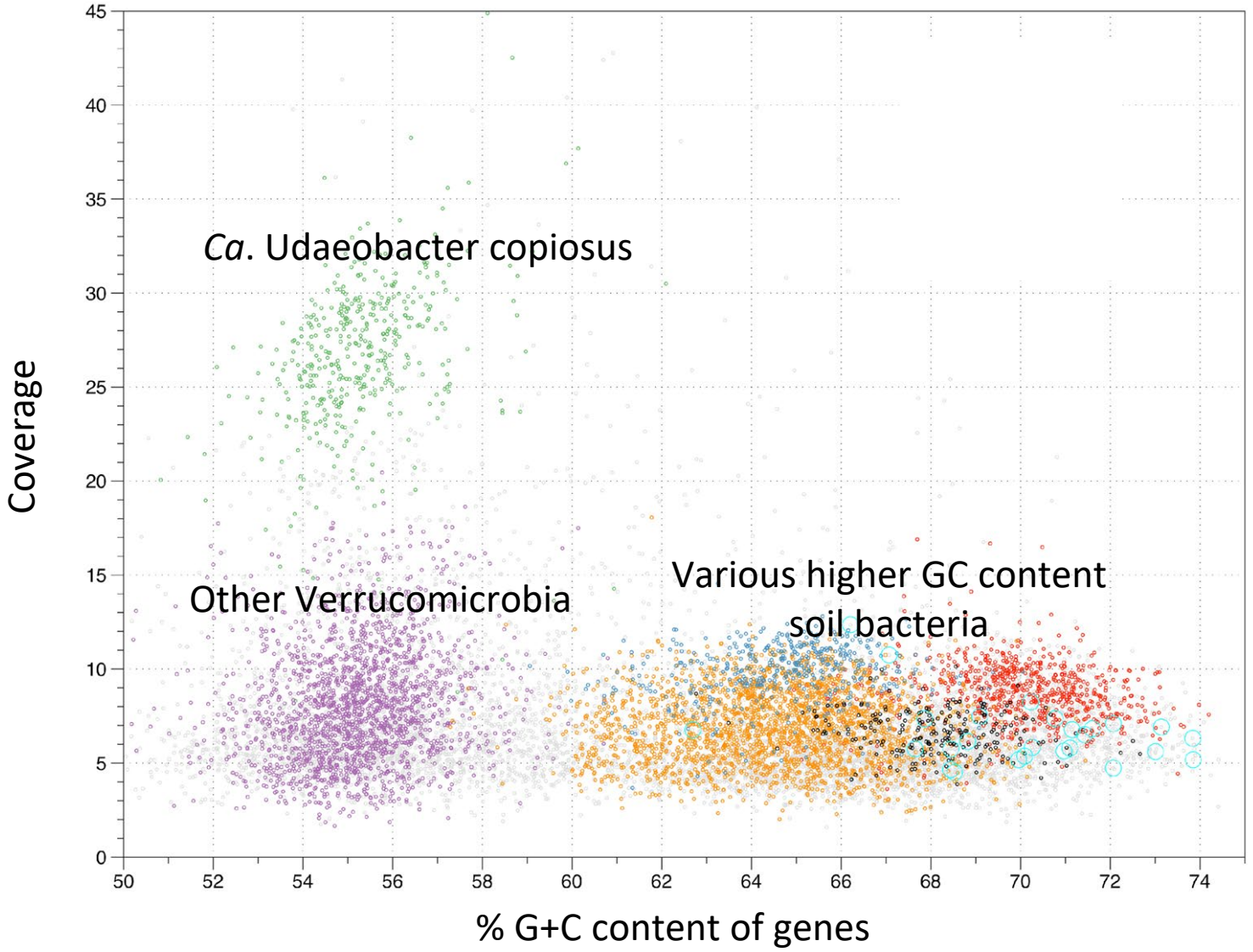
Grassland soil community



Separation of genomes by:

- % GC content of each gene
- Average contig coverage (determined by mapping reads back to contigs)

Grassland soil community



Separation of genomes by:

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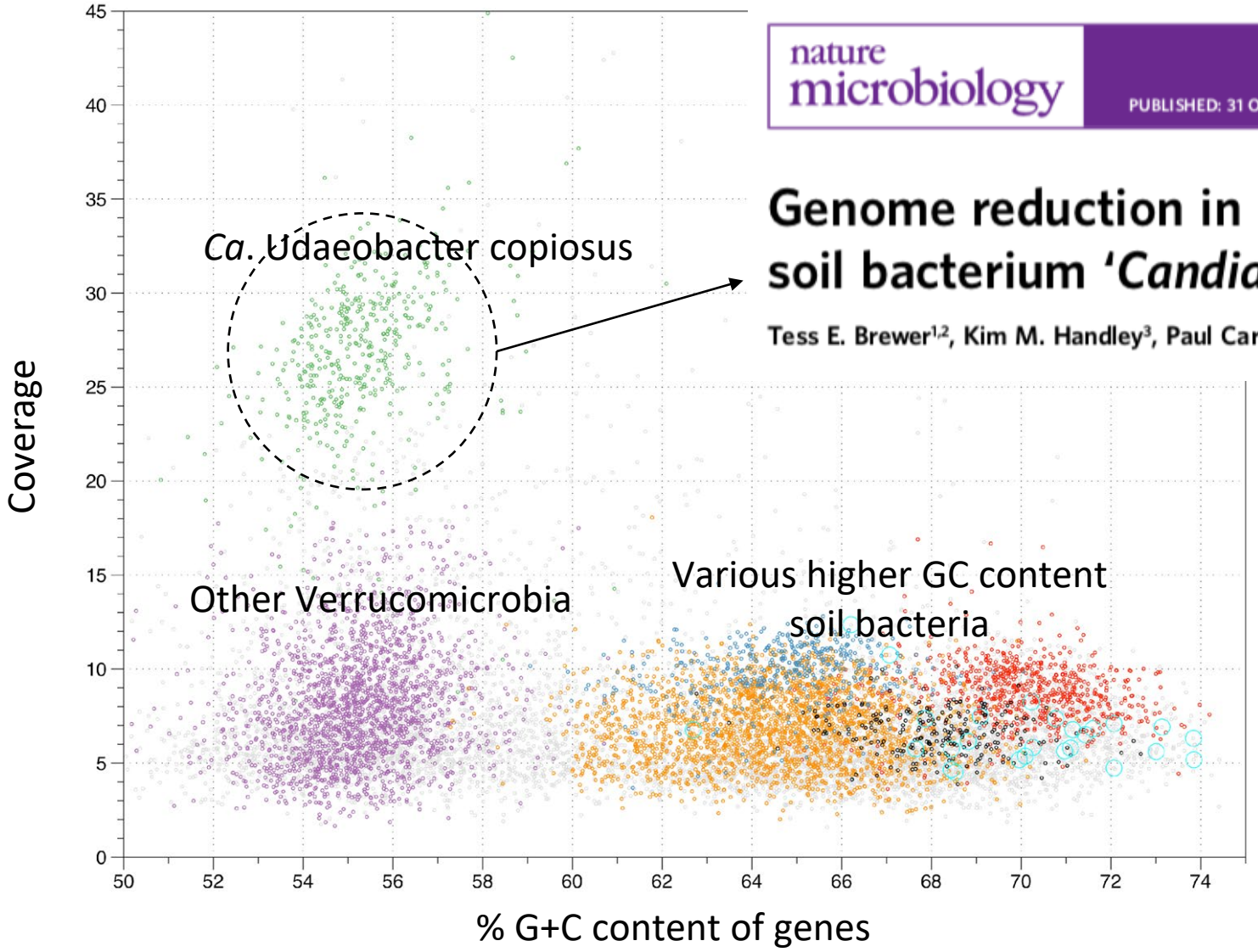
Grassland soil community



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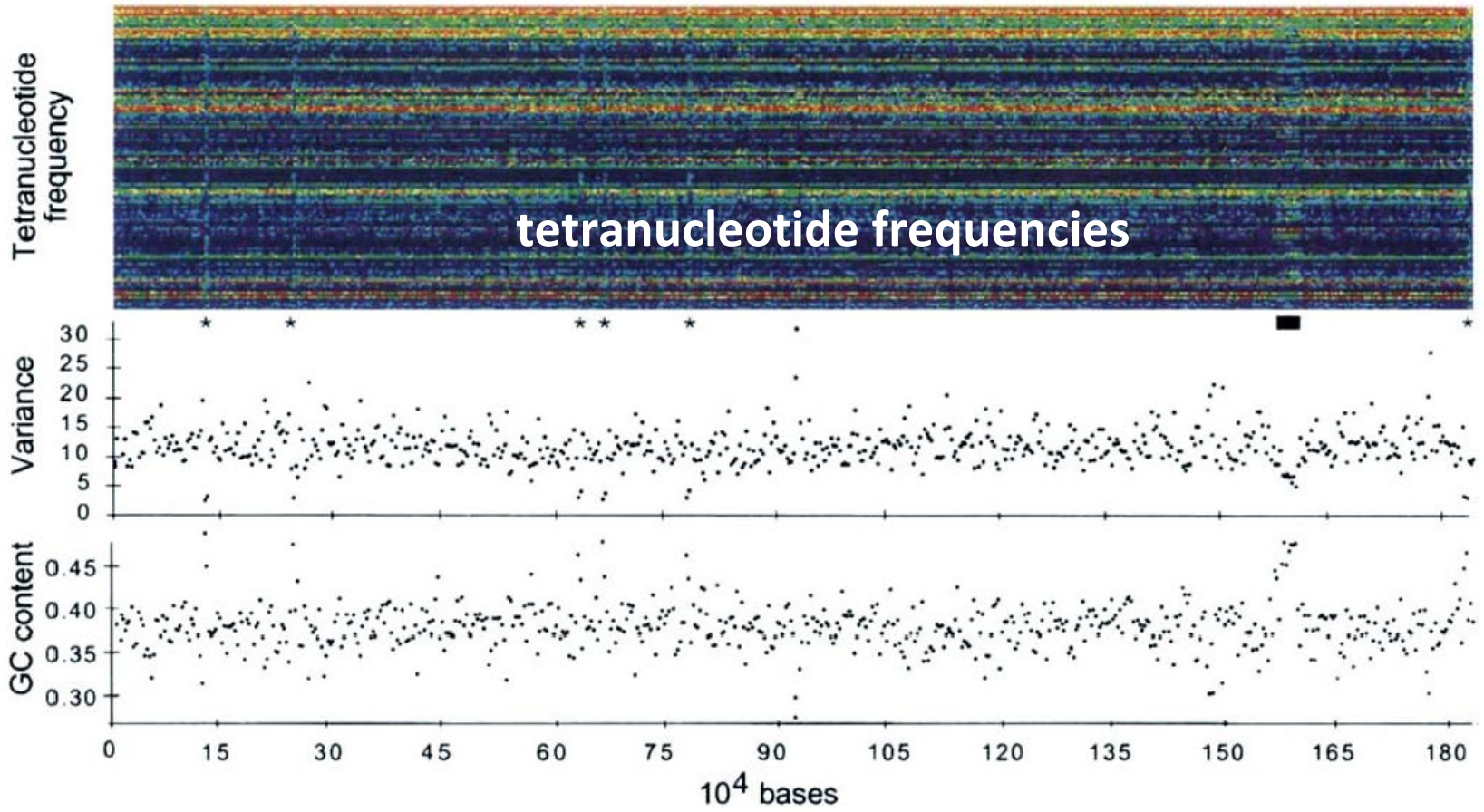
Genome reduction in an abundant and ubiquitous soil bacterium '*Candidatus* Udaeobacter copiosus'

Tess E. Brewer[1,2], Kim M. Handley[3], Paul Carini[1], Jack A. Gilbert[4,5] and Noah Fierer[1,6]*

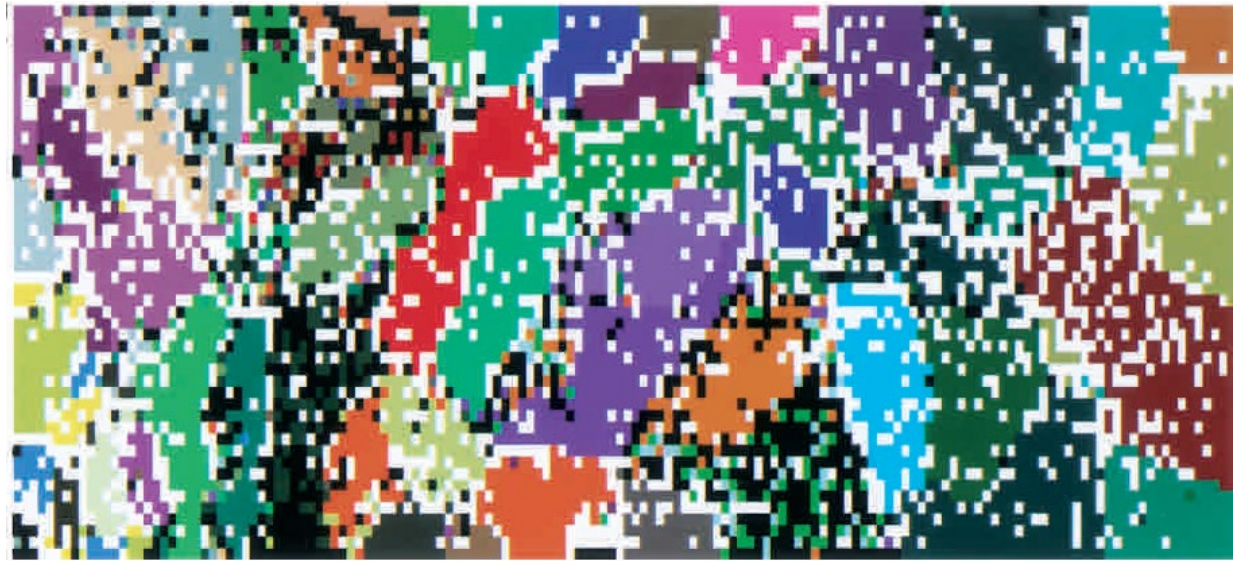
Tetranucleotide frequencies



(Noble et al., 1998, Electrophoresis)

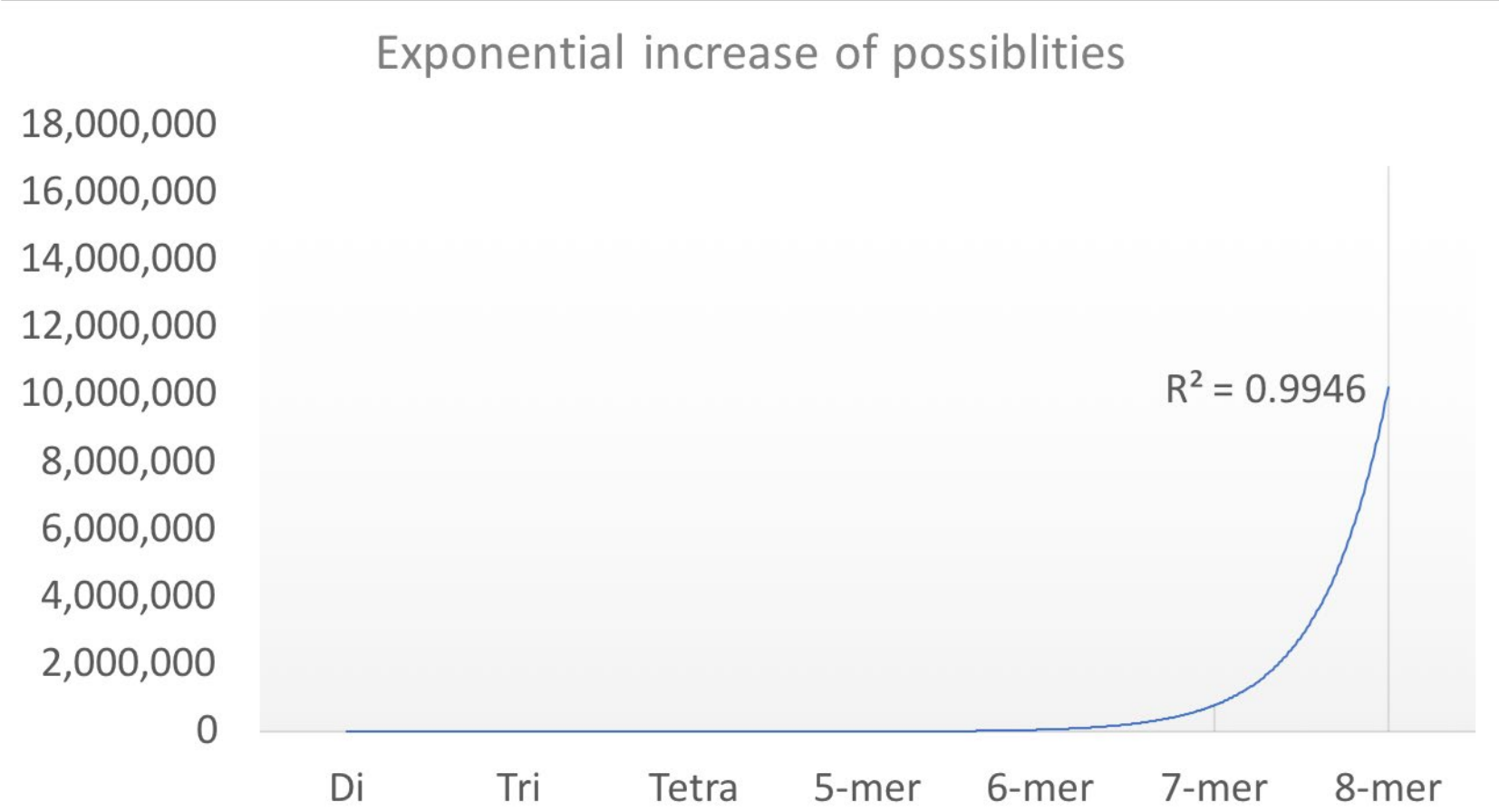
Tetranucleotide frequencies

- Tend to be conserved within genomes
- Can use 2 to 8 mers to detect genomic signatures
- Longer oligomers have increased sensitivity
- Longer oligomers require longer lengths of sequence for sampling saturation
- The number of possibilities rises exponentially with length

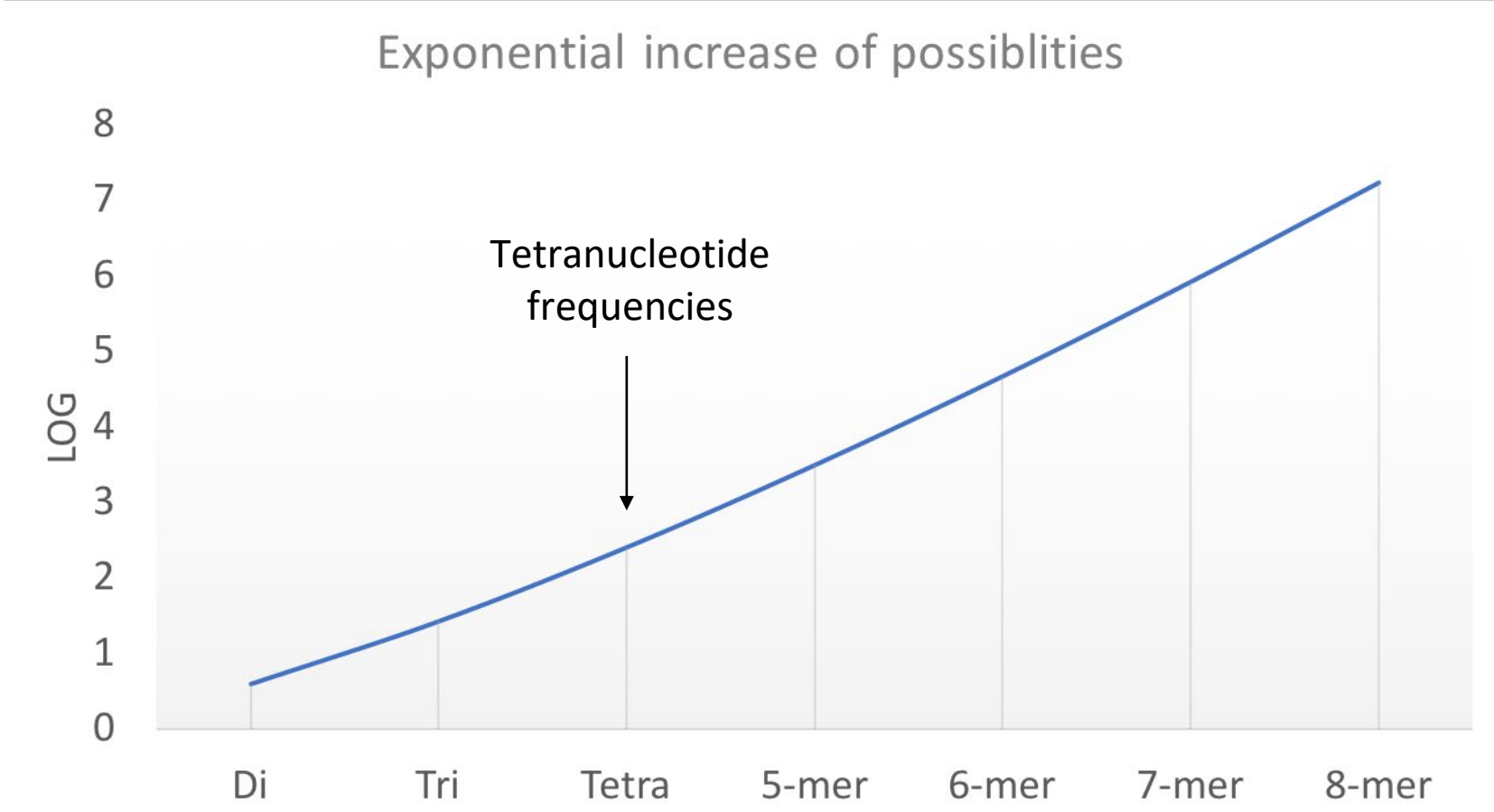


Tetra-SOM of prokaryotic and eukaryotic genomes artificially combined, yield inter and intra species separation (Abe et al., 2003, Genome Research).

Discrimination with different *k*-mers



Discrimination with different *k*-mers



Possible tetranucleotide freqs

4 x 4 x 4 x 4 = 256 possible tetranucleotides in DNA

16 are palindromic (e.g. reverse complement of AATT is AATT)

240 include distinct reverse complement sequences (e.g. AAAA = TTTT)

(240 / 2) + (16) = 136 unique tetranucleotides (when removing reverse complements with distinct sequences)

Tetranucleotide frequencies

Procedure:

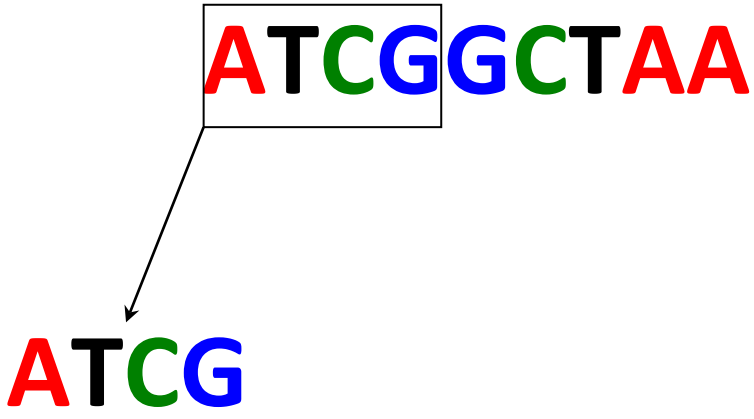
- **Fragment contigs to uniform lengths (e.g. 5 kb), use a sliding window for calculations, or normalized frequencies by contig length**

- **Count fragment tetranucleotide frequencies for forward or reverse complement**

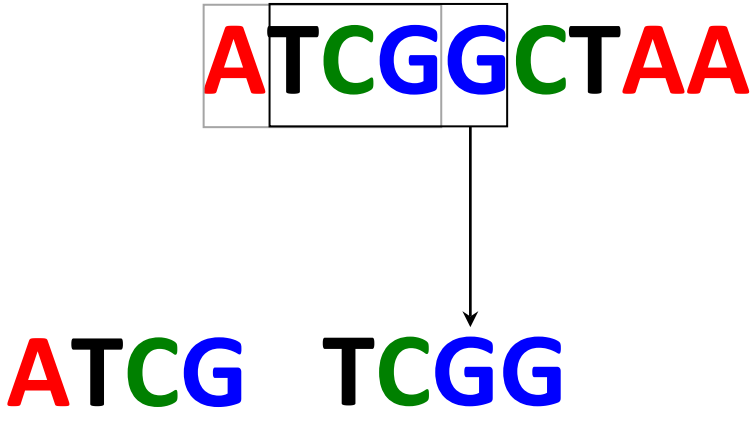
Tetranucleotide frequencies

ATCGGCTAA

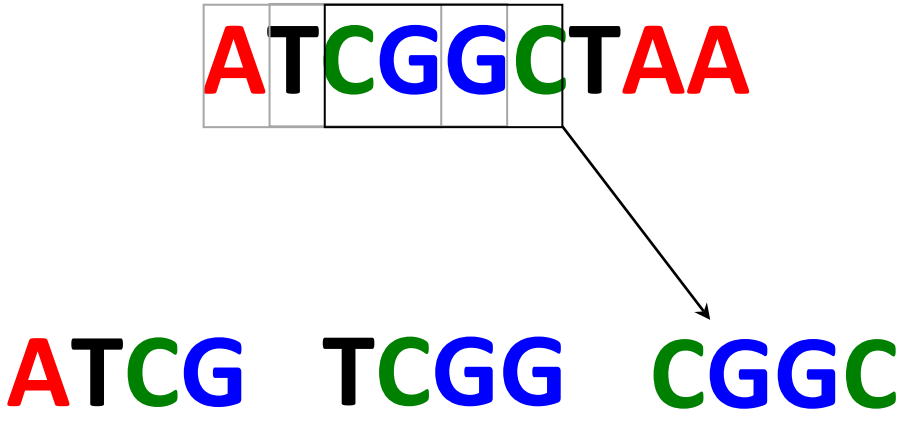
Tetranucleotide frequencies



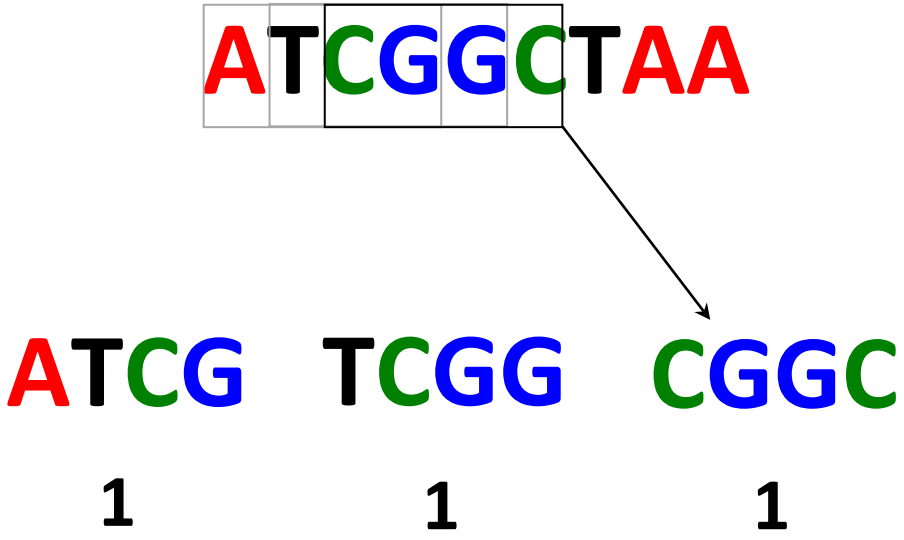
Tetranucleotide frequencies



Tetranucleotide frequencies



Tetranucleotide frequencies



Possible tetranucleotide freqs

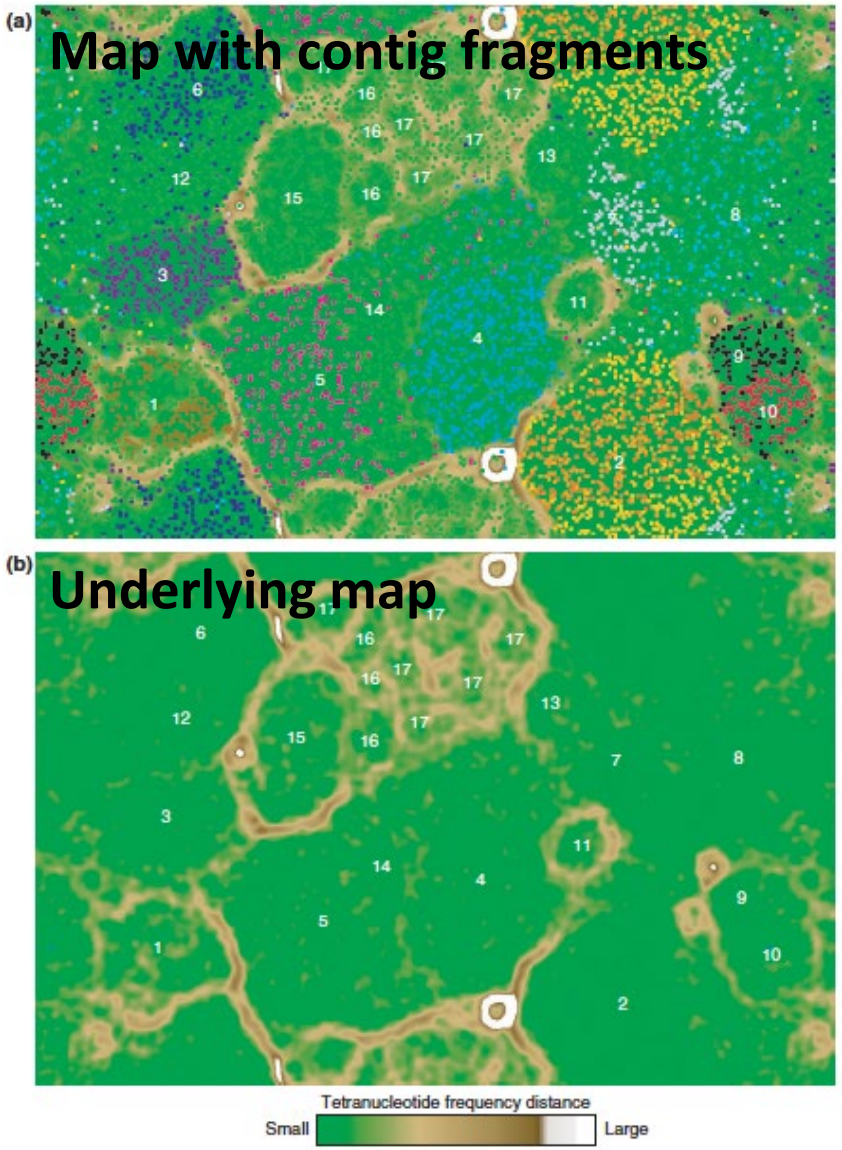
Example for ESOM using esomana software

	A	B	C	D	E	F	G	H	I	J	K	L	M	
1	% Key	AAAA	AAAT	AAAC	AAAG	AATA	AATT	AATC	AATG	AACA	AACT	AACC	AACG	AAG
2	1	0.0225338	0.01151728	0.01402103	0.01552328	0.00751127	0.00350526	0.01452178	0.00901352	0.01101652	0.00550826	0.00751127	0.00751127	0.00
3	2	0.01452178	0.01502253	0.01402103	0.01301953	0.00901352	0.00751127	0.00751127	0.01352028	0.01301953	0.00851277	0.00751127	0.00650976	0.00
4	3	0.01702554	0.01301953	0.01802704	0.01001502	0.01352028	0.00650976	0.00701052	0.00650976	0.01101652	0.00851277	0.01201803	0.00951427	0.00
5	4	0.01652479	0.01101652	0.01051577	0.01452178	0.01352028	0.00400601	0.00751127	0.00901352	0.00951427	0.00951427	0.00951427	0.00650976	0.00
6	5	0.00801202	0.01101652	0.01001502	0.00951427	0.00901352	0.00300451	0.00801202	0.01151728	0.00901352	0.00901352	0.00901352	0.00500751	0.00
7	6	0.01251878	0.01352028	0.01151728	0.01051577	0.01201803	0.00300451	0.00851277	0.01001502	0.01402103	0.00250376	0.01101652	0.00801202	0.00
8	7	0.01402103	0.01101652	0.01251878	0.01802704	0.01151728	0.00150225	0.01251878	0.01402103	0.01151728	0.00400601	0.00901352	0.00600901	0.01
9	8	0.01402103	0.01101652	0.01001502	0.01352028	0.00851277	0.00500751	0.00801202	0.01251878	0.01201803	0.00500751	0.01001502	0.00901352	0.00
10	9	0.01552328	0.01301953	0.01402103	0.01301953	0.00801202	0.00450676	0.01051577	0.01201803	0.00550826	0.00400601	0.01352028	0.01001502	0.01
11	10	0.01201803	0.00701052	0.01402103	0.01201803	0.00350526	0.00500751	0.00951427	0.01151728	0.00450676	0.00650976	0.01301953	0.01051577	0.01
12	11	0.02704056	0.01652479	0.01201803	0.01552328	0.00751127	0.00500751	0.00751127	0.01001502	0.00901352	0.00650976	0.01051577	0.00801202	0.01
13	12	0.01352028	0.01452178	0.01151728	0.01352028	0.00901352	0.00851277	0.01151728	0.01151728	0.01051577	0.00751127	0.00901352	0.00751127	0.01
14	13	0.01702554	0.01201803	0.01051577	0.01301953	0.00901352	0.00600901	0.00801202	0.01051577	0.00901352	0.00851277	0.01201803	0.00901352	0.00
15	14	0.03204807	0.02303455	0.01402103	0.01352028	0.01051577	0.00550826	0.01301953	0.01201803	0.00951427	0.00650976	0.00951427	0.00751127	0.00
16	15	0.01602404	0.01101652	0.01502253	0.01001502	0.00701052	0.00400601	0.00751127	0.01151728	0.01151728	0.00751127	0.01051577	0.00500751	0.00
17	16	0.01051577	0.01251878	0.01352028	0.00751127	0.01301953	0.00650976	0.00801202	0.00951427	0.01201803	0.00650976	0.00901352	0.00851277	0.00
18	17	0.01552328	0.01502253	0.01301953	0.01251878	0.01201803	0.00500751	0.01101652	0.00801202	0.01101652	0.00600901	0.01151728	0.00450676	0.00
19	18	0.0225338	0.01952929	0.01151728	0.01151728	0.00650976	0.00600901	0.01101652	0.01251878	0.00801202	0.00650976	0.01101652	0.01151728	0.01
20	19	0.0235353	0.01452178	0.01502253	0.01602404	0.01001502	0.00150225	0.01301953	0.01301953	0.01001502	0.00801202	0.01201803	0.00951427	0.01
21	20	0.02203305	0.01352028	0.01101652	0.01251878	0.00801202	0.00400601	0.01001502	0.01402103	0.01151728	0.00550826	0.00901352	0.00901352	0.00
22	21	0.01852779	0.01402103	0.01352028	0.01301953	0.00650976	0.00500751	0.00851277	0.01101652	0.00851277	0.00600901	0.00951427	0.00751127	0.00
23	22	0.01502253	0.01101652	0.01352028	0.00901352	0.01151728	0.00550826	0.01101652	0.01352028	0.01101652	0.00801202	0.01201803	0.00450676	0.00
24	23	0.02403605	0.01652479	0.01051577	0.01702554	0.00901352	0.00650976	0.01101652	0.01352028	0.00650976	0.00801202	0.00550826	0.00851277	0.00
25	24	0.01452178	0.01402103	0.00951427	0.01151728	0.01301953	0.00801202	0.00851277	0.01051577	0.01151728	0.00951427	0.01352028	0.00500751	0.00
26	25	0.01802704	0.00951427	0.01201803	0.01251878	0.01301953	0.00550826	0.00550826	0.01001502	0.01201803	0.00901352	0.01051577	0.00851277	0.00
27	26	0.01301953	0.01201803	0.00851277	0.01552328	0.00751127	0.00500751	0.01101652	0.01352028	0.00951427	0.00801202	0.00650976	0.00751127	0.01
28	27	0.02954432	0.01902854	0.01301953	0.01552328	0.01402103	0.00701052	0.01101652	0.01301953	0.00951427	0.00801202	0.00751127	0.00400601	0.01
29	28	0.02103155	0.01502253	0.01301953	0.01502253	0.01352028	0.00701052	0.00951427	0.01151728	0.00600901	0.00751127	0.01101652	0.00851277	0.00

ESOM and Tetranucleotide frequencies

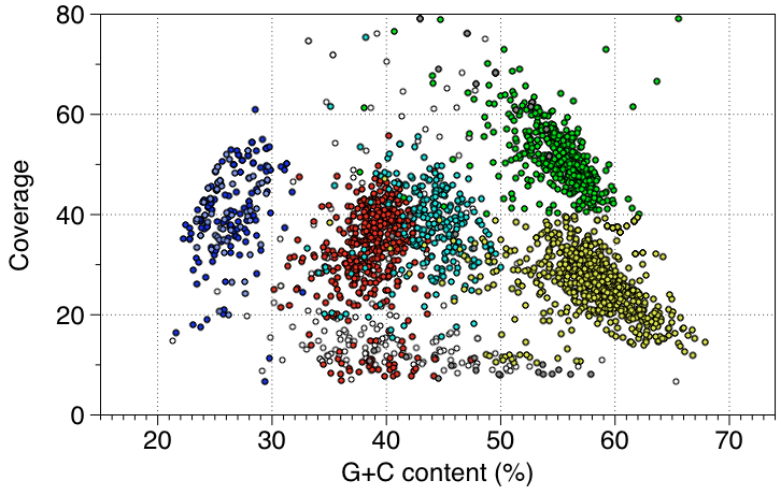
Acid mine drainage genomes

(Dick et al., 2009, Genome Biology)

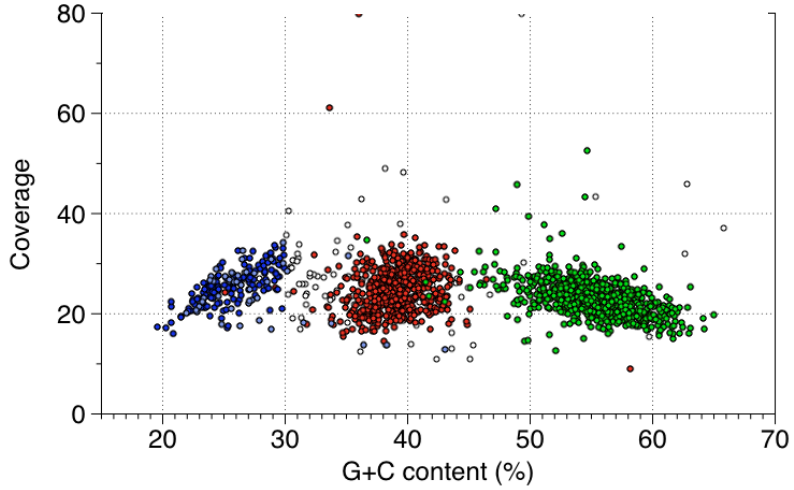


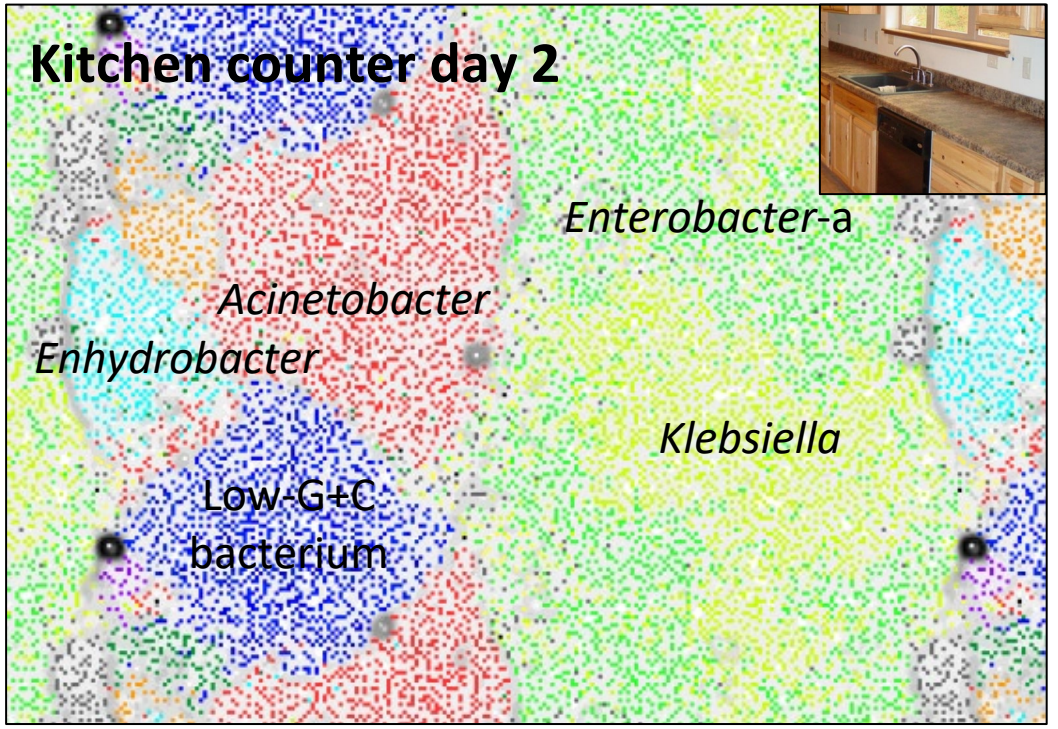
- Emergent Self Organizing Map (ESOM)
- Created using the Databionics tool esomana (http://databionic-esom.sourceforge.net/)
- Unsupervised neural network algorithm
- Allows outliers (e.g. partially sampled genomes)
- Determines clusters as supported by the data

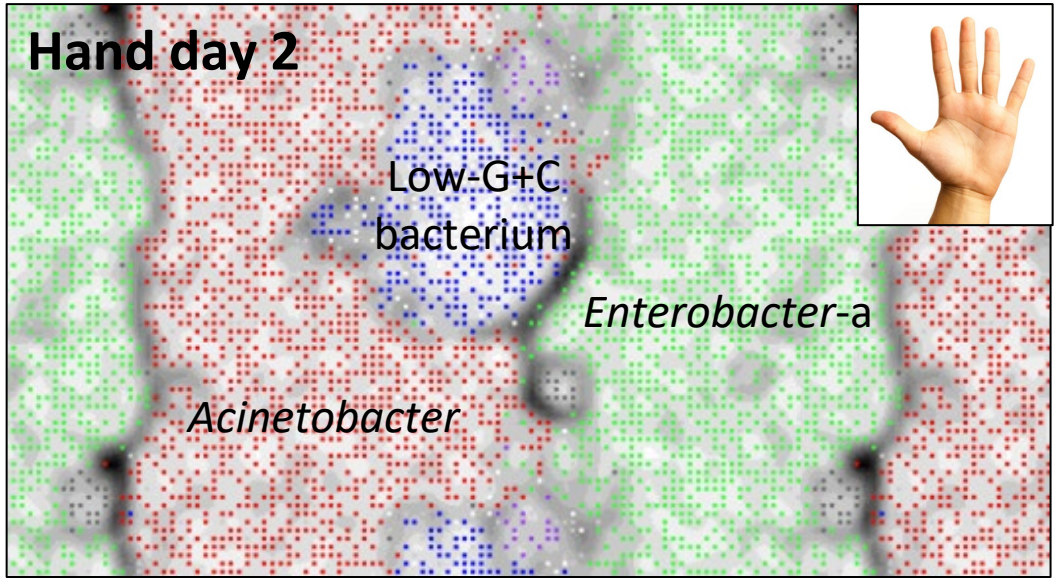
Kitchen counter day 2



Hand day 2



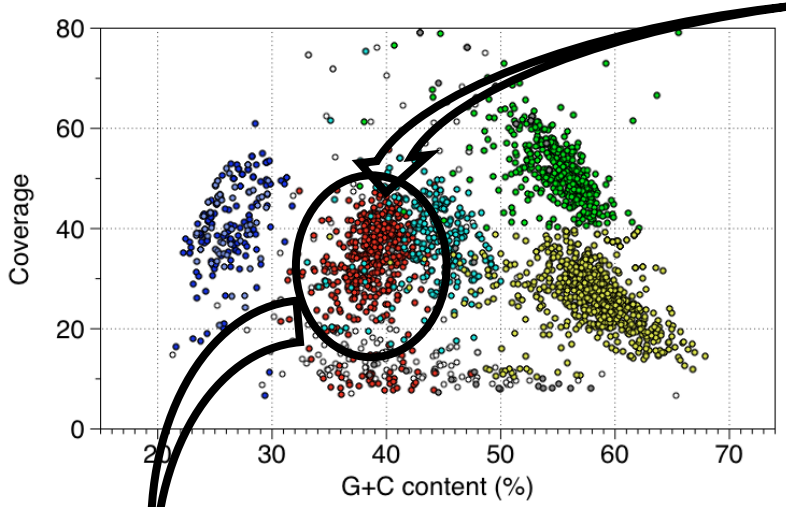




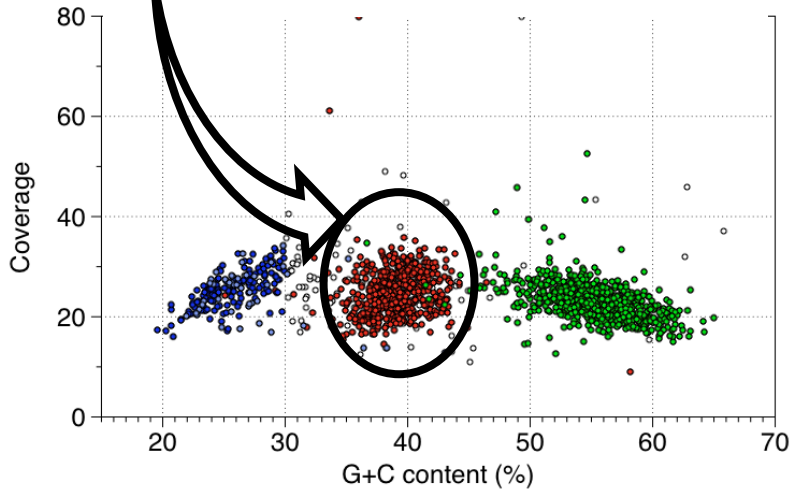
Home microbiome study

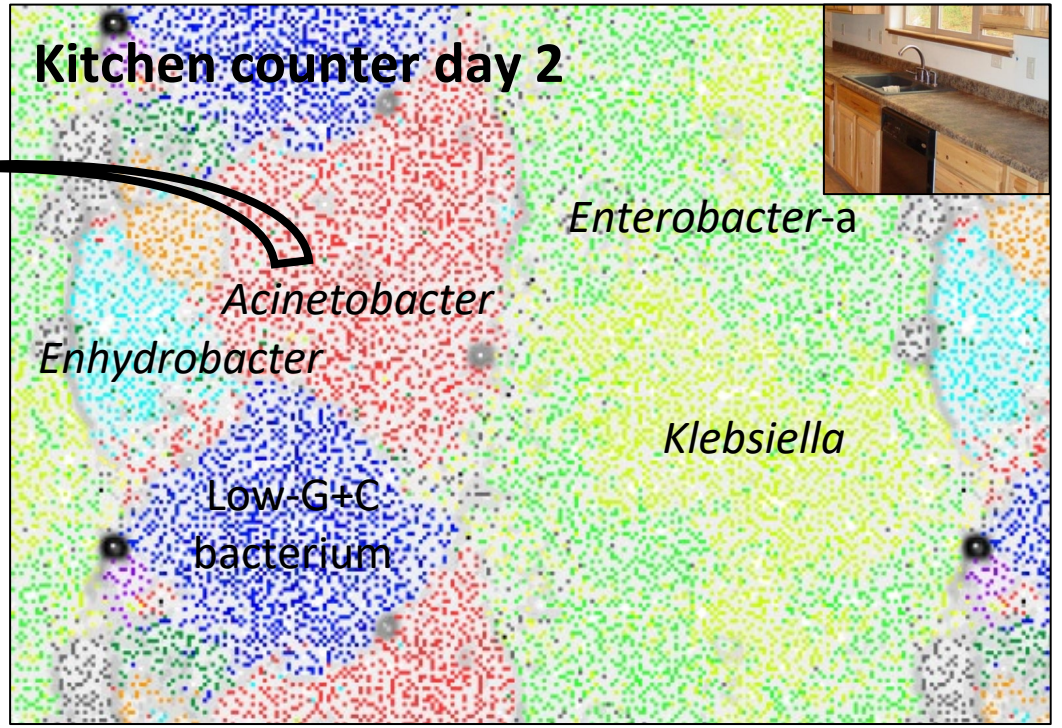
Cluster separation of distinct genera or species

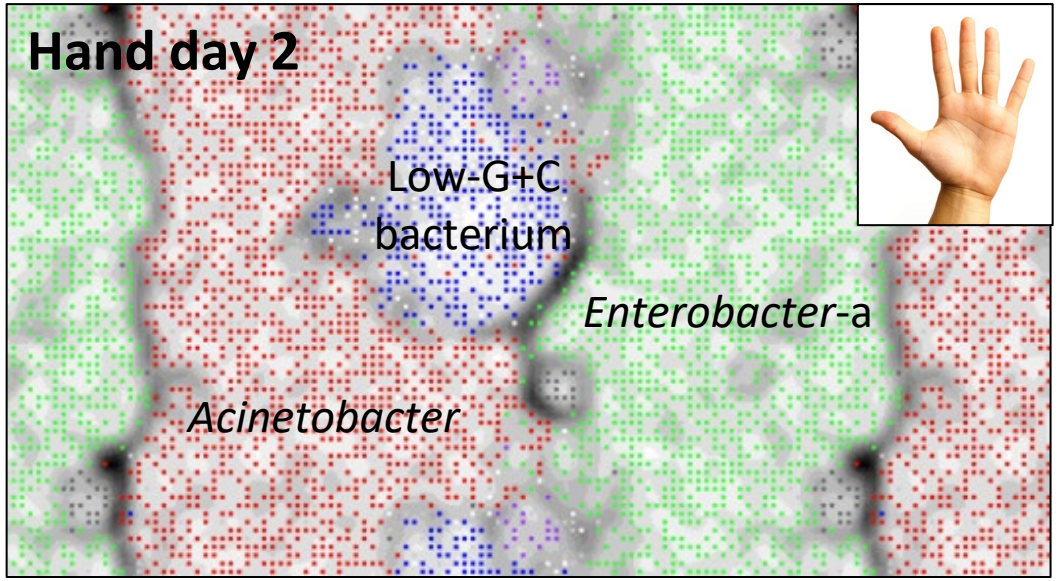
Kitchen counter day 2



Hand day 2



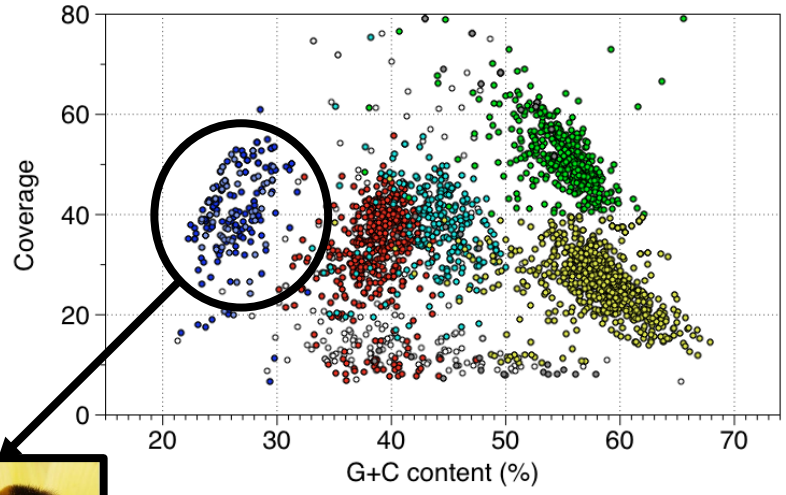




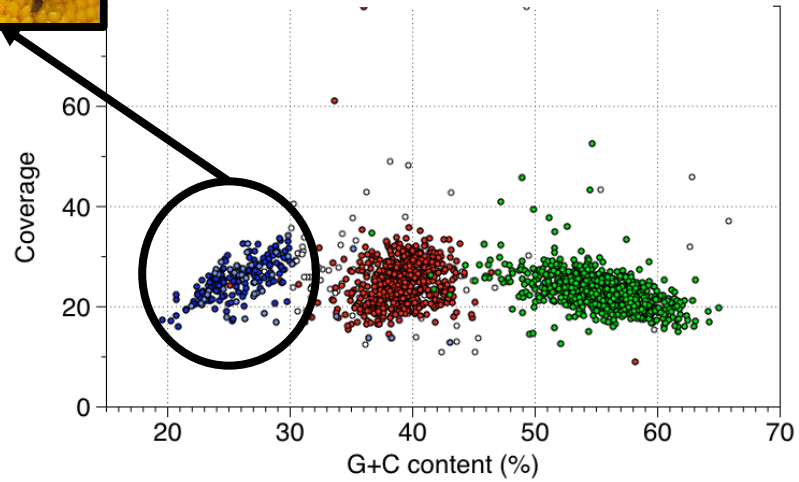
Home microbiome study

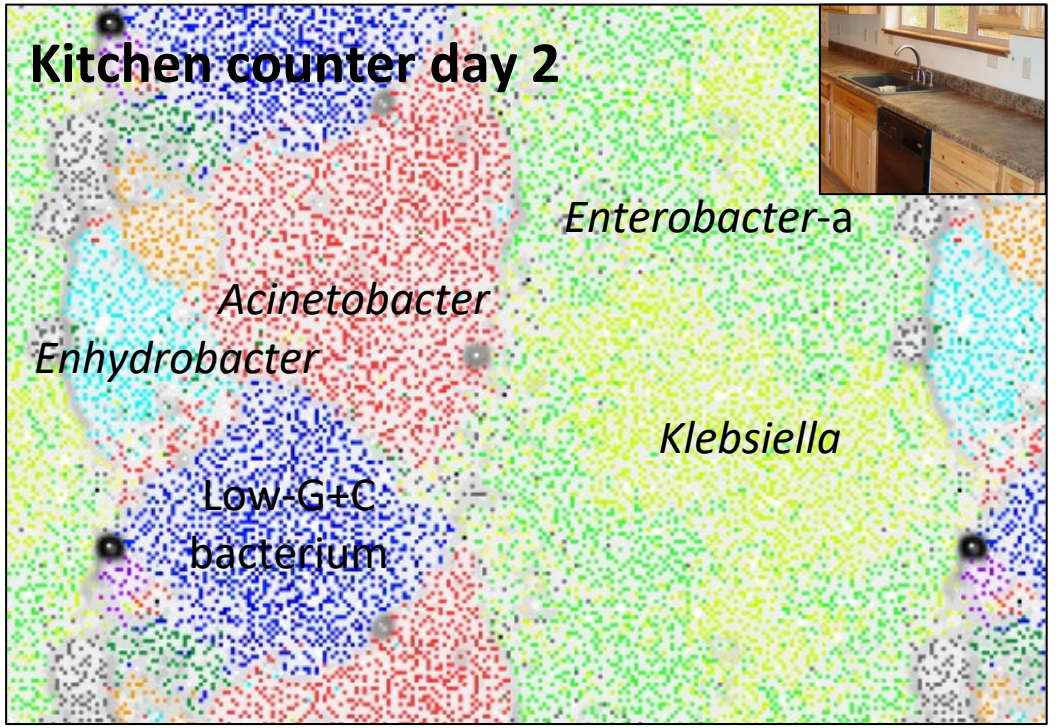
Cluster separation of distinct genera or species

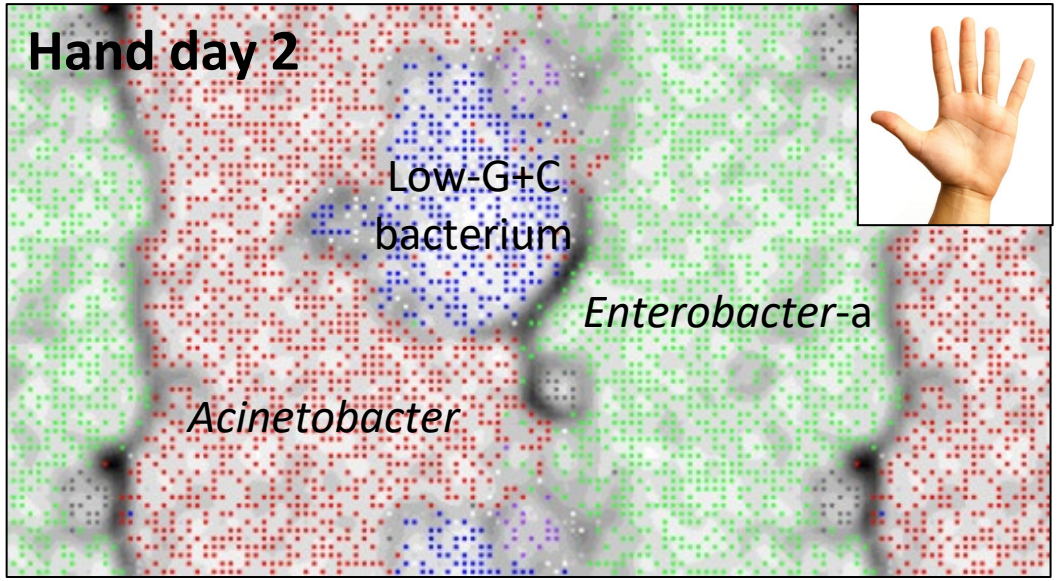
Kitchen counter day 2



Hand day 2





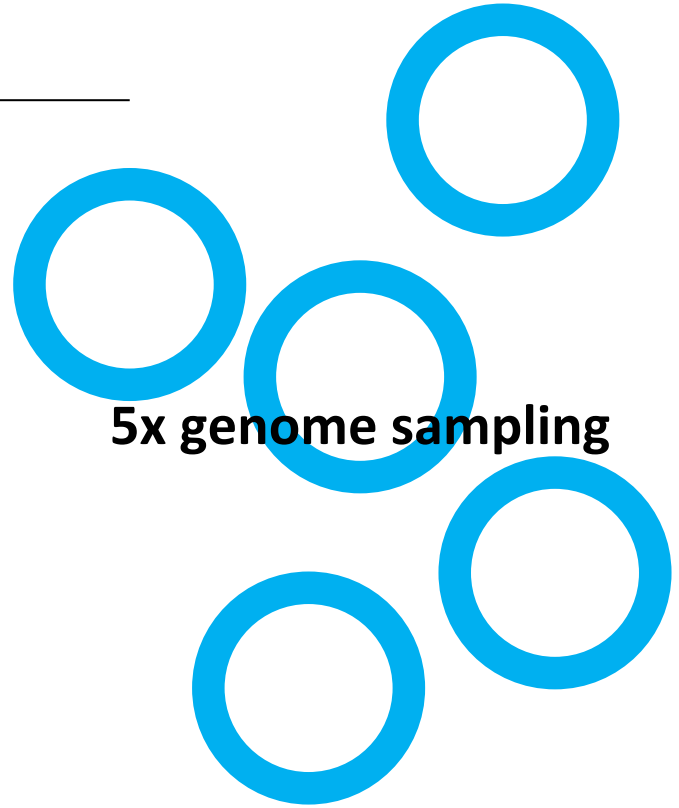


Cluster separation of distinct genera or species

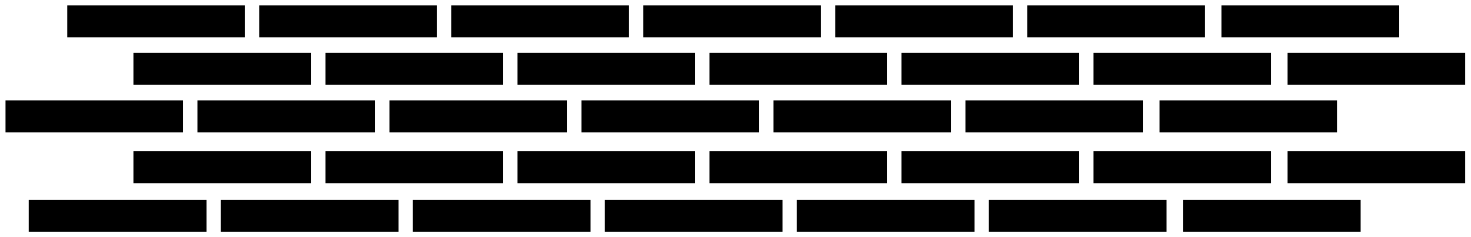
Recombinase A (RecA) ~70% ID to *Gilliamella apicola*

Coverage

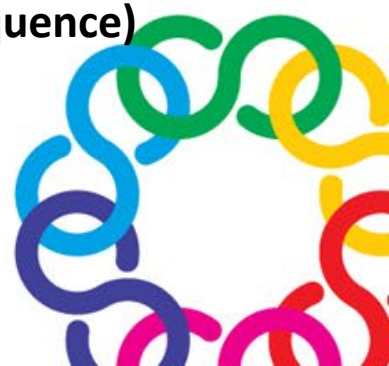
- Coverage = Number of times genome sequenced
- Map reads to genome



ATGGGCTAAAGATAGGCCTAATAGGTACTGGGATCCAAG Assembled contig (consensus sequence)

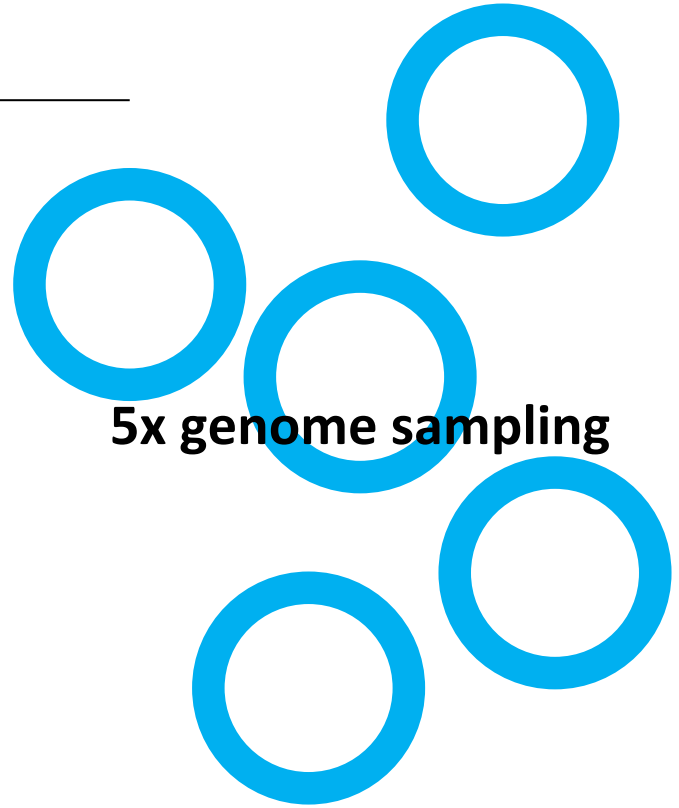


Mapped reads at 5x coverage

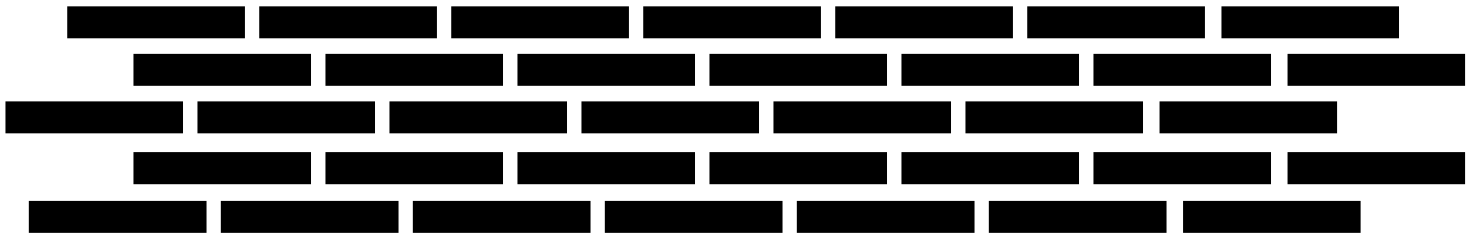


Coverage

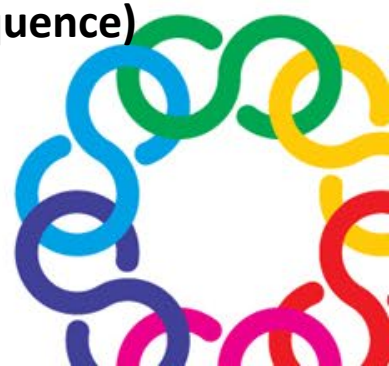
- Use mapping file (e.g. bowtie sam file)
- Count reads per mapped per contig
- Calculate contig (or bin) coverage:
  - (read length * number reads) / contig (or bin) length
  - Normalize to library size (if multiple samples)



ATGGGCTAAAGATAGGCCTAATAGGTACTGGGATCCAAG Assembled contig (consensus sequence)



Mapped reads at 5x coverage



Coverage viewing: Tablet

overview of reads mapped to contig

consensus

zoomed in view of reads mapped to part of contig

(http://tablet.hutton.ac.uk/en/latest/index.html#)

Read mapping: sam file contents

```
HWI-ST1035:107:C0LBYACXX:3:2308:18248:182773          163          NODE_2076_length_3089_cov_9.764973          1497          255          101M          =
          1511          115          GTAATGCCACGTCCCACCCATGCCACAAGGCCGAGCACTTGCCCTCTGGGAGCACGGCGCATTCCAACCGCCTTTCCGGACGCAACGAATGAGGCAAGGTG
          CCCFFFFFHHHHHIJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJGIJIJJJJJFHHHFFDDDDDDFFDDDDDDDDDDDDDDDDDDDDDDDDDDDDCDDDDDDDCC          XA:i:1          MD:Z:2G42T55          NM:i:2

HWI-ST1035:107:C0LBYACXX:3:2308:18248:182773          83          NODE_2076_length_3089_cov_9.764973          1511          255          101M          =
          1497          -115          CACCCATGCCACAAGGCCGAGCACTTGCCCTCTGGGAGCACGGCGCATTCCAACCGCCTTTCCGGACGCAACGAATGAGGCAAGGTGTGCGAGAGCAGCAA
          BDDDDDDDDDDDDDDDDDDEDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDCDDFEHJJIJJJJJJJJJIJJJJJJJJIJJJJJJJJJJJJHHHHHFFFFFCCC          XA:i:0          MD:Z:31T69          NM:i:1

HWI-ST1035:107:C0LBYACXX:3:2308:13412:183356          99          NODE_389_length_2884_cov_10.890083          2682          255          101M          =
          2735          154          ATCTCGGGCTTTCCGAGATCCAGCAGCTGGCCGGCTTGGGCGAGCTATTCGACAGCTTGGTGGTGTTTGAGAACTATCCGGTGGAACGCAGCAGCCTGTCG
          @@@DFFFFHHHHHJJJJJIIJJJIJJJJIIJJJJJJJIIJGIEHFFEDEDCEDDDBCCCD@BB;@ABDDCDDDDDDDDDDDBDDDDCDDDDDDDDDDDDDDD          XA:i:0          MD:Z:46G54          NM:i:1

HWI-ST1035:107:C0LBYACXX:3:2308:13412:183356          147          NODE_389_length_2884_cov_10.890083          2735          255          101M          =
          2682          -154          AGCTTGGTGGTGTTTGAGAACTATCCGGTGGAACGCAGCAGCCTGTCGGCGACTGCCGCGGGTGTTCGGCTCTGCCAAGTCAGCGGGCACGACGCGACACA
          DDBDDDDDBBA>DDCDEEDCDDDDDDDDDA<25DDDDDDDDDDDDDDDDDCDDDDDDFFHHJJIIJIJIJJJJJJJJJJJJIJIJJJJIHHHGHFFFFFCCC          XA:i:0          MD:Z:101          NM:i:0

HWI-ST1035:107:C0LBYACXX:3:2308:16904:185061          163          NODE_1144_length_1992_cov_11.747992          579          255          101M          =
          592          114          TCTACCTTATCGGGCACGAGCCGGTAAGTGATGCCGCGCGGGATGACGATGTAATCCTCCGCGCGATAACGTTGCTTTCCGAAAAAAGTTTCAACTGTACC
          @??DBD?          DF<CCCBDEHBH@AF?D0?DD0?DBFFGFHGHHFCB'8@C@BD?5@C>CCDDDDDD<BB@DDDD<9ABCCA@CDDDD@?<B::::@>@>34>@>          XA:i:0
          MD:Z:85T0C14          NM:i:2

HWI-ST1035:107:C0LBYACXX:3:2308:16904:185061          83          NODE_1144_length_1992_cov_11.747992          592          255          101M          =
          579          -114          GCCGGAGCCGGTAAGTGATGCCGCGCGGGATGACGATGTAATCCTCCGCGCGATAACGTTGCTTTCCGAAAAAAGTTTCAACTGTACCGCCGCCTTTGAAG
          ##########?          CC@@ACCB@BB<5>><?>CBBBBBCECCC@?B;95BBB9<<C?CC9@?C?<?@AACECD@GGEIHFCHF@GIG@;7IGFDHBDDDA;D@?@          XA:i:1          MD:Z:2A0C68T0C27          NM:i:4
```

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<tr><th>Col</th><th>Field</th><th>Type</th><th>Regexp/Range</th><th>Brief description</th></tr>
<tr><td>1</td><td>QNAME</td><td>String</td><td>[!-?A-~]{1,255}</td><td>Query template NAME</td></tr>
<tr><td>2</td><td>FLAG</td><td>Int</td><td>$[0,2^{16}-1]$</td><td>bitwise FLAG</td></tr>
<tr><td>3</td><td>RNAME</td><td>String</td><td>*|[!-()+-<>-~][!-~]*</td><td>Reference sequence NAME</td></tr>
<tr><td>4</td><td>POS</td><td>Int</td><td>$[0,2^{31}-1]$</td><td>1-based leftmost mapping POSition</td></tr>
<tr><td>5</td><td>MAPQ</td><td>Int</td><td>$[0,2^{8}-1]$</td><td>MAPping Quality</td></tr>
<tr><td>6</td><td>CIGAR</td><td>String</td><td>*|([0-9]+[MIDNSHPX=])+</td><td>CIGAR string</td></tr>
<tr><td>7</td><td>RNEXT</td><td>String</td><td>*|=|[!-()+-<>-~][!-~]*</td><td>Ref. name of the mate/next read</td></tr>
<tr><td>8</td><td>PNEXT</td><td>Int</td><td>$[0,2^{31}-1]$</td><td>Position of the mate/next read</td></tr>
<tr><td>9</td><td>TLEN</td><td>Int</td><td>$[-2^{31}+1,2^{31}-1]$</td><td>observed Template LENgth</td></tr>
<tr><td>10</td><td>SEQ</td><td>String</td><td>*|[A-Za-z=.]+</td><td>segment SEQuence</td></tr>
<tr><td>11</td><td>QUAL</td><td>String</td><td>[!-~]+</td><td>ASCII of Phred-scaled base QUALity+33</td></tr>
</table>

Task: Binning - Read mapping

Go to Github MGSS webpage

Tasks:

- ✓ **Short contig removal**
- **Read mapping**
- **Multi-binning strategy (Metabat and Maxbin)**
- **Bin dereplication via DAS_Tool**
  - **Pick-the-winner bin dereplication**
- **Evaluating bins using CheckM**

NOTE: short contig removal was done in previous exercise (Day 1 - Evaluating the assemblies)

Binning (part 2)

Task: Binning - Multi-binning strategy

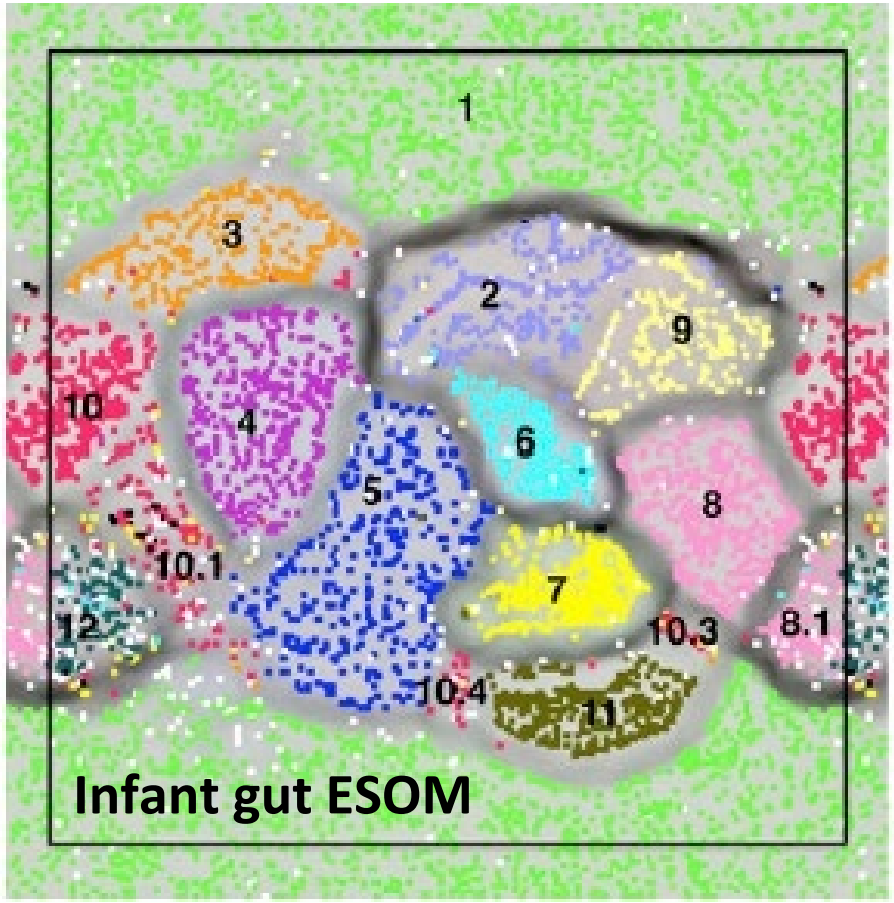
Go to Github MGSS webpage

Tasks:

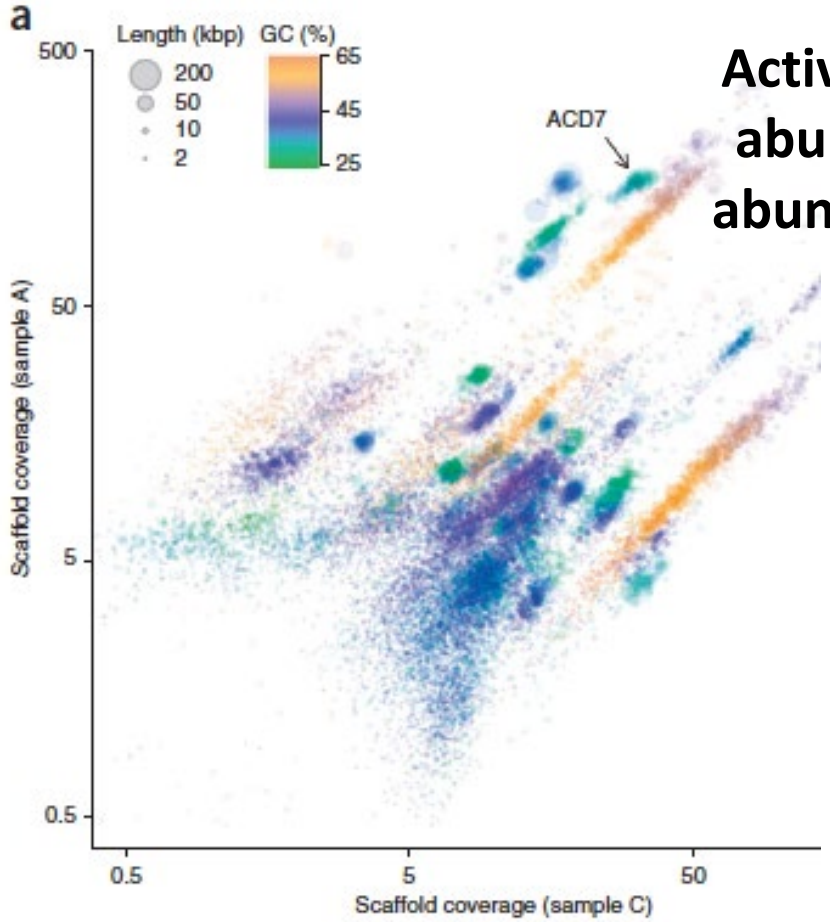
- ✓ Short contig removal
- ✓ Read mapping
- Multi-binning strategy (Metabat and Maxbin)
- Bin dereplication via DAS_Tool
  - Pick-the-winner bin dereplication
- Evaluating bins using CheckM

NOTE: we usually also use CONCOCT which tends to produce a smaller number of high quality bins. Running it is a little more involved, so it's excluded from the MGSS, but we encourage everyone to consider using this tool.

Differential coverage



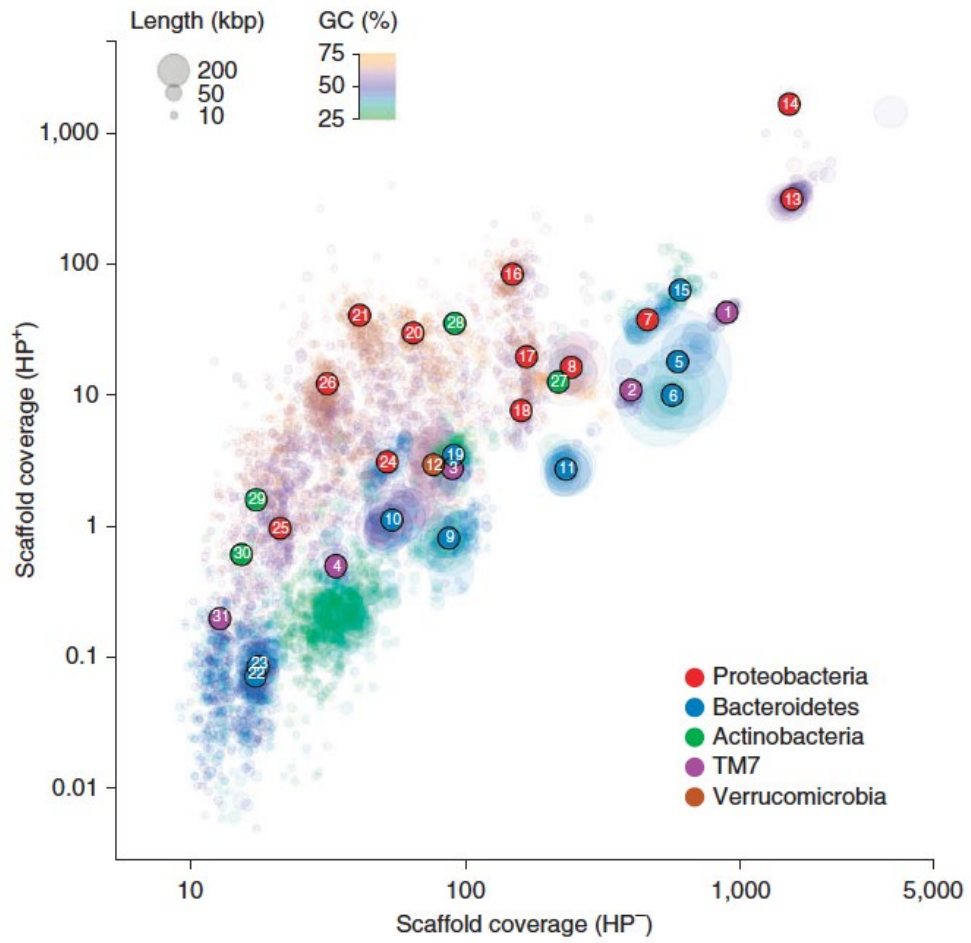
(Time series differential coverage ESOM, Sharon et al., 2013, Genome Research)



Activated sludge abundance 1 vs abundance 2 plot

(Extraction bias differential coverage, Albertson et al., 2013, Nature Biotechnology)

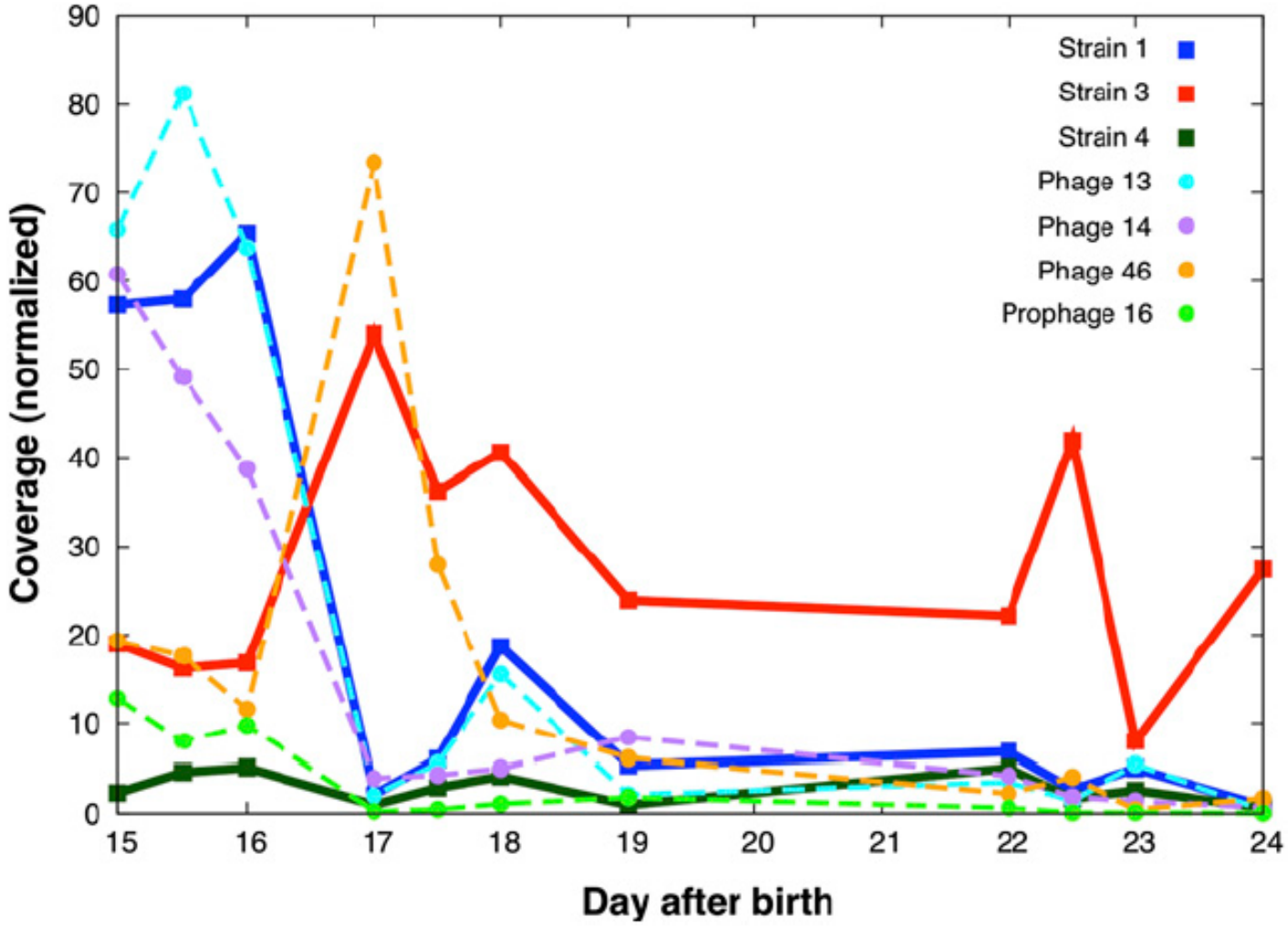
Differential coverage: Extraction bias



(Albertson et al., 2013, Nature Biotechnology)

- Activated sludge DNA was extracted 2 different ways
- Extraction bias resulted in different relative abundances of microbial DNA
- Differential coverage helped separate 12 genomes, including 4 TM7 candidate phyla
- How to guide: https://github.com/MadsAlbertsen/multi-metagenome

Differential coverage: Time series

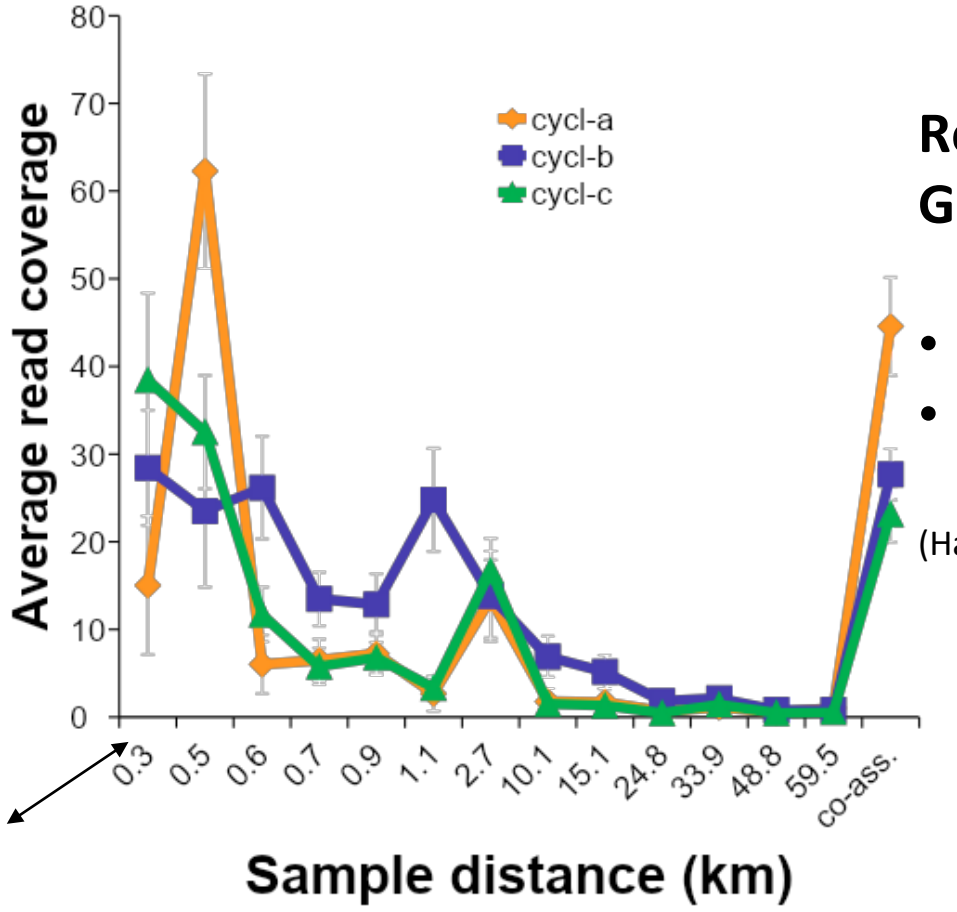


Strain separation

(Sharon et al., 2013, Genome Research)

Differential coverage: Spatial gradient

Hydrocarbon degrading *Cycloclasticus* genome abundances across the Gulf of Mexico seafloor following the 2010 Deepwater Horizon oil spill



Macondo wellhead

Resolved dozens of highly similar Gammaproteobacteria using ESOM with:

- **tetranucleotides frequencies**
- **+ differential coverage**

(Handley et al., 2017, ISME J)

Phylogeny

- Classify bins based on core gene matches (16S rRNA genes are typically not recovered!!!)

- Manual binning or inspection:
  - use protein sequence matches to reference genome database
  - e.g. uniref90 predicted protein database from UniProt (http://www.uniprot.org/uniref/)
  - many are not phylogenetically conserved
  - works best for longer contigs with many genes

node	gene	g+C	coverage	length	%ID	e-value	bin	taxa	lineage
contig-75_1_	2	0.49	32.34137	118,738	92	1.10E-144	alpha-Rhodobacterales	Rhodobacteraceae RepID=Q169T6_	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	82	0.5	32.34137	118,738	65	5.50E-79	alpha-Rhodobacterales	Roseobacter RepID=Q16CQ2_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	38	0.5	32.34137	118,738	57	4.50E-95	alpha-Rhodobacterales	Silicibacter lacuscaerulensis ITI-115	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	21	0.49	32.34137	118,738	78	2.70E-213	alpha-Rhodobacterales	Roseovarius nubinhibens ISM RepID	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	83	0.47	32.34137	118,738	68	1.80E-76	alpha-Rhodobacterales	Pseudoxanthomonas spadix (strain	Bacteria; Proteobacteria; Gammaproteobacteri
contig-75_1_	100	0.46	32.34137	118,738	60	3.50E-70	alpha-Rhodobacterales	Oceanicola batsensis HTCC2597 Rep	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	104	0.5	32.34137	118,738	55	1.90E-57	alpha-Rhodobacterales	Roseovarius sp. 217 RepID=A3VXP4_	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	113	0.52	32.34137	118,738	79	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	39	0.51	32.34137	118,738	79	5.20E-107	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 700	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	59	0.5	32.34137	118,738	66	5.70E-177	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 700	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	64	0.51	32.34137	118,738	77	2.40E-153	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 700	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	65	0.49	32.34137	118,738	77	2.00E-155	alpha-Rhodobacterales	Roseobacter sp. SK209-2-6 RepID=A	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	49	0.48	32.34137	118,738	83	5.70E-120	alpha-Rhodobacterales	Rhodobacteraceae bacterium HTCC	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	14	0.5	32.34137	118,738	37	5.80E-43	alpha-Rhodobacterales	Limnobacter sp. MED105 RepID=A6	Bacteria; Proteobacteria; Betaproteobacteria; B
contig-75_1_	32	0.47	32.34137	118,738	55	5.20E-134	alpha-Rhodobacterales	Silicibacter lacuscaerulensis ITI-115	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	80	0.52	32.34137	118,738	49	4.20E-94	alpha-Rhodobacterales	Thalassiobium sp. R2A62 RepID=C7I	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	110	0.52	32.34137	118,738	63	2.70E-134	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	55	0.5	32.34137	118,738	73	1.50E-184	alpha-Rhodobacterales	Celeribacter baekdonensis B30 Repl	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	81	0.49	32.34137	118,738	63	4.50E-73	alpha-Rhodobacterales	Acaryochloris marina (strain MBIC 1	Bacteria; Cyanobacteria; Oscillatoriophycideae;
contig-75_1_	16	0.5	32.34137	118,738	80	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	47	0.51	32.34137	118,738	76	3.40E-123	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	48	0.52	32.34137	118,738	79	9.60E-127	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	30	0.49	32.34137	118,738	67	0	alpha-Rhodobacterales	Dinoroseobacter shibae (strain DFL	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	77	0.5	32.34137	118,738	63	2.30E-136	alpha-Rhodobacterales	Rhodobacter sp. SW2 RepID=C8RZY	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	28	0.5	32.34137	118,738	75	1.40E-195	alpha-Rhodobacterales	Rhodobacteraceae RepID=I7ESI9_PI	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	106	0.5	32.34137	118,738	87	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	67	0.49	32.34137	118,738	91	5.50E-139	alpha-Rhodobacterales	Alphaproteobacteria RepID=Q1GDV	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	70	0.44	32.34137	118,738	43	2.30E-82	alpha-Rhodobacterales	Roseobacter RepID=Q160N7_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_6_	32	0.41	45.95991	106,871	47	1.60E-91	delta-a1	Clostridium thermocellum RepID=A	Bacteria; Firmicutes; Clostridia; Clostridiales; Cl
contig-75_6_	47	0.34	45.95991	106,871	48	8.60E-56	delta-a1	Nostoc sp. PCC 7107 RepID=K9QA8	Bacteria; Cyanobacteria; Nostocales; Nostocace
contig-75_6_	62	0.42	45.95991	106,871	41	1.40E-41	delta-a1	Sulfurihydrogenibium RepID=B2V76	Bacteria; Aquificae; Aquificales; Hydrogenother
contig-75_6_	48	0.37	45.95991	106,871	49	2.70E-63	delta-a1	Candidatus Kuenenia stuttgartiensis	Bacteria; Planctomycetes; Planctomycetia; Can
contig-75_6_	44	0.35	45.95991	106,871	35	5.00E-26	delta-a1	uncultured bacterium RepID=K2CVX	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	5	0.42	45.95991	106,871	58	9.40E-234	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	18	0.45	45.95991	106,871	66	1.90E-168	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	60	0.4	45.95991	106,871	61	2.30E-147	delta-a1	Desulfatibacillum alkenivorans (stra	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	6	0.37	45.95991	106,871	41	2.00E-147	delta-a1	uncultured bacterium RepID=K2D67	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	10	0.38	45.95991	106,871	31	2.90E-128	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	58	0.37	45.95991	106,871	50	2.90E-102	delta-a1	Cyanothece RepID=B1WVA7_CYAA5	Bacteria; Cyanobacteria; Oscillatoriophycideae;
contig-75_6_	63	0.4	45.95991	106,871	55	1.60E-77	delta-a1	Methylomicrobium album BG8 Repl	Bacteria; Proteobacteria; Gammaproteobacteri
contig-75_6_	7	0.43	45.95991	106,871	29	3.80E-66	delta-a1	Cyanothece sp. (strain PCC 7822) Re	Bacteria; Cyanobacteria; Oscillatoriophycideae;
contig-75_6_	20	0.39	45.95991	106,871	32	9.30E-26	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	34	0.43	45.95991	106,871	69	5.70E-23	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	35	0.42	45.95991	106,871	64	2.70E-276	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	12	0.39	45.95991	106,871	53	4.20E-31	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	14	0.38	45.95991	106,871	50	3.70E-16	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	15	0.42	45.95991	106,871	48	3.70E-126	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	13	0.41	45.95991	106,871	58	9.70E-45	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;

contig 1

contig 2

node	gene	g+C	coverage	length	%ID	e-value	bin	taxa	lineage
contig-75_1_	2	0.49	32.34137	118,738	92	1.10E-144	alpha-Rhodobacterales	Rhodobacteraceae RepID=Q169T6_	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	82	0.5	32.34137	118,738	65	5.50E-79	alpha-Rhodobacterales	Roseobacter RepID=Q16CQ2_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	38	0.5	32.34137	118,738	57	4.50E-95	alpha-Rhodobacterales	Silicibacter lacuscaerulensis ITI-115	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	21	0.49	32.34137	118,738	78	2.70E-213	alpha-Rhodobacterales	Roseovarius nubinhibens ISM RepID	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	83	0.47	32.34137	118,738	68	1.80E-76	alpha-Rhodobacterales	Pseudoxanthomonas spadix (strain	Bacteria; Proteobacteria; Gammaproteobacteri
contig-75_1_	100	0.46	32.34137	118,738	60	3.50E-70	alpha-Rhodobacterales	Oceanicola batsensis HTCC2597 Rep	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	104	0.5	32.34137	118,738	55	1.90E-57	alpha-Rhodobacterales	Roseovarius sp. 217 RepID=A3VXP4_	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	113	0.52	32.34137	118,738	79	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	39	0.51	32.34137	118,738	79	5.20E-107	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 700	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	59	0.5	32.34137	118,738	66	5.70E-177	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 700	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	64	0.51	32.34137	118,738	77	2.40E-153	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 700	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	65	0.49	32.34137	118,738	77	2.00E-155	alpha-Rhodobacterales	Roseobacter sp. SK209-2-6 RepID=A	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	49	0.48	32.34137	118,738	83	5.70E-120	alpha-Rhodobacterales	Rhodobacteraceae bacterium HTCC	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	14	0.5	32.34137	118,738	37	5.80E-43	alpha-Rhodobacterales	Limnobacter sp. MED105 RepID=A6	Bacteria; Proteobacteria; Betaproteobacteria; B
contig-75_1_	32	0.47	32.34137	118,738	55	5.20E-134	alpha-Rhodobacterales	Silicibacter lacuscaerulensis ITI-115	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	80	0.52	32.34137	118,738	49	4.20E-94	alpha-Rhodobacterales	Thalassiobium sp. R2A62 RepID=C7I	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	110	0.52	32.34137	118,738	63	2.70E-134	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	55	0.5	32.34137	118,738	73	1.50E-184	alpha-Rhodobacterales	Celeribacter baekdonensis B30 Repl	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	81	0.49	32.34137	118,738	63	4.50E-73	alpha-Rhodobacterales	Acaryochloris marina (strain MBIC 1	Bacteria; Cyanobacteria; Oscillatoriophycideae;
contig-75_1_	16	0.5	32.34137	118,738	80	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	47	0.51	32.34137	118,738	76	3.40E-123	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	48	0.52	32.34137	118,738	79	9.60E-127	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	30	0.49	32.34137	118,738	67	0	alpha-Rhodobacterales	Dinoroseobacter shibae (strain DFL	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	77	0.5	32.34137	118,738	63	2.30E-136	alpha-Rhodobacterales	Rhodobacter sp. SW2 RepID=C8RZY	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	28	0.5	32.34137	118,738	75	1.40E-195	alpha-Rhodobacterales	Rhodobacteraceae RepID=I7ESI9_PI	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	106	0.5	32.34137	118,738	87	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	67	0.49	32.34137	118,738	91	5.50E-139	alpha-Rhodobacterales	Alphaproteobacteria RepID=Q1GDV	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	70	0.44	32.34137	118,738	43	2.30E-82	alpha-Rhodobacterales	Roseobacter RepID=Q160N7_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_6_	32	0.41	45.95991	106,871	47	1.60E-91	delta-a1	Clostridium thermocellum RepID=A	Bacteria; Firmicutes; Clostridia; Clostridiales; Cl
contig-75_6_	47	0.34	45.95991	106,871	48	8.60E-56	delta-a1	Nostoc sp. PCC 7107 RepID=K9QA8	Bacteria; Cyanobacteria; Nostocales; Nostocace
contig-75_6_	62	0.42	45.95991	106,871	41	1.40E-41	delta-a1	Sulfurihydrogenibium RepID=B2V76	Bacteria; Aquificae; Aquificales; Hydrogenother
contig-75_6_	48	0.37	45.95991	106,871	49	2.70E-63	delta-a1	Candidatus Kuenenia stuttgartiensis	Bacteria; Planctomycetes; Planctomycetia; Can
contig-75_6_	44	0.35	45.95991	106,871	35	5.00E-26	delta-a1	uncultured bacterium RepID=K2CVX	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	5	0.42	45.95991	106,871	58	9.40E-234	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	18	0.45	45.95991	106,871	66	1.90E-168	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	60	0.4	45.95991	106,871	61	2.30E-147	delta-a1	Desulfatibacillum alkenivorans (stra	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	6	0.37	45.95991	106,871	41	2.00E-147	delta-a1	uncultured bacterium RepID=K2D67	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	10	0.38	45.95991	106,871	31	2.90E-128	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	58	0.37	45.95991	106,871	50	2.90E-102	delta-a1	Cyanothece RepID=B1WVA7_CYAA5	Bacteria; Cyanobacteria; Oscillatoriophycideae;
contig-75_6_	63	0.4	45.95991	106,871	55	1.60E-77	delta-a1	Methylomicrobium album BG8 Repl	Bacteria; Proteobacteria; Gammaproteobacteri
contig-75_6_	7	0.43	45.95991	106,871	29	3.80E-66	delta-a1	Cyanothece sp. (strain PCC 7822) Re	Bacteria; Cyanobacteria; Oscillatoriophycideae;
contig-75_6_	20	0.39	45.95991	106,871	32	9.30E-26	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	34	0.43	45.95991	106,871	69	5.70E-23	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	35	0.42	45.95991	106,871	64	2.70E-276	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	12	0.39	45.95991	106,871	53	4.20E-31	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	14	0.38	45.95991	106,871	50	3.70E-16	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	15	0.42	45.95991	106,871	48	3.70E-126	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	13	0.41	45.95991	106,871	58	9.70E-45	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;

node	gene	g+C	coverage	length	%ID	e-value	bin	taxa	lineage
contig-75_1_	2	0.49	32.34137	118,738	92	1.10E-144	alpha-Rhodobacterales	Rhodobacteraceae RepID=Q169T6_	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	82	0.5	32.34137	118,738	65	5.50E-79	alpha-Rhodobacterales	Roseobacter RepID=Q16CQ2_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	38	0.5	32.34137	118,738	57	4.50E-95	alpha-Rhodobacterales	Silicibacter lacuscaerulensis ITI-115	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	21	0.49	32.34137	118,738	78	2.70E-213	alpha-Rhodobacterales	Roseovarius nubinhibens ISM RepID	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	83	0.47	32.34137	118,738	68	1.80E-76	alpha-Rhodobacterales	Pseudoxanthomonas spadix (strain	Bacteria; Proteobacteria; Gammaproteobacter
contig-75_1_	100	0.46	32.34137	118,738	60	3.50E-70	alpha-Rhodobacterales	Oceanicola batsensis HTCC2597 Re	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	104	0.5	32.34137	118,738	55	1.90E-57	alpha-Rhodobacterales	Roseovarius sp. 217 RepID=A3VXP4	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	113	0.52	32.34137	118,738	79	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC2	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	39	0.51	32.34137	118,738	79	5.20E-107	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 70	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	59	0.5	32.34137	118,738	66	5.70E-177	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 70	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	64	0.51	32.34137	118,738	77	2.40E-153	alpha-Rhodobacterales	Ruegeria pomeroyi (strain ATCC 70	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	65	0.49	32.34137	118,738	77	2.00E-155	alpha-Rhodobacterales	Roseobacter sp. SK209-2-6 RepID=A	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	49	0.48	32.34137	118,738	83	5.70E-120	alpha-Rhodobacterales	Rhodobacteraceae bacterium HTCC	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	14	0.5	32.34137	118,738	37	5.80E-43	alpha-Rhodobacterales	Limnobacter sp. MED105 RepID=A6	Bacteria; Proteobacteria; Betaproteobacteria;
contig-75_1_	32	0.47	32.34137	118,738	55	5.20E-134	alpha-Rhodobacterales	Silicibacter lacuscaerulensis ITI-115	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	80	0.52	32.34137	118,738	49	4.20E-94	alpha-Rhodobacterales	Thalassiobium sp. R2A62 RepID=C7	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	110	0.52	32.34137	118,738	63	2.70E-134	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC2	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	55	0.5	32.34137	118,738	73	1.50E-184	alpha-Rhodobacterales	Celeribacter baekdonensis B30 Rep	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	81	0.49	32.34137	118,738	63	4.50E-73	alpha-Rhodobacterales	Acaryochloris marina (strain MBIC	Bacteria; Cyanobacteria; Oscillatoriophycideae
contig-75_1_	16	0.5	32.34137	118,738	80	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC2	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	47	0.51	32.34137	118,738	76	3.40E-123	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC2	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	48	0.52	32.34137	118,738	79	9.60E-127	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC2	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	30	0.49	32.34137	118,738	67	0	alpha-Rhodobacterales	Dinoroseobacter shibae (strain DFL	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	77	0.5	32.34137	118,738	63	2.30E-136	alpha-Rhodobacterales	Rhodobacter sp. SW2 RepID=C8RZY	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	28	0.5	32.34137	118,738	75	1.40E-195	alpha-Rhodobacterales	Rhodobacteraceae RepID=I7ESI9_P	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	106	0.5	32.34137	118,738	87	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC2	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	67	0.49	32.34137	118,738	91	5.50E-139	alpha-Rhodobacterales	Alphaproteobacteria RepID=Q1GDV	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	70	0.44	32.34137	118,738	43	2.30E-82	alpha-Rhodobacterales	Roseobacter RepID=Q160N7_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_6_	32	0.41	45.95991	106,871	47	1.60E-91	delta-a1	Clostridium thermocellum RepID=A	Bacteria; Firmicutes; Clostridia; Clostridiales; Cl
contig-75_6_	47	0.34	45.95991	106,871	48	8.60E-56	delta-a1	Nostoc sp. PCC 7107 RepID=K9QA8	Bacteria; Cyanobacteria; Nostocales; Nostocac
contig-75_6_	62	0.42	45.95991	106,871	41	1.40E-41	delta-a1	Sulfurihydrogenibium RepID=B2V7	Bacteria; Aquificae; Aquificales; Hydrogenothe
contig-75_6_	48	0.37	45.95991	106,871	49	2.70E-63	delta-a1	Candidatus Kuenenia stuttgartiensi	Bacteria; Planctomycetes; Planctomycetia; Can
contig-75_6_	44	0.35	45.95991	106,871	35	5.00E-26	delta-a1	uncultured bacterium RepID=K2CV	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	5	0.42	45.95991	106,871	58	9.40E-234	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	18	0.45	45.95991	106,871	66	1.90E-168	delta-a1	Desulfobacterium autotrophicum (	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	60	0.4	45.95991	106,871	61	2.30E-147	delta-a1	Desulfatibacillum alkenivorans (stra	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	6	0.37	45.95991	106,871	41	2.00E-147	delta-a1	uncultured bacterium RepID=K2D6	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	10	0.38	45.95991	106,871	31	2.90E-128	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	58	0.37	45.95991	106,871	50	2.90E-102	delta-a1	Cyanothece RepID=B1WVA7_CYAA	Bacteria; Cyanobacteria; Oscillatoriophycideae
contig-75_6_	63	0.4	45.95991	106,871	55	1.60E-77	delta-a1	Methylomicrobium album BG8 Rep	Bacteria; Proteobacteria; Gammaproteobacter
contig-75_6_	7	0.43	45.95991	106,871	29	3.80E-66	delta-a1	Cyanothece sp. (strain PCC 7822) R	Bacteria; Cyanobacteria; Oscillatoriophycideae
contig-75_6_	20	0.39	45.95991	106,871	32	9.30E-26	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	34	0.43	45.95991	106,871	69	5.70E-23	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	35	0.42	45.95991	106,871	64	2.70E-276	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	12	0.39	45.95991	106,871	53	4.20E-31	delta-a1	Desulfobacterium autotrophicum (	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	14	0.38	45.95991	106,871	50	3.70E-16	delta-a1	Desulfobacterium autotrophicum (	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	15	0.42	45.95991	106,871	48	3.70E-126	delta-a1	Desulfobacterium autotrophicum (	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	13	0.41	45.95991	106,871	58	9.70E-45	delta-a1	Desulfobacterium autotrophicum (	Bacteria; Proteobacteria; Deltaproteobacteria;

contig 1

Alphaproteobacteria
G+C = 50%
Coverage = 32 x

contig 2

Deltaproteobacteria
G+C = 40%
Coverage = 46 x

node	gene	g+C	coverage	length	%ID	e-value	bin	taxa	lineage
contig-75_1_	2	0.49	32.34137	118,738	92	1.10E-144	alpha-Rhodobacterales	Rhodobacteraceae RepID=Q169T6_	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	82	0.5	32.34137	118,738	65	5.50E-79	alpha-Rhodobacterales	Roseobacter RepID=Q16CQ2_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	38	0.5	32.34137	118,738	57	4.50E-95	alpha-Rhodobacterales	Silicibacter lacuscaerulensis ITI-115	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	21	0.49	32.34137	118,738	78	2.70E-213	alpha-Rhodobacterales	Roseovarius nubinhibens ISM RepID	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	83	0.47	32.34137	118,738	68	1.80E-76	alpha-Rhodobacterales	Pseudoxanthomonas spadix (strain	Bacteria; Proteobacteria; Gammaproteobacter
contig-75_1_	100	0.46	32.34137	118,738	60	3.50E-70	alpha-Rhodobacterales	Oceanicola batsensis HTCC2597 Re	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	104	0.5	32.34137	118,738	55	1.90E-57	alpha-Rhodobacterales	Roseovarius sp. 217 RepID=A3VXP4	Bacteria; Proteobacteria; Alphaproteobacteria;

contig 1

contig-75_1_	47	0.51	32.34137	118,738	76	3.40E-123	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	48	0.52	32.34137	118,738	79	9.60E-127	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	30	0.49	32.34137	118,738	67	0	alpha-Rhodobacterales	Dinoroseobacter shibae (strain DFL	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	77	0.5	32.34137	118,738	63	2.30E-136	alpha-Rhodobacterales	Rhodobacter sp. SW2 RepID=C8RZY	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	28	0.5	32.34137	118,738	75	1.40E-195	alpha-Rhodobacterales	Rhodobacteraceae RepID=I7ESI9_PI	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	106	0.5	32.34137	118,738	87	0	alpha-Rhodobacterales	Rhodobacterales bacterium HTCC22	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	67	0.49	32.34137	118,738	91	5.50E-139	alpha-Rhodobacterales	Alphaproteobacteria RepID=Q1GDV	Bacteria; Proteobacteria; Alphaproteobacteria;
contig-75_1_	70	0.44	32.34137	118,738	43	2.30E-82	alpha-Rhodobacterales	Roseobacter RepID=Q160N7_ROSD	Bacteria; Proteobacteria; Alphaproteobacteria;

contig 2

contig-75_6_	32	0.41	45.95991	106,871	47	1.60E-91	delta-a1	Clostridium thermocellum RepID=A	Bacteria; Firmicutes; Clostridia; Clostridiales; Cl
contig-75_6_	47	0.34	45.95991	106,871	48	8.60E-56	delta-a1	Nostoc sp. PCC 7107 RepID=K9QA8	Bacteria; Cyanobacteria; Nostocales; Nostocace
contig-75_6_	62	0.42	45.95991	106,871	41	1.40E-41	delta-a1	Sulfurihydrogenibium RepID=B2V76	Bacteria; Aquificae; Aquificales; Hydrogenothe
contig-75_6_	48	0.37	45.95991	106,871	49	2.70E-63	delta-a1	Candidatus Kuenenia stuttgartiensis	Bacteria; Planctomycetes; Planctomycetia; Can
contig-75_6_	44	0.35	45.95991	106,871	35	5.00E-26	delta-a1	uncultured bacterium RepID=K2CVX	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	5	0.42	45.95991	106,871	58	9.40E-234	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	18	0.45	45.95991	106,871	66	1.90E-168	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	60	0.4	45.95991	106,871	61	2.30E-147	delta-a1	Desulfatibacillum alkenivorans (stra	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	6	0.37	45.95991	106,871	41	2.00E-147	delta-a1	uncultured bacterium RepID=K2D67	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	10	0.38	45.95991	106,871	31	2.90E-128	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;

contig-75_6_	60	0.4	45.95991	106,871	61	2.30E-147	delta-a1	Desulfatibacillum alkenivorans (stra	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	6	0.37	45.95991	106,871	41	2.00E-147	delta-a1	uncultured bacterium RepID=K2D6	Bacteria; Firmicutes; Clostridia; Clostridiales
contig-75_6_	10	0.38	45.95991	106,871	31	2.90E-128	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	58	0.37	45.95991	106,871	50	2.90E-102	delta-a1	Cyanothece RepID=B1WVA7_CYAA	Bacteria; Cyanobacteria; Oscillatoriophycideae
contig-75_6_	63	0.4	45.95991	106,871	55	1.60E-77	delta-a1	Methylomicrobium album BG8 Rep	Bacteria; Proteobacteria; Gammaproteobacter
contig-75_6_	7	0.43	45.95991	106,871	29	3.80E-66	delta-a1	Cyanothece sp. (strain PCC 7822) R	Bacteria; Cyanobacteria; Oscillatoriophycideae
contig-75_6_	20	0.39	45.95991	106,871	32	9.30E-26	delta-a1	Desulfobacula toluolica (strain DSM	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	34	0.43	45.95991	106,871	69	5.70E-23	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	35	0.42	45.95991	106,871	64	2.70E-276	delta-a1	Desulfobacter postgatei 2ac9 RepID	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	12	0.39	45.95991	106,871	53	4.20E-31	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	14	0.38	45.95991	106,871	50	3.70E-16	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	15	0.42	45.95991	106,871	48	3.70E-126	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;
contig-75_6_	13	0.41	45.95991	106,871	58	9.70E-45	delta-a1	Desulfobacterium autotrophicum (s	Bacteria; Proteobacteria; Deltaproteobacteria;

Short contig removal

Shorter contigs are harder to bin based on:

- **Compositional signatures**
- **Phylogeny affiliation to reference databases**

There is no rule, but we typically remove contigs <1 kbp long. This tends to remove a large number of contigs, which can be:

- **problematic to bin, or**
- **contain truncated genes**

Manual vs automated binning

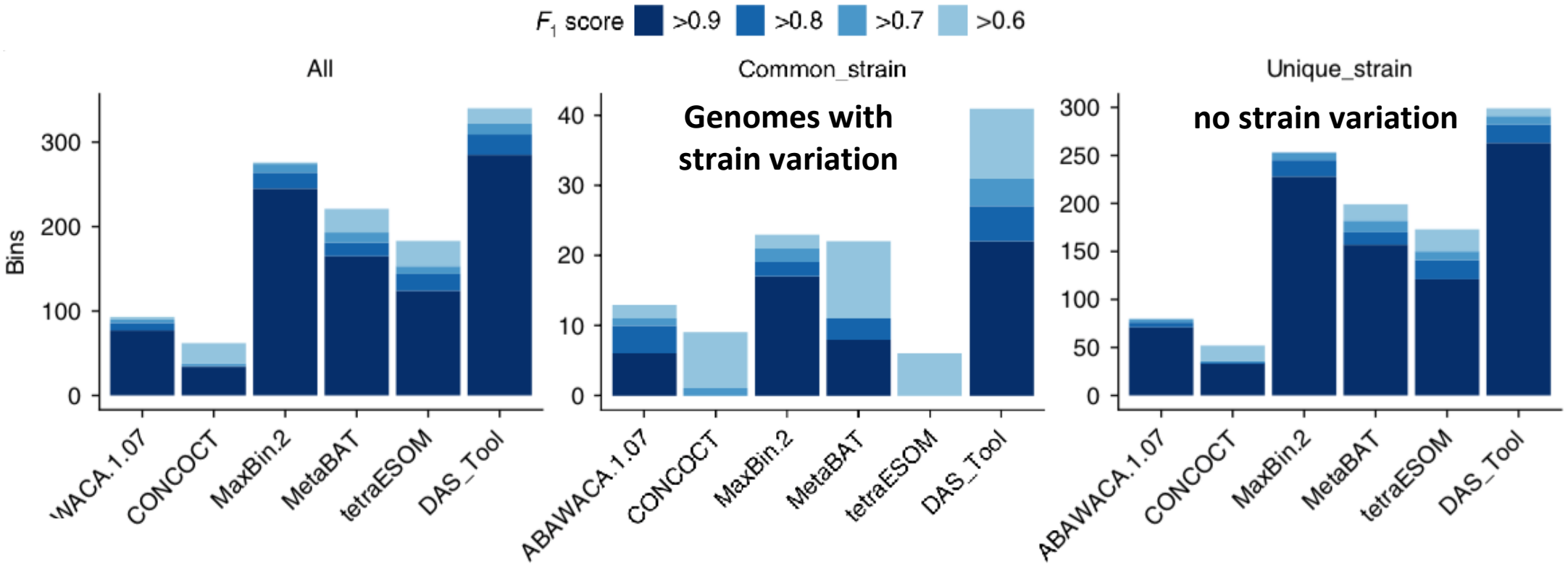
Manual:

- **traditional**
- **laborious**
- **highly effective**

Automated:

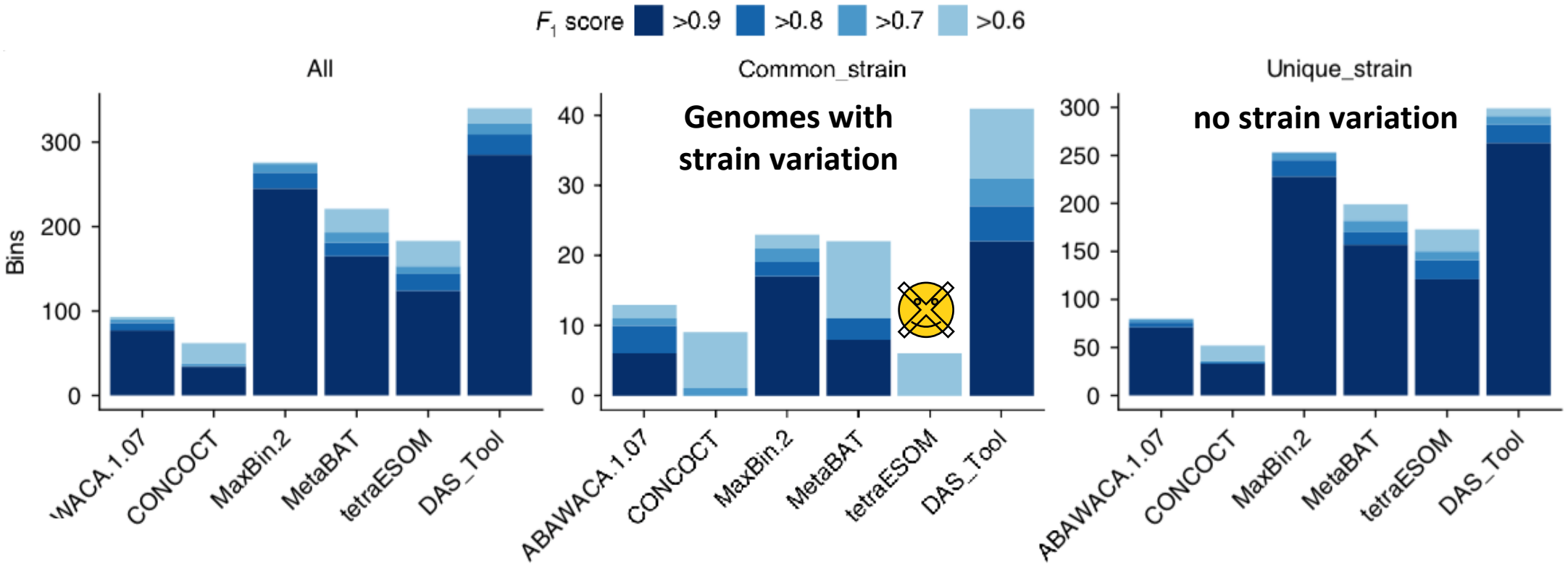
- **fast**
- **highly variable results between tools**
- **can co-bin highly abundant contigs from distinct genomes**
- **tend to discard small genomes (e.g. viruses)**

Multi-binning strategy



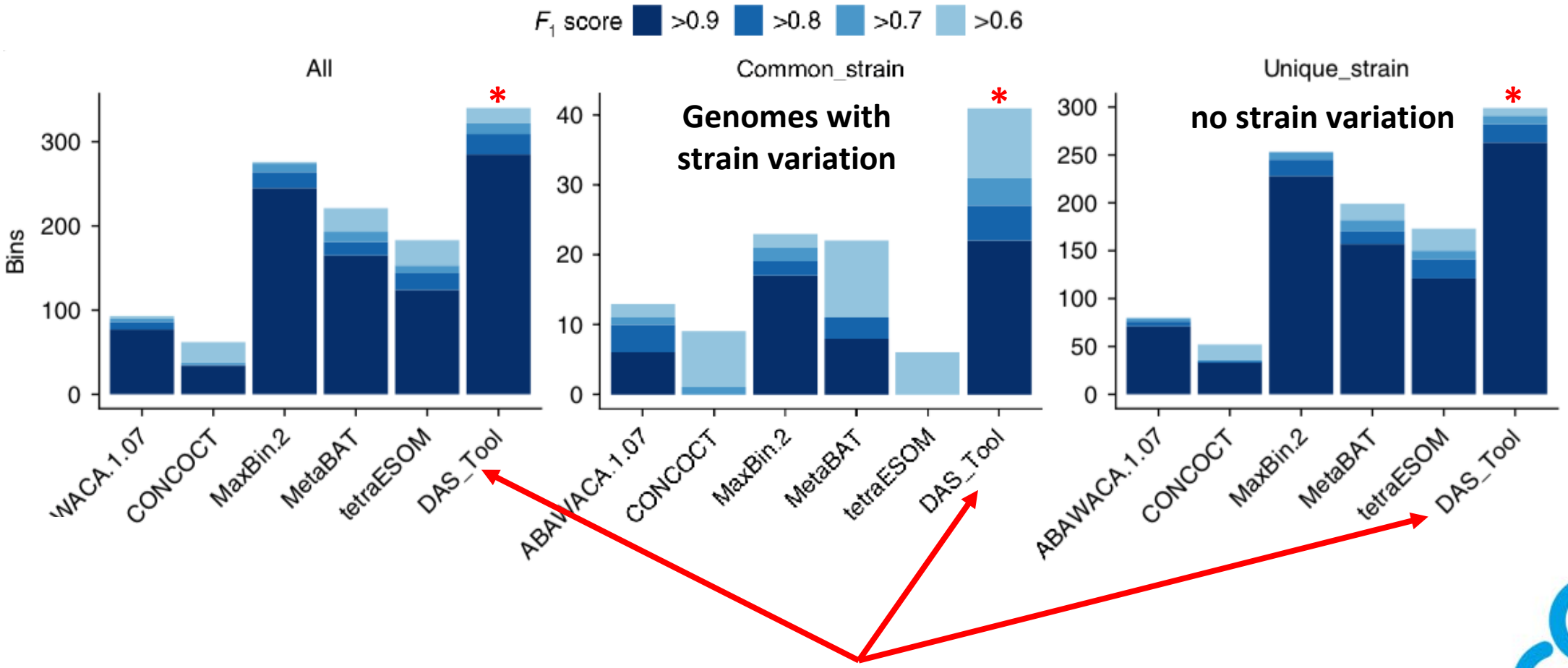
(Sieber et al., 2018, Nature Microbiology)

Multi-binning strategy



(Sieber et al., 2018, Nature Microbiology)

Multi-binning strategy



Pick-the-winner of multiple strategies

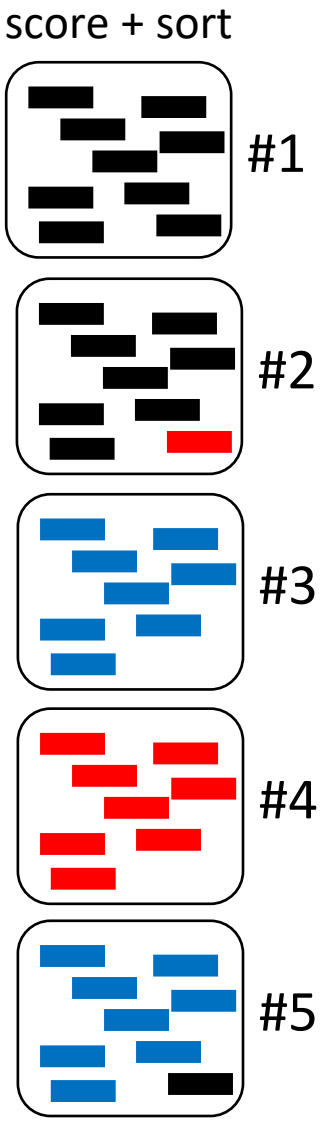
(Sieber et al., 2018, Nature Microbiology)

Multi-binning strategy: pick winner

DAS Tool:

repeat

1. **Score bins**
2. **Sort from best to worst**
3. **Pick top bin**
4. **Delete identical contigs in other bins**



Multi-binning strategy: pick winner

DAS Tool:

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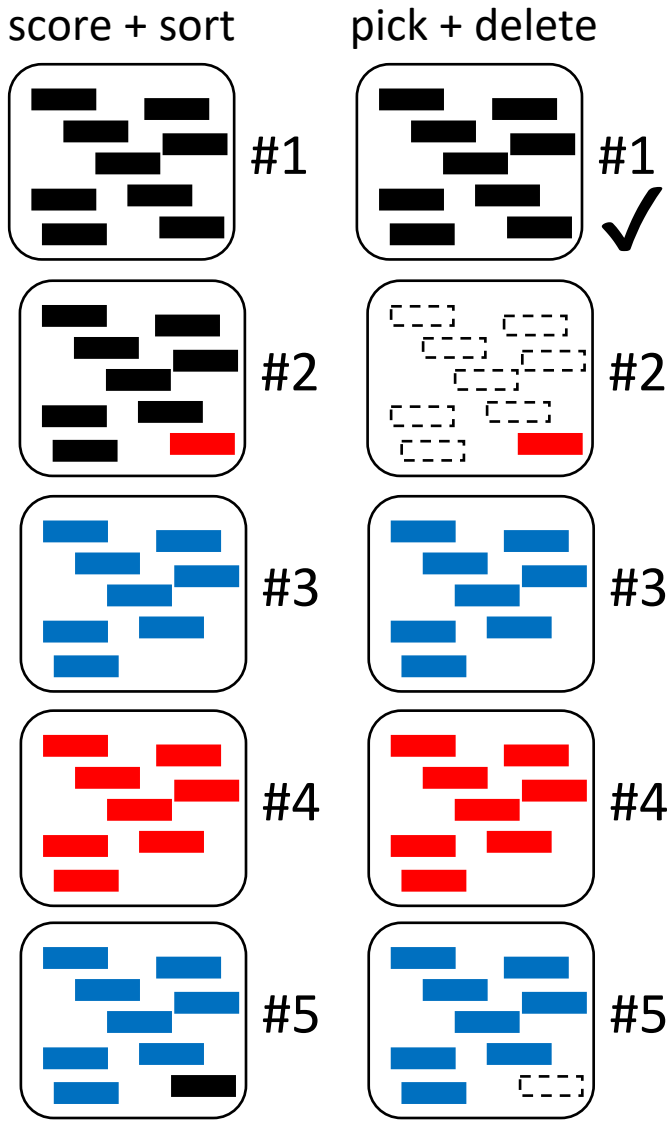

Multi-binning strategy: pick winner

DAS Tool:

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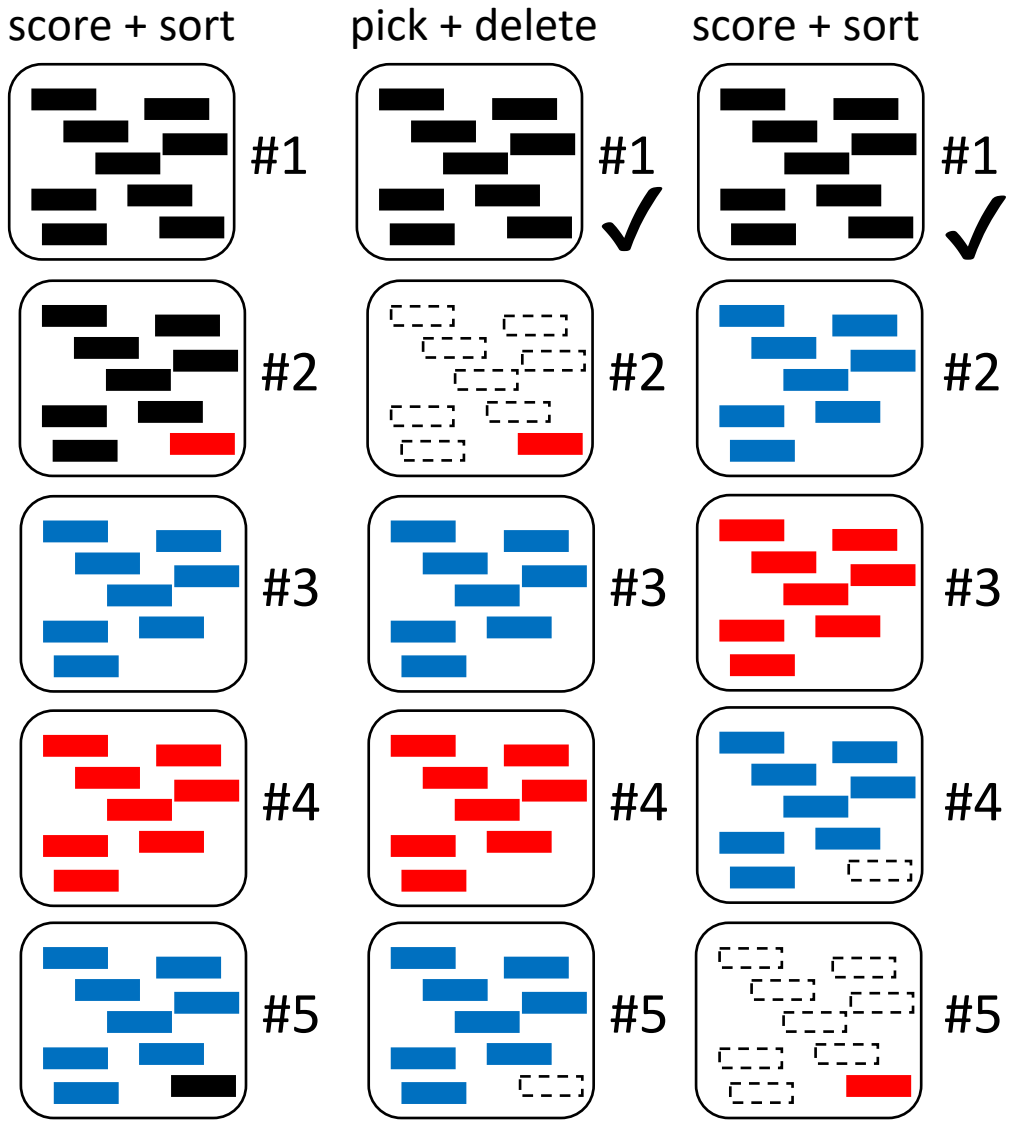

Multi-binning strategy: pick winner

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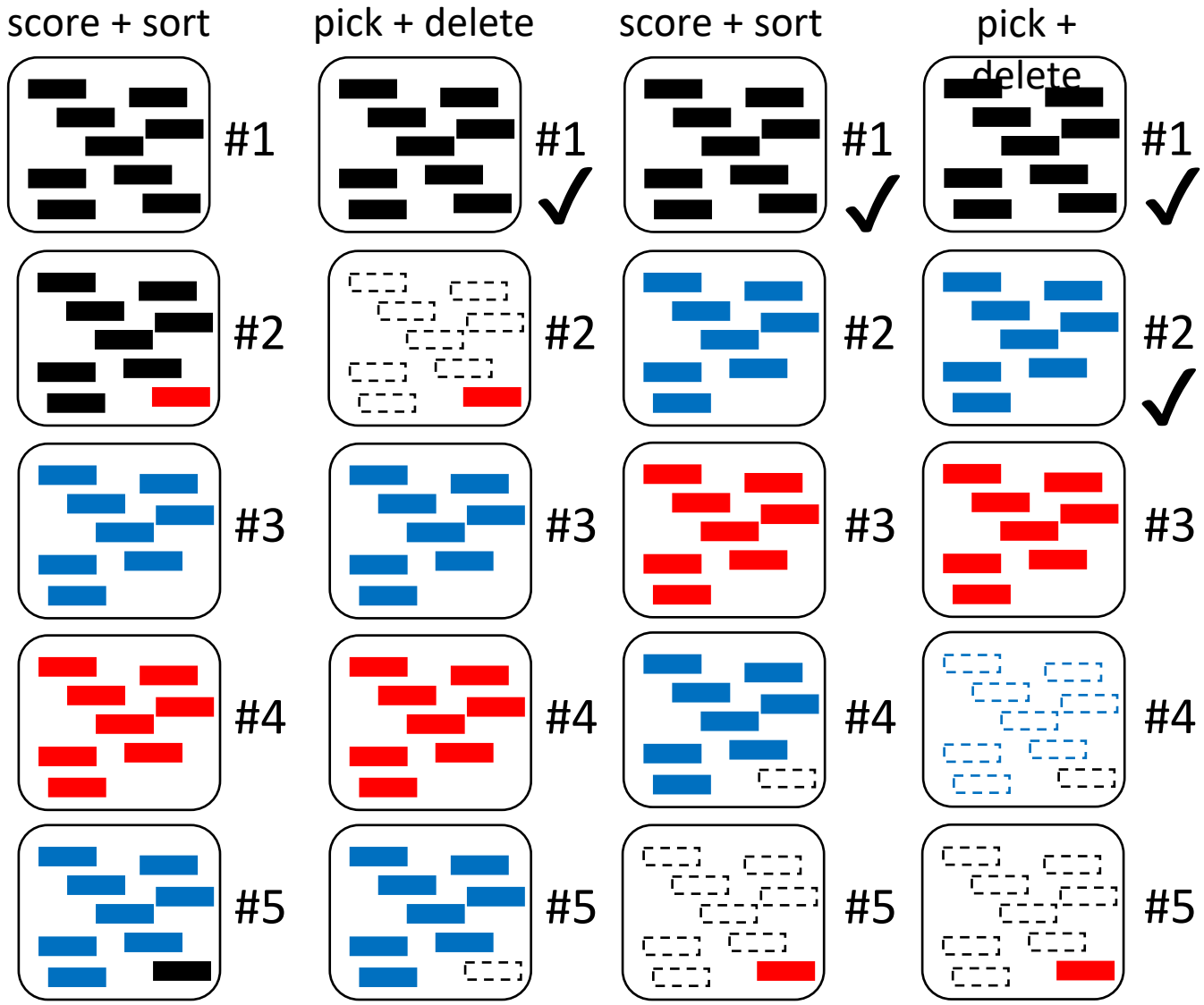


Bin evaluation

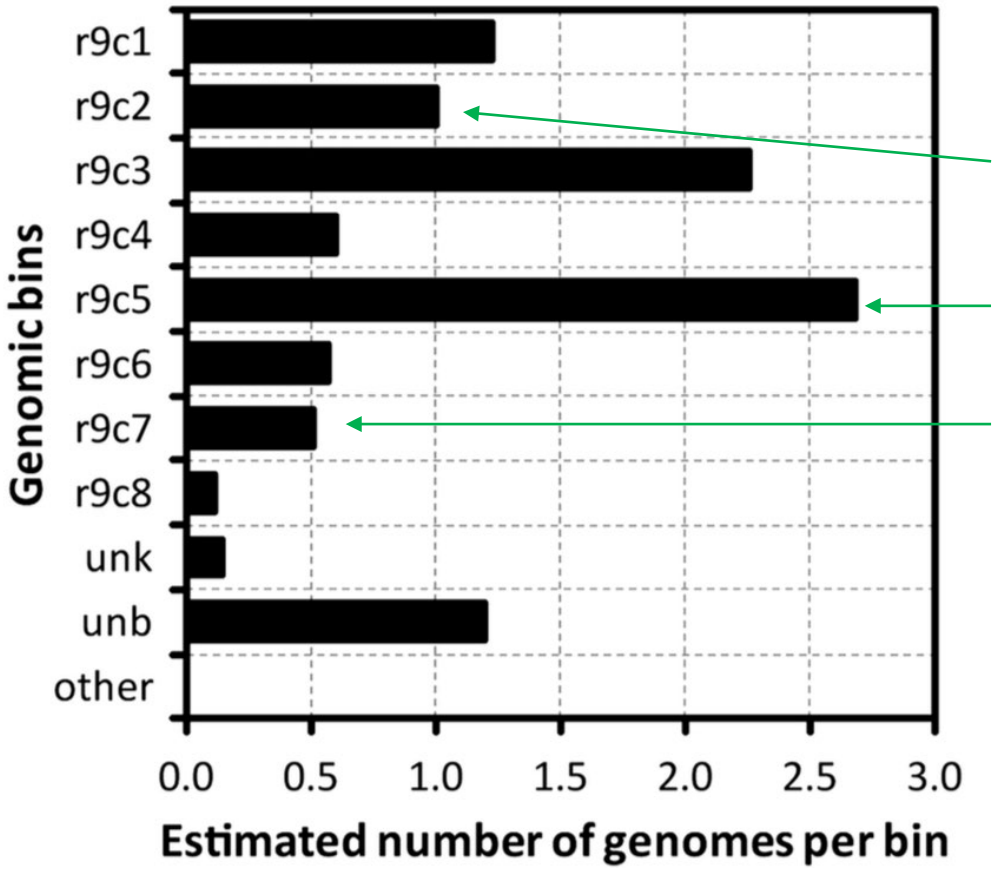
Determine presence of a set of single copy core genes:

- **Present/Missing = completeness/incompleteness**
- **Duplicates = strain heterogeneity (co-binned closely-related genomes)**
- **Phyla mismatches (duplicates or unique core genes) = contamination**

Example: CheckM (https://ecogenomics.github.io/CheckM/)

Estimating genome completeness

- **Completeness =** percentage of single copy core genes present out of a given set



Groundwater community in Colorado

Bin with exactly 1 genome

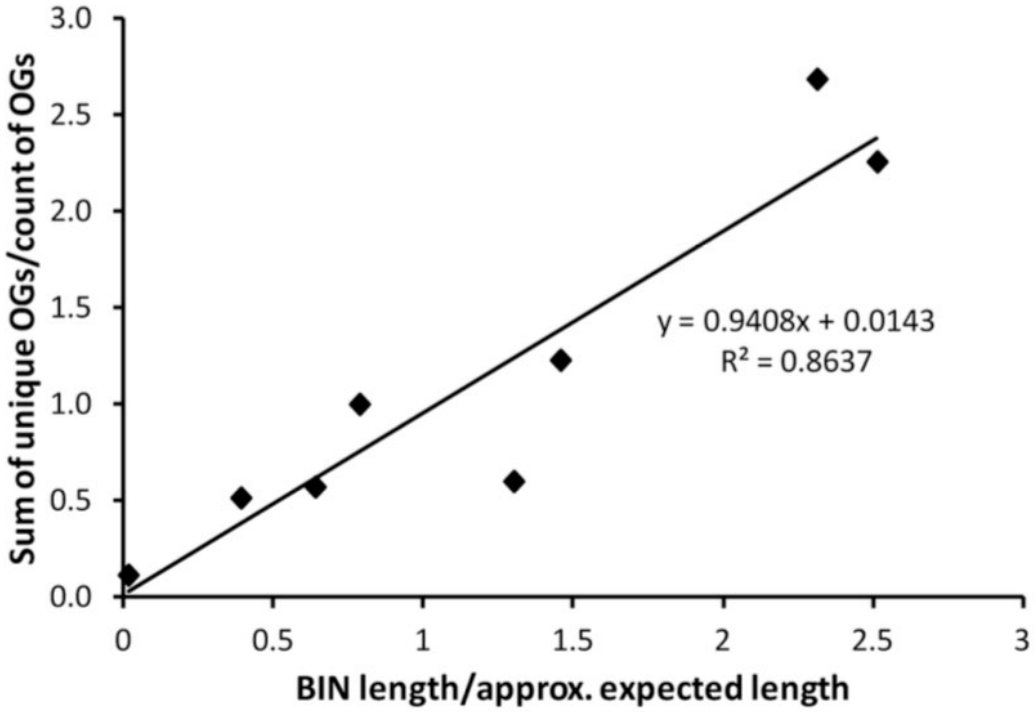
Bin with >2.5 genomes

Bin with ½ a genome

(Handley et al., 2013, ISME J)

Estimating genome completeness

- **Completeness = percentage of single copy core genes present out of a given set**
- **Is “often” equivalent to expected genome size based on close relatives**
- **CAVEAT: genome sizes can vary substantially between close relatives**



(Handley et al., 2013, ISME J)

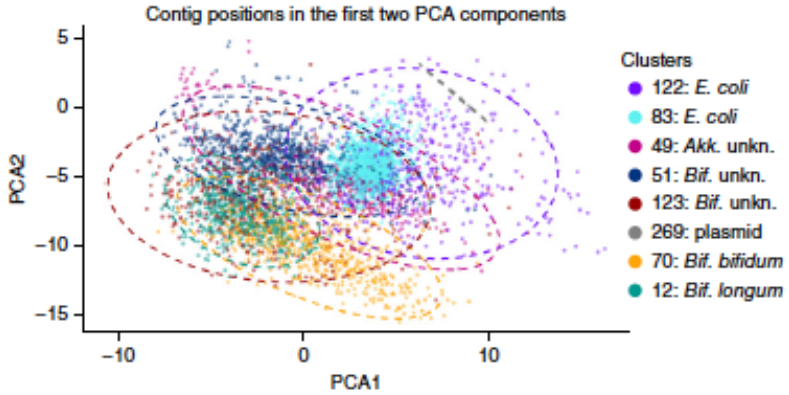
Automated binning!!!

MetaBAT (https://bitbucket.org/berkeleylab/metabat):

- **Partial multi-parameter binning tool**
- **Combines:**
  - **tetranucleotide frequencies**
  - **(Differential) coverage**
- **Iterative binning using a modified k-medoid (like k-means) clustering algorithm: iteratively forms clusters, keeps large clusters, dissolves small clusters, and then attempts to recruit ex-small cluster contigs to large clusters**
- **Does not use taxonomy**

Automated binning!!!

CONCOCT (https://github.com/BinPro/CONCOCT):



- **Partial multi-parameter binning tool**
- **Combines:**
  - **tetranucleotide frequencies**
  - **(Differential) coverage**
- **Gaussian mixture models used to cluster contigs**
- **Cluster evaluation: checks bins for a set of single copy core genes to determine bin completeness and purity**
- **Does not use taxonomy**

Group discussion

Thinking about your project and things you have learned:

- What is your biological question?
- What is your experimental design?
- How much read depth do you need?
- Are you going to assemble?
- Do you have enough replicates?
- What computational resources do you have access to?

Binning (part 3)

Task: Binning - DAS_Tool and CheckM

Go to Github MGSS webpage

Tasks:

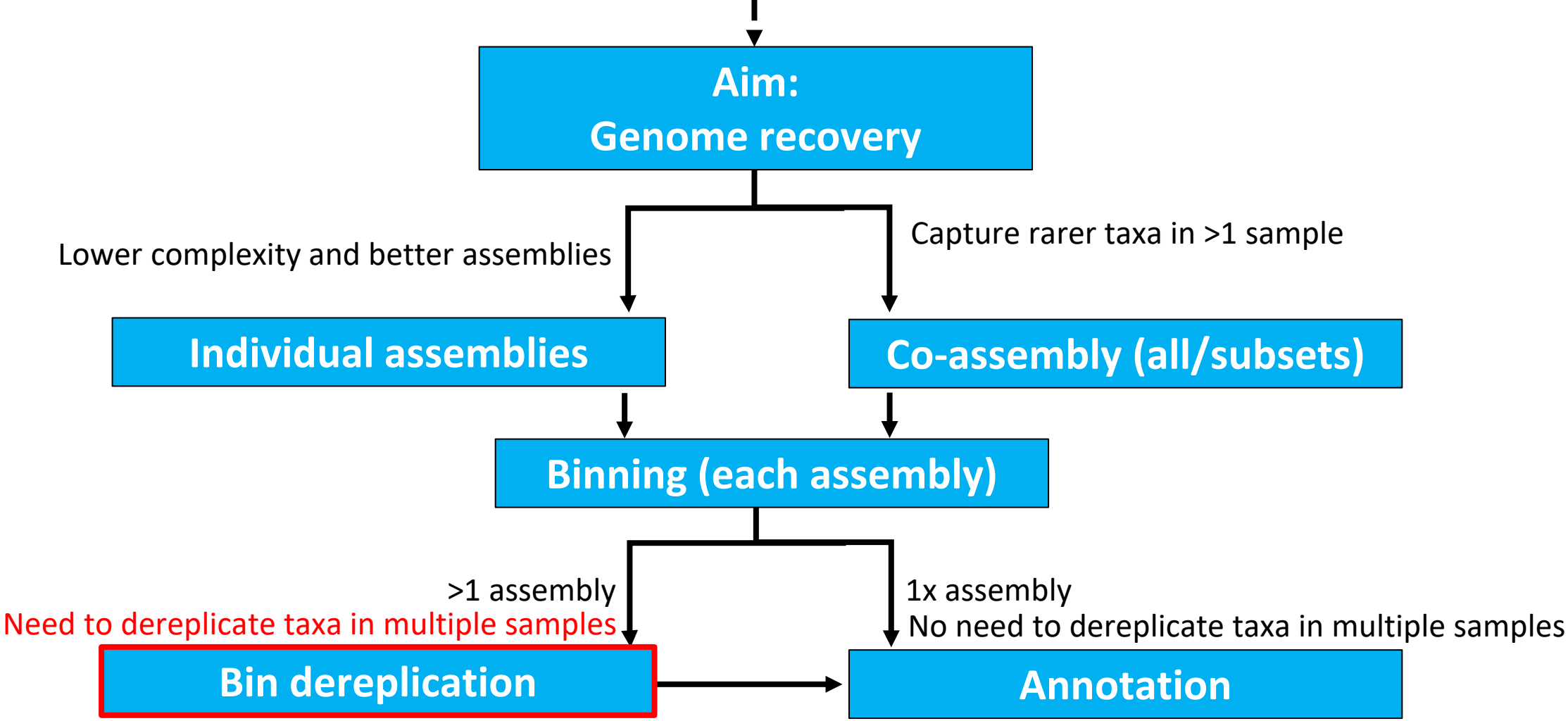
- ✓ Short contig removal
- ✓ Read mapping
- ✓ Multi-binning strategy (Metabat and Maxbin)
- Bin dereplication via DAS_Tool
  - Pick-the-winner bin dereplication
- Evaluating bins using CheckM

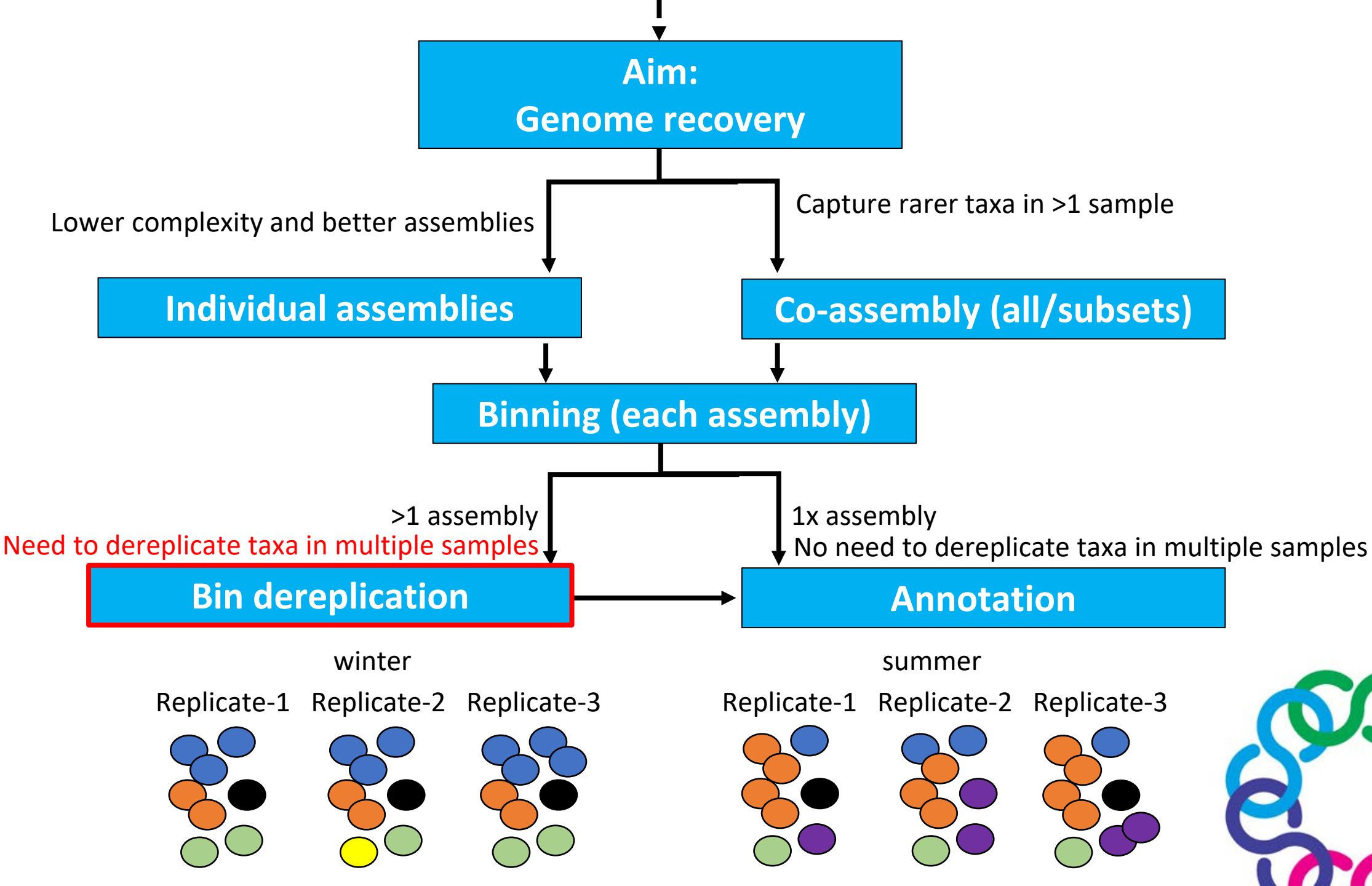
Binning (part 4)

Binning: other considerations

Dealing with:

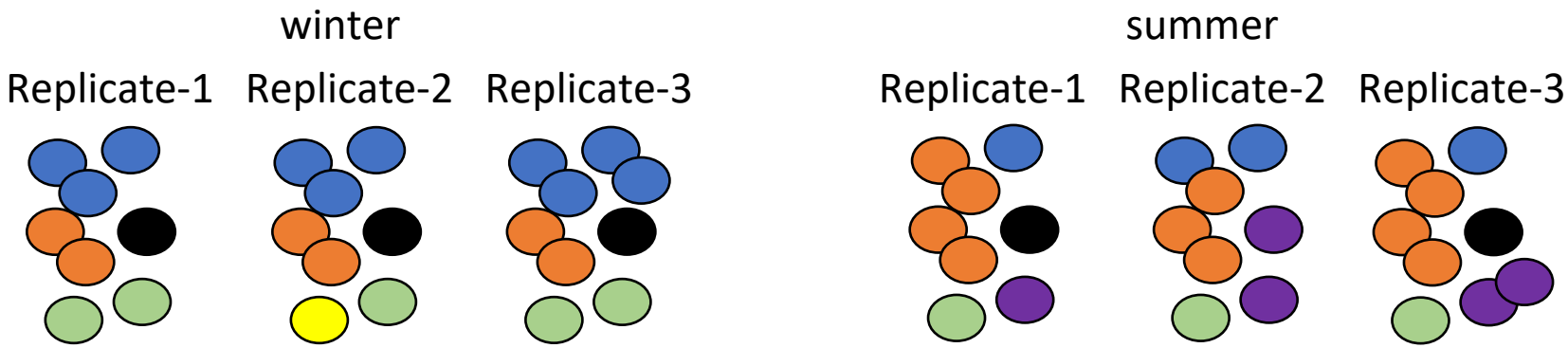
- **Duplicate bins across multiple assemblies (samples)**
- **Viral and eukaryotic bins**
- **Unassembled data**
- **Organisms that possess minimal genomes**


Aim:
Genome recovery
Lower complexity and better assemblies
Capture rarer taxa in >1 sample
Individual assemblies
Co-assembly (all/subsets)
Binning (each assembly)
>1 assembly
Need to dereplicate taxa in multiple samples
1x assembly
No need to dereplicate taxa in multiple samples
Bin dereplication
Annotation

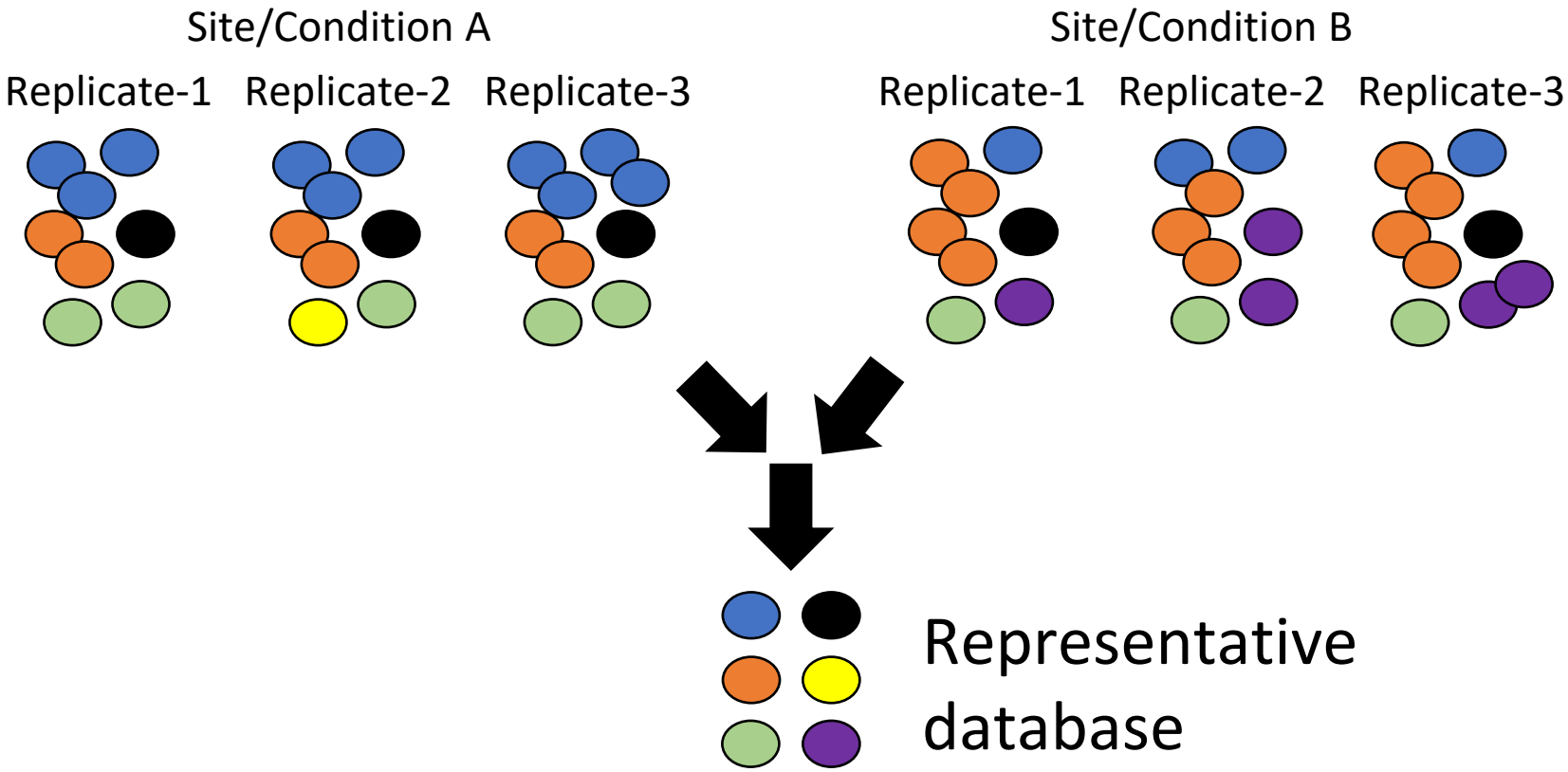

Aim:
Genome recovery
Lower complexity and better assemblies
Capture rarer taxa in >1 sample
Individual assemblies
Co-assembly (all/subsets)
Binning (each assembly)
>1 assembly
Need to dereplicate taxa in multiple samples
1x assembly
No need to dereplicate taxa in multiple samples
Bin dereplication
Annotation
winter
Replicate-1
Replicate-2
Replicate-3
summer
Replicate-1
Replicate-2
Replicate-3

Multiple samples: duplicate genomes

- **Remove repeat genomes**
- **For example, separate assemblies of the communities below would yield duplicate genomes for:** 



Multiple samples: duplicate genomes



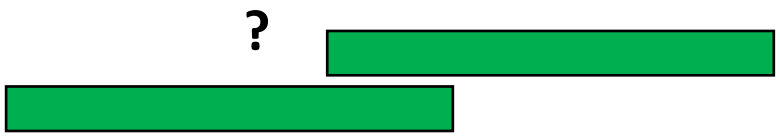
Goal:

- **work with 1 genomic database**
- **generate sample-genome coverage table**
- **if applicable, map transcript data and generate counts table**

Multiple samples: duplicate genomes

Option 1: search contigs between datasets

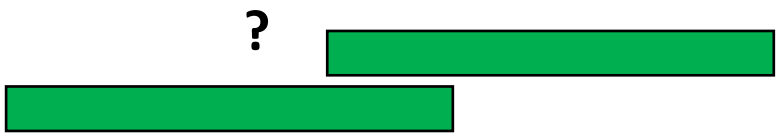
- Problem: dealing with partially overlapping contigs



Multiple samples: duplicate genomes

Option 1: search contigs between datasets

- Problem: dealing with partially overlapping contigs

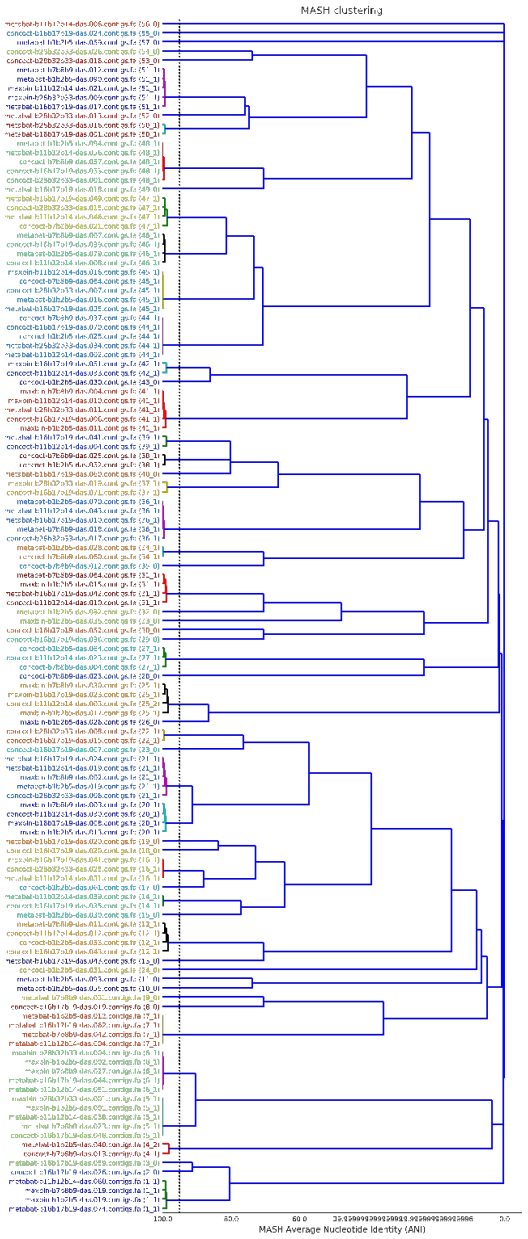


Option 2: search bins between datasets (e.g. dRep, Olm et al., 2017, ISME J)

- Step 1: Fast pairwise MASH search of bins identify clusters
- Step 2: Slow gANI (average nucleotide identity) pairwise search of bins in primary clusters

STEP 1

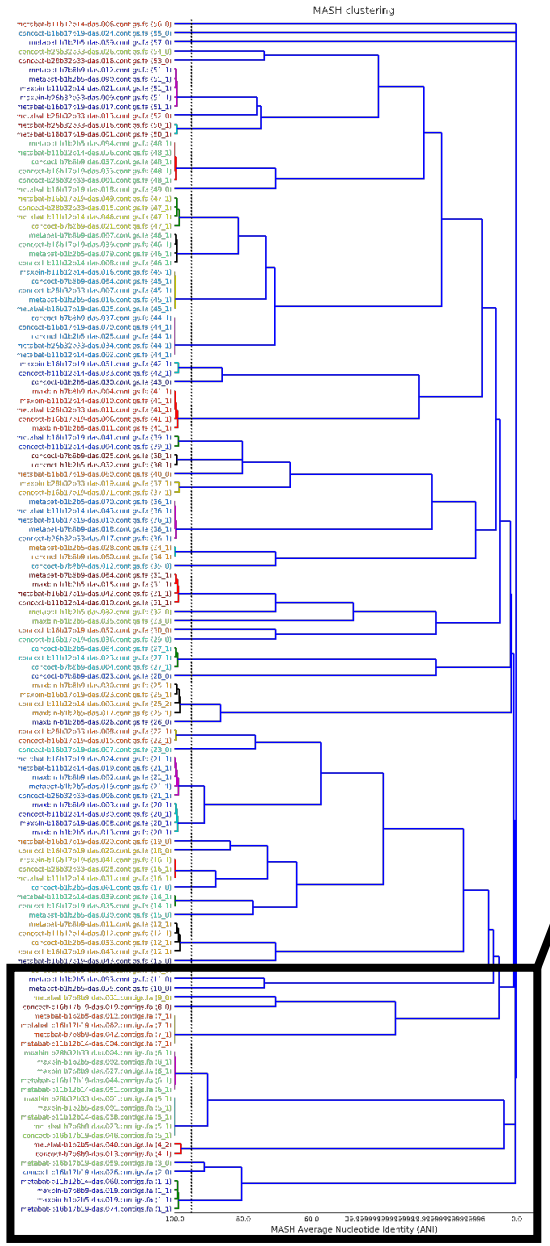
MASH primary cluster creation



Genomes from a cyanobacterial bloom (Wai-iti River, Nelson)

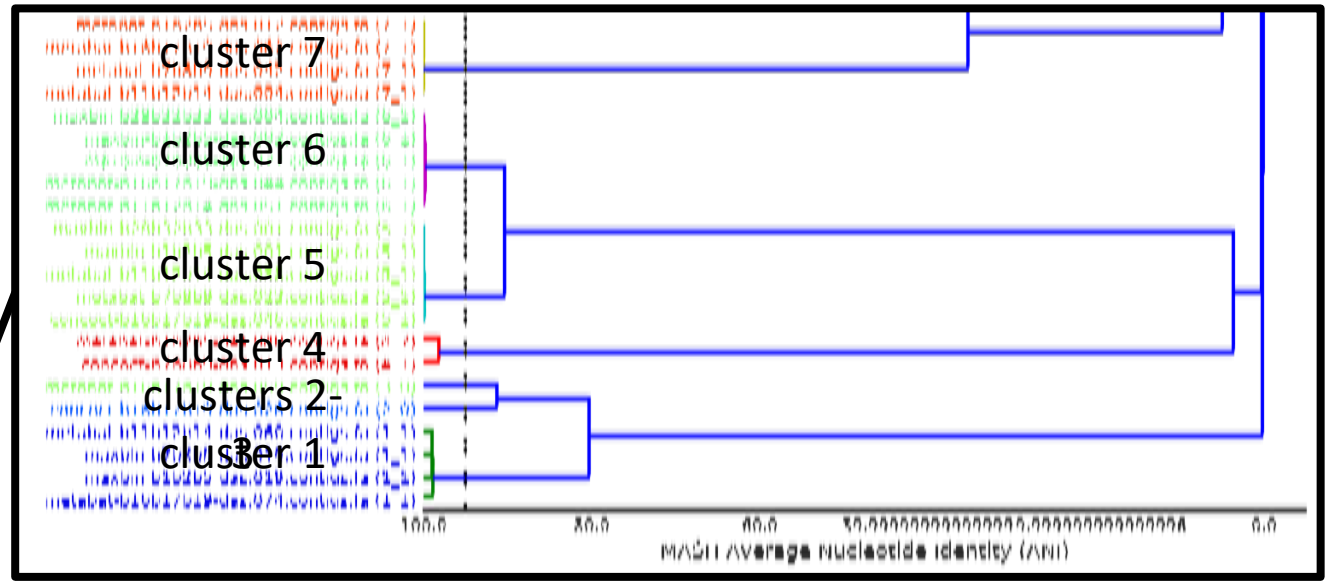
STEP 1

MASH primary cluster creation



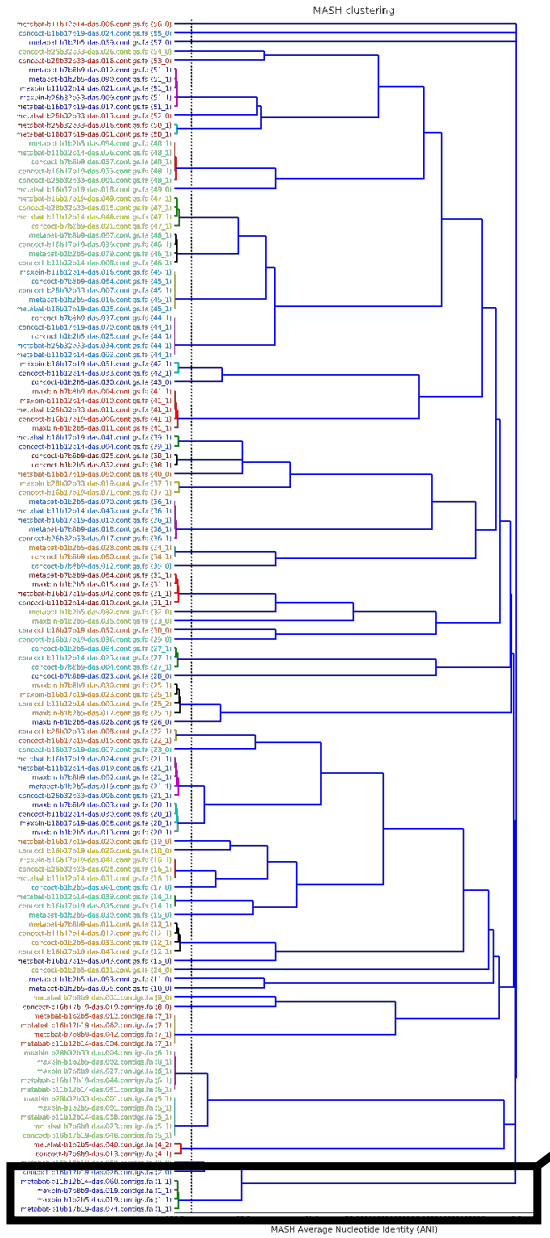
Genomes from a cyanobacterial bloom (Wai-iti River, Nelson)

90% ANI cluster cutoff



STEP 1

MASH primary cluster creation



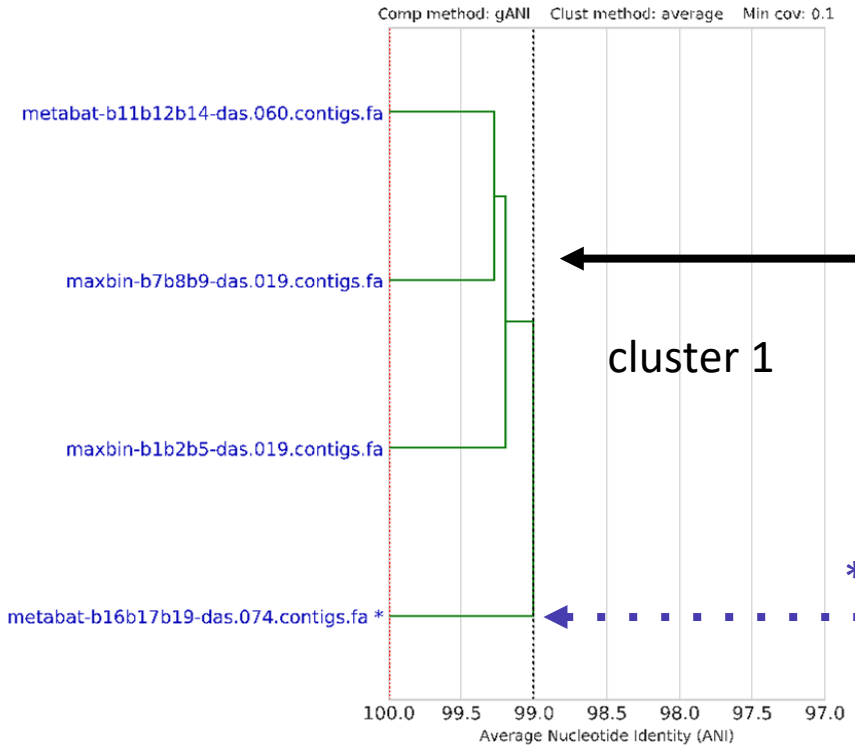
Re-cluster cluster (gANI method)

Genomes from a cyanobacterial bloom (Wai-iti River, Nelson)

STEP 2

Secondary clustering

99% gANI cluster cutoff



All >99% similar

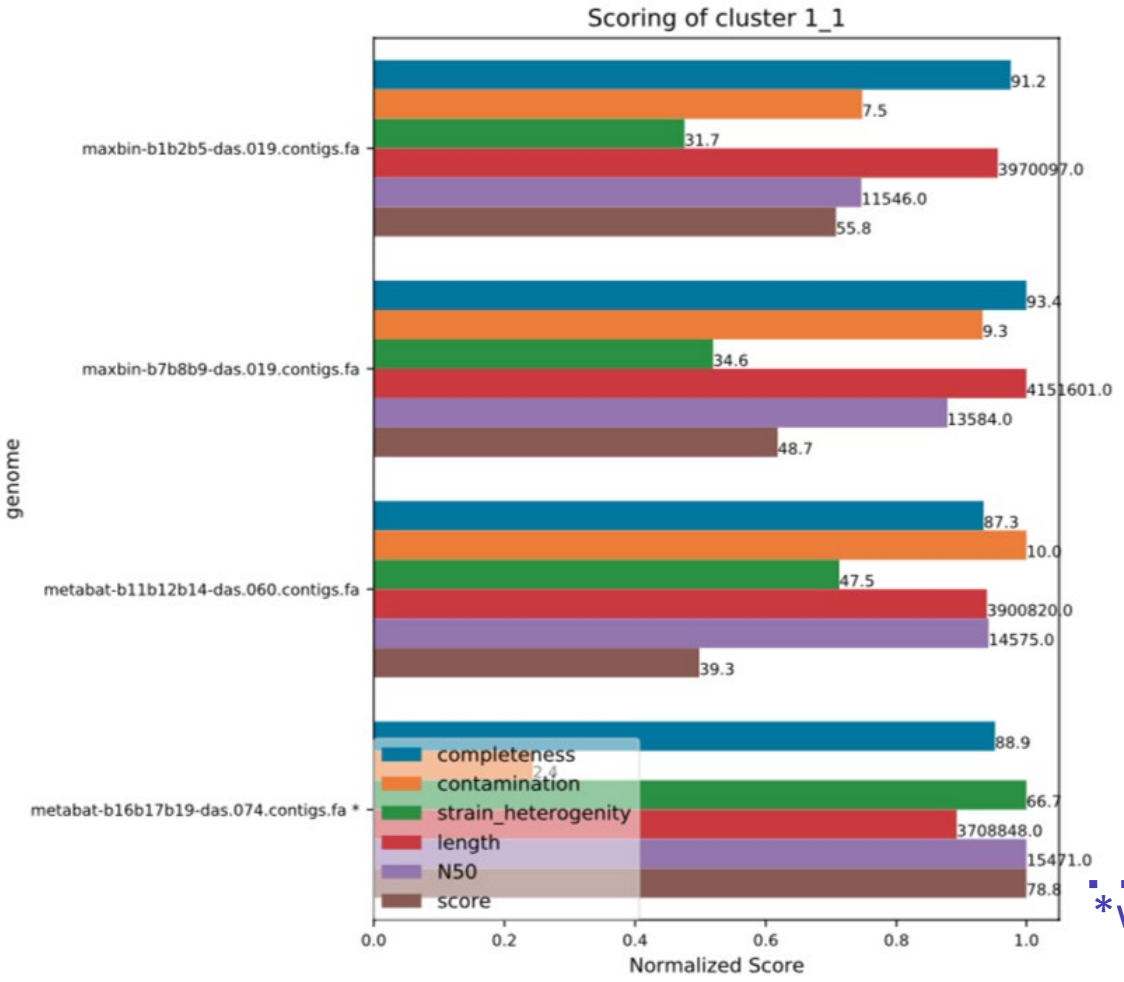
- Dereplicate
- Pick best scoring bin

cluster 1

*winner

Genomes from a cyanobacterial bloom (Wai-iti River, Nelson)

scoring cluster 1 bins

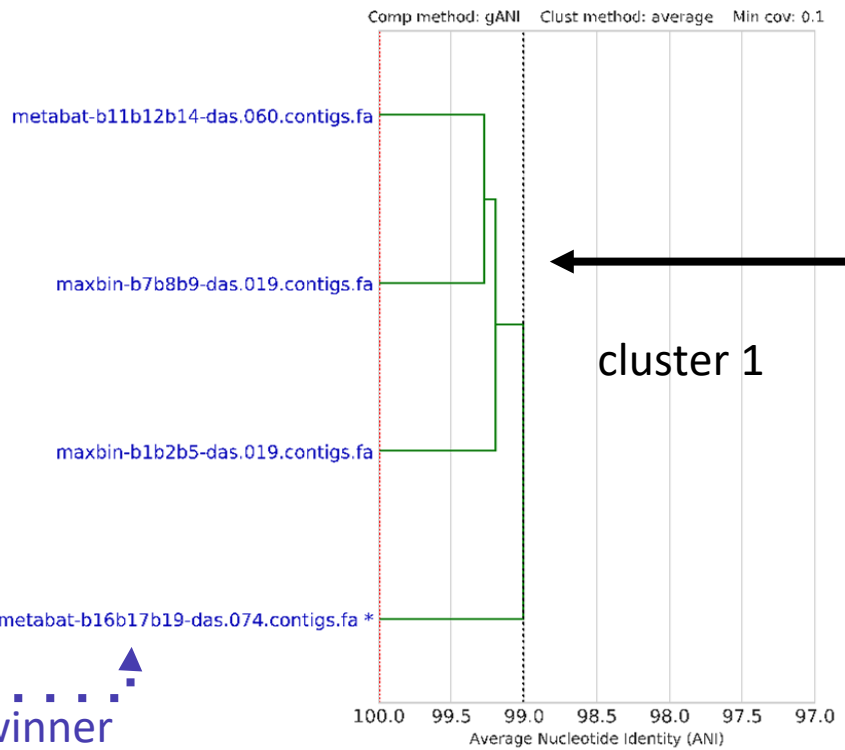


*winner

STEP 2

Secondary clustering

99% ANI cluster cutoff



cluster 1

All >99% similar

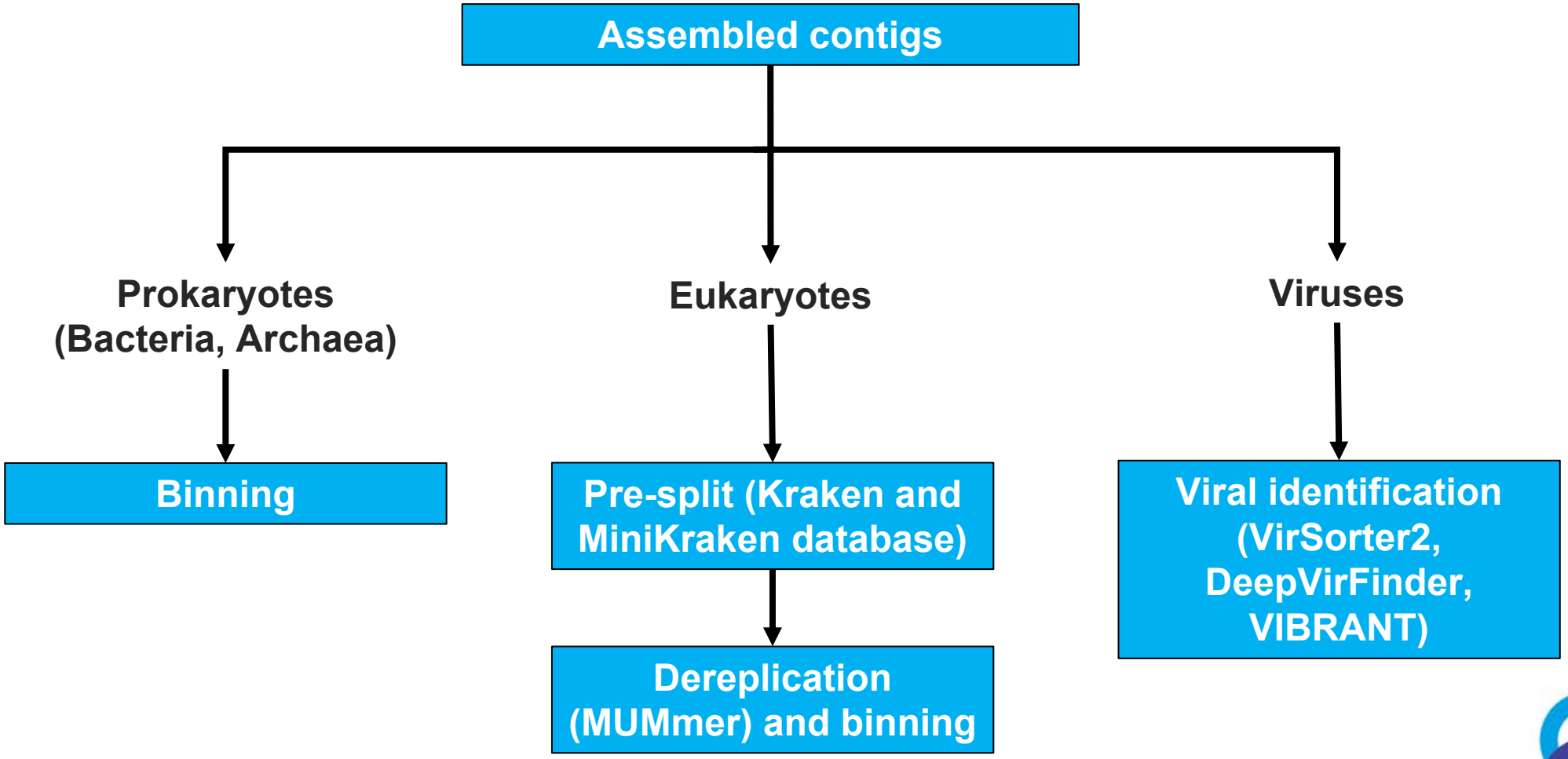
- Dereplicate
- Pick best scoring bin

Eukaryotic and viral bins

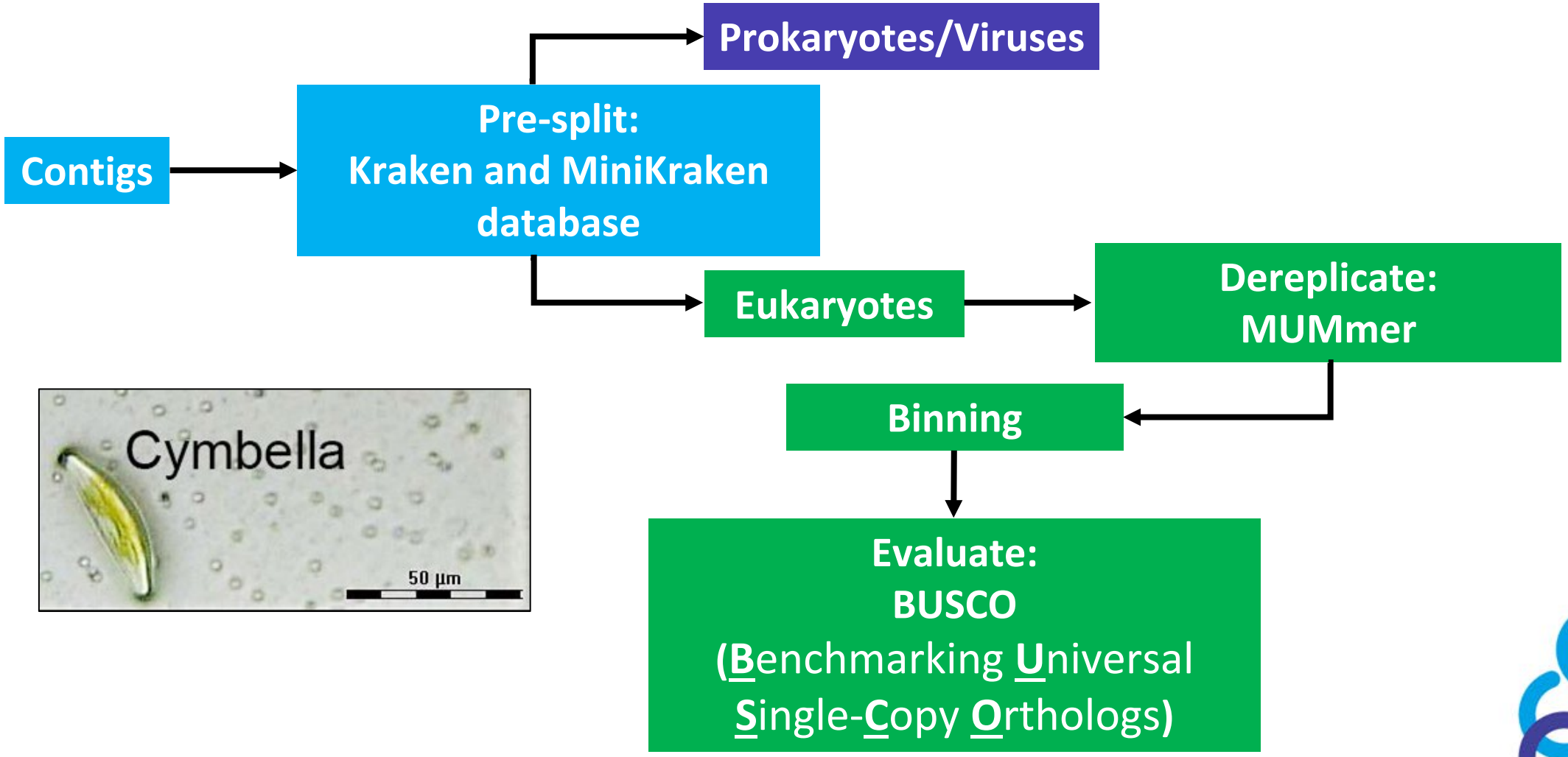
Not included in output of tools using prokaryotic single copy core genes for bin evaluation:

- **CheckM**
- **DAS Tool**
- **dRep**
- **(CONCOCT)**

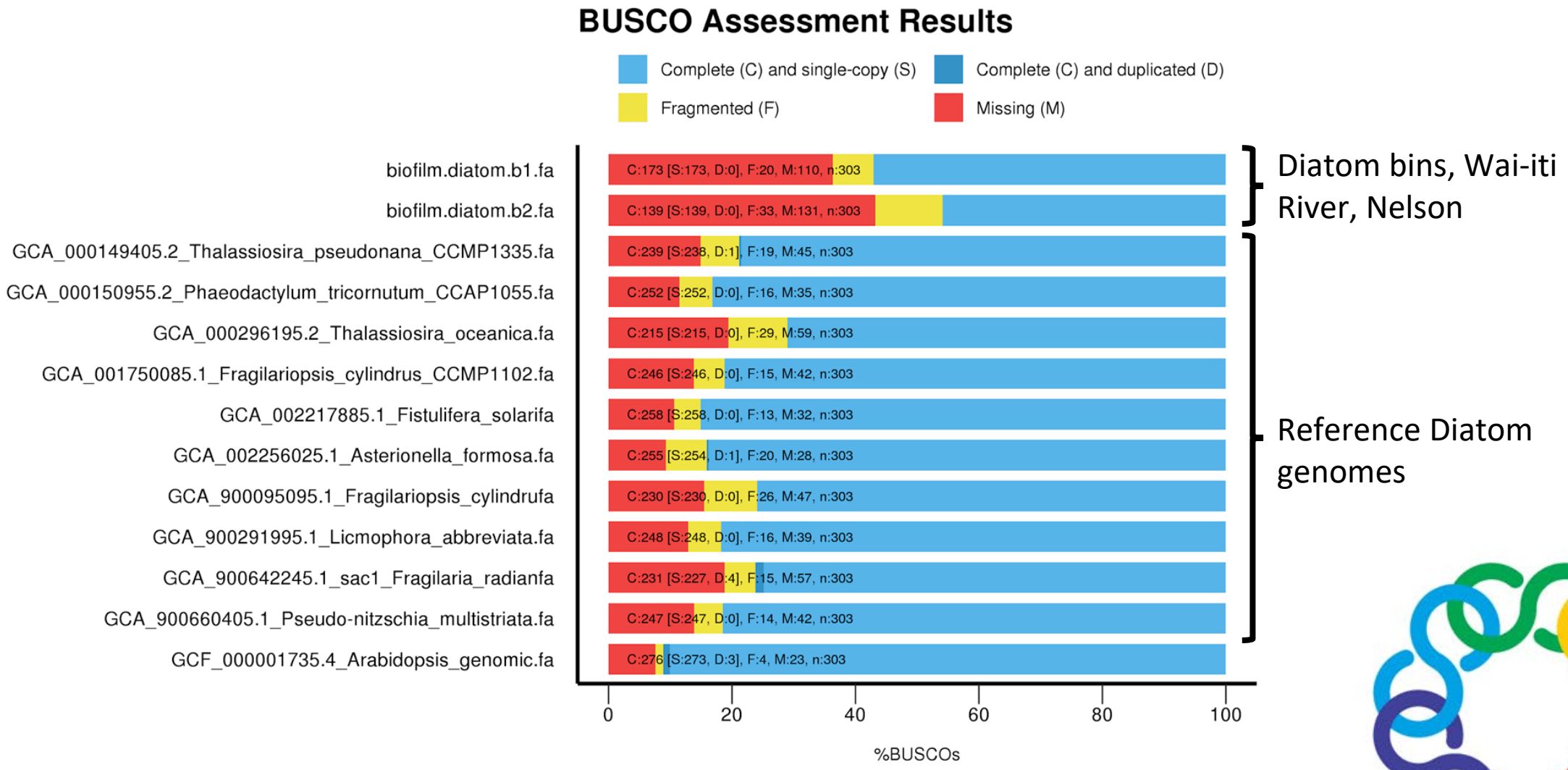
Recovering genomes



Eukaryotic and viral bins

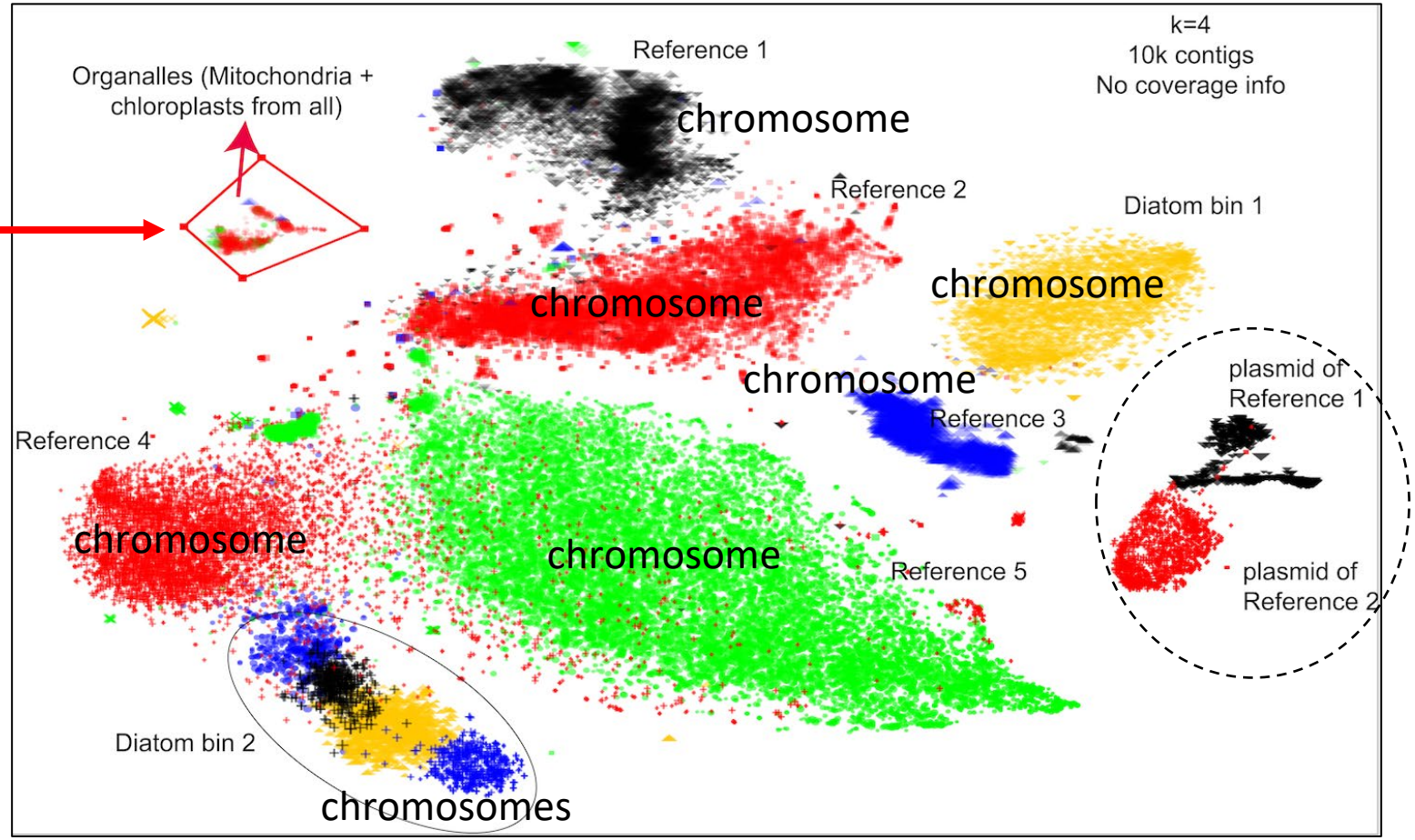


SCG assessment of diatom genomes



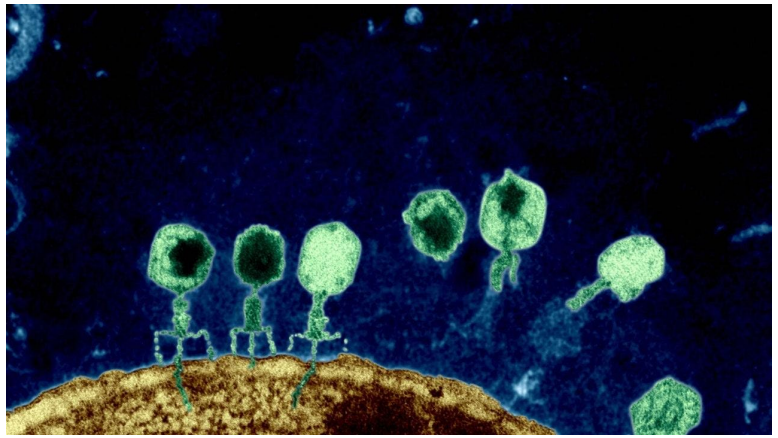
Caveat: Organelles and plasmids

Mitochondria and chloroplast contigs have distinct compositional signatures from chromosomes



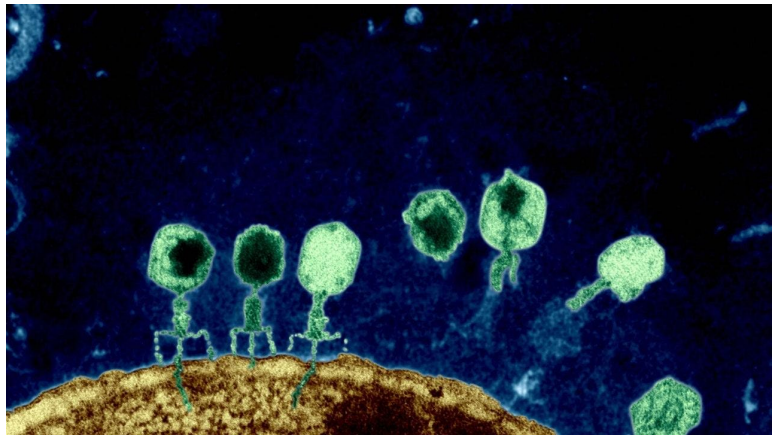
Plasmids

Viruses: Identification



- Viral identification tools:
  - **VirSorter2**
    - Reference database-based + viral genomic features
  - **DeepVirFinder**
    - Kmer frequency-based (machine learning approach)
  - **VIBRANT**
    - Protein similarity-based (machine learning approach)

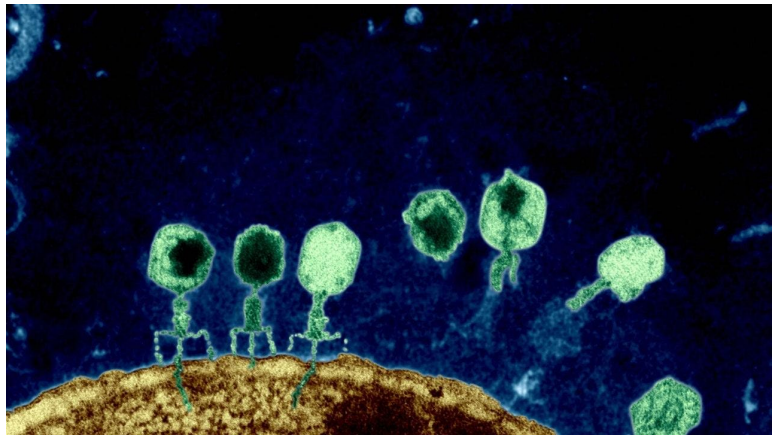
Viruses: Evaluation



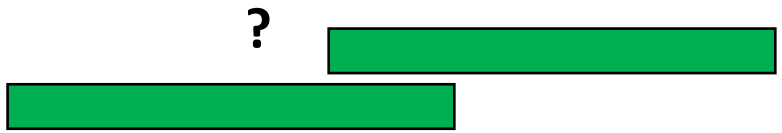
- **VIBRANT**
  - Includes **v-score**
    - proxy for quality and completeness

- **CheckV**
  - Proviruses: Trims retained host sequence
  - Estimates genome completeness
  - Predicts closed genomes (direct terminal repeats)
  - Outputs completeness score consistent with **MIUViG**
    - Minimum Information about an Uncultivated Virus Genome standard
    - Roux et al. (2019) *Nature Biotechnology* 37(1):29-37

Viruses: Dereplication



- **BBMap's dedupe.sh**
  - Based on min identity fully duplicate or *contained* contigs
  - Problem: overlapping contigs

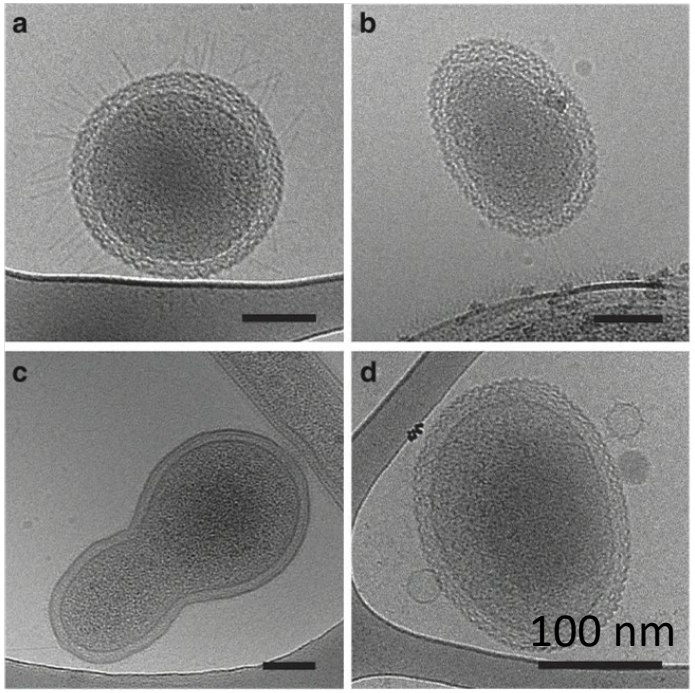


- **Roux group's Cluster_genomes.pl**
  - cluster viral/phage genomes based on ANI (Average Nucleotide Identity) and AF (Alignment Fraction)

- **CheckV's anicalc and aniclust**

Minimal genomes

- **What if you're working with tiny bacteria or archaea, with small genomes?**
- **Some lack certain single copy core genes (SCCGs), e.g. certain ribosomal proteins**



Tiny groundwater bacteria

(Luef et al., 2015, Nature Communications)

Minimal genomes

- Potential outcome:
  - unexpectedly poor completeness scores!
  - can be eliminated during dereplication steps due to poor scores

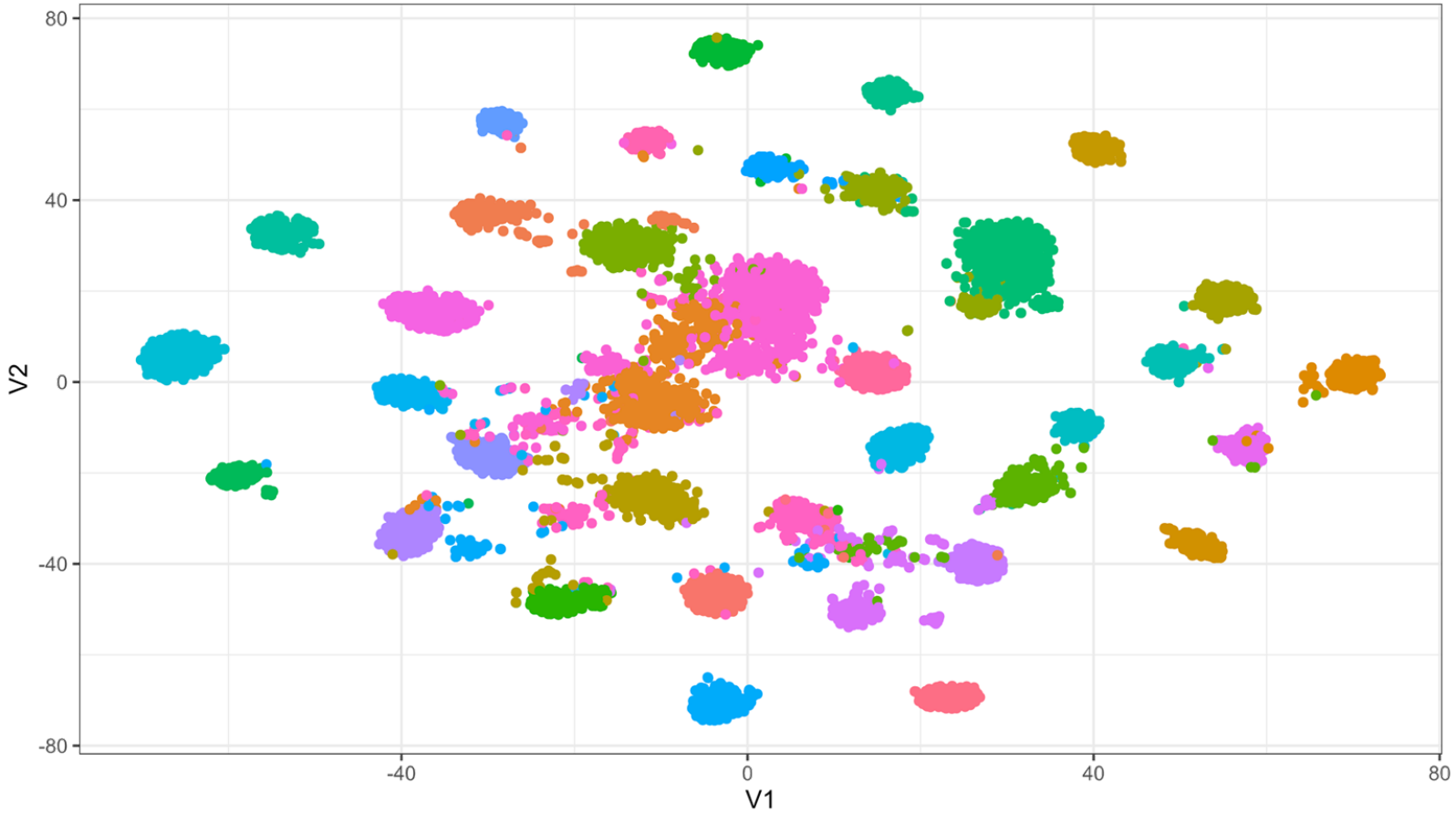
- Solution:
  - recalculate scores
  - exclude troublesome SCCGs

- Example: Patescibacteria superphylum
  - identify reduced set of 43 single copy genes (Brown et al., 2015, Nature)
  - 100 NZ groundwater Patescibacteria genomes: average completeness increased from 60% to 85%

Bin refinement

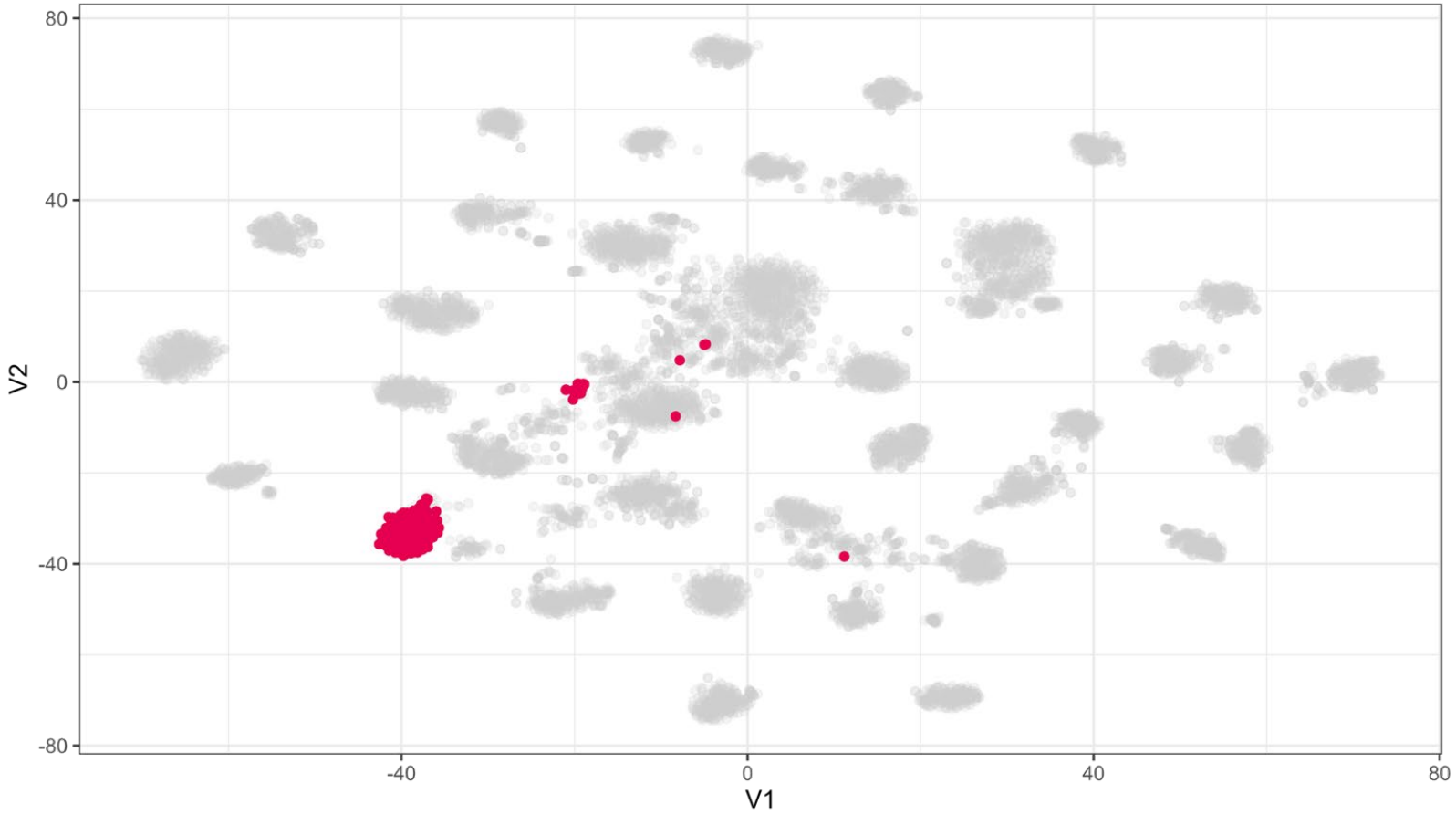
VizBin

- **Inspect bin assignments**



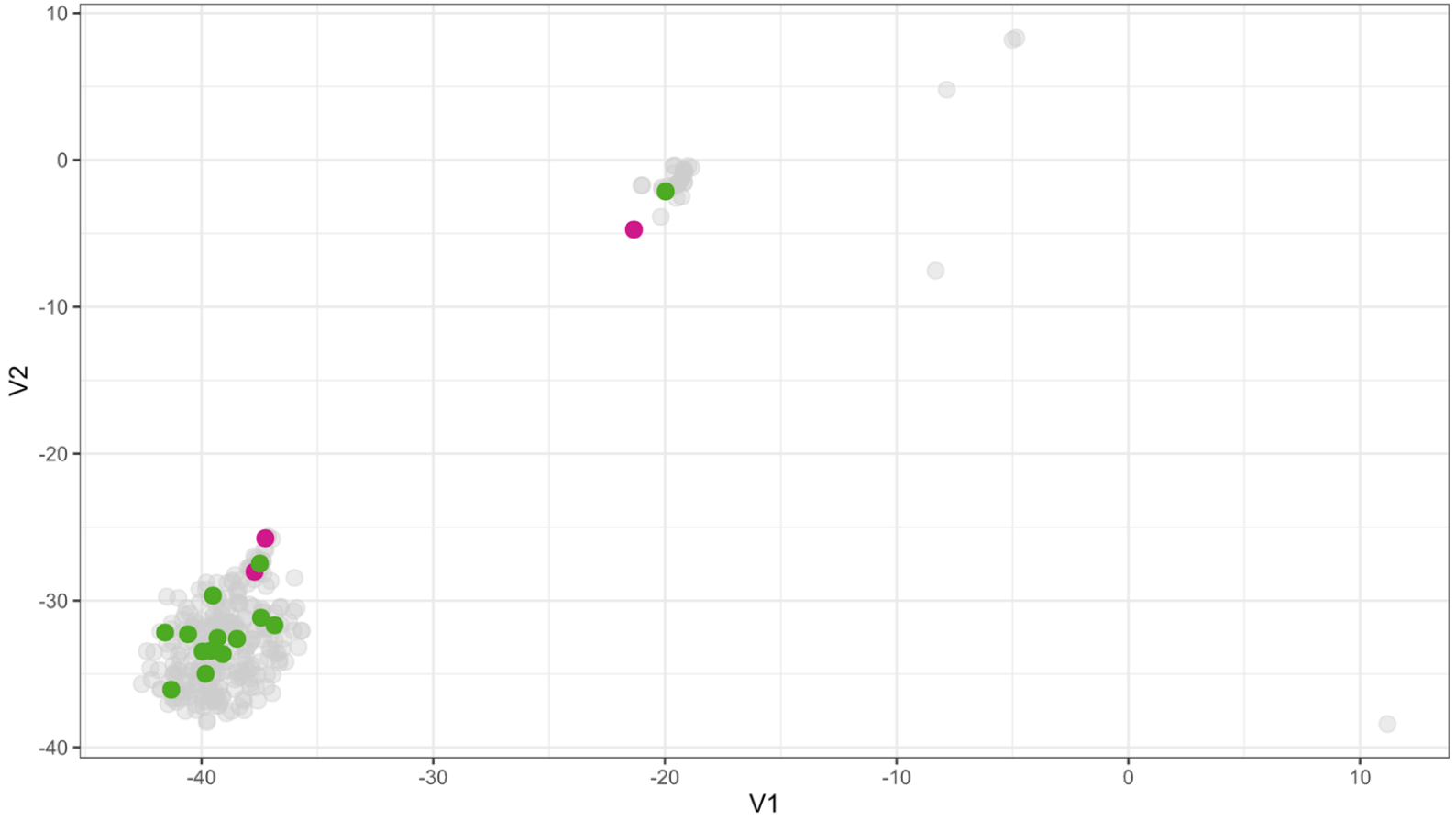
VizBin

- **Use graphical user interface to assign bins or reassign contigs**



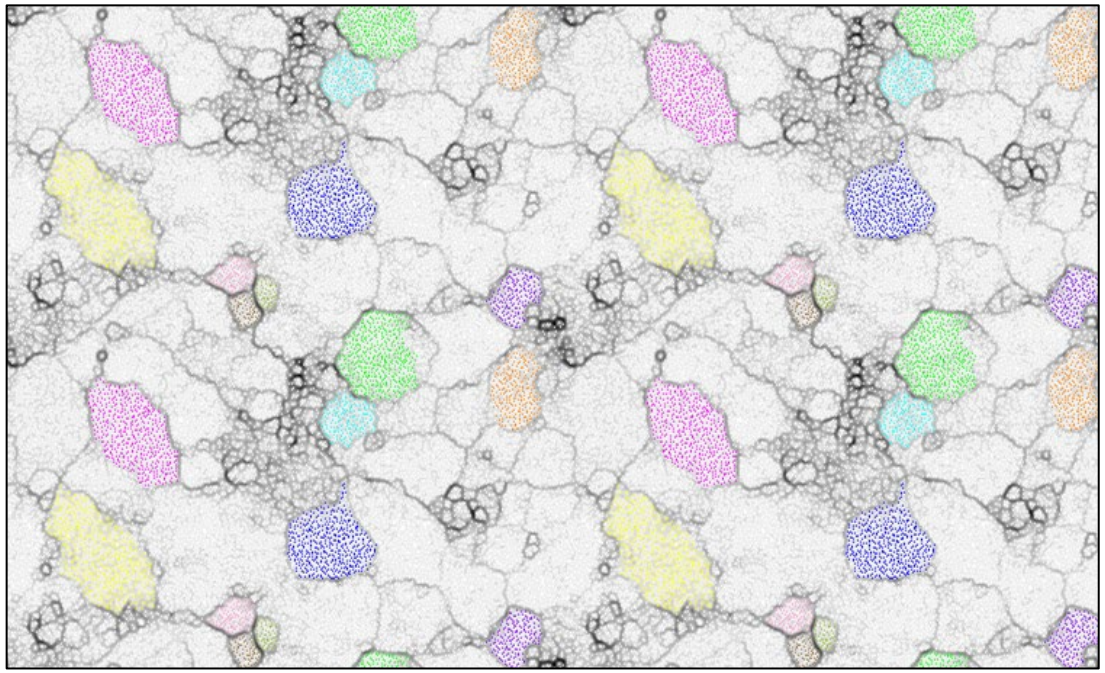
VizBin

- **Identify contigs with unstable placement**



Alternative: ESOM

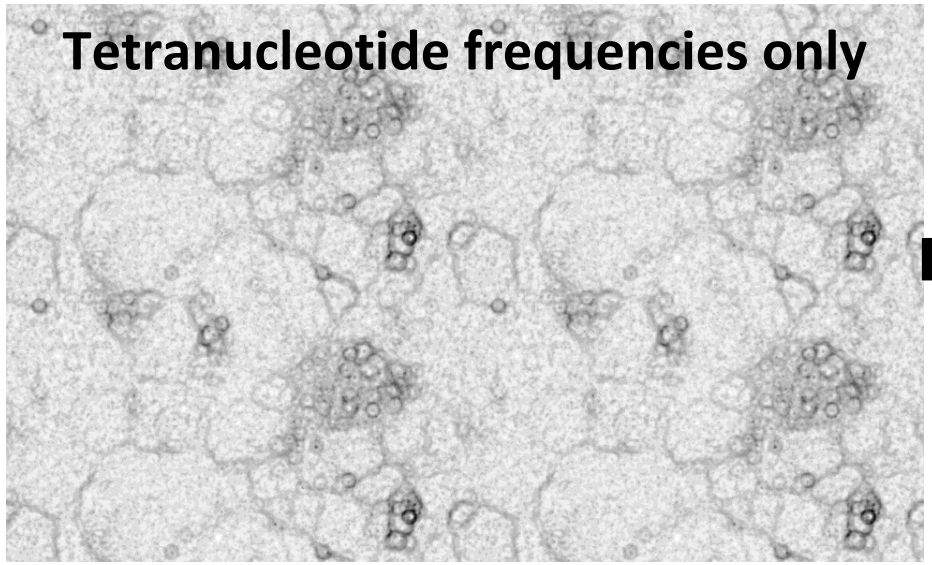
- **Emergent Self Organizing Map (ESOM)**
- **esomana: http://databionic-esom.sourceforge.net/user.html**



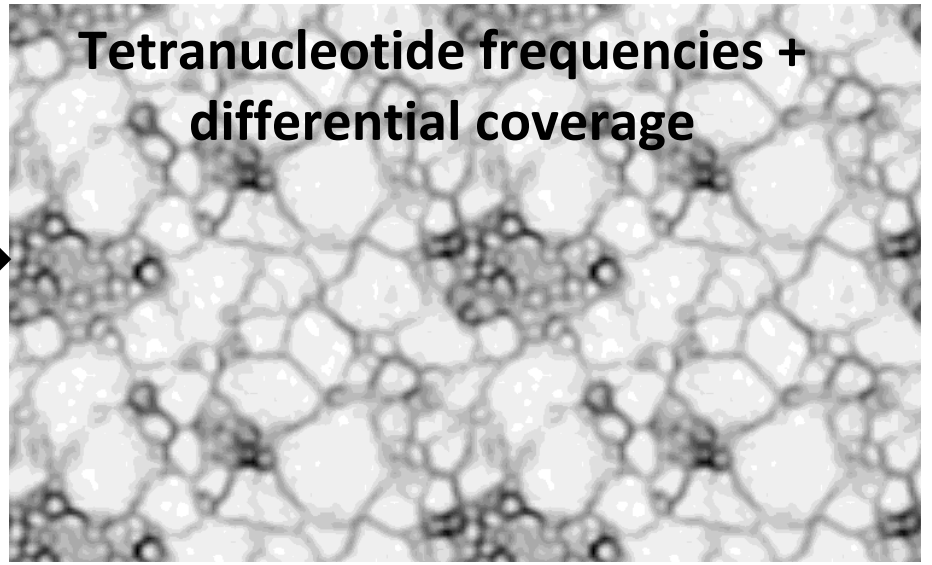
Gulf of Mexico seafloor communities (Handley et al., 2017, ISME J)

Alternative: ESOM

- **Flexible: use whatever data you want, e.g.: tetranucleotide frequencies, coverage, both**



Indistinct bin boundaries between highly similar genomes

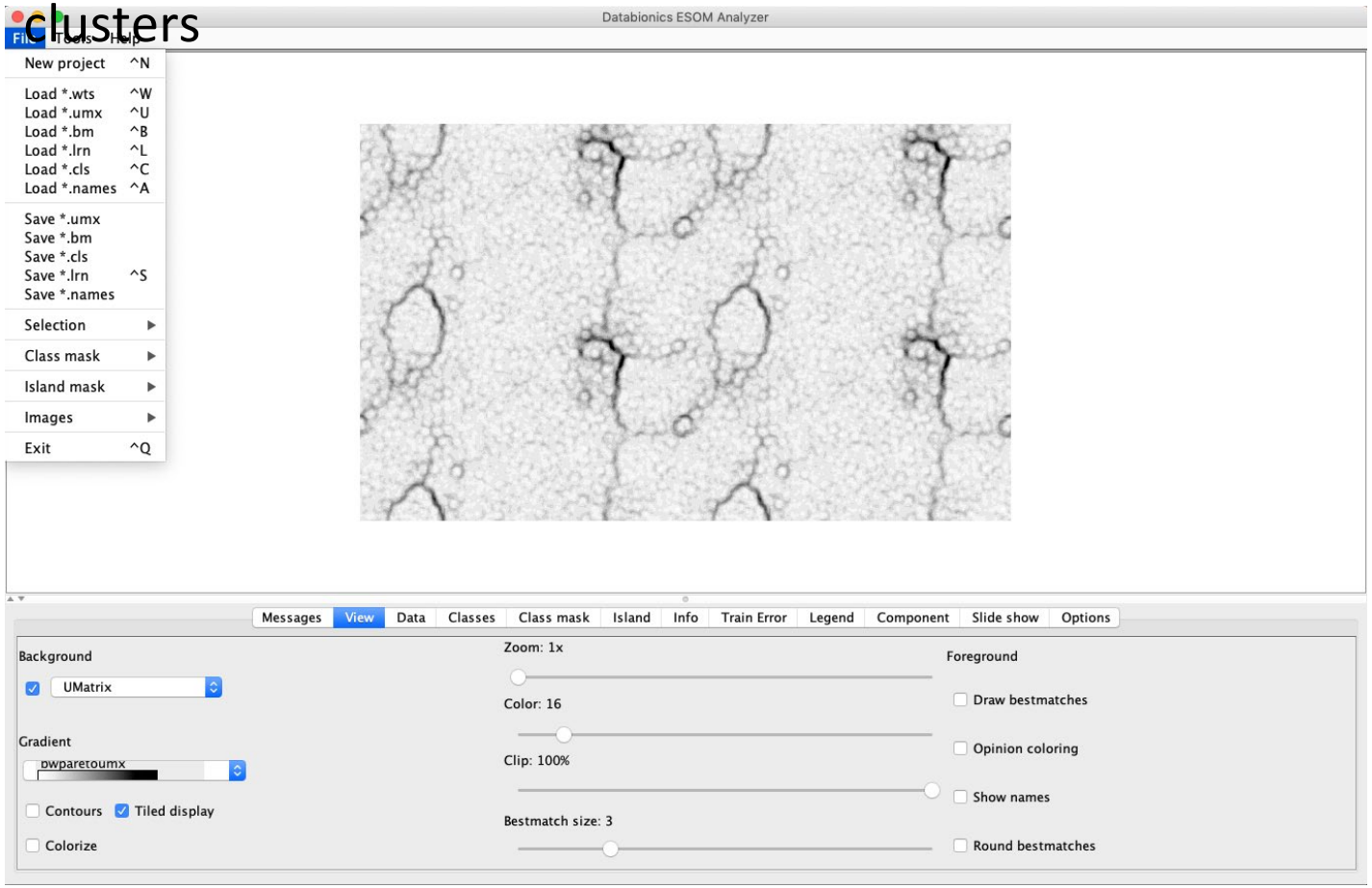


Clear bin boundaries between highly similar genomes using spatial gradient data

Gulf of Mexico seafloor communities (Handley et al., 2017, ISME J)

Alternative: ESOM

Topographic map of clusters



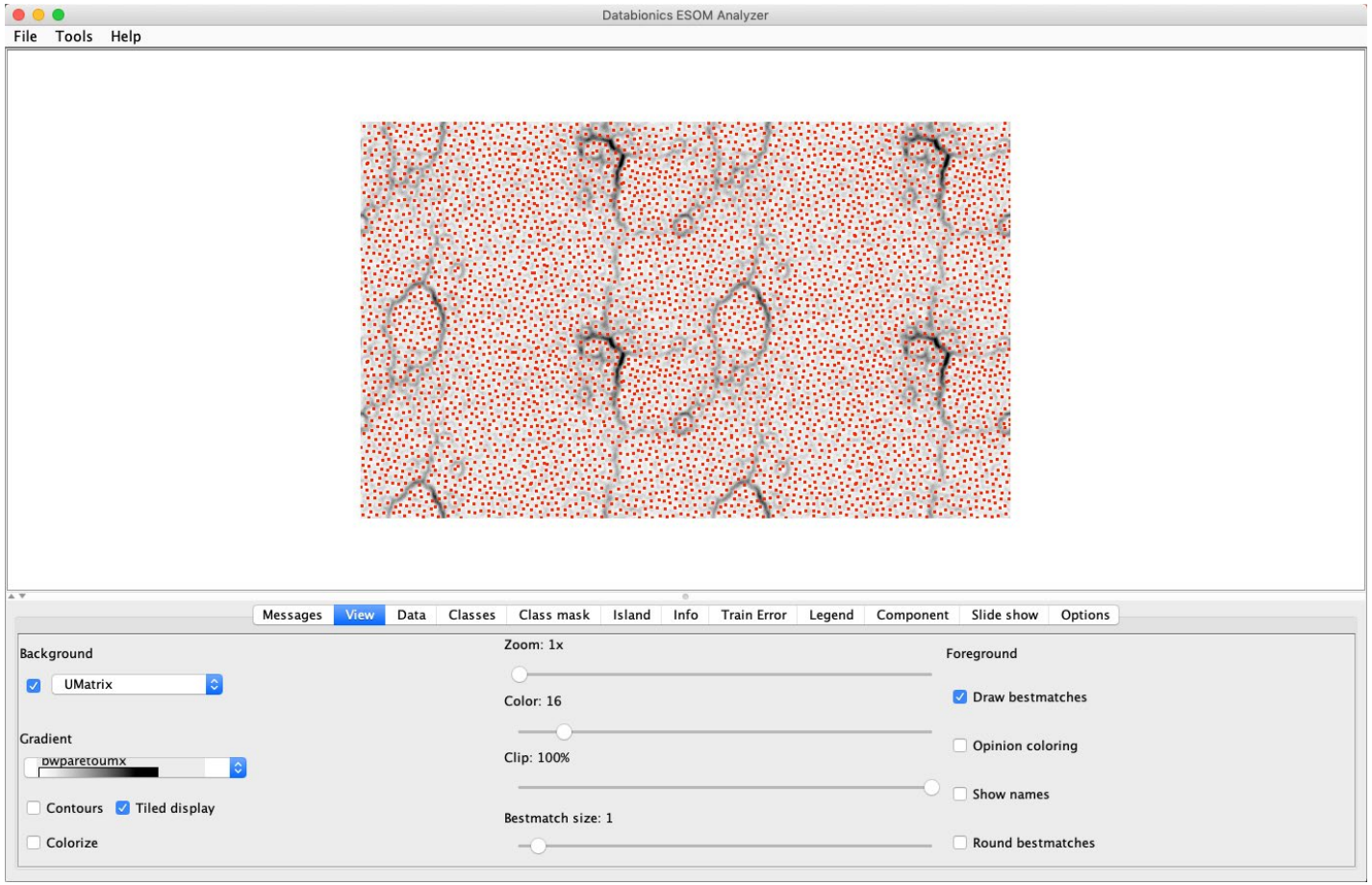
- Uses graphical user interface
- Must supply own prepared data (e.g. pre-calculate tetranucleotide frequencies)

- Dark lines = bin boundaries
- Strong lines = strong bin divisions

Colorado groundwater communities (Handley et al., 2013, ISME J)

Alternative: ESOM

Map with contig fragments shown in red



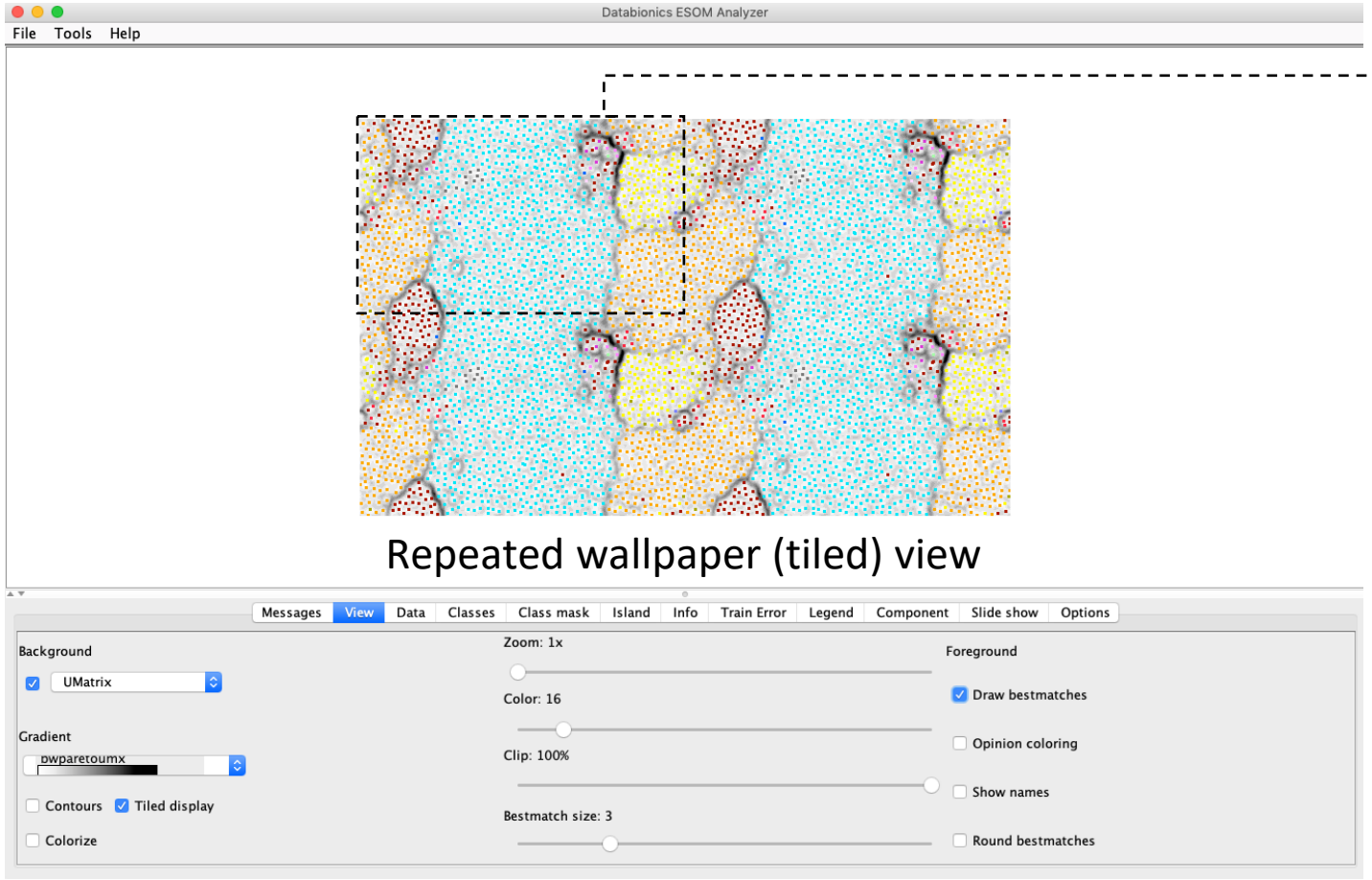
- Uses graphical user interface
- Must supply own prepared data (e.g. pre-calculate tetranucleotide frequencies)

- Dark lines = bin boundaries
- Strong lines = strong bin divisions

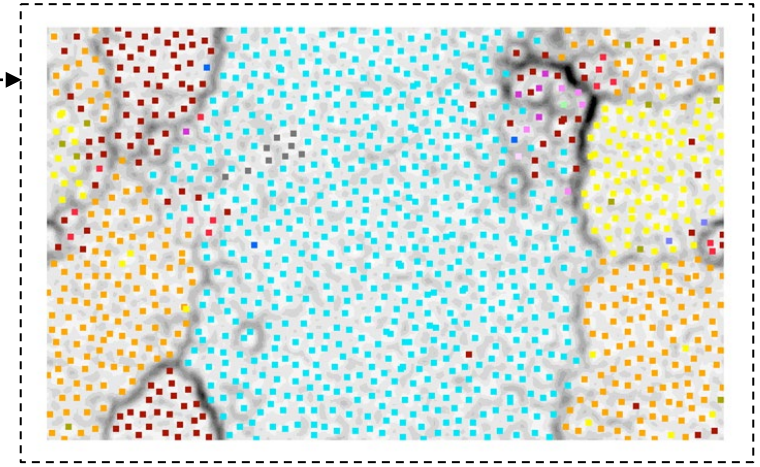
Colorado groundwater communities (Handley et al., 2013, ISME J)

Alternative: ESOM

Map with contig fragments shown and coloured by bin assignment



Repeated wallpaper (tiled) view

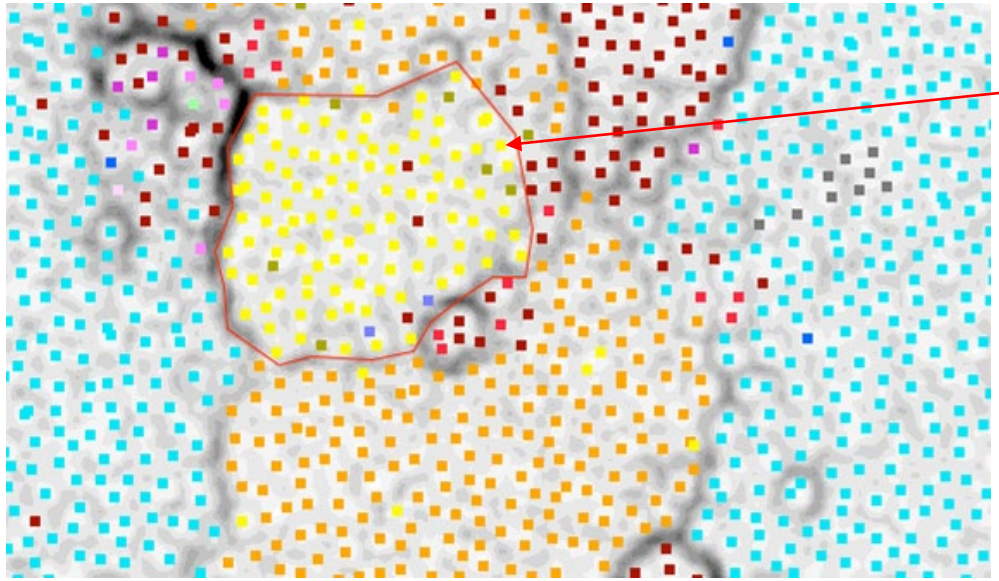


Non-repeating (un-tiled) view

Colorado groundwater communities (Handley et al., 2013, ISME J)

Alternative: ESOM

Map with contig fragments shown and coloured by bin assignment

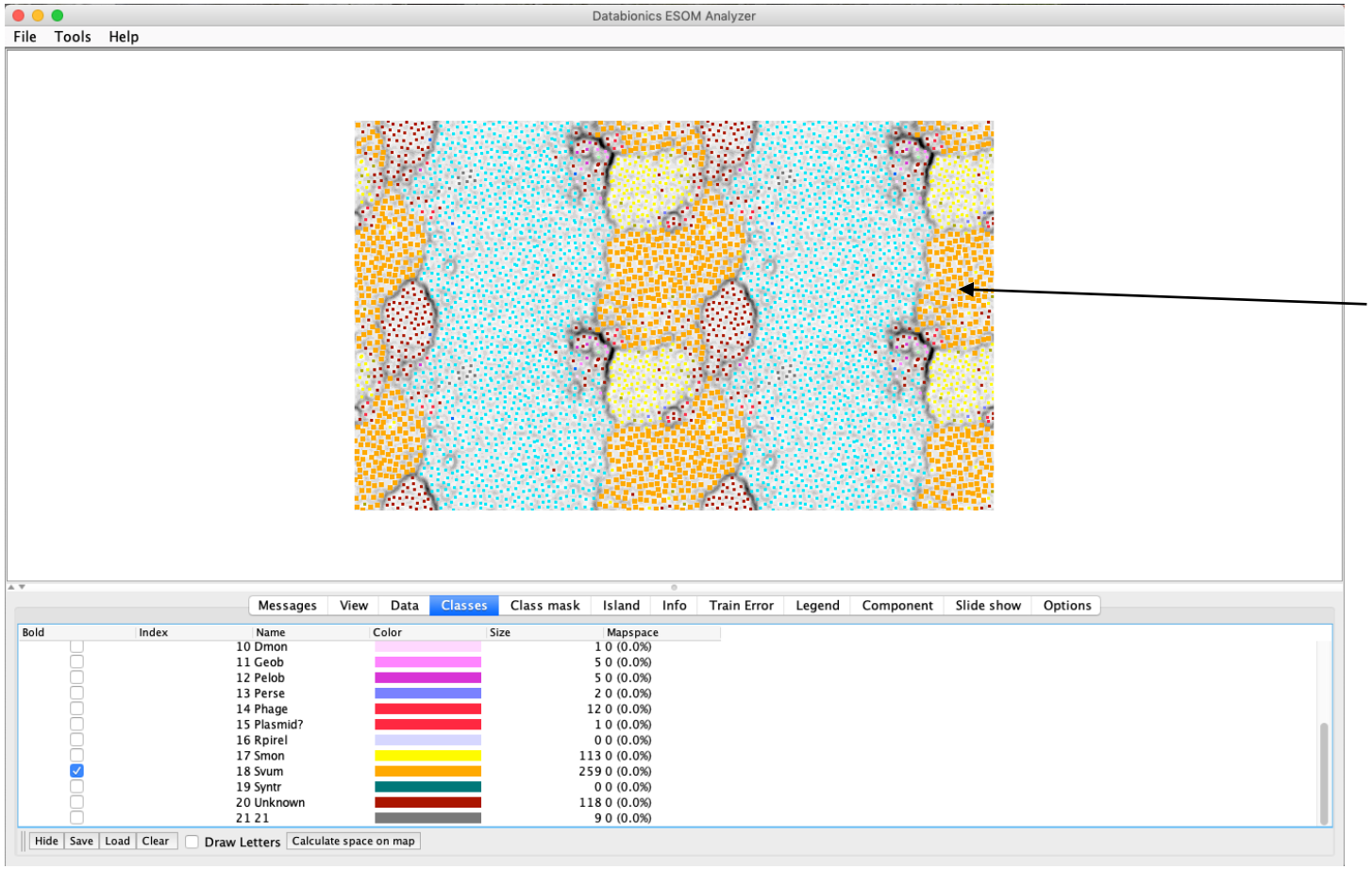


Like VizBin, bins are selected by manually drawing around boundary

Colorado groundwater communities (Handley et al., 2013, ISME J)

Alternative: ESOM

Map with contig fragments shown and coloured by bin assignment



- Select pre-assigned bin to highlight contig fragments
- Choose/change bin colours
- Example: *Sulfurovum* bin highlighted

Colorado groundwater communities (Handley et al., 2013, ISME J)

Task: Working with VizBin

Go to Github MGSS webpage

Tasks:

- **Prepare input files for VizBin**
- **Project high-dimensional data down into a 2D plot**
- **Picking refined bins**
- **(Optional) Refine and filter problematic contigs from bins**
- **(Optional) Comparing pre- and post- filtered bins via CheckM**

Recorded Talk:
Genetic exchange in ultra-small Patescibacteria
(Emilie Gios)

Task: Identifying viral contigs and QC

(Prep for tomorrow!)

Go to Github MGSS webpage (*Day 3: Identifying viral contigs in metagenomic data*)

Tasks:

- **Identifying viral contigs using VIBRANT**
- **QC of viral contigs using CheckV**

End of day wrap up