SANS serif: alignment-free, whole-genome based phylogenetic reconstruction

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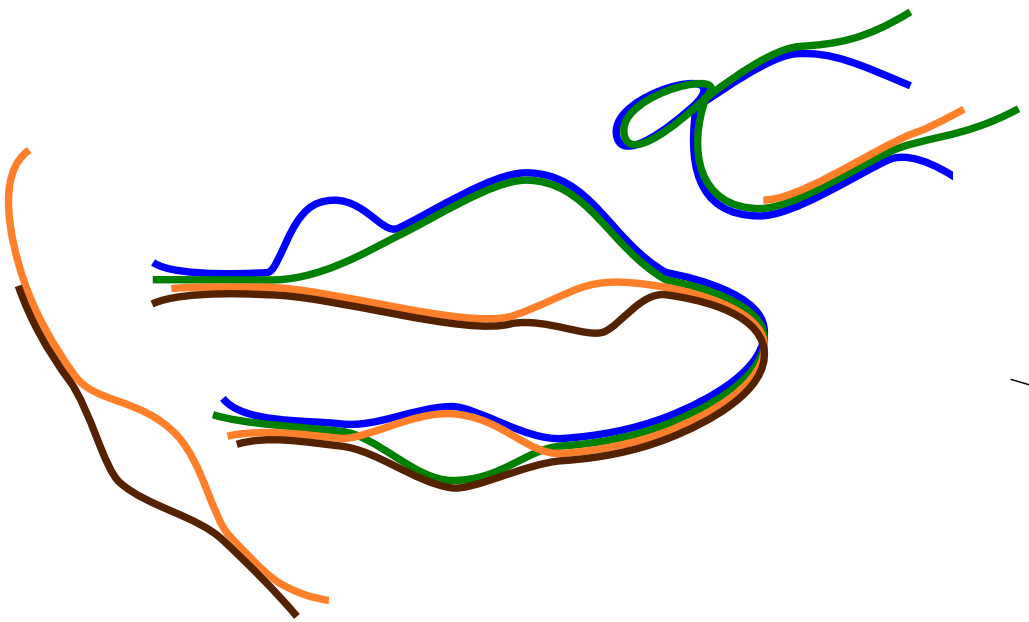
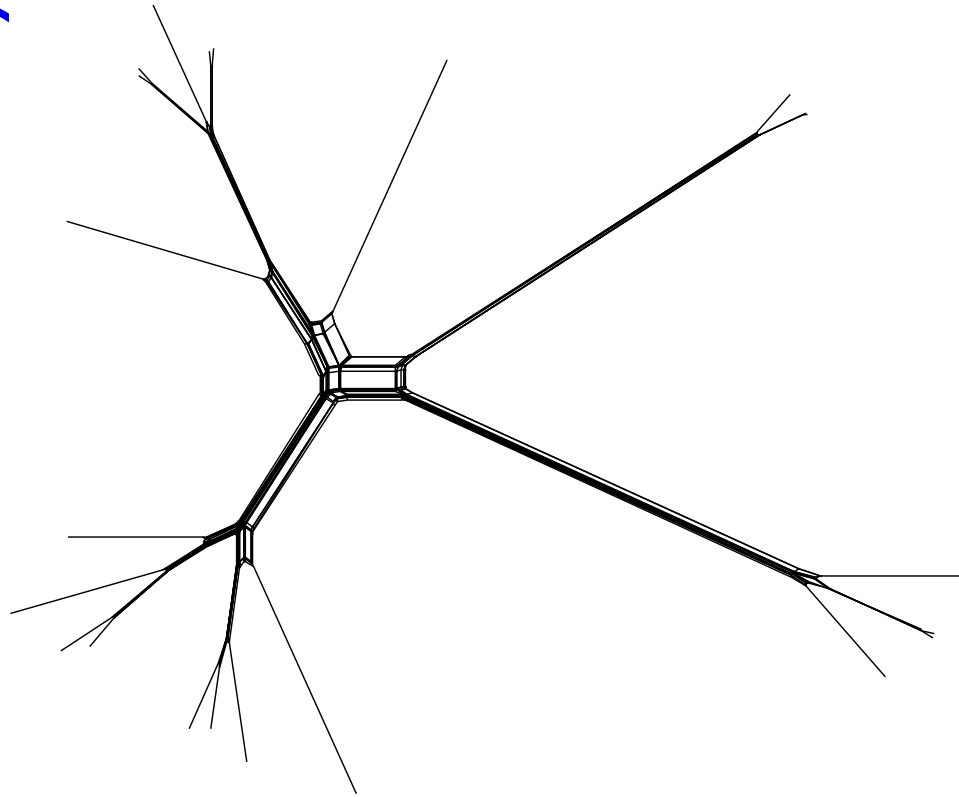
Bielefeld University

DSB 2021

Outline





Introduction — SANS

Classical approaches

- mult. alignment of marker genes
- alignment to reference $\rightarrow$ SNPs
- pairwise distances or ML
- tree

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SANS: Symmetric Alignment-free phylogeNomic Splits

- whole genome (no markers, no alignment, no reference)

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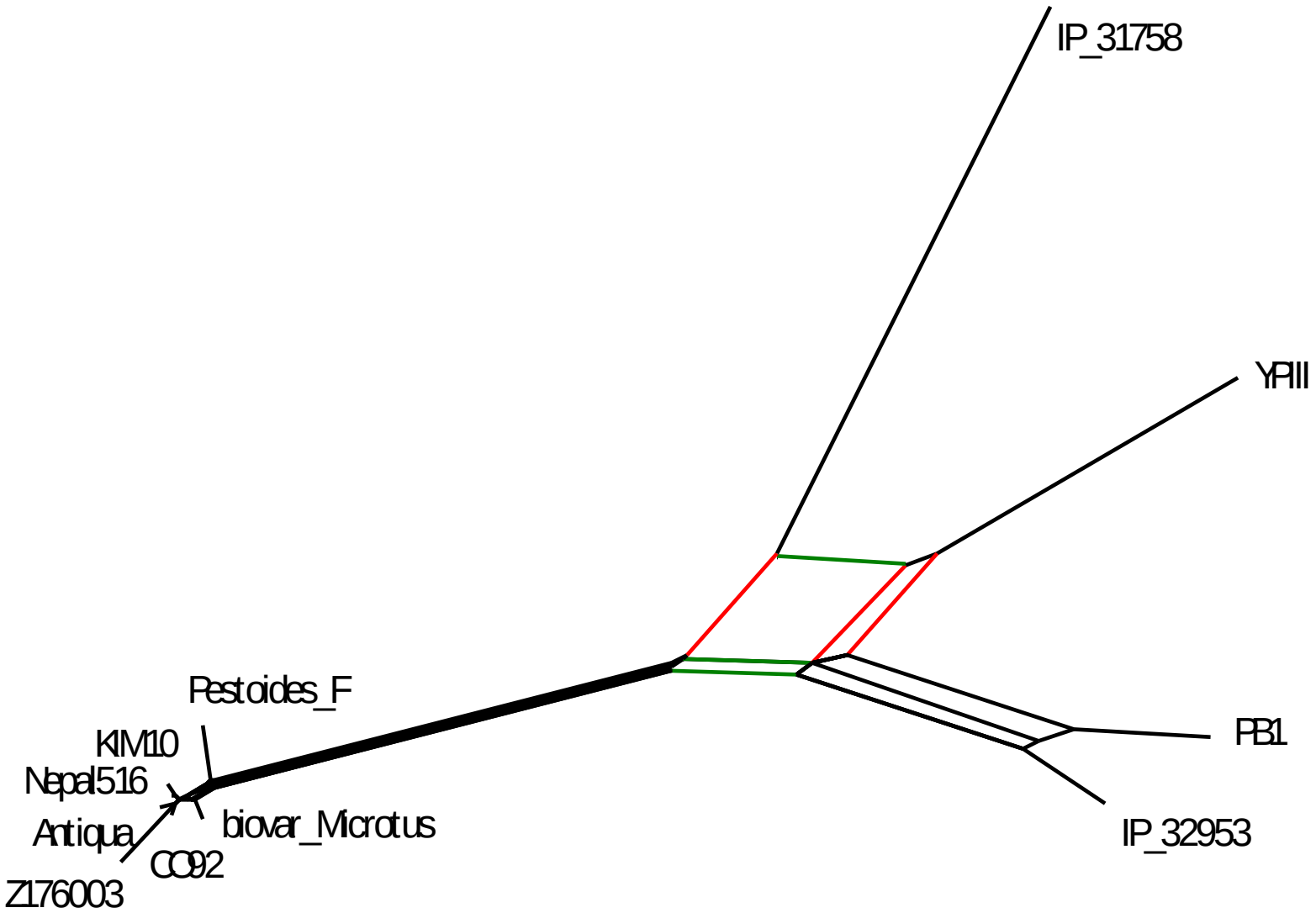
SANS: Symmetric Alignment-free phylogeNomic Splits

- whole genome (no markers, no alignment, no reference)
- k-mers $\rightarrow$ splits (no pairwise comparison)

Introduction — SANS



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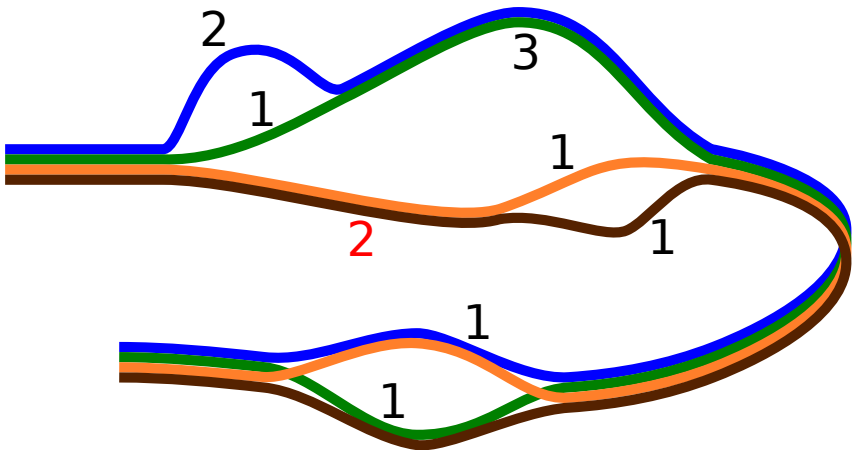

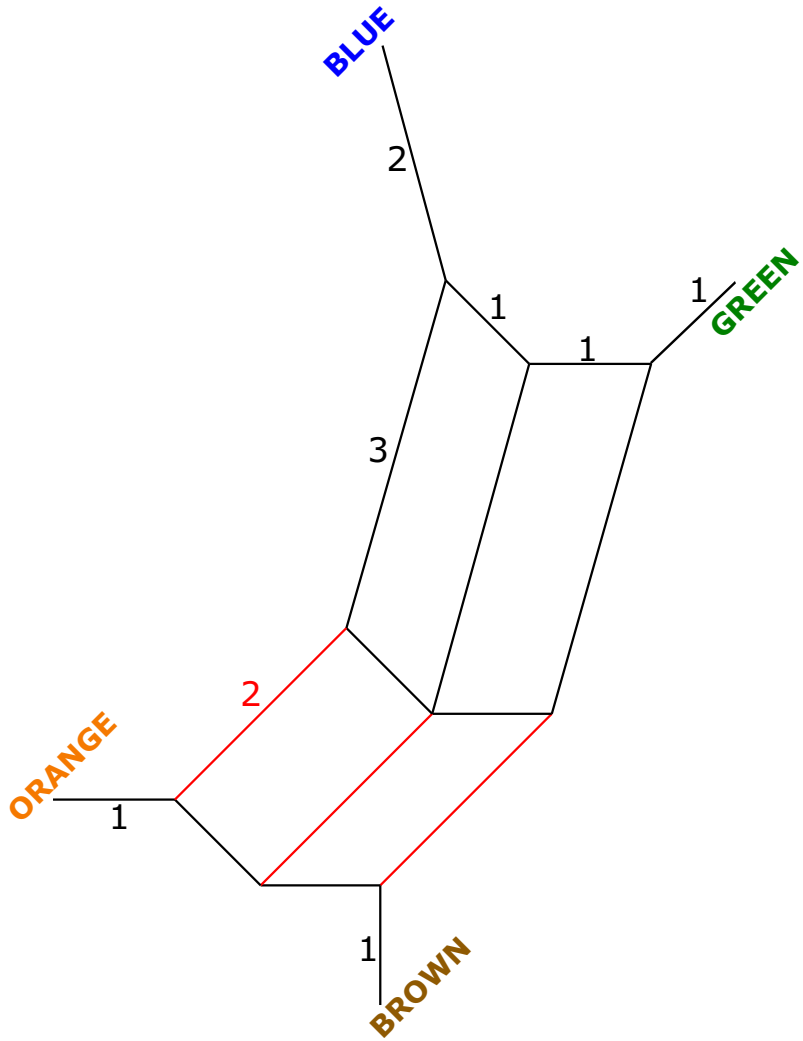

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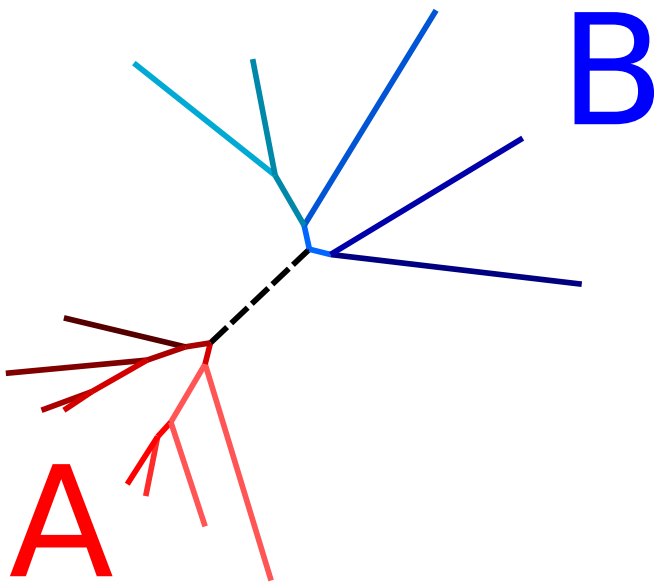

A and B separated in phylogeny

$\Rightarrow$ mutations on that edge

$\Rightarrow$ sequences unique to A and
sequences unique to B

$\Rightarrow$ A and B k-mers
of about same total amount $w \approx w'$

$\Rightarrow$ $\sqrt{w \cdot w'}$ to downweight asymmetry
or $\sqrt{(w+1) \cdot (w'+1)}$ to not loose any

Outline

SANS serif

Andreas Rempel

First SANS version [DSB 2019, WABI 2019]:

1. build C-DBG (Bifrost, Melsted&Holley)
2. extract splits from unitigs and store in trie

Re-implementation:

- hash tables
- no dependencies
- space and time efficient
- includes filtering of splits (e.g. tree filter)

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Space and time **E**fficient **R**e-**I**mplementation incl. **F**ilters: SANS **serif**

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SANS serif

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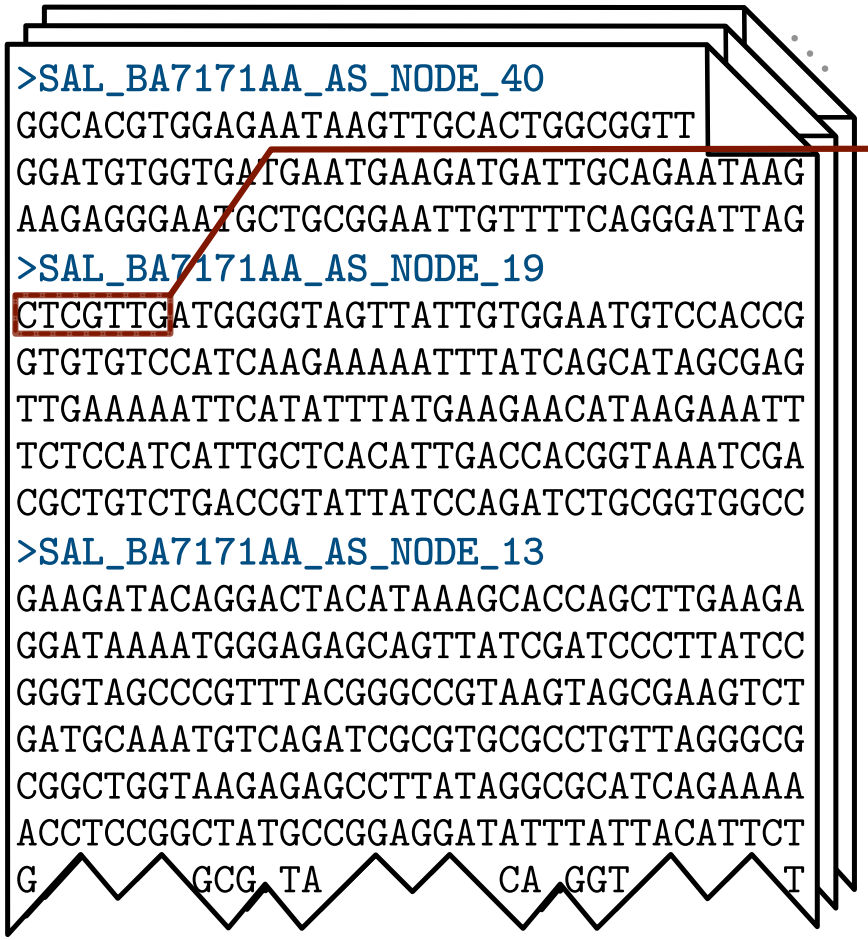

k-mer	files
0b10010010100000	0b10000000000000
0b01110110111110	0b11000000000000
0b11000101111000	0b11000000000000
0b01001010000001	0b01011101100000
0b01000011000110	0b00111111111111
0b00010001101001	0b00111111111111
0b00011101010101	0b00111111111111
0b00011111010111	0b01011101100000
0b10100011101001	0b11000000000000
0b01100011111101	0b00001001111111
. . . . .	. . . . .

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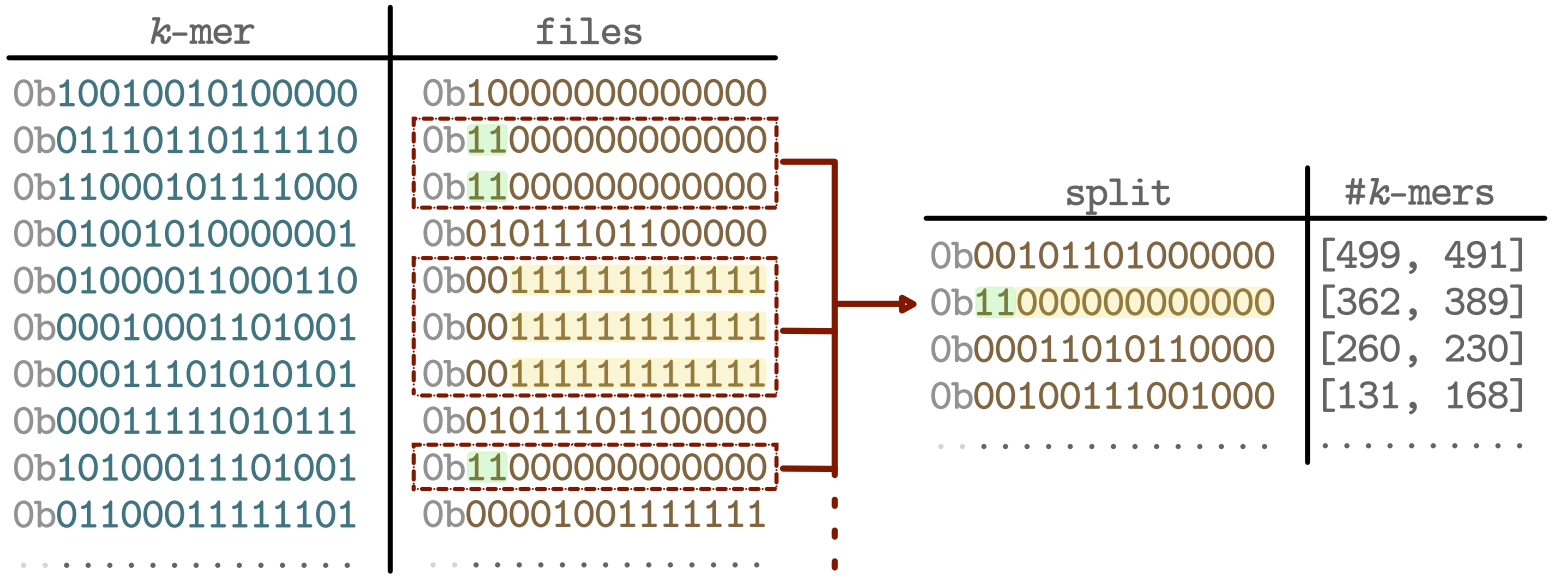

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split	#k-mers
0b00101101000000	[499, 491]
0b11000000000000	[362, 389]
0b00011010110000	[260, 230]
0b00100111001000	[131, 168]
.....	.....

weight
494.983
375.257
244.540
148.350
.....

SANS serif

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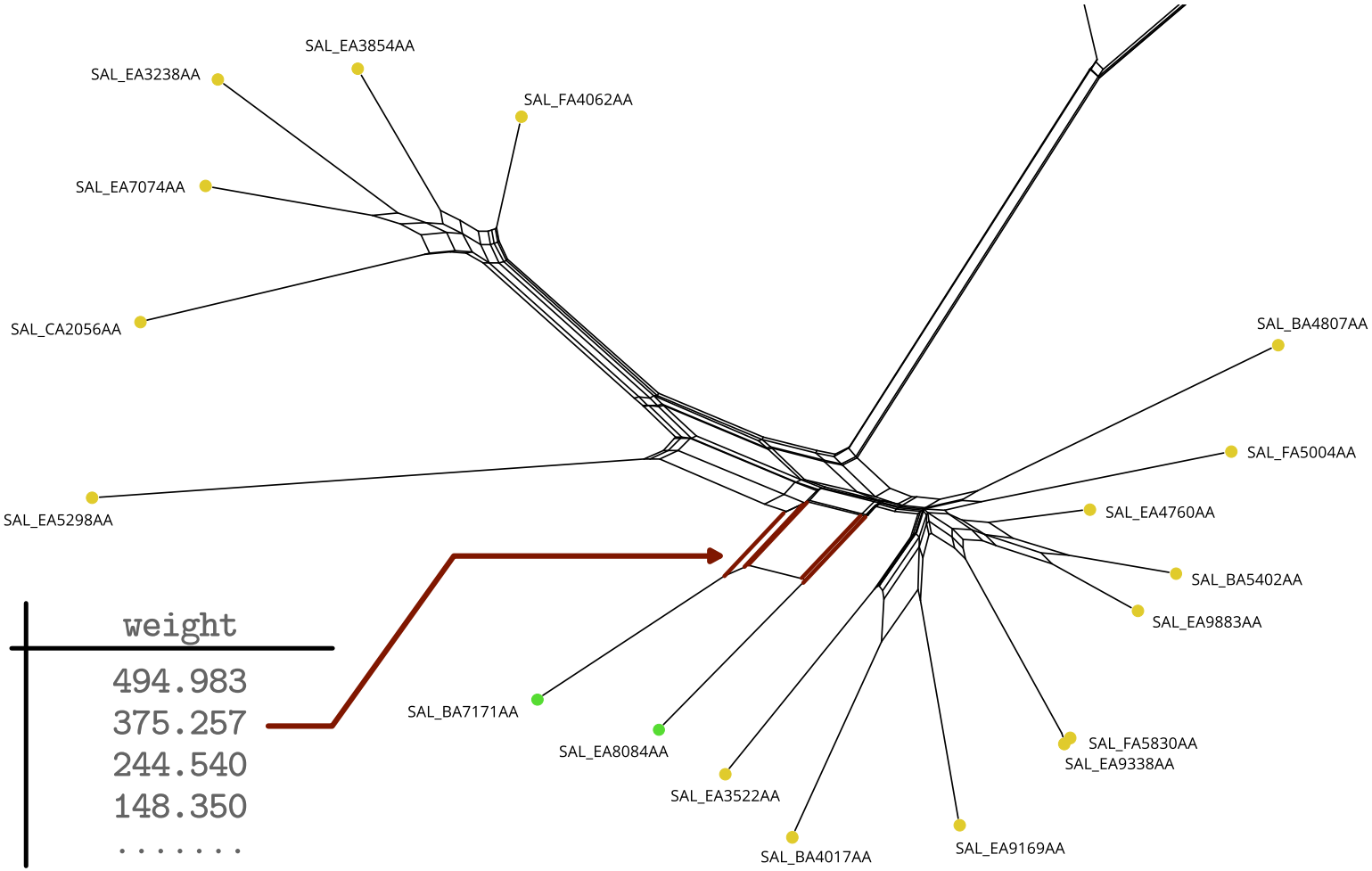
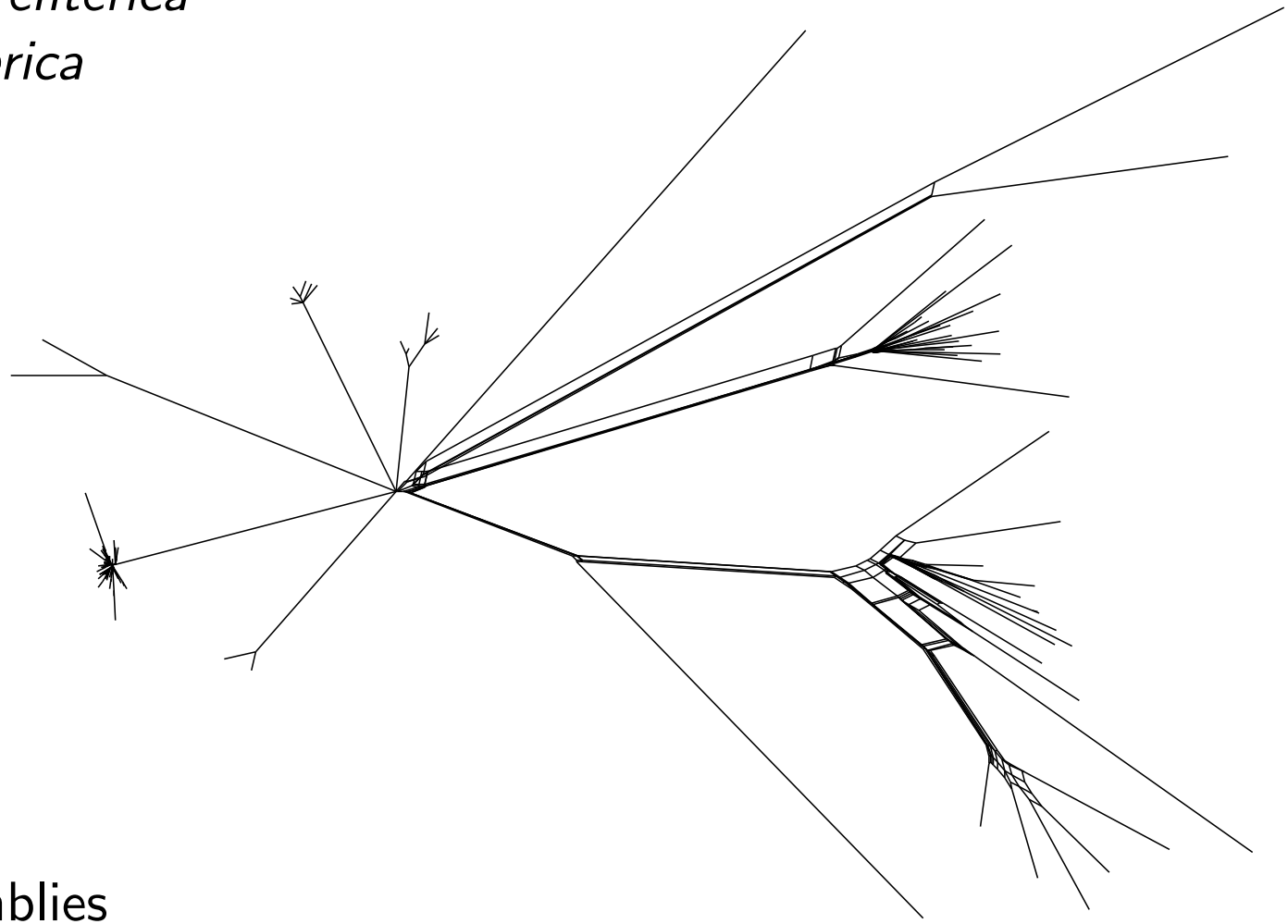


[SplitsTree]

Salmonella enterica
subsp. *enterica*



2964 assemblies
[Zhou et al., 2017]

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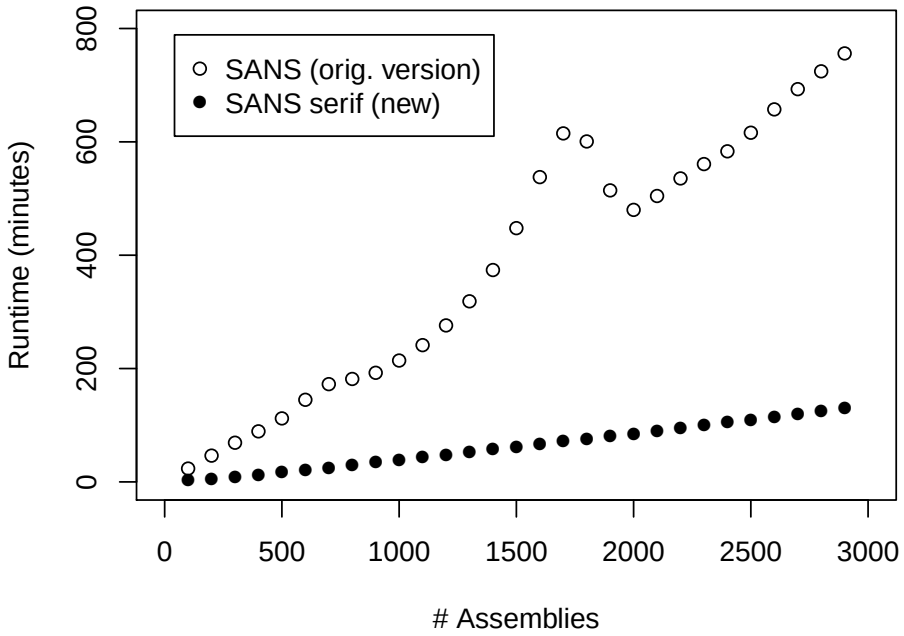

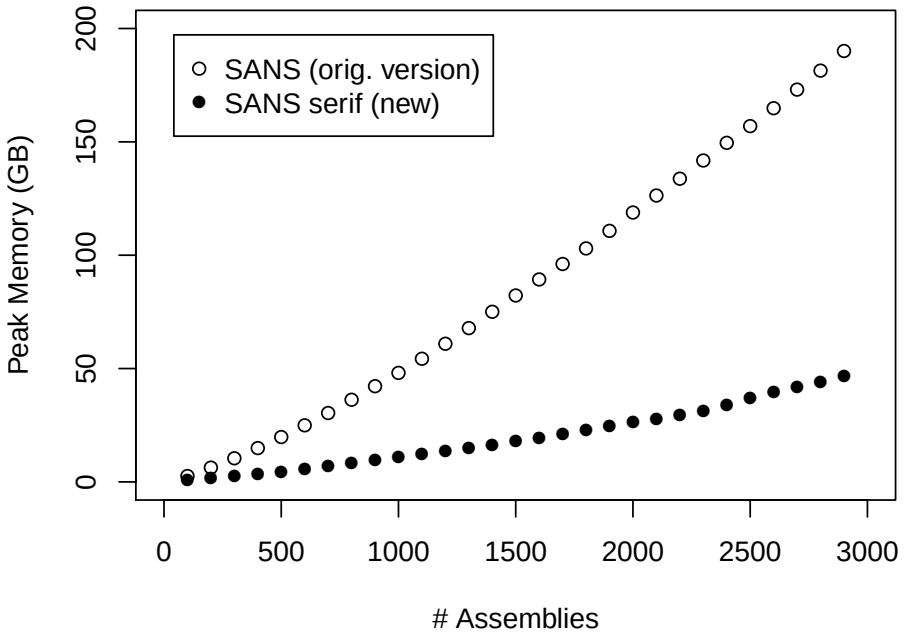

250 assemblies: andi: 110 min, Co-phylog: 9 h, FSWM: 50 h, ...

Outline

IUPAC Characters

Andreas Rempel

R → A or G
Y → C or T
S → G or C
W → A or T
K → G or T
M → A or C
B → C or G or T
D → A or G or T
H → A or C or T
V → A or C or G
N → any base

Consider all variants:

$$\text{AARCGYA} \Rightarrow \begin{cases} \text{AAACGCA} \\ \text{AAACGTA} \\ \text{AAGCGCA} \\ \text{AAGCGTA} \end{cases}$$

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True variant:

supports / is supported by further k-mers

False variant:

cancelled out due to missing inverse split

IUPAC Characters

Andreas Rempel

Simulated phylogeny, 100 leaf genomes, length ∼96 kb, 5 PAM to the root
simulated with ALF [Dalquen et al., 2012]

	original	with 0.1 % `N`'s	
		skipped	replaced
unweighted			
precision	0.90	0.63	0.78
recall	0.70	0.38	0.58
weighted			
precision	0.98	0.95	0.98
recall	0.88	0.68	0.88

Outline

Coding Sequences
Marco Sohn

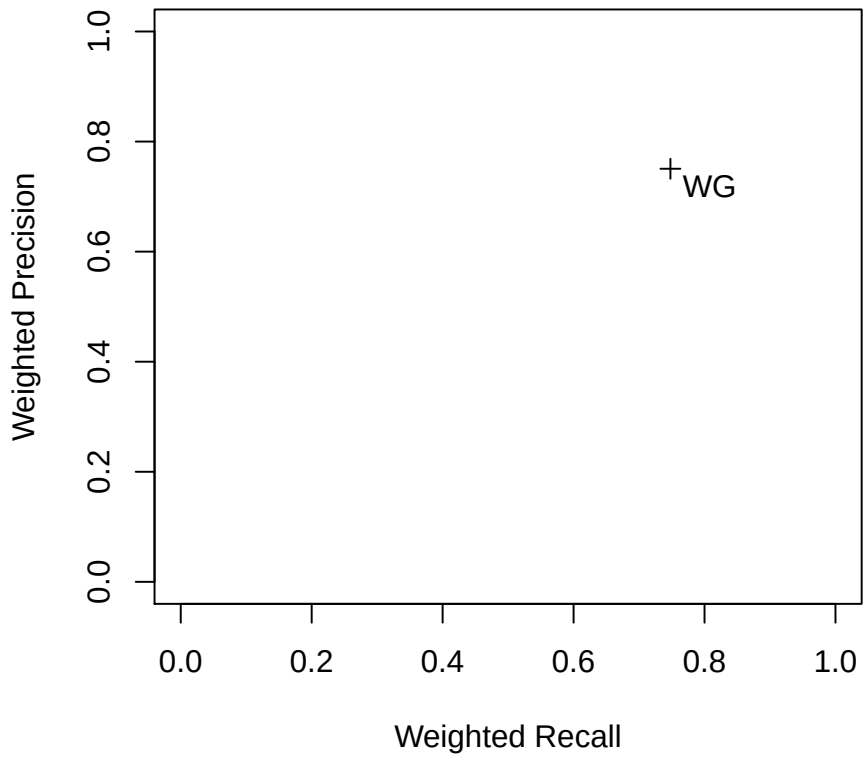
whole genome	$\Rightarrow$	coding sequnences
DNA	$\Rightarrow$	amino acids

```
-a, --amino Consider amino acids:
            --input provides amino acid sequences
            Implies --norev and a default k of 10

-c, --code  Translate DNA: --input provides coding sequences
            Implies --norev and a default k of 10
            optional: ID of the genetic code to be used
            Default: 1 (The Standard Code)
            Use 11 for Bacterial, Archaeal, and Plant Plastid Code
```

Coding Sequences
Marco Sohn

90 Pseudomonas genomes



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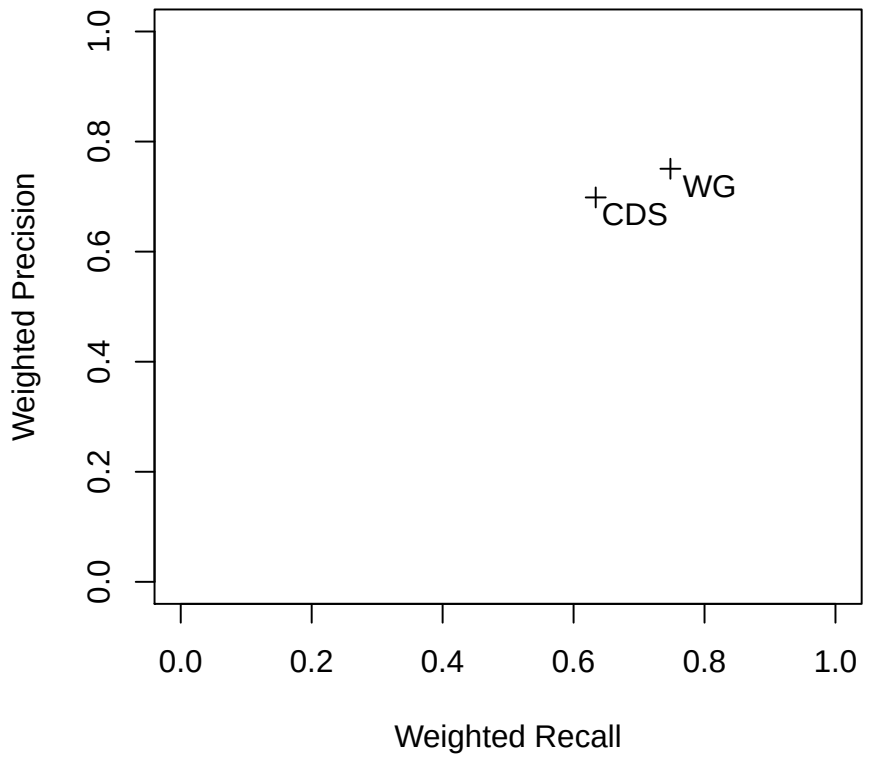

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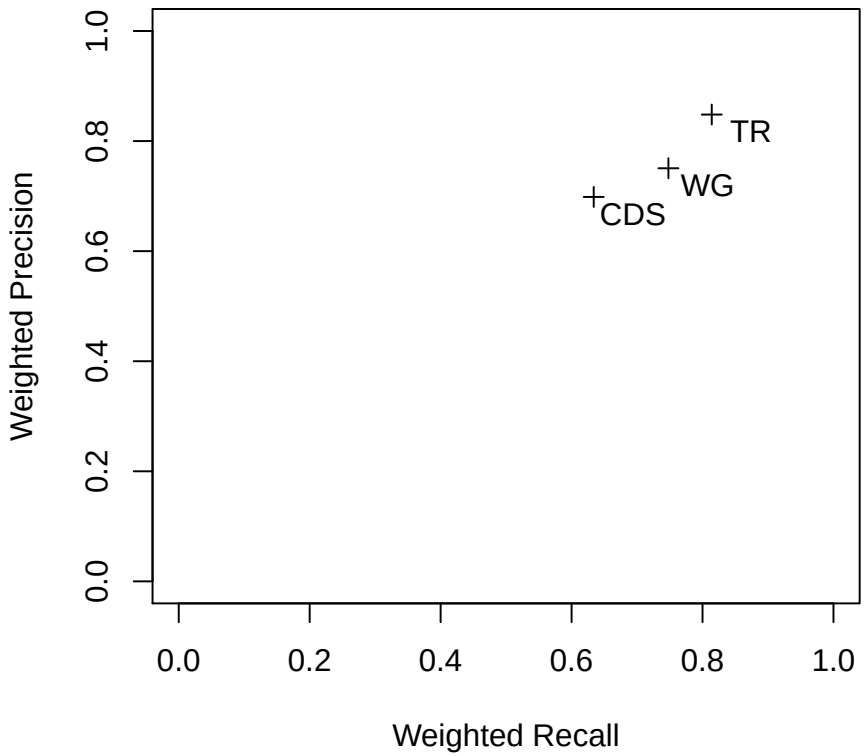
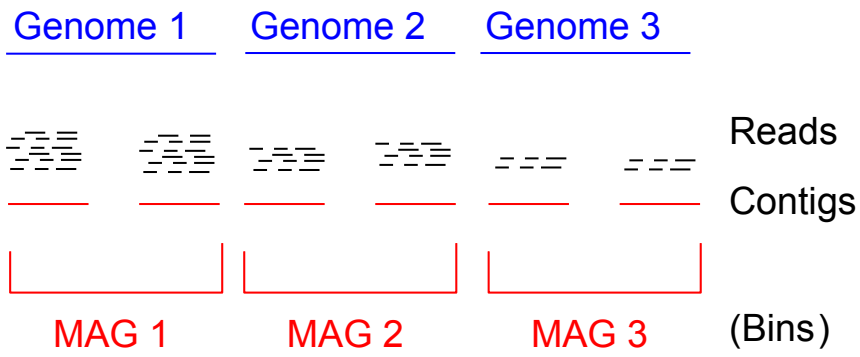


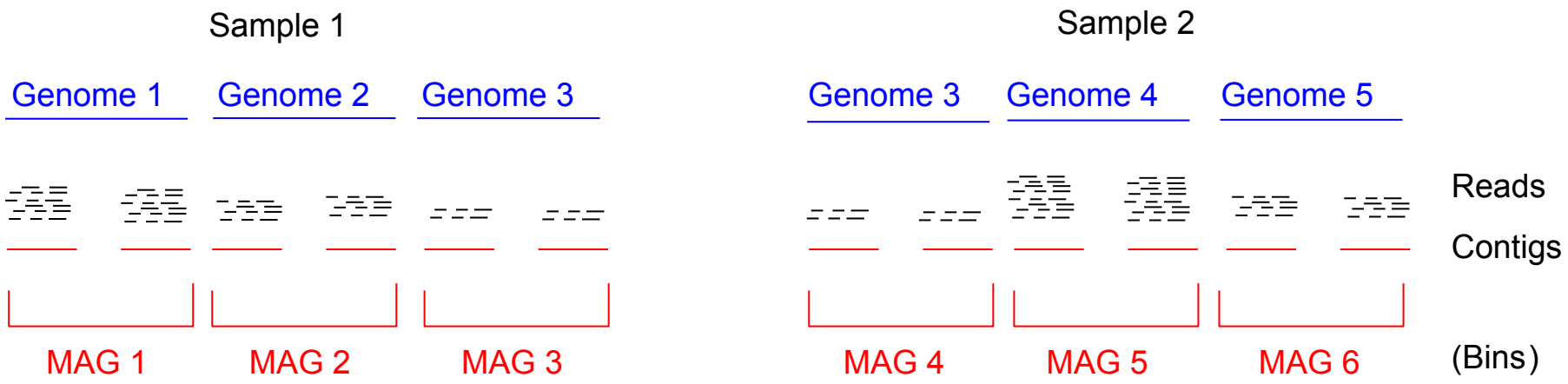
Outline

Clustering MAGs

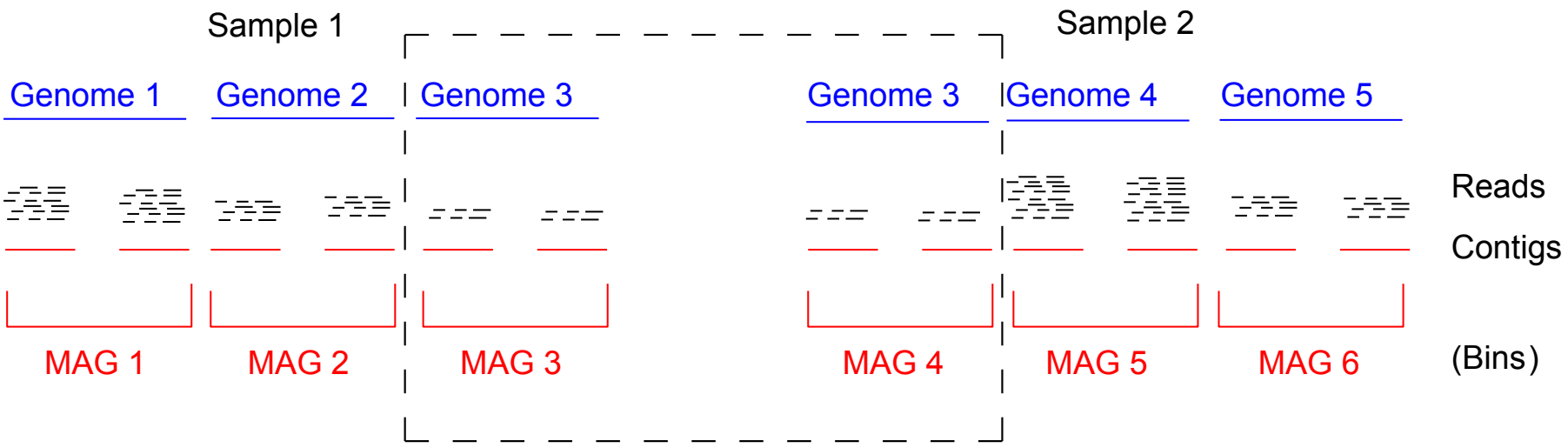


Metagenome Assembled Genomes

Clustering MAGs



Clustering MAGs



Given: Many MAGs from many samples.

Problem: Cluster MAGs such that: cluster = genome

Clustering MAGs

1. infer phylogeny using SANS
2. chop tree into clusters

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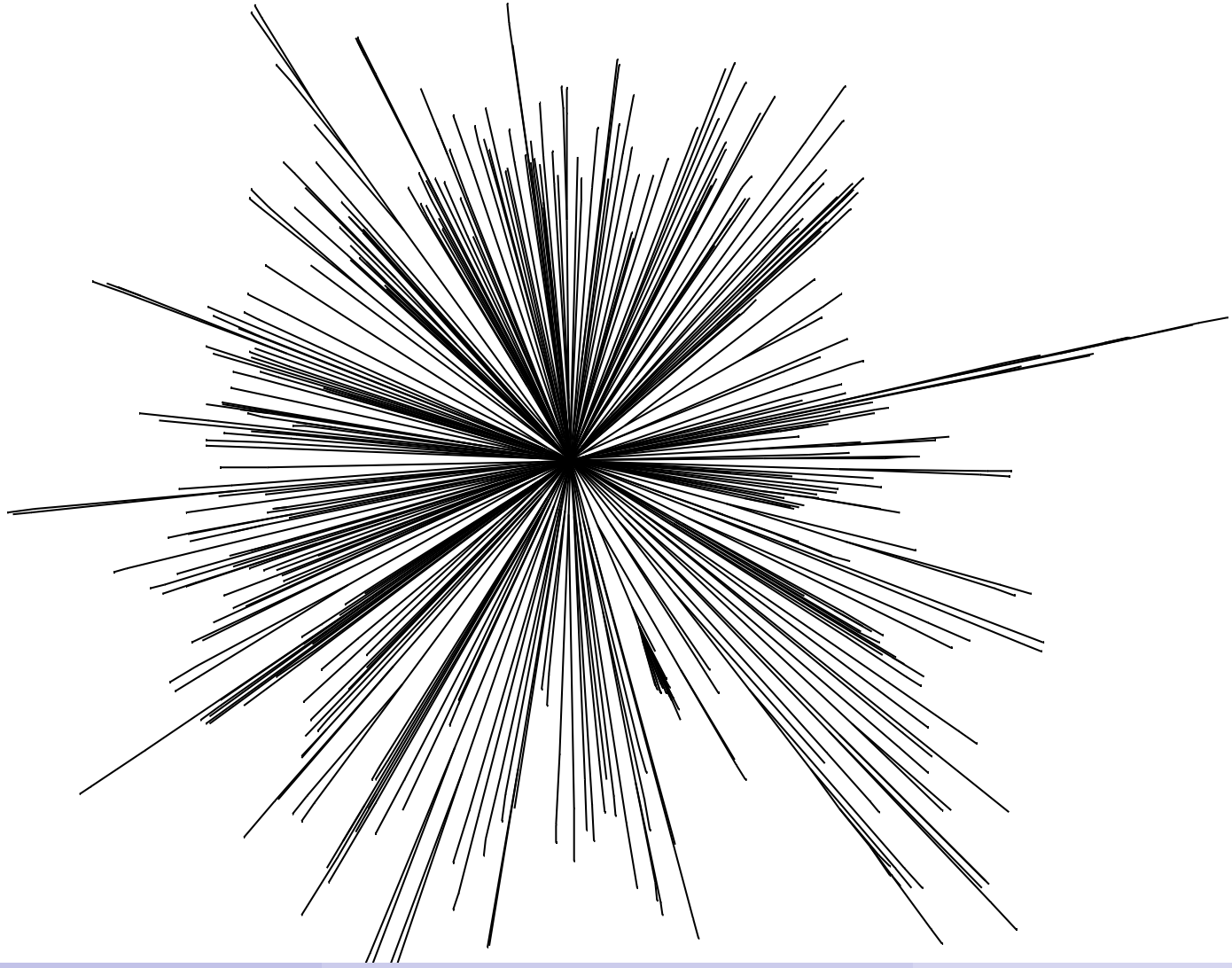

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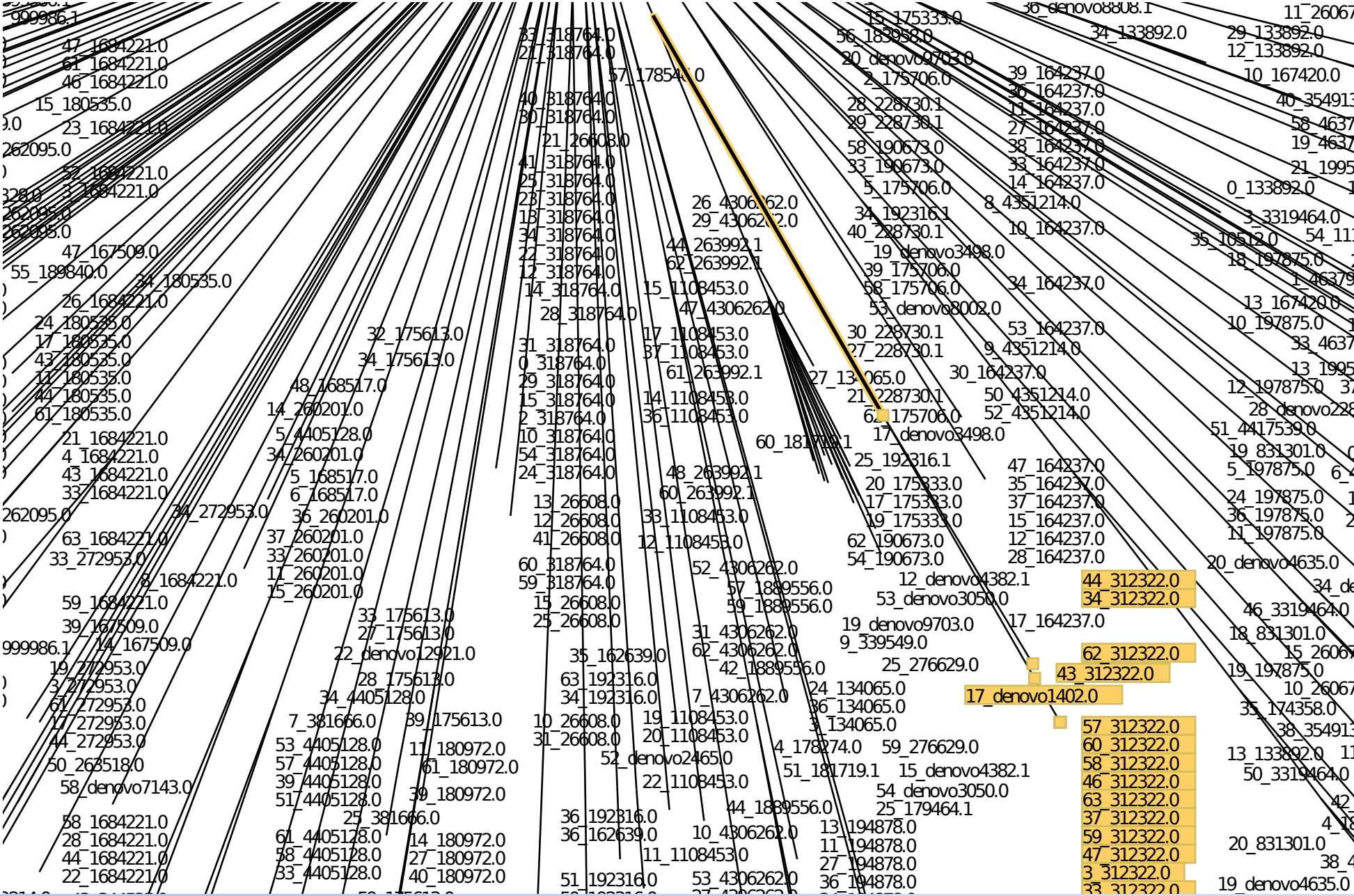

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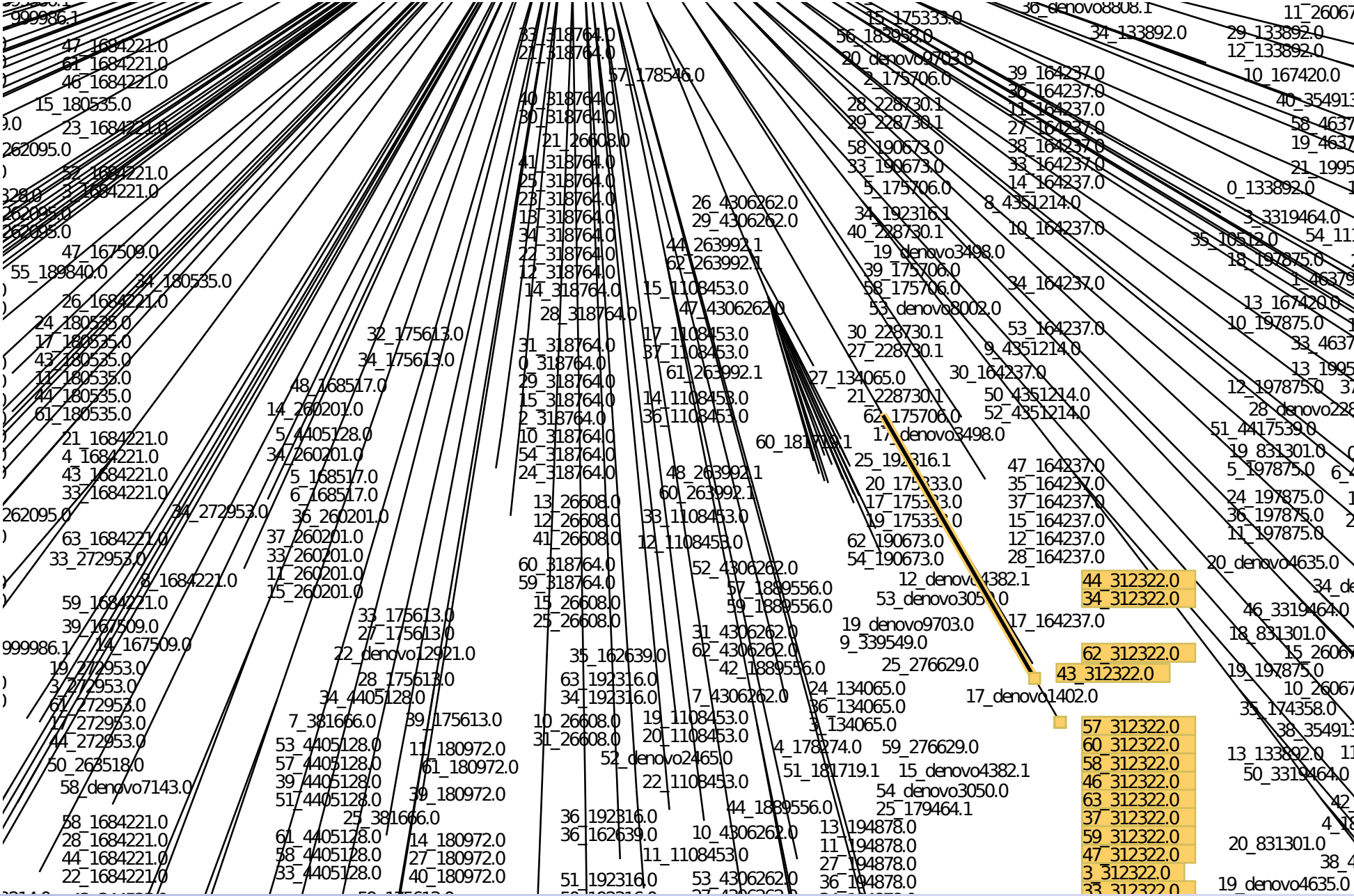

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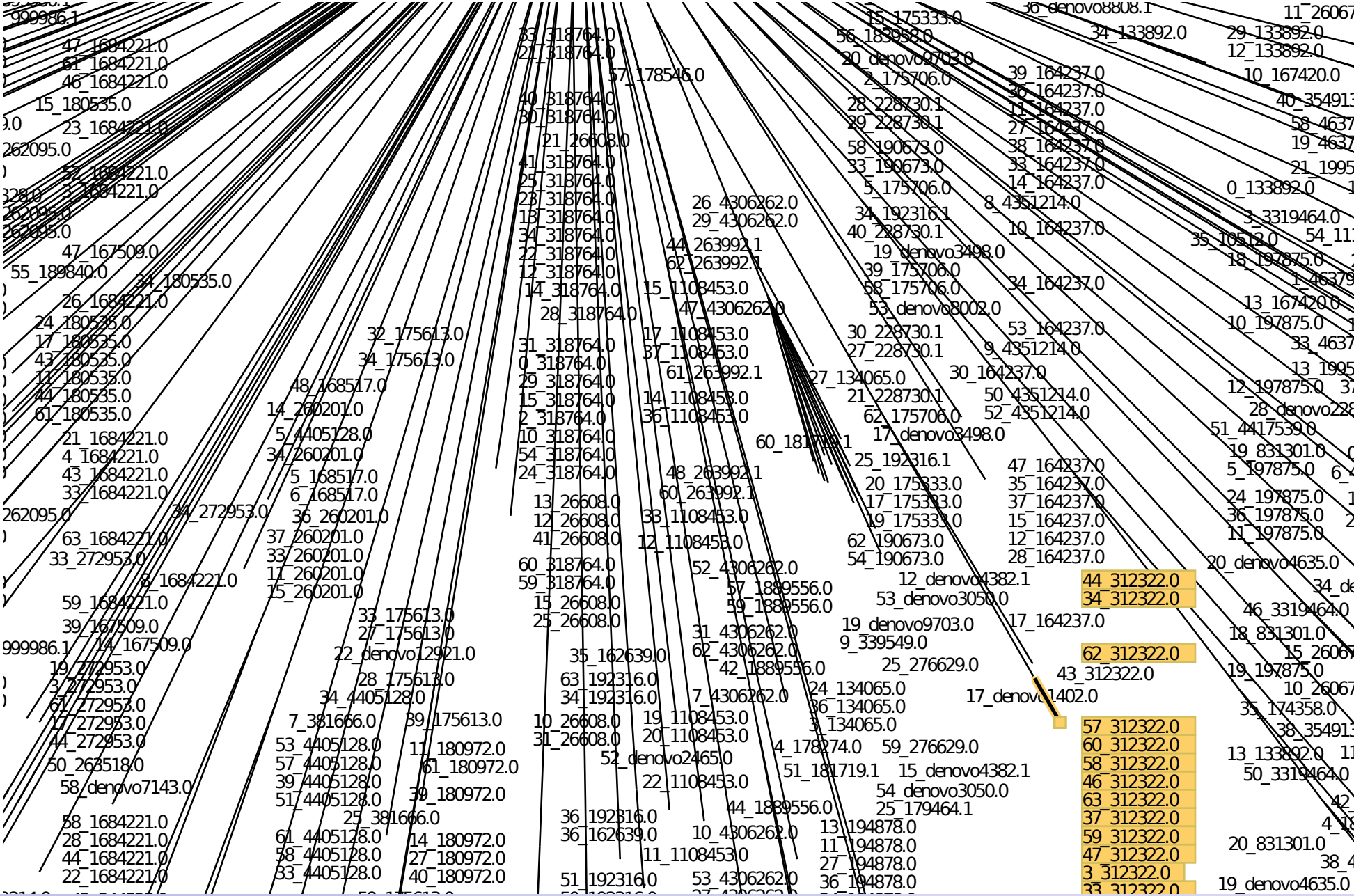
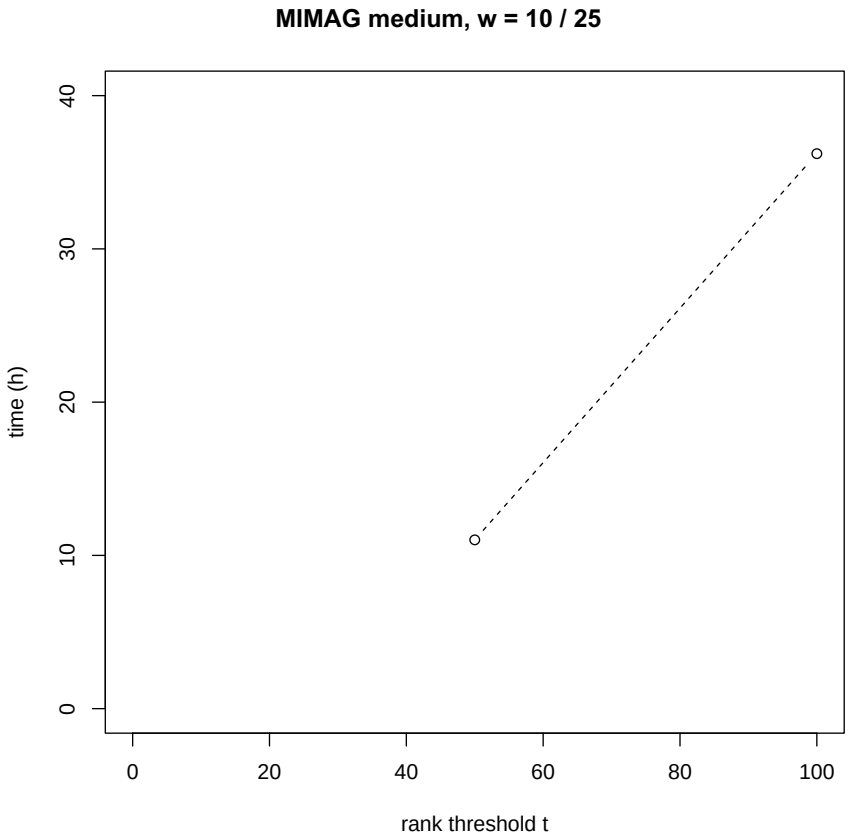
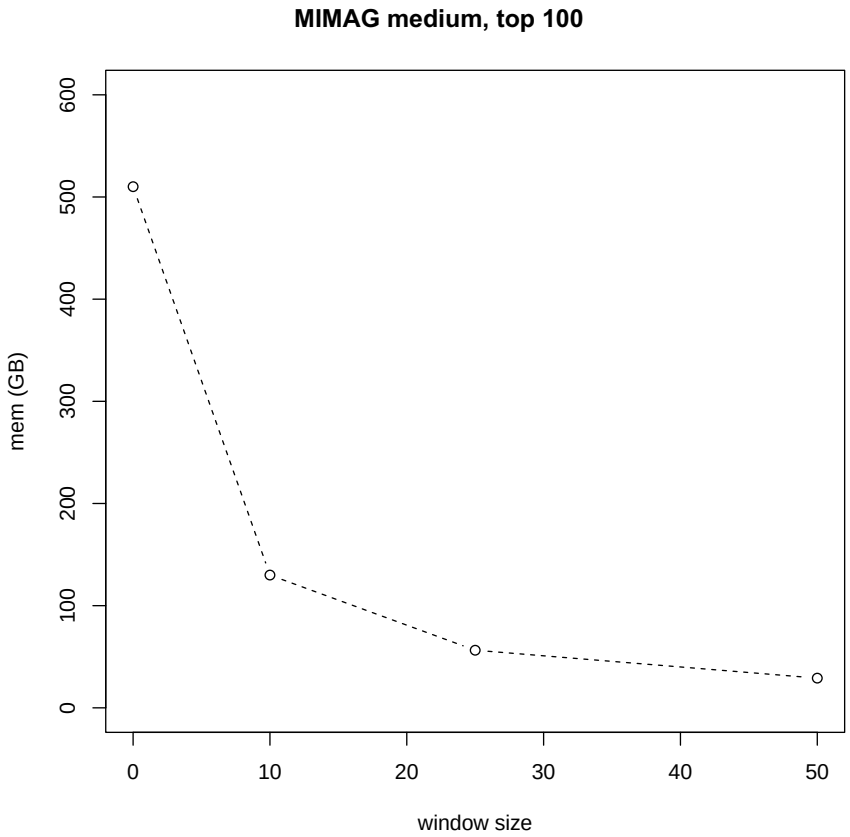


Clustering MAGs

Rank threshold



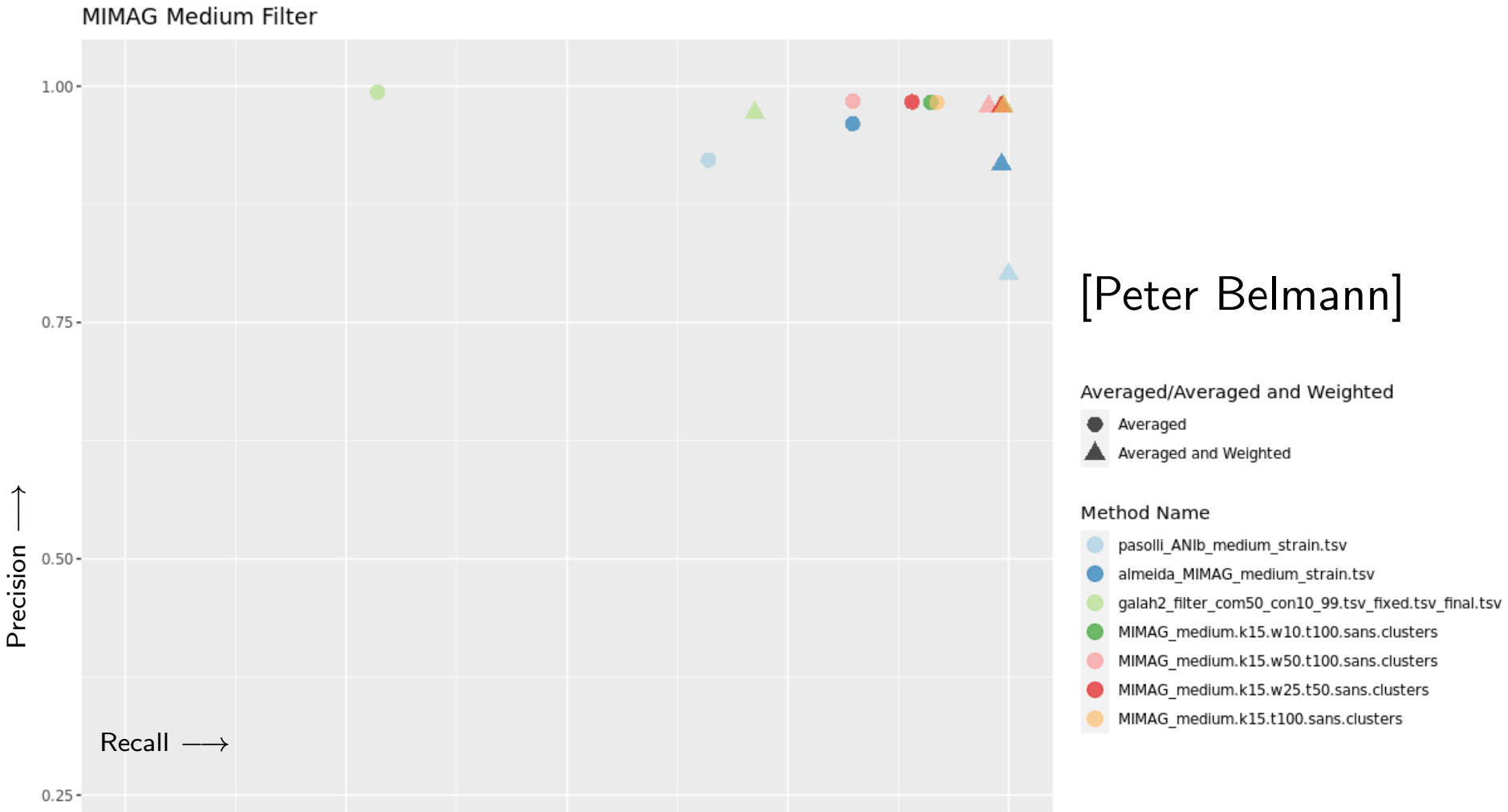
Minimizers *[Andreas Rempel]*



Clustering MAGs

CAMI mouse gut metagenome [Sczyrba et al.]

5 786 MAGs, 686 genomes (simulated)



[Peter Belmann]

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  in particular reading the input files

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Thank you!

Appendix

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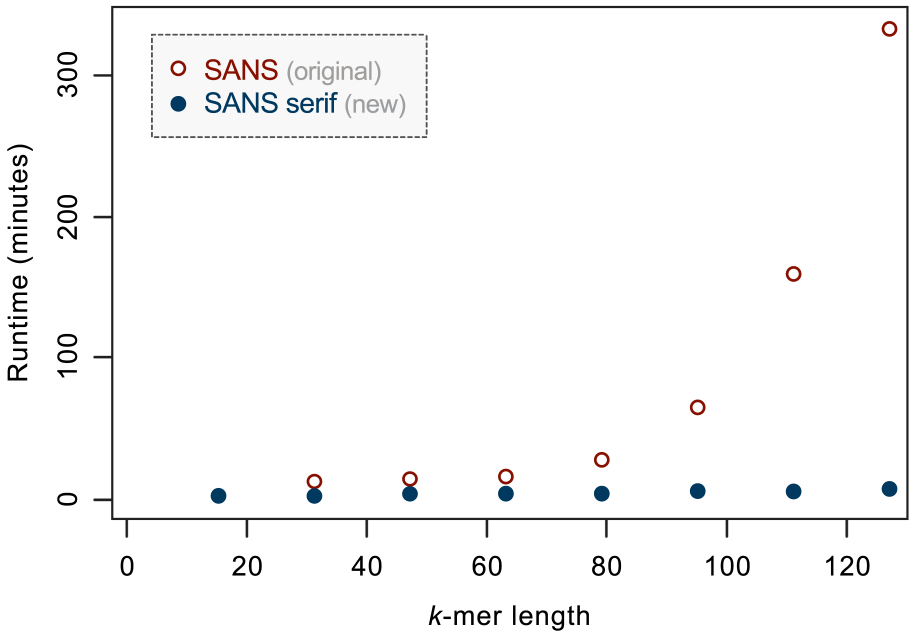
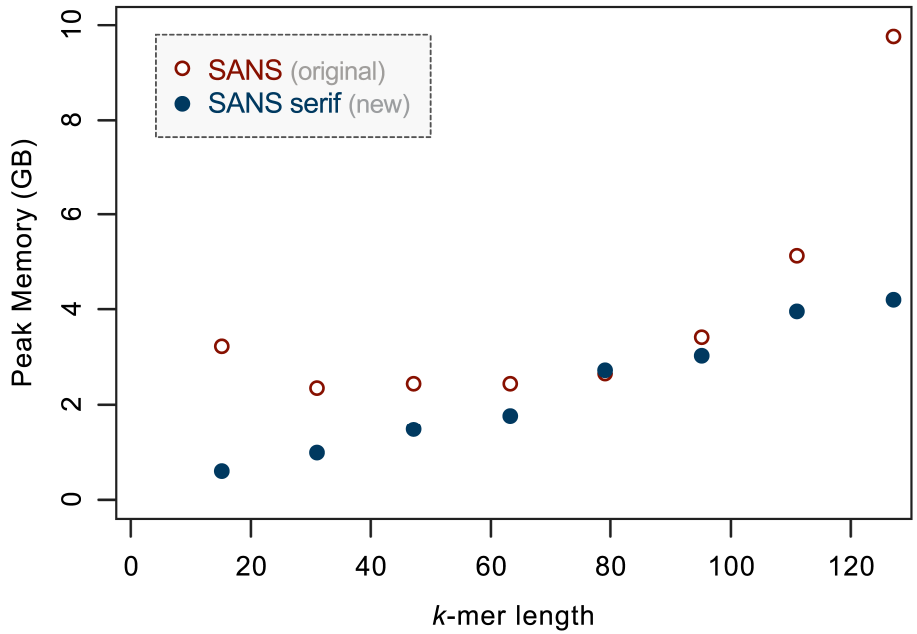
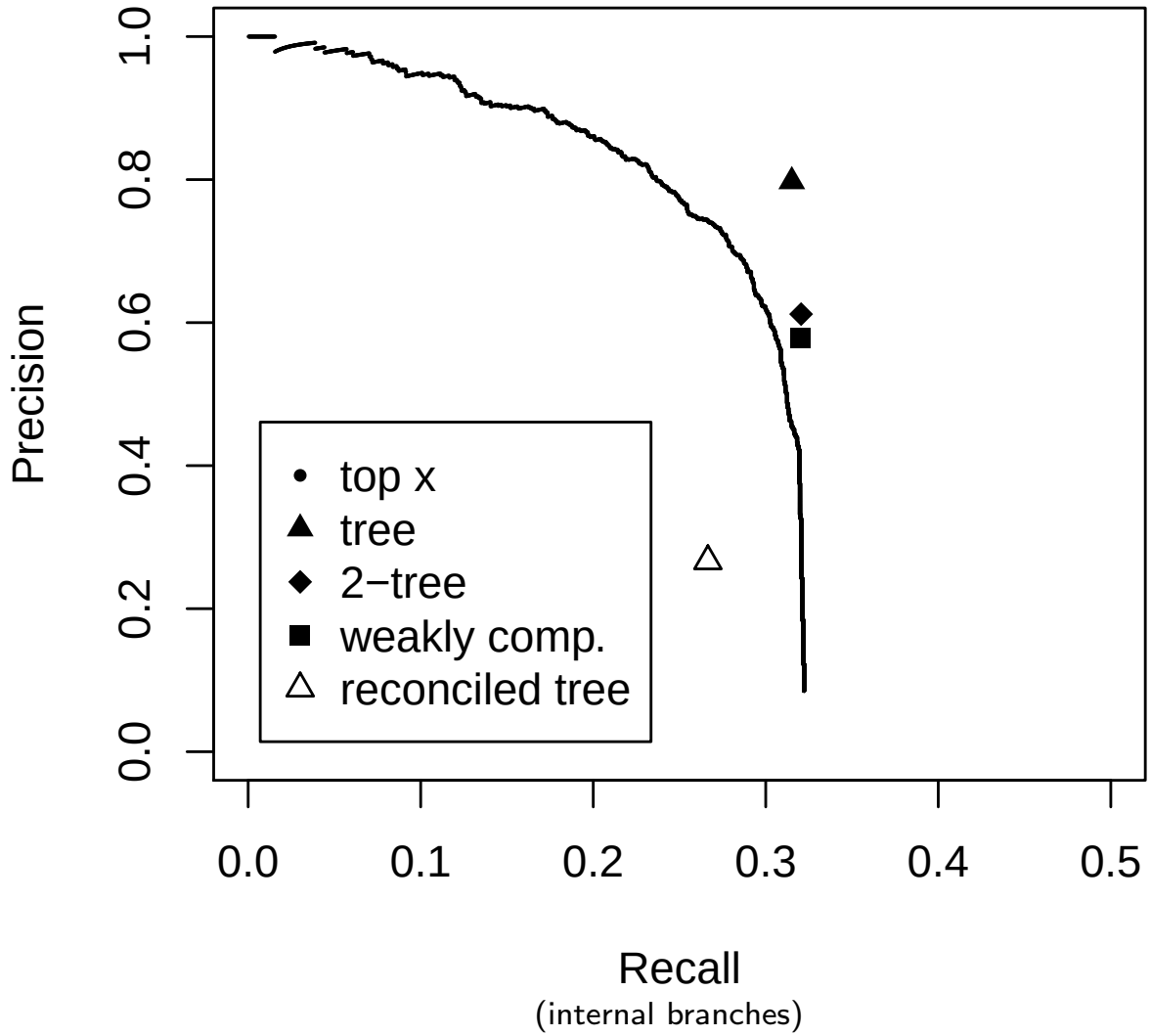




(100 assemblies)

Appendix


Precision
1.0
0.8
0.6
0.4
0.2
0.0
top x
tree
2−tree
weakly comp.
reconciled tree
0.0
0.1
0.2
0.3
0.4
0.5
Recall
(internal branches)

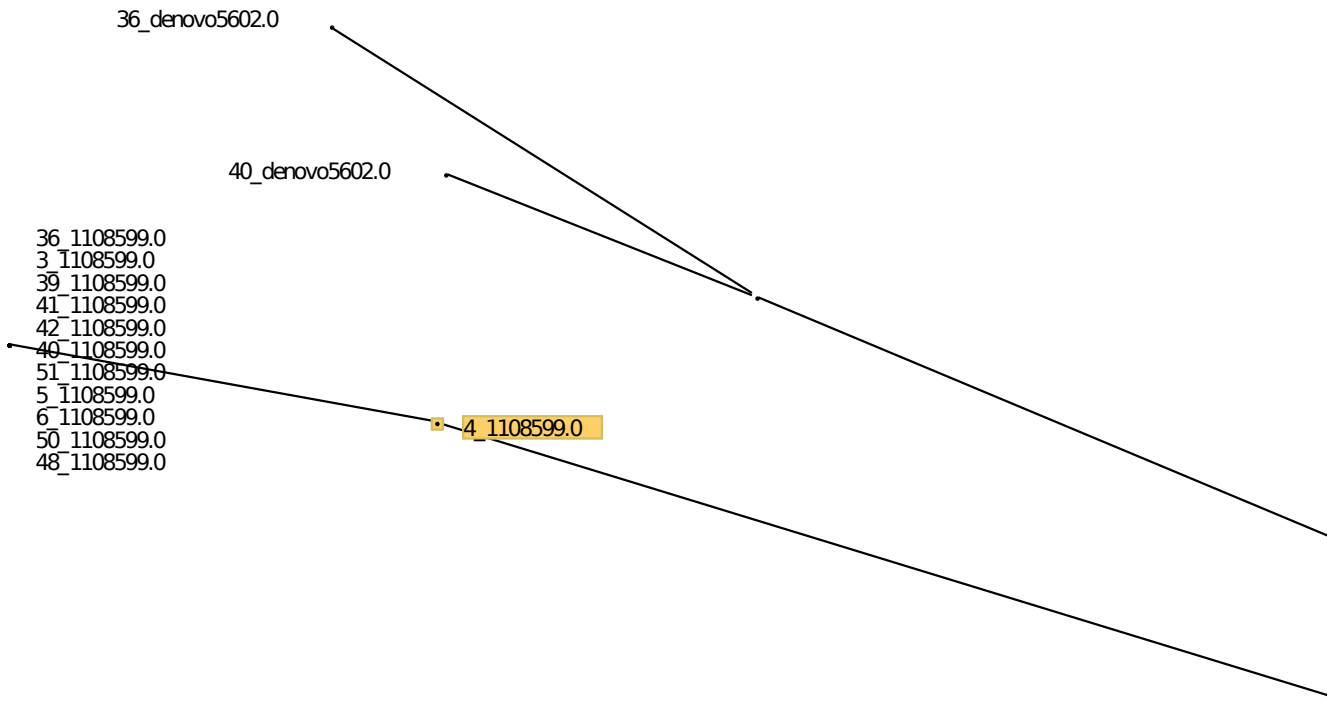
Appendix

90 Pseudomonas genomes

	Whole Genome	Coding Sequences	
	DNA	DNA	AA
k	25	25	8
time	17min	6.9s	1.5s
memory	12G	140M	17M

Appendix

1. SANS, filter for tree, re-root
2. post-order traversal:
   - 2.1 ignore non-branching node
   - 2.2 get clusters from sub-trees (recursively)
     item if edge $\geq$ parent edge:
     remove found clusters from current leaf set
     remaining leaf set $=:$ new cluster



Appendix

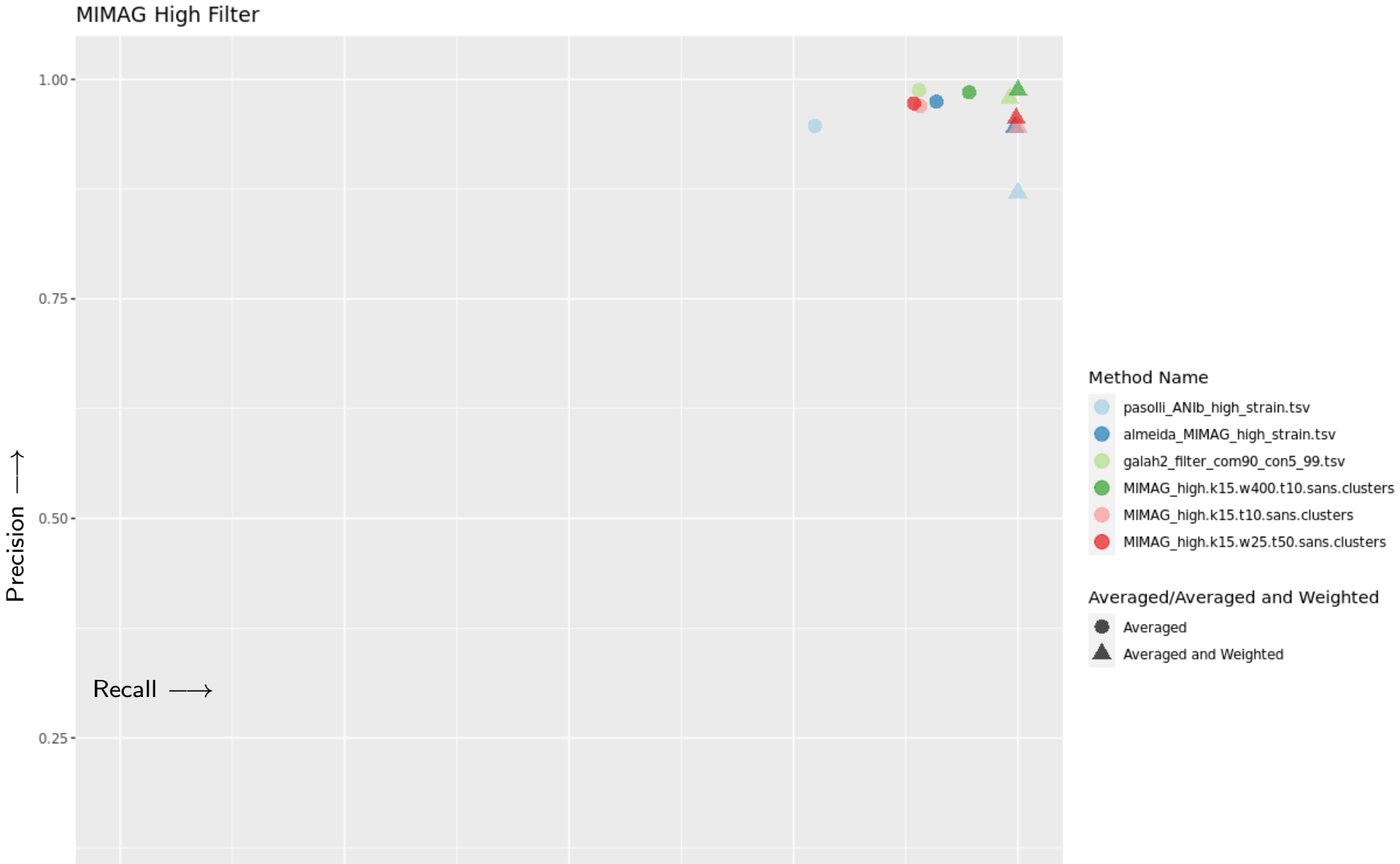
CAMI mouse gut metagenome [Sczyrba et al.]

Simulated dataset representing 64 metagenome samples.

	All	MIMAG medium	MIMAG high
contamination		$< 10\,\%$	$< \ 5\,\%$
completeness		$\geq 50\,\%$	$> 90\,\%$
# MAGs	11 602 (23 GB)	5 786	2 510
# genomes	791	686	349
# species	509	448	271

Clustering MAGS – Strain level

[Peter Belmann]



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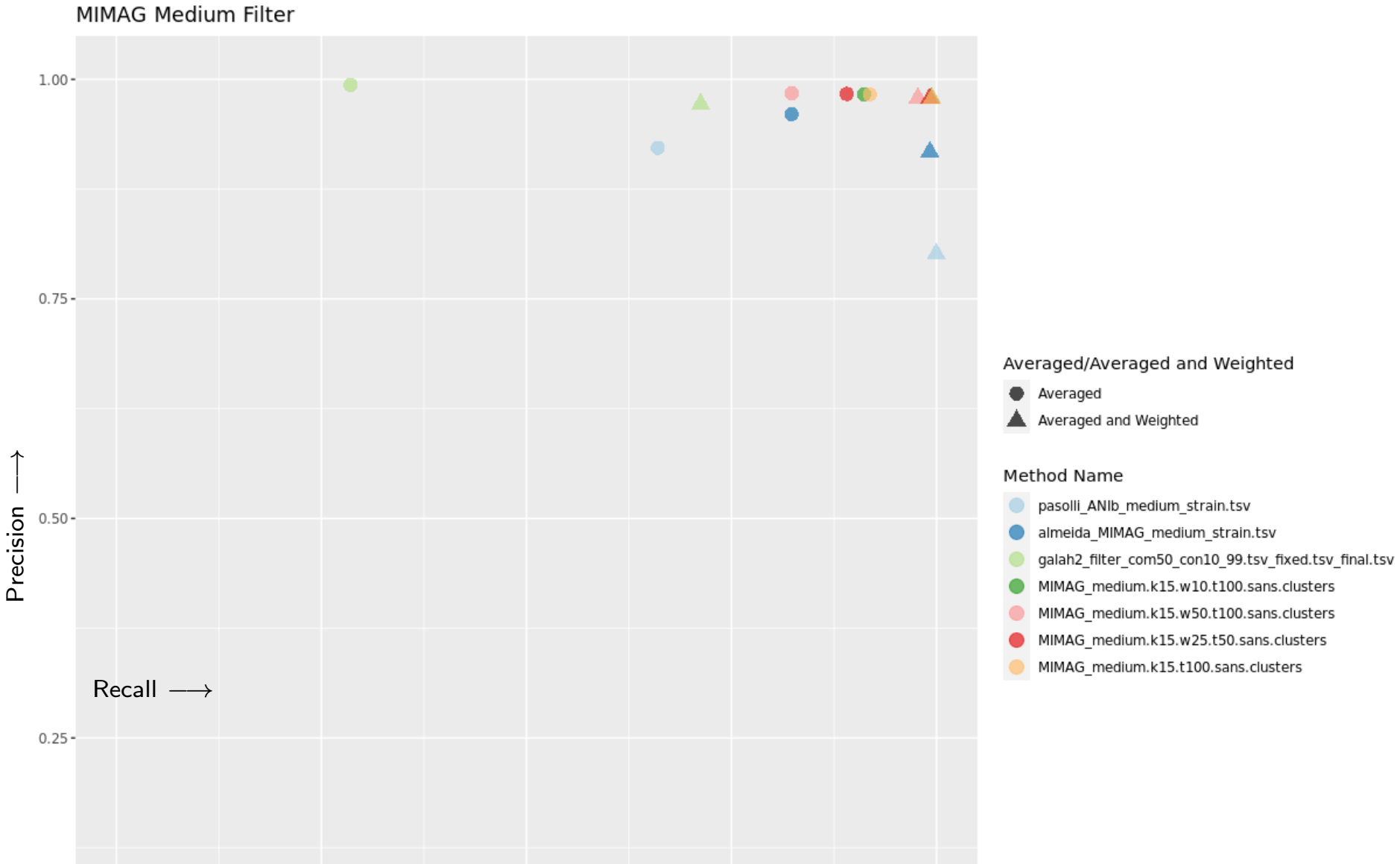

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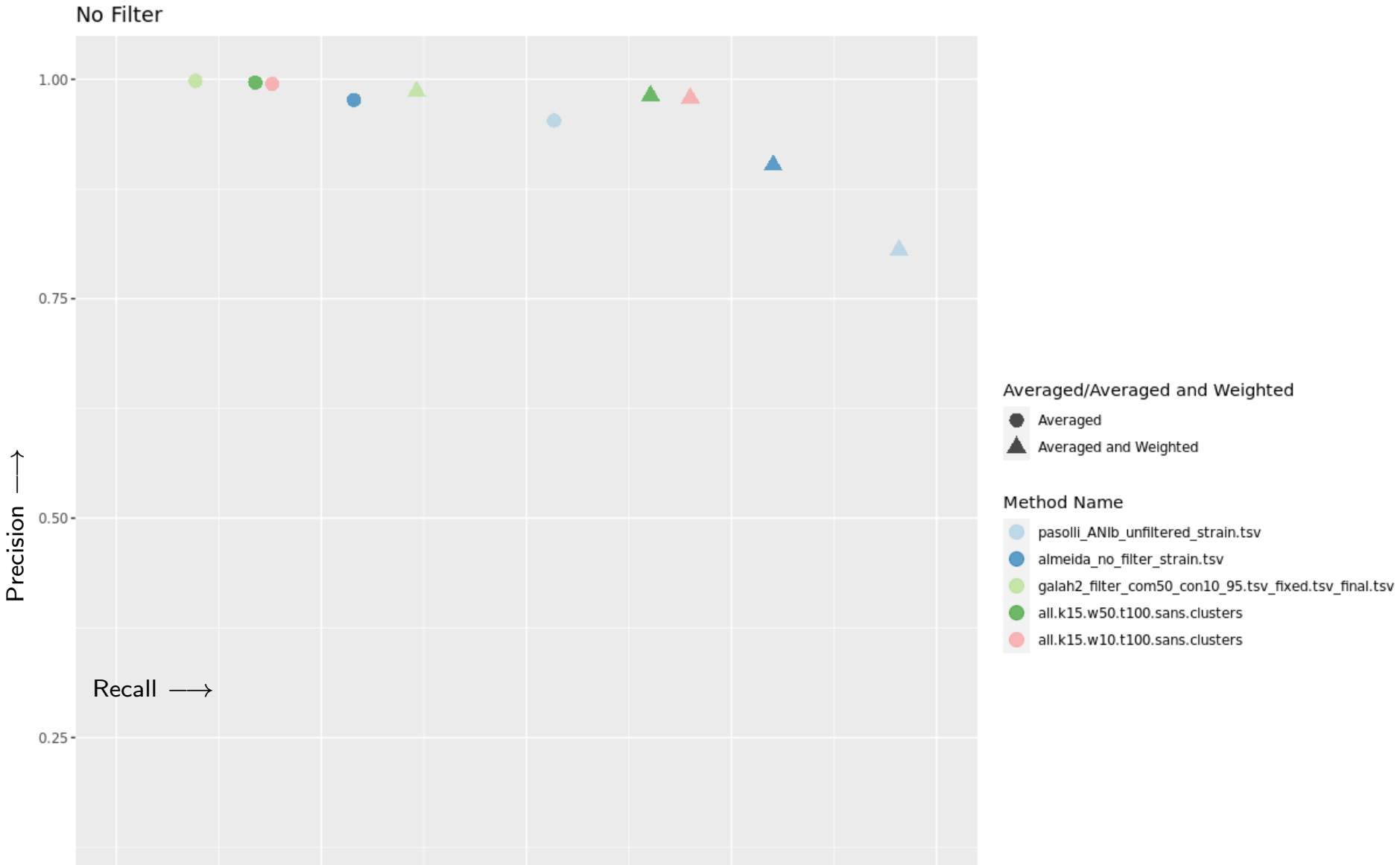

Clustering MAGS – Species level

[Peter Belmann]

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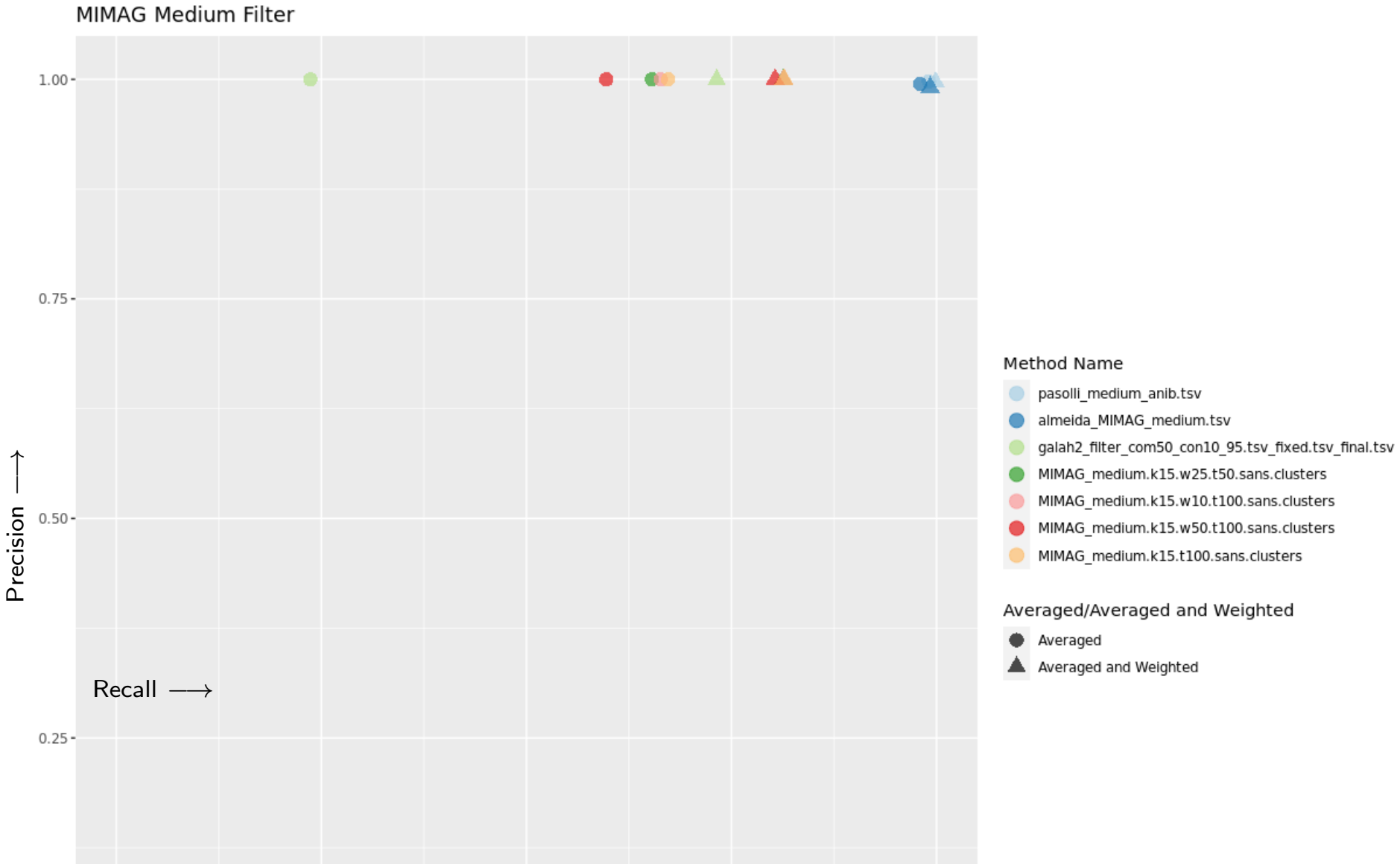

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