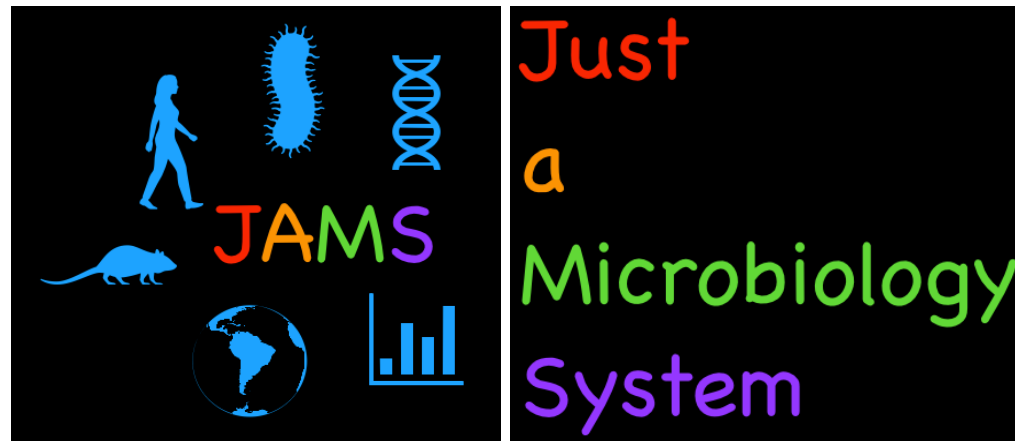




Streamlining microbial shotgun analysis with JAMS - from fastqs to pdfs

John A. McCulloch

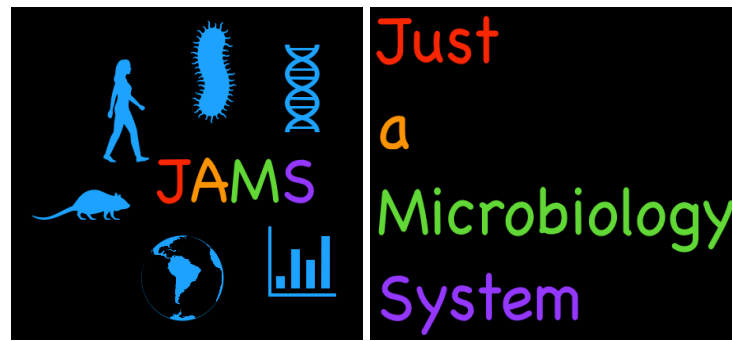
Staff Scientist

Microbiome and Genetics Core CCR, NCI

Laboratory for Integrative Cancer Immunology (LICI) – NCI – NIH

john.mcculloch@nih.gov

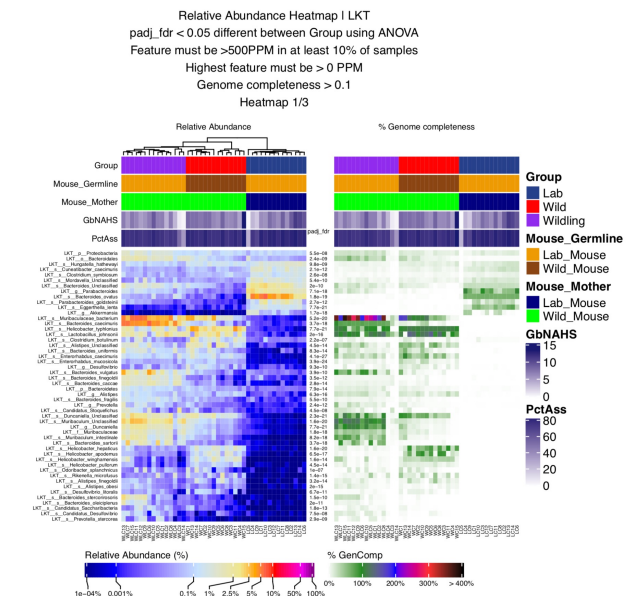
github.com/johnmcculloch

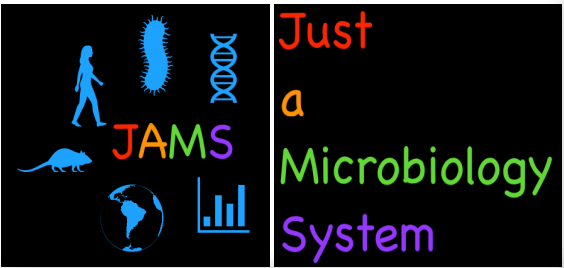


JAMS – a comprehensive framework for advanced microbiome analysis

- A self-contained publicly available software package
https://github.com/johnmcculloch/JAMS_BW
 Biorxiv: <https://doi.org/10.1101/2023.03.03.531026>
- Deals with every step in microbiome analysis (fastqs to stats and plotting)
- **Current version is 1.9.8 (March 2024) – Check for updates, as they are frequent at this stage!**
- Can handle shotgun metagenomic data, but also isolate sequencing, metatranscriptomics and even 16S data
- Requires minimal user input

raw fastqs





nature metabolism

Explore content ▾ About the journal ▾ Publish with us ▾

nature > nature metabolism > articles > article

Article | [Published: 20 August 2021](#)

Neonatal exposure to a wild-derived microbiome protects mice against diet-induced obesity

[Benedikt Hild](#), [Matthew S. Dreier](#), [Ji Hoon Oh](#), [John A. McCulloch](#), [Jonathan H. Badger](#), [Juen Guo](#), [Claire E. Thefaïne](#), [Regina Umarova](#), [Kevin D. Hall](#), [Oksana Gavrilova](#), [Stephan P. Rosshart](#), [Barbara Rehmann](#) 

Nature Metabolism **3**, 1042–1057 (2021) | [Cite this article](#)

4250 Accesses | 16 Citations | 70 Altmetric | [Metrics](#)

Science

Current Issue First release papers Archive About ▾ Submit manuscript

HOME > SCIENCE > VOL. 365, NO. 6452 > LABORATORY MICE BORN TO WILD MICE HAVE NATURAL MICROBIOTA AND MODEL HUMAN IMMUNE RESPONSES

 | RESEARCH ARTICLE

[f](#) [t](#) [in](#) [r](#) [w](#) [e](#)

Laboratory mice born to wild mice have natural microbiota and model human immune responses

[STEPHAN P. ROSSHART](#) , [JASMIN HERZ](#) , [BRIAN G. VASSALLO](#) , [ASHLI HUNTER](#) , [MORGAN K. WALL](#) , [JONATHAN H. BADGER](#) , [JOHN A. MCCULLOCH](#) , [DIMITRIOS G. ANASTASAKIS](#) , [AISHE A. SARSHAD](#) , [IRINA LEONARDI](#) , [NICHOLAS COLLINS](#) , [JOSHUA A. BLATTER](#) , [SEONG-JI HAN](#) , [SAMIRA TAMOUTOUNOUR](#) , [SVETLANA POTAPOVA](#) , [MARK B. FOSTER](#) , [ST. CLAIRE, WUXING YUAN](#) , [SHURJO K. SEN](#) , [MATTHEW S. DREIER](#) , [BENEDIKT HILD](#) , [MARKUS HAFNER](#) , [ILIJAN D. ILIEV](#) , [YASMINE BELKAID](#) , [GIORGIO TRINCHIERI](#) , AND [BARBARA REHERMANN](#)  fewer [Authors Info & Affiliations](#)

SCIENCE • 2 Aug 2019 • Vol 365, Issue 6452 • DOI: 10.1126/science.aaw4361

nature medicine

Explore content ▾ About the journal ▾ Publish with us ▾

nature > nature medicine > articles > article

Article | [Published: 28 February 2022](#)

Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1

[John A. McCulloch](#), [Diwakar Davar](#), [Richard R. Rodrigues](#), [Jonathan H. Badger](#), [Jennifer R. Fang](#), [Alicia M. Cole](#), [Ascharya K. Balaji](#), [Marie Vetizou](#), [Stephanie M. Prescott](#), [Miriam R. Fernandes](#), [Raquel G. F. Costa](#), [Wuxing Yuan](#), [Rosalba Salcedo](#), [Erol Bahadiroglu](#), [Soumen Roy](#), [Richelle N. DeBlasio](#), [Robert M. Morrison](#), [Ding, Bochra Zidi](#), [Ava Lowin](#), [Saranya Chakka](#), [Wentao Gao](#), [Yasmine Belkaid](#) 

+ Show authors

[Cite this article](#)

Altmetric | [Metrics](#)

Cell

Volume 184, Issue 3, 4 February 2021, Pages 615–627.e17

Article

Infection trains the host for microbiota-enhanced resistance to pathogens

[Apollo Stacy](#) ^{1 2 3}  , [Vinicius Andrade-Oliveira](#) ^{1 7}, [John A. McCulloch](#) ⁴, [Benedikt Hild](#) ⁵, [Ji Hoon Oh](#) ⁵, [P. Juliana Perez-Chaparro](#) ², [Choon K. Sim](#) ^{6 8}, [Ai Ing Lim](#) ¹, [Verena M. Link](#) ¹, [Michel Enamorado](#) ¹, [Giorgio Trinchieri](#) ⁴, [Julia A. Segre](#) ⁶, [Barbara Rehmann](#) ⁵, [Yasmine Belkaid](#) ^{1 2 9}  



Science

Current Issue First release papers Archive About ▾ Submit manuscript

HOME > SCIENCE > VOL. 371, NO. 6529 > FECAL MICROBIOTA TRANSPLANT OVERCOMES RESISTANCE TO ANTI-PD-1 THERAPY IN MELANOMA PATIENTS

 | RESEARCH ARTICLE

[f](#) [t](#) [in](#) [r](#) [w](#) [e](#)

Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients

[DIWAKAR DAVAR](#) , [AMIRAN K. DZUTSEV](#) , [JOHN A. MCCULLOCH](#) , [RICHARD R. RODRIGUES](#) , [JOE-MARC CHAUVIN](#) , [ROBERT M. MORRISON](#) , [RICHELLE N. DEBLASIO](#) , [CARMINE MENNA](#) , [QUANQUAN DING](#) , [ORNELLA PAGLIANO](#) , [BOCHRA ZIDI](#) , [SHUOWEN ZHANG](#) , [JONATHAN H. BADGER](#) , [MARIE VETIZOU](#) 

Science

Current Issue First release papers Archive About ▾ Submit manuscript

 | REPORT | IMMUNOTHERAPY

[f](#) [t](#) [in](#) [r](#) [w](#) [e](#)

Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response

[CHRISTINE N. SPENCER](#) , [JENNIFER L. MCQUADE](#) , [VANCHESWARAN GOPALAKRISHNAN](#) , [JOHN A. MCCULLOCH](#) , [MARIE VETIZOU](#) , [ALEXANDRIA P. COGDILL](#) , [MD A. WADUD KHAN](#) , [XIAOTAO ZHANG](#) , [MICHAEL G. WHITE](#) , [CHRISTINE B. PETERSON](#) , [MATTHEW C. WONG](#) , [GOLNAZ MORAD](#) , [THERESA RODGERS](#) , [JONATHAN H. BADGER](#) , [BETH A. HELMINK](#) , [MILES C. ANDREWS](#) , [RICHARD R. RODRIGUES](#) , [ANDREY MORGUN](#) , [YOUNG S. KIM](#) , [JASON ROSZIK](#) , [KRISTI L. HOFFMAN](#) , [JIALI ZHENG](#) , [YIFAN ZHOU](#) , [YUSRA B. MEDIK](#) , [LAURA M. KAHN](#) , [SARAH JOHNSON](#) , [COURTNEY W. HUDGENS](#) 

nature communications



Article

<https://doi.org/10.1038/s41467-024-45107-3>

Deep phenotyping of post-infectious myalgic encephalomyelitis/chronic fatigue syndrome

Received: 24 June 2023

Accepted: 16 January 2024

Published online: 21 February 2024

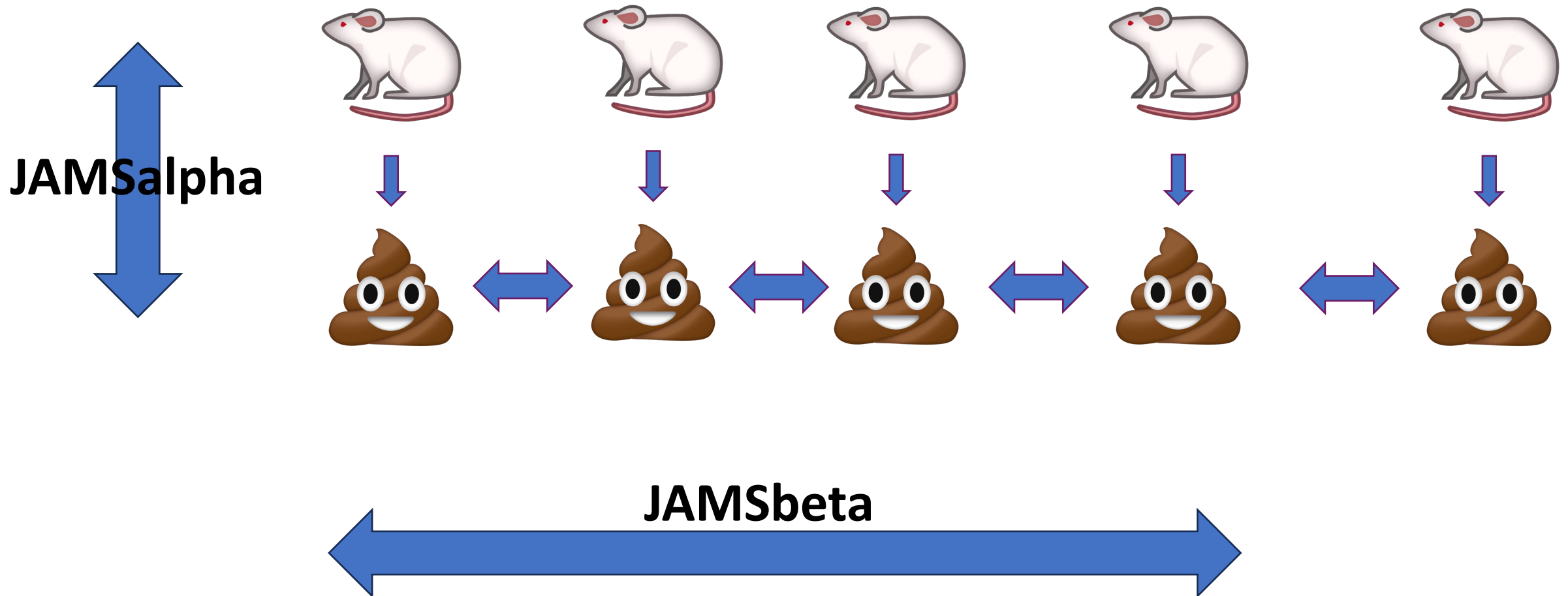
 Check for updates

A list of authors and their affiliations appears at the end of the paper

Post-infectious myalgic encephalomyelitis/chronic fatigue syndrome (PI-ME/CFS) is a disabling disorder, yet the clinical phenotype is poorly defined, the pathophysiology is unknown, and no disease-modifying treatments are available. We used rigorous criteria to recruit PI-ME/CFS participants with matched controls to conduct deep phenotyping. Among the many physical and cog-

Metagenomics: The two phases of analysis

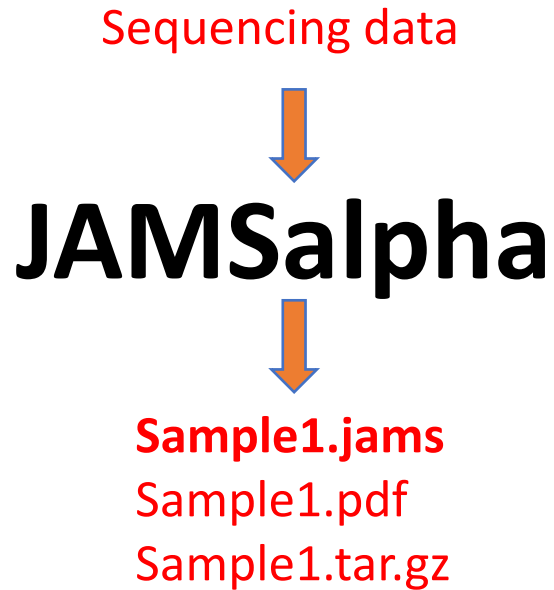
A unique characteristic of the JAMS philosophy is the dissociation between the two main phases of microbiome sample analysis



Microbial analysis made easy

Within sample analysis

```
JAMSalpha -f Sample1_R1.fastq.gz -r Sample1_R2.fastq.gz  
-d ./JAMSdb/JAMSdb202212 -A metagenome -H human -p Sample1
```



Input sequences:	Reads <i>or</i> SRA accession number <i>or</i> contigs <i>or</i> GenBank assembly accession number
Path to database:	JAMS-formatted database (available to download)
Information about host:	Human, mouse, none or something else (taxid or name)
Information about sample:	Prefix and what to do – metagenome, metatranscriptome or isolate?

Microbial analysis made easy

```
JAMStbeta -y /path/to/jamsfiles -x metadata.xlsx -p MyProjectName
```

*.jams metadata

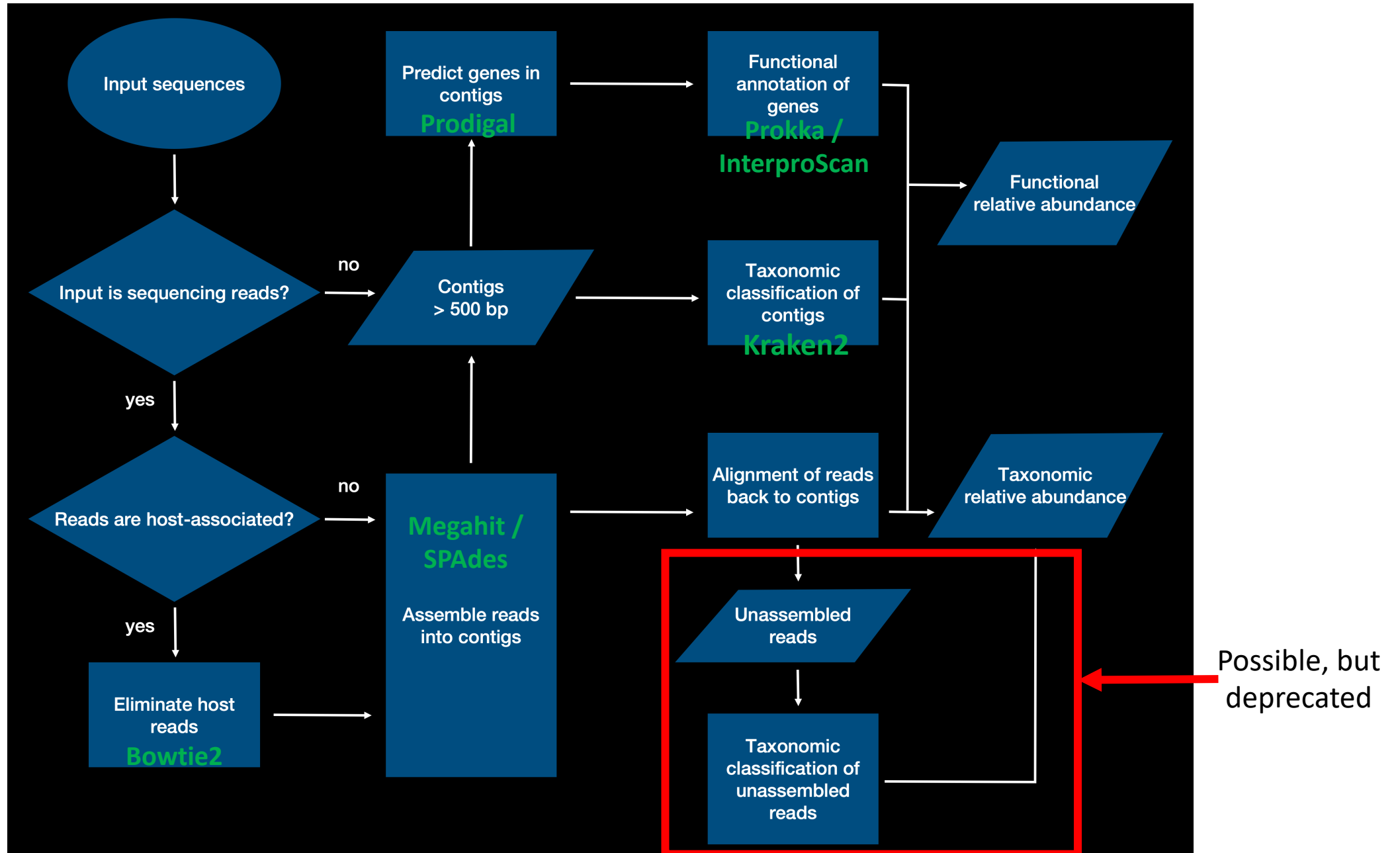
JAMStbeta

MyProjectName.RData

Ordination plots
Heatmaps
Statistics

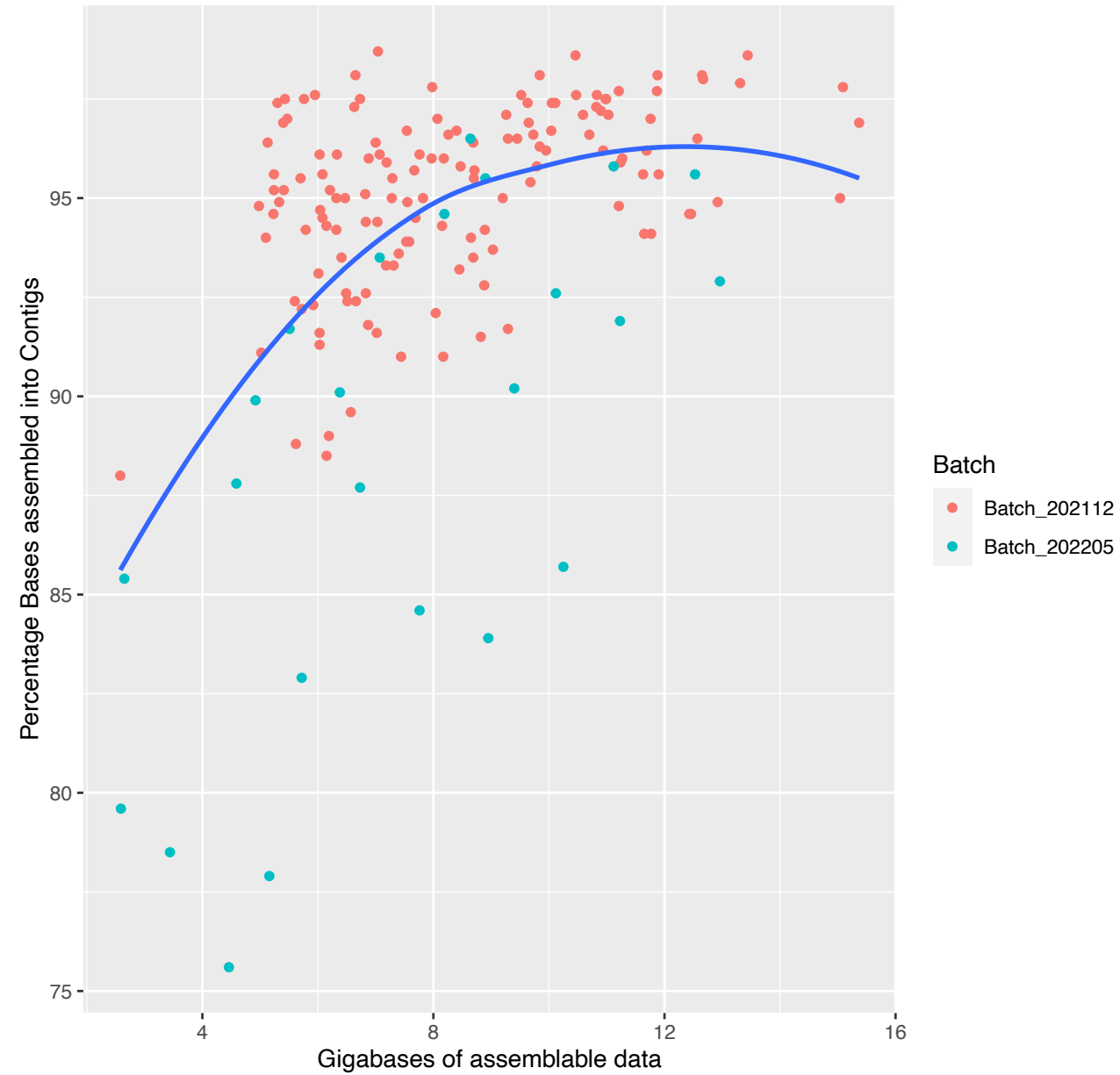
	A	B	C	D	E	F	G	H
1	Sample	Well	Group	Timepoint_days	Mouse_number	Treatment	Vancomycin_used	Neomycin_used
2	MR50G1M1D1dna202212	A1	G1	1	Mouse_1	none	Not_Used	Not_Used
3	MR50G1M2D1dna202212	B1	G1	1	Mouse_2	none	Not_Used	Not_Used
4	MR50G1M3D1dna202212	C1	G1	1	Mouse_3	none	Not_Used	Not_Used
5	MR50G1M4D1dna202212	D1	G1	1	Mouse_4	none	Not_Used	Not_Used
6	MR50G1M5D1dna202212	E1	G1	1	Mouse_5	none	Not_Used	Not_Used
7	MR50G2M1D1dna202212	F1	G2	1	Mouse_1	vancomycin	Used	Not_Used
8	MR50G2M2D1dna202212	G1	G2	1	Mouse_2	vancomycin	Used	Not_Used
9	MR50G2M3D1dna202212	H1	G2	1	Mouse_3	vancomycin	Used	Not_Used
10	MR50G2M4D1dna202212	A2	G2	1	Mouse_4	vancomycin	Used	Not_Used
11	MR50G2M5D1dna202212	B2	G2	1	Mouse_5	vancomycin	Used	Not_Used
12	MR50G3M1D1dna202212	C2	G3	1	Mouse_1	vancomycin+neomycin	Used	Used
13	MR50G3M2D1dna202212	D2	G3	1	Mouse_2	vancomycin+neomycin	Used	Used
14	MR50G3M3D1dna202212	E2	G3	1	Mouse_3	vancomycin+neomycin	Used	Used
15	MR50G3M4D1dna202212	F2	G3	1	Mouse_4	vancomycin+neomycin	Used	Used
16	MR50G3M5D1dna202212	G2	G3	1	Mouse_5	vancomycin+neomycin	Used	Used
17	MR50G1M1D5dna202212	H2	G1	5	Mouse_1	none	Not_Used	Not_Used
18	MR50G1M2D5dna202212	A3	G1	5	Mouse_2	none	Not_Used	Not_Used
19	MR50G1M3D5dna202212	B3	G1	5	Mouse_3	none	Not_Used	Not_Used
20	MR50G1M4D5dna202212	C3	G1	5	Mouse_4	none	Not_Used	Not_Used
21	MR50G1M5D5dna202212	D3	G1	5	Mouse_5	none	Not_Used	Not_Used
22	MR50G2M1D5dna202212	E3	G2	5	Mouse_1	vancomycin	Used	Not_Used
23	MR50G2M2D5dna202212	F3	G2	5	Mouse_2	vancomycin	Used	Not_Used
24	MR50G2M3D5dna202212	G3	G2	5	Mouse_3	vancomycin	Used	Not_Used
25	MR50G2M4D5dna202212	H3	G2	5	Mouse_4	vancomycin	Used	Not_Used
26	MR50G2M5D5dna202212	A4	G2	5	Mouse_5	vancomycin	Used	Not_Used
27	MR50G3M1D5dna202212	B4	G3	5	Mouse_1	vancomycin+neomycin	Used	Used
28	MR50G3M2D5dna202212	C4	G3	5	Mouse_2	vancomycin+neomycin	Used	Used
29	MR50G3M3D5dna202212	D4	G3	5	Mouse_3	vancomycin+neomycin	Used	Used
30	MR50G3M4D5dna202212	E4	G3	5	Mouse_4	vancomycin+neomycin	Used	Used
31	MR50G3M5D5dna202212	F4	G3	5	Mouse_5	vancomycin+neomycin	Used	Used
32	MR50G1M1D15dna202212	G4	G1	15	Mouse_1	none	Not_Used	Not_Used
33	MR50G1M2D15dna202212	H4	G1	15	Mouse_2	none	Not_Used	Not_Used
34	MR50G1M3D15dna202212	A5	G1	15	Mouse_3	none	Not_Used	Not_Used
35	MR50G1M4D15dna202212	B5	G1	15	Mouse_4	none	Not_Used	Not_Used

JAMSalpha pipeline flowchart

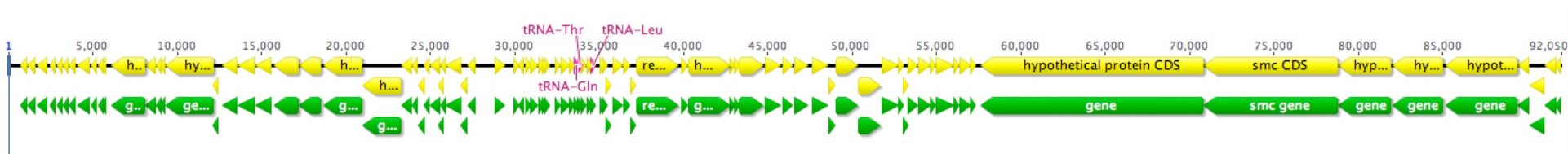
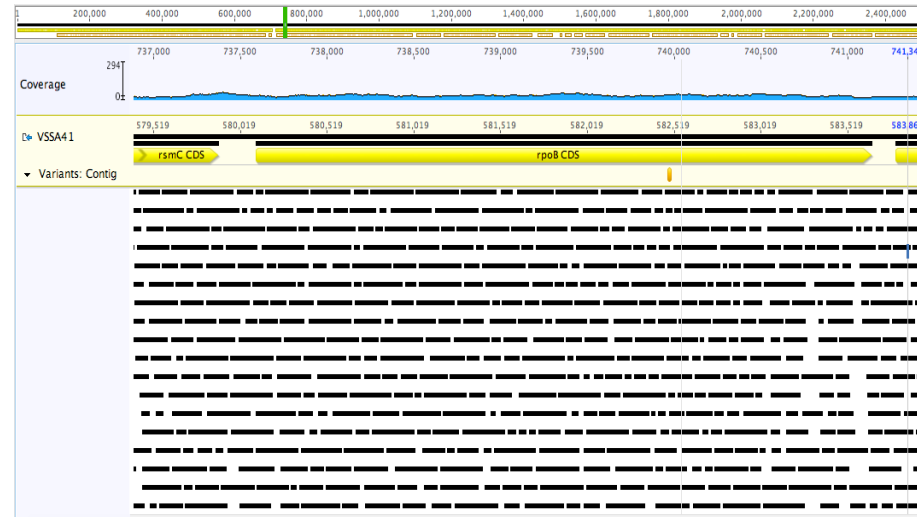


Stool

PD1CpG2batches Contig Assembly Statistics



Contig assembly



Contigs are larger contiguous sequences assembled from read redundancy
JAMS considers anything larger than 500 bp

Information from contigs: taxonomy + predicted proteome
Ultimately: **Last Known Taxon** = lowest ranking classification of sequence

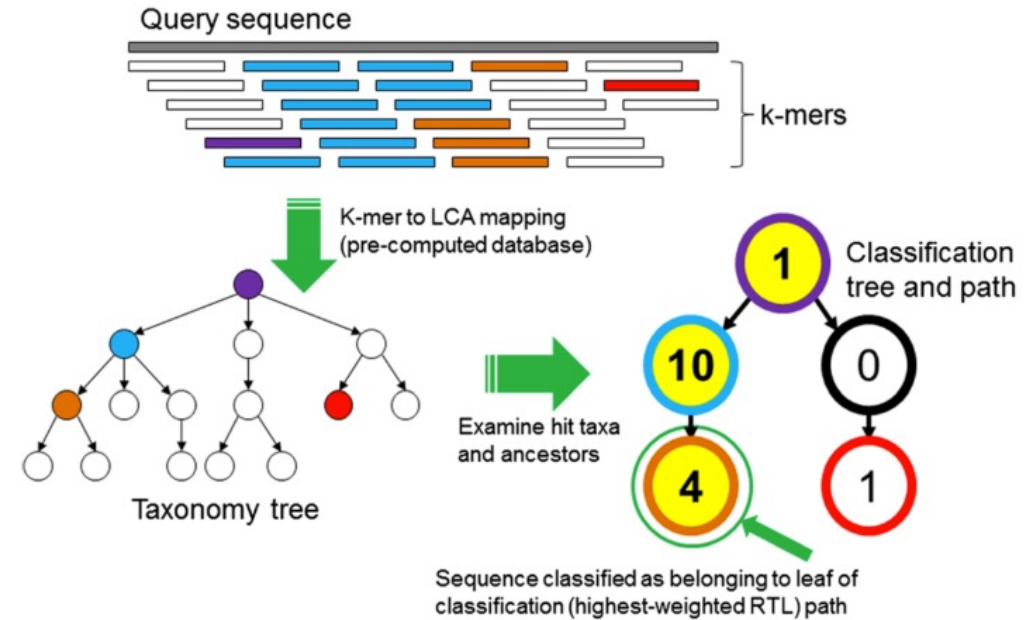
Alignment independent taxonomic classification

Taxonomic Contig Classification

Wood and Salzberg. Genome Biology 2014, 15:R46

Taxonomic identification of contigs using Kraken

- Counts k-mers in query sequences and matches to exact k-mers in database;
- Is ultrafast and accurate;
- Custom k-mer databases can be built;
- Result is hierarchical



Alignment independent taxonomic classification

Taxonomic Contig Classification

Wood, D.E., Lu, J. & Langmead, B. Improved metagenomic analysis with Kraken 2. *Genome Biol* **20**, 257 (2019).
<https://doi.org/10.1186/s13059-019-1891-0>

The JAMS **kraken2** database is built through a dedicated script which will download, curate and build a kraken2 database with non-flagged complete genomes of all:

- Bacteria
- Archaea
- Protozoa
- Viruses
- Fungi
- *Homo sapiens* (1 genome: GRCh38)
- *Mus musculus* (1 genome: GRCm38)

Kingdom	Freq
k__Archaea	1426
k__Bacteria	66345
k__Fungi	6001
k__Metazoa	11
k__Missing	1136
k__Viruses	34058

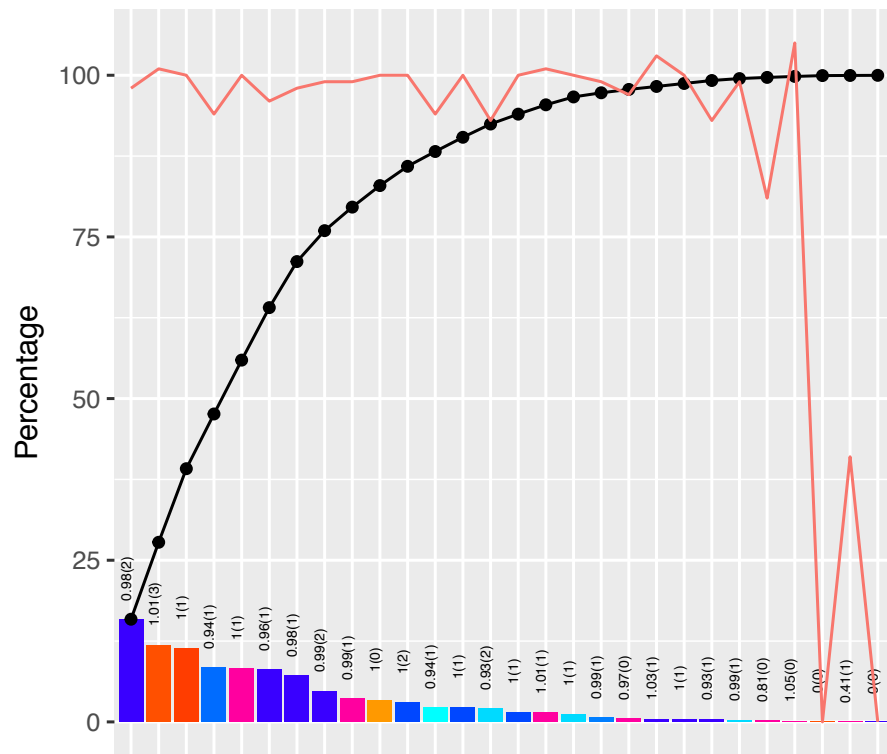
Latest build from
December 2022

NCBI Taxonomy IDs – Taxid

Curated taxonomy compiled using the JAMSbuildk2db script

Taxid	Kingdom	Phylum	Class	Order	Family	Genus	Species	LKT
1297742	k_Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Myxococcaceae	g__Myxococcus	s__Myxococcus_hansupus	LKT_s_Myxococcus_hansupus
1297750	k_Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Bacteroidaceae	g__Bacteroides	s__Bacteroides_luti	LKT_s_Bacteroides_luti
1298855	k_Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Novosphingobium	s__Novosphingobium_Unclassified_B_7	LKT_s_Novosphingobium_Unclassified
320599	k_Bacteria	p__Actinobacteria	c__Thermoleophila	o__Solirubrobacterales	f__Solirubrobacteraceae	g__Unclassified	s__Unclassified	LKT_f_Solirubrobacteraceae
207599	k_Bacteria	p__Actinobacteria	c__Thermoleophila	o__Solirubrobacterales	f__Solirubrobacteraceae	g__Solirubrobacter	s__Unclassified	LKT_g_Solirubrobacter
2640968	k_Bacteria	p__Actinobacteria	c__Thermoleophila	o__Solirubrobacterales	f__Solirubrobacteraceae	g__Solirubrobacter	s__Unclassified	LKT_g_Solirubrobacter
1298857	k_Bacteria	p__Actinobacteria	c__Thermoleophila	o__Solirubrobacterales	f__Solirubrobacteraceae	g__Solirubrobacter	s__Solirubrobacter_Unclassified_URHD0082	LKT_s_Solirubrobacter_Unclassified
1298858	k_Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Mesorhizobium	s__Mesorhizobium_Unclassified_URHA0056	LKT_s_Mesorhizobium_Unclassified
1298860	k_Bacteria	p__Actinobacteria	c__Actinobacteria	o__Micrococcales	f__Microbacteriaceae	g__Microbacterium	s__Microbacterium_Unclassified_URHA0036	LKT_s_Microbacterium_Unclassified
1298861	k_Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Lysobacter	s__Lysobacter_Unclassified_URHA0019	LKT_s_Lysobacter_Unclassified
1298862	k_Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Caulobacter	s__Caulobacter_Unclassified_URHA0033	LKT_s_Caulobacter_Unclassified
1298863	k_Bacteria	p__Actinobacteria	c__Actinobacteria	o__Propionibacteriales	f__Nocardiodiaceae	g__Marmoricola	s__Marmoricola_Unclassified_URHB0036	LKT_s_Marmoricola_Unclassified
1298864	k_Bacteria	p__Actinobacteria	c__Actinobacteria	o__Corynebacteriales	f__Mycobacteriaceae	g__Mycobacterium	s__Mycobacterium_Unclassified_URHD0025	LKT_s_Mycobacterium_Unclassified
1298866	k_Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	s__Bacillus_Unclassified_URHB0009	LKT_s_Bacillus_Unclassified
1298867	k_Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	g__Bradyrhizobium	s__Bradyrhizobium_Unclassified_URHA0002	LKT_s_Bradyrhizobium_Unclassified
1298868	k_Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__Flavobacterium_Unclassified_URHB0058	LKT_s_Flavobacterium_Unclassified
1298879	k_Bacteria	p__Actinobacteria	c__Actinobacteria	o__Streptomycetales	f__Streptomyetaceae	g__Streptomyces	s__Streptomyces_Unclassified_CNQ329	LKT_s_Streptomyces_Unclassified
1298880	k_Bacteria	p__Actinobacteria	c__Actinobacteria	o__Streptomycetales	f__Streptomyetaceae	g__Streptomyces	s__Streptomyces_Unclassified_TAA486	LKT_s_Streptomyces_Unclassified
946227	k_Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Idiomarinaceae	g__Unclassified	s__Unclassified	LKT_f_Idiomarinaceae
1298881	k_Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Idiomarinaceae	g__Missing	s__Idiomarinaceae_bacterium_HL_53	LKT_s_Idiomarinaceae_bacterium
1298913	k_Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Missing	s__Comamonadaceae_bacterium_URHA0028	LKT_s_Comamonadaceae_bacterium
1300914	k_Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	g__Mucilaginibacter	s__Mucilaginibacter_Unclassified_PAMC_2664	LKT_s_Mucilaginibacter_Unclassified
1301032	k_Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Rhizobium	s__Rhizobium_Unclassified_IE4771	LKT_s_Rhizobium_Unclassified
1301086	k_Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	s__Bacillus_Unclassified_GeD10	LKT_s_Bacillus_Unclassified
1301088	k_Bacteria	p__Actinobacteria	c__Actinobacteria	o__Corynebacteriales	f__Nocardiaceae	g__Rhodococcus	s__Rhodococcus_Unclassified_EsD8	LKT_s_Rhodococcus_Unclassified
1301100	k_Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptostreptococcaceae	g__Romboutsia	s__Clostridium_dakarense	LKT_s_Clostridium_dakarense
1302308	k_Bacteria	p__Actinobacteria	c__Actinobacteria	o__Corynebacteriales	f__Nocardiaceae	g__Rhodococcus	s__Rhodococcus_Unclassified_P1Y	LKT_s_Rhodococcus_Unclassified
1302376	k_Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__Candidatus_Pseudomonas_adelgestsugas	LKT_s_Candidatus_Pseudomonas
1302410	k_Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Candidatus_Annaditia	s__Unclassified	LKT_g_Candidatus_Annaditia

Taxonomic relative abundance – LKT in SRR3656745



Confidence stats

Expected Genome Completeness

PhylumInfo

- p__Acidobacteria
- p__Actinobacteria
- p__Bacteroidetes
- p__Deinococcus_Thermus
- p__Euryarchaeota
- p__Firmicutes
- p__Proteobacteria
- p__Spirochaetes
- p__Thermotogae
- p__Verrucomicrobia

MBARC-26 consists of 23 bacterial and 3 archaeal strains spanning 10 phyla and 14 classes, a range of GC contents, genome sizes, and repeat content

SCIENTIFIC DATA

OPEN

Data Descriptor: Next generation sequencing data of a defined microbial mock community

SUBJECT CATEGORIES

» Next-generation sequencing

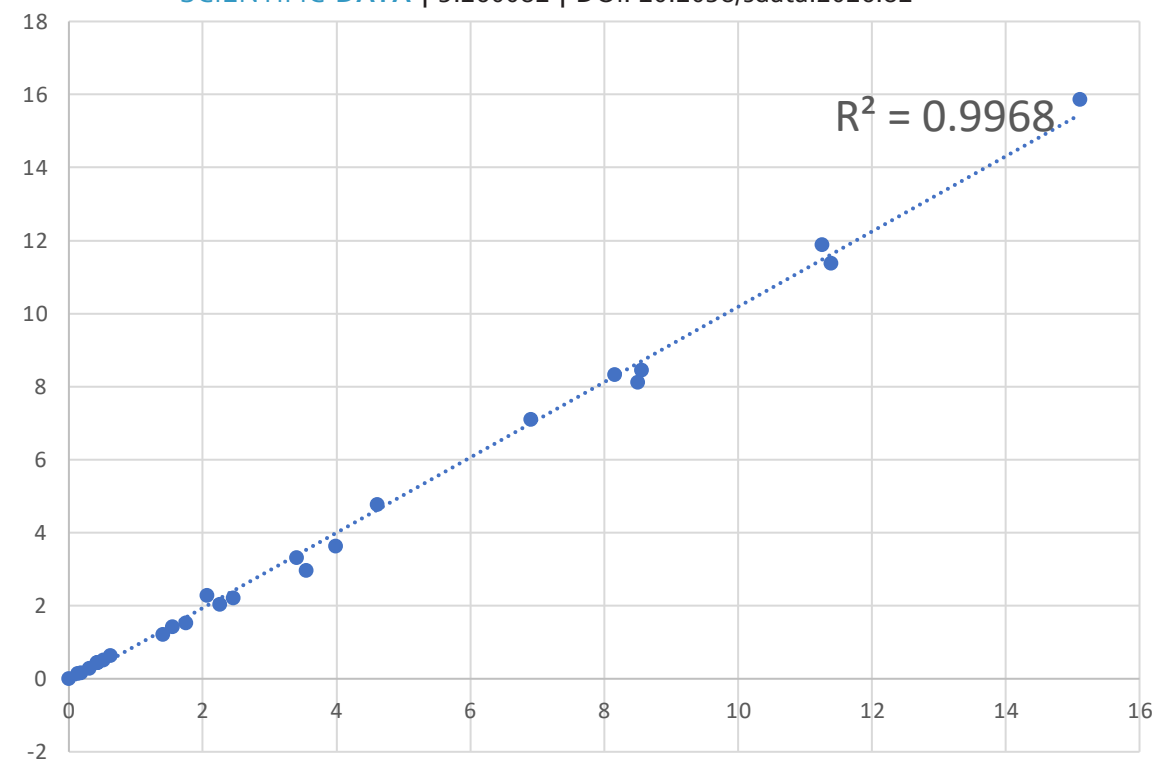
» Microbial communities

» DNA sequencing

» Metagenomics

Esther Singer¹, Bill Andreopoulos¹, Robert M. Bowers¹, Janey Lee¹, Shweta Deshpande¹, Jennifer Chiniquy¹, Doina Ciobanu¹, Hans-Peter Klenk², Matthew Zane¹, Christopher Daum¹, Alicia Clum¹, Jan-Fang Cheng¹, Alex Copeland¹ & Tanja Woyke²

SCIENTIFIC DATA | 3:160081 | DOI: 10.1038/sdata.2016.81

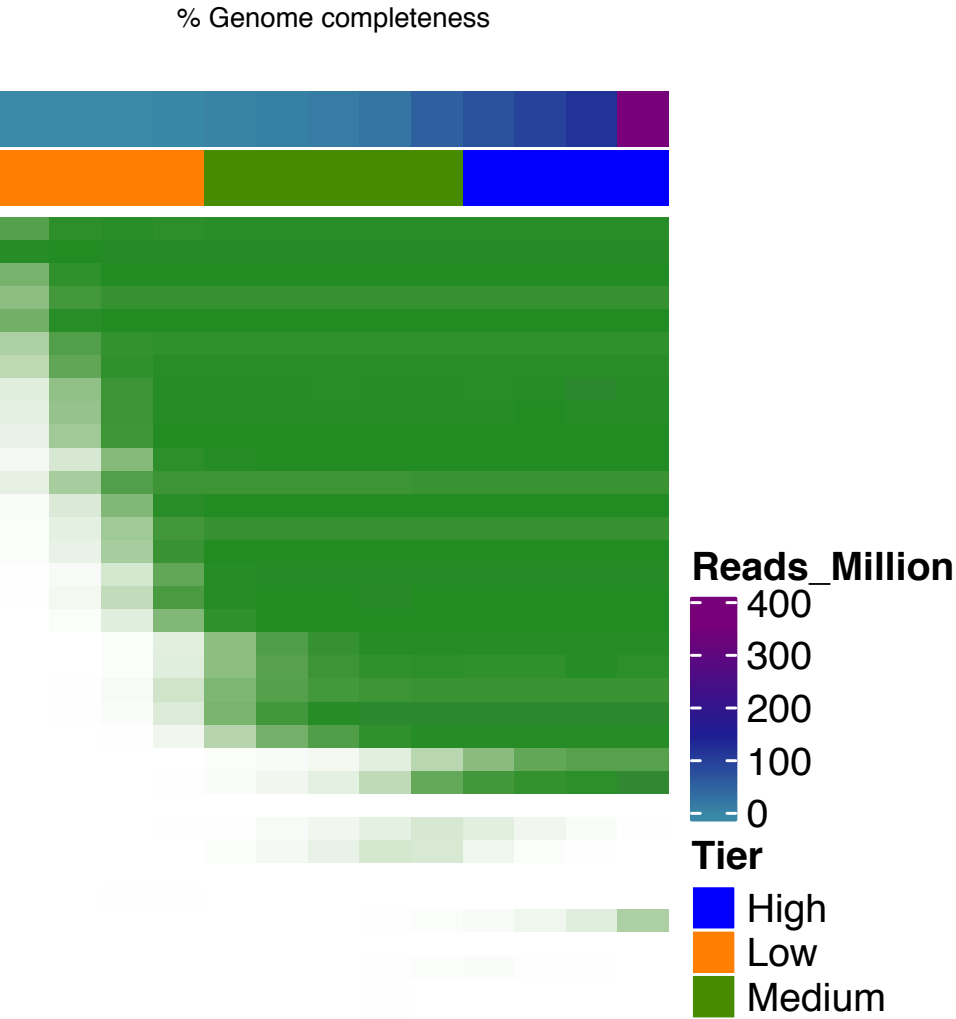
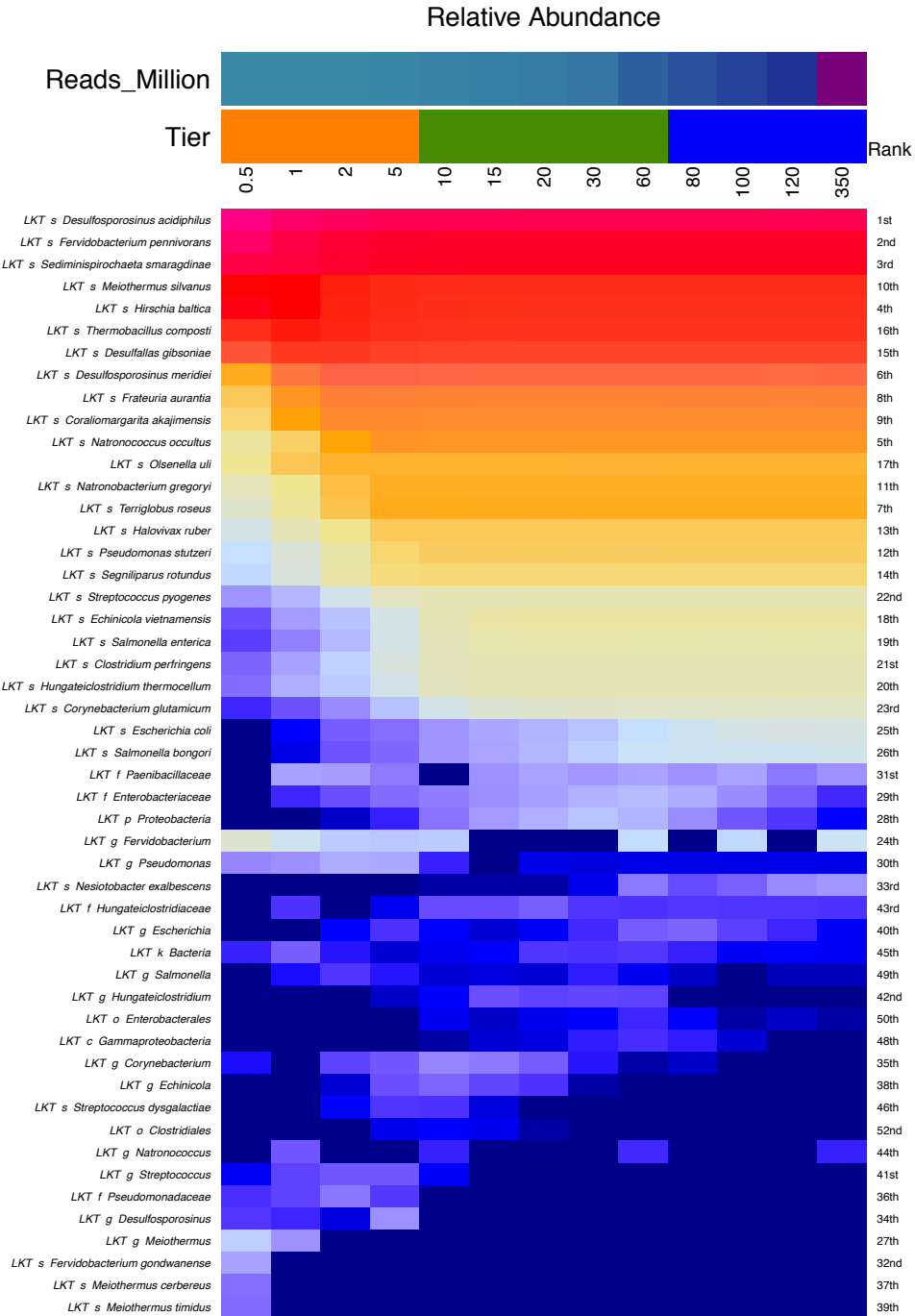
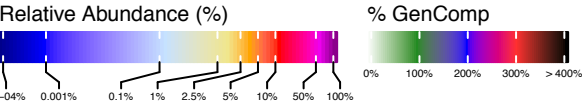


LKT_s_Desulfosporosinus_acidiphilus=15.88%
 LKT_s_Fervidobacterium_pennivorans=11.9%
 LKT_s_Sediminispirochaeta_smaragdinae=11.38%
 LKT_s_Meiothermus_silvanus=8.46%
 LKT_s_Hirschia_baltica=8.33%
 LKT_s_Thermobacillus_composti=8.12%
 LKT_s_Desulfallas_gibsoniae=7.12%
 LKT_s_Desulfosporosinus_meridiei=4.78%
 LKT_s_Frateuria_aurantia=3.64%
 LKT_s_Coralimargarita_akajimensis=3.33%
 LKT_s_Natronococcus_occultus=2.98%
 LKT_s_Terriglobus_roseus=2.29%
 LKT_s_Natronobacterium_gregoryi=2.2%
 LKT_s_Olsenella_uli=2.05%
 LKT_s_Halovivax_ruber=1.53%
 LKT_s_Pseudomonas_stutzeri=1.44%
 LKT_s_Segniliparus_rotundus=1.22%
 LKT_s_Echinicola_vietnamensis=0.64%
 LKT_s_Salmonella_enterica=0.52%
 LKT_s_Hungateiclostridium_thermocellum=0.46%
 LKT_s_Streptococcus_pyogenes=0.46%
 LKT_s_Clostridium_perfringens=0.46%
 LKT_s_Corynebacterium_glutamicum=0.3%
 LKT_s_Escherichia_coli=0.18%
 LKT_s_Salmonella_bongori=0.15%
 LKT_g_Fervidobacterium=0.13%
 LKT_s_Nesiotobacter_exalbescens=0.02%
 LKT_f_Paenibacillaceae=0.02%

LKT

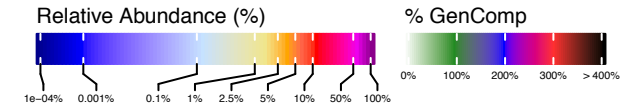
JAMS

No filtration



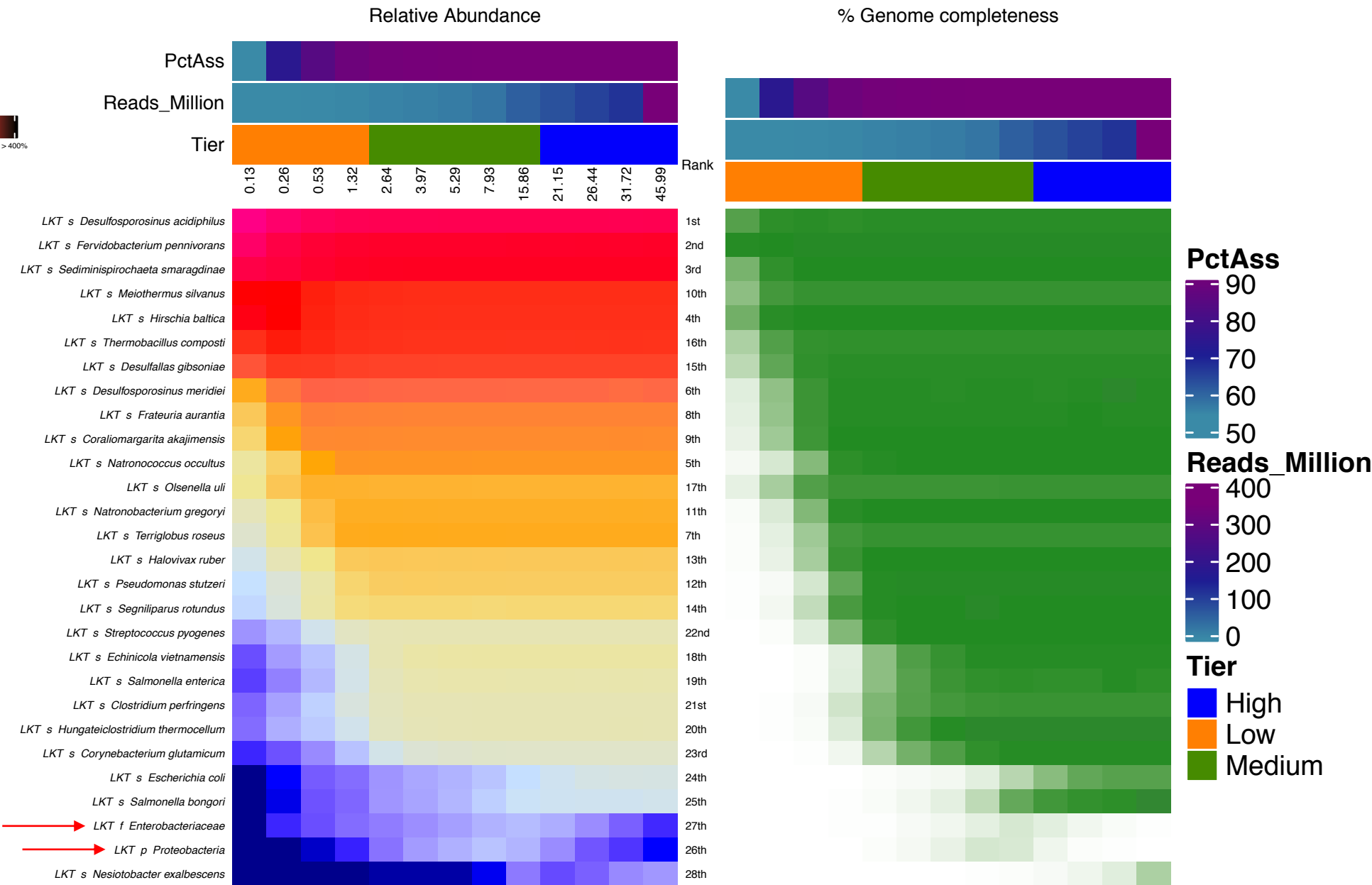
JAMS

Light filtering (<0.005%)



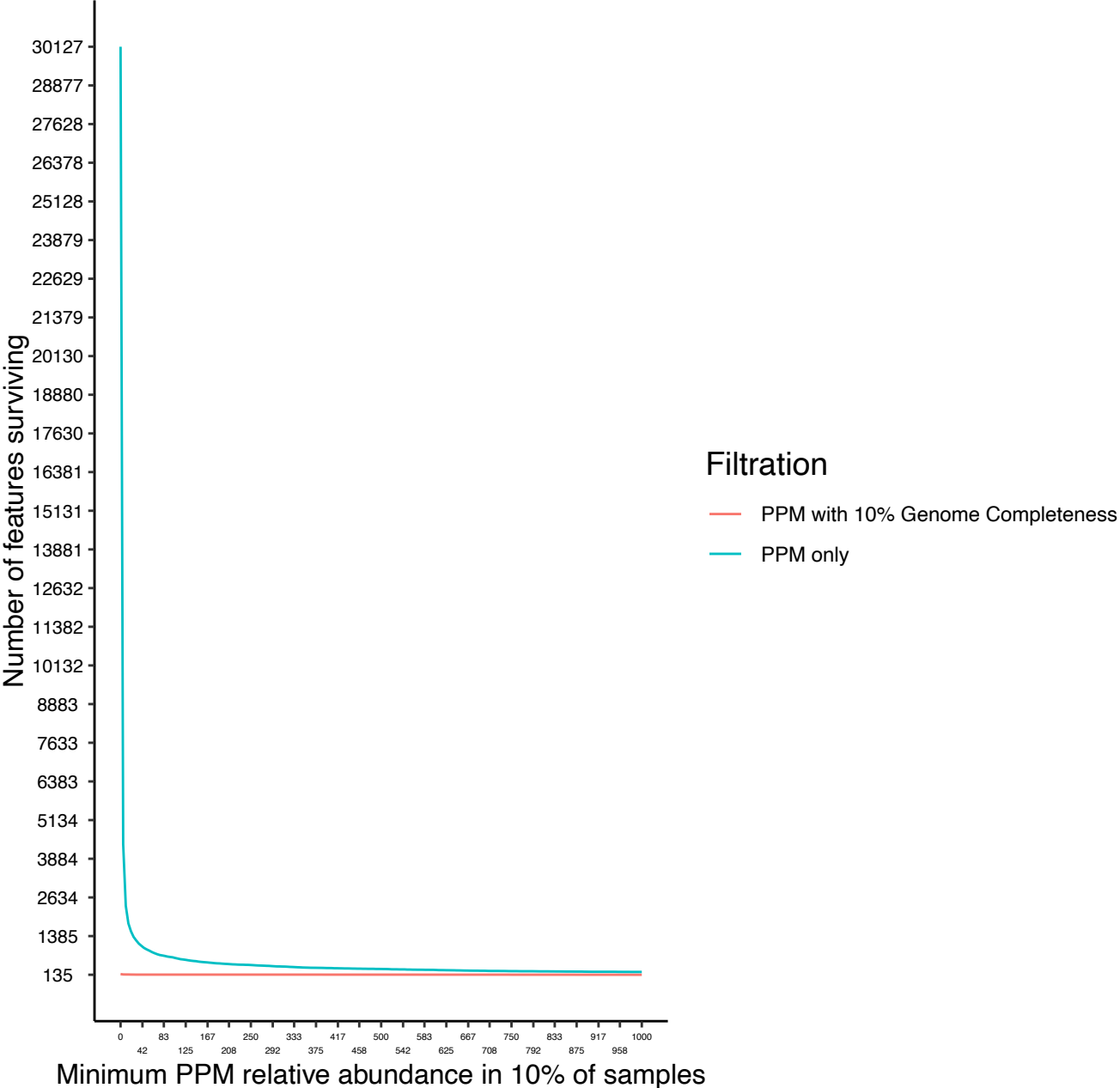
→ = False positive

Feature must be >50PPM in at least 5% of samples
Taxon genome completeness must be >5% in at least 5% of samples
Number of samples in heatmap = 13 | Number of features assessed = 28



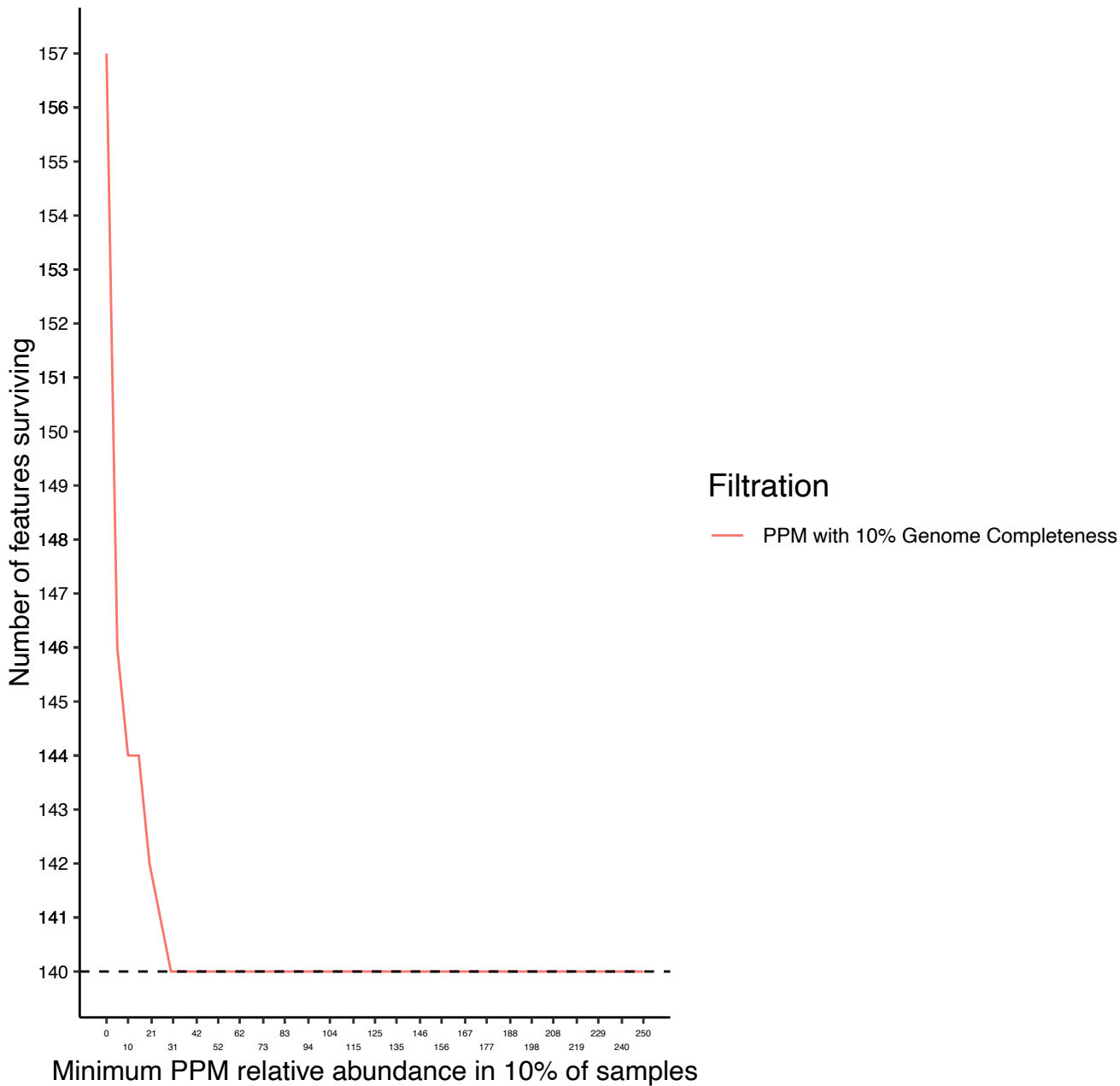
JAMS filtration

Filtering by relative abundance and Genome Completeness



JAMS filtration

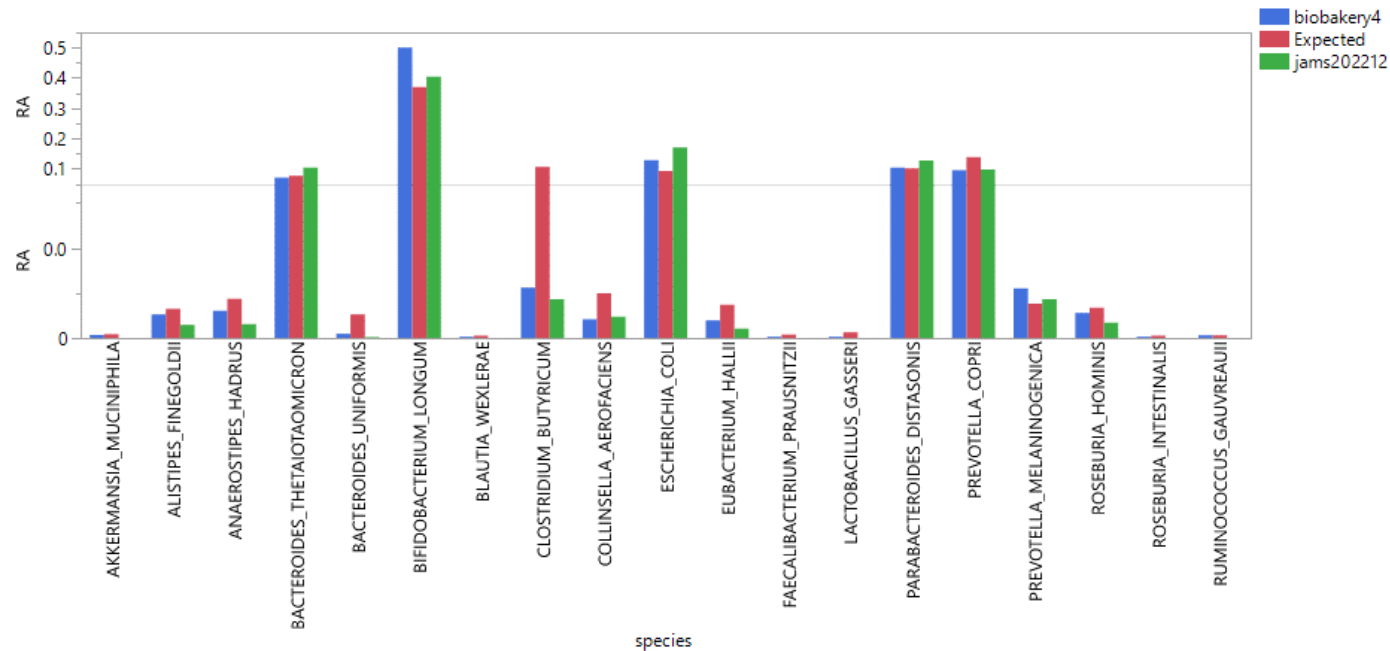
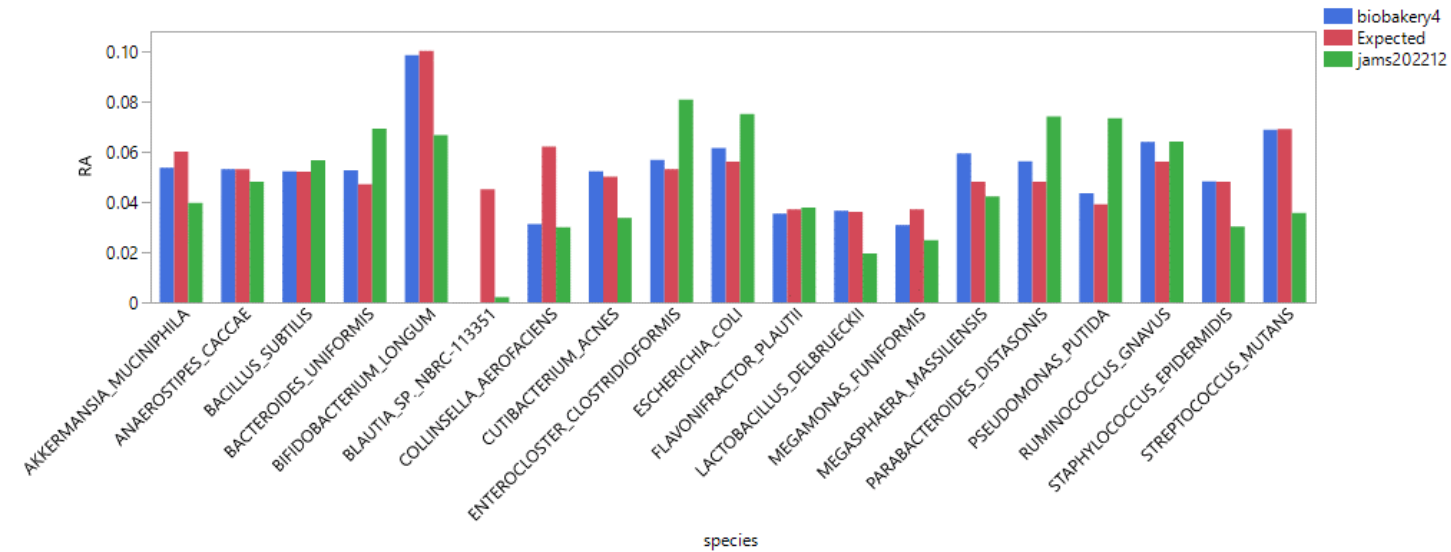
Filtering by relative abundance and Genome Completeness



**Translational Biobehavioral
and Health Disparities**
Branch NIH

Jennifer J. Barb
Michael Valencia

Species level comparison
between observed and
expected for Turlousse



Species level comparison between
observed and Expected for Amos
HiLo

Functional analysis

Taxonomy independent analyses

InterProScan

InterProScan is the software package that allows sequences (protein and nucleic) to be scanned against InterPro's signatures. Signatures are predictive models, provided by several different databases, that make up the InterPro consortium.

ECNumber: hierarchical classification of enzymes

Pfam: Protein Families

Phobius: Protein localization within cell

PRINTS: Functional domain fingerprints

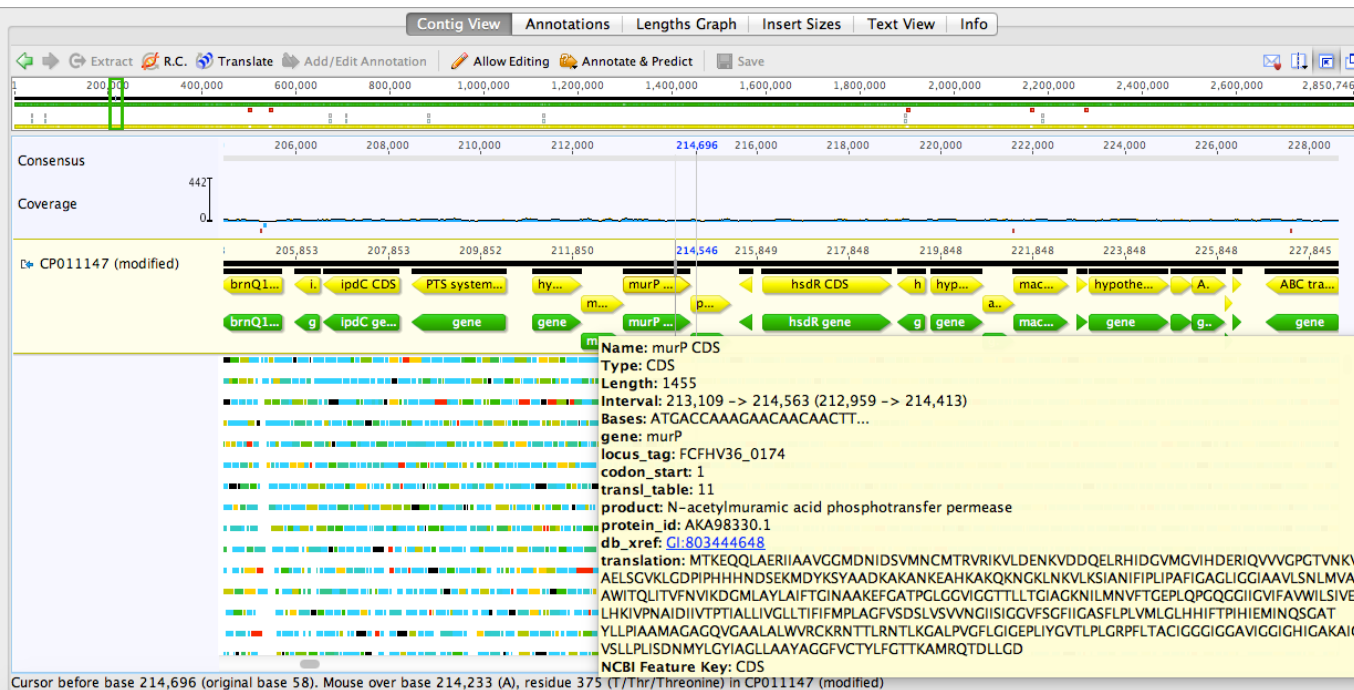
GO: Gene Ontology terms

MetaCyc: Metabolic Pathways

Curated BLASTp databases:

Resfinder: Antibiotic resistance genes

VFDB: Virulence Finder database



Microbial analysis made easy

```
JAMStbeta -y /path/to/jamsfiles -x metadata.xlsx -p MyProjectName
```

*.jams metadata

JAMStbeta

MyProjectName.RData

Ordination plots
Heatmaps
Statistics

	A	B	C	D	E	F	G	H	I	J
1	Sample	Well	Host_species	Group	Timepoint	Timepoint_days	Mouse_number	Treatment	Vancomycin_used	Neomycin_used
2	MR50G1M1D1dna202212	A1	mouse	G1	Day_0	0	Mouse_1G1	none	Not_Used	Not_Used
3	MR50G1M2D1dna202212	B1	mouse	G1	Day_0	0	Mouse_2G1	none	Not_Used	Not_Used
4	MR50G1M3D1dna202212	C1	mouse	G1	Day_0	0	Mouse_3G1	none	Not_Used	Not_Used
5	MR50G1M4D1dna202212	D1	mouse	G1	Day_0	0	Mouse_4G1	none	Not_Used	Not_Used
6	MR50G1M5D1dna202212	E1	mouse	G1	Day_0	0	Mouse_5G1	none	Not_Used	Not_Used
7	MR50G2M1D1dna202212	F1	mouse	G2	Day_0	0	Mouse_1G2	vancomycin	Used	Not_Used
8	MR50G2M2D1dna202212	G1	mouse	G2	Day_0	0	Mouse_2G2	vancomycin	Used	Not_Used
9	MR50G2M3D1dna202212	H1	mouse	G2	Day_0	0	Mouse_3G2	vancomycin	Used	Not_Used
10	MR50G2M4D1dna202212	A2	mouse	G2	Day_0	0	Mouse_4G2	vancomycin	Used	Not_Used
11	MR50G2M5D1dna202212	B2	mouse	G2	Day_0	0	Mouse_5G2	vancomycin	Used	Not_Used
12	MR50G3M1D1dna202212	C2	mouse	G3	Day_0	0	Mouse_1G3	vancomycin+neomycin	Used	Used
13	MR50G3M2D1dna202212	D2	mouse	G3	Day_0	0	Mouse_2G3	vancomycin+neomycin	Used	Used
14	MR50G3M3D1dna202212	E2	mouse	G3	Day_0	0	Mouse_3G3	vancomycin+neomycin	Used	Used
15	MR50G3M4D1dna202212	F2	mouse	G3	Day_0	0	Mouse_4G3	vancomycin+neomycin	Used	Used
16	MR50G3M5D1dna202212	G2	mouse	G3	Day_0	0	Mouse_5G3	vancomycin+neomycin	Used	Used
17	MR50G1M1D5dna202212	H2	mouse	G1	Day_5	5	Mouse_1G1	none	Not_Used	Not_Used
18	MR50G1M2D5dna202212	A3	mouse	G1	Day_5	5	Mouse_2G1	none	Not_Used	Not_Used
19	MR50G1M3D5dna202212	B3	mouse	G1	Day_5	5	Mouse_3G1	none	Not_Used	Not_Used
20	MR50G1M4D5dna202212	C3	mouse	G1	Day_5	5	Mouse_4G1	none	Not_Used	Not_Used
21	MR50G1M5D5dna202212	D3	mouse	G1	Day_5	5	Mouse_5G1	none	Not_Used	Not_Used
22	MR50G2M1D5dna202212	E3	mouse	G2	Day_5	5	Mouse_1G2	vancomycin	Used	Not_Used
23	MR50G2M2D5dna202212	F3	mouse	G2	Day_5	5	Mouse_2G2	vancomycin	Used	Not_Used
24	MR50G2M3D5dna202212	G3	mouse	G2	Day_5	5	Mouse_3G2	vancomycin	Used	Not_Used
25	MR50G2M4D5dna202212	H3	mouse	G2	Day_5	5	Mouse_4G2	vancomycin	Used	Not_Used
26	MR50G2M5D5dna202212	A4	mouse	G2	Day_5	5	Mouse_5G2	vancomycin	Used	Not_Used
27	MR50G3M1D5dna202212	B4	mouse	G3	Day_5	5	Mouse_1G3	vancomycin+neomycin	Used	Used
28	MR50G3M2D5dna202212	C4	mouse	G3	Day_5	5	Mouse_2G3	vancomycin+neomycin	Used	Used
29	MR50G3M3D5dna202212	D4	mouse	G3	Day_5	5	Mouse_3G3	vancomycin+neomycin	Used	Used
30	MR50G3M4D5dna202212	E4	mouse	G3	Day_5	5	Mouse_4G3	vancomycin+neomycin	Used	Used
31	MR50G3M5D5dna202212	F4	mouse	G3	Day_5	5	Mouse_5G3	vancomycin+neomycin	Used	Used
32	MR50G1M1D15dna202212	G4	mouse	G1	Day_15	15	Mouse_1G1	none	Not_Used	Not_Used
33	MR50G1M2D15dna202212	H4	mouse	G1	Day_15	15	Mouse_2G1	none	Not_Used	Not_Used
34	MR50G1M3D15dna202212	A5	mouse	G1	Day_15	15	Mouse_3G1	none	Not_Used	Not_Used
35	MR50G1M4D15dna202212	B5	mouse	G1	Day_15	15	Mouse_4G1	none	Not_Used	Not_Used
36	MR50G1M5D15dna202212	C5	mouse	G1	Day_15	15	Mouse_5G1	none	Not_Used	Not_Used
37	MR50G2M1D15dna202212	D5	mouse	G2	Day_15	15	Mouse_1G2	vancomycin	Used	Not_Used
38	MR50G2M2D15dna202212	E5	mouse	G2	Day_15	15	Mouse_2G2	vancomycin	Used	Not_Used

Beta diversity analysis made easy

```
$LKT
class: SummarizedExperiment
dim: 31580 59
metadata(4): TotalBasesSequenced TotalBasesSequencedinAnalysis analysis
             ctable
assays(3): BaseCounts PctFromCtgs GenomeCompleteness
rownames(31580): LKT__s__Ligilactobacillus_murinus
                 LKT__s__Lactobacillus_johnsonii ...
                 LKT__s__Pantoea_Unclassified_OXW06B1
                 LKT__s__Alkalihalobacillus_Unclassified_LMS6
rowData names(10): Gram Domain ... Species LKT
colnames(59): MR50G1M1D1dna202212 MR50G1M2D1dna202212 ...
              MR50G3M4CECdna202212 MR50G3M5CECdna202212
colData names(15): Sample Well ... GbNAHS PctAss

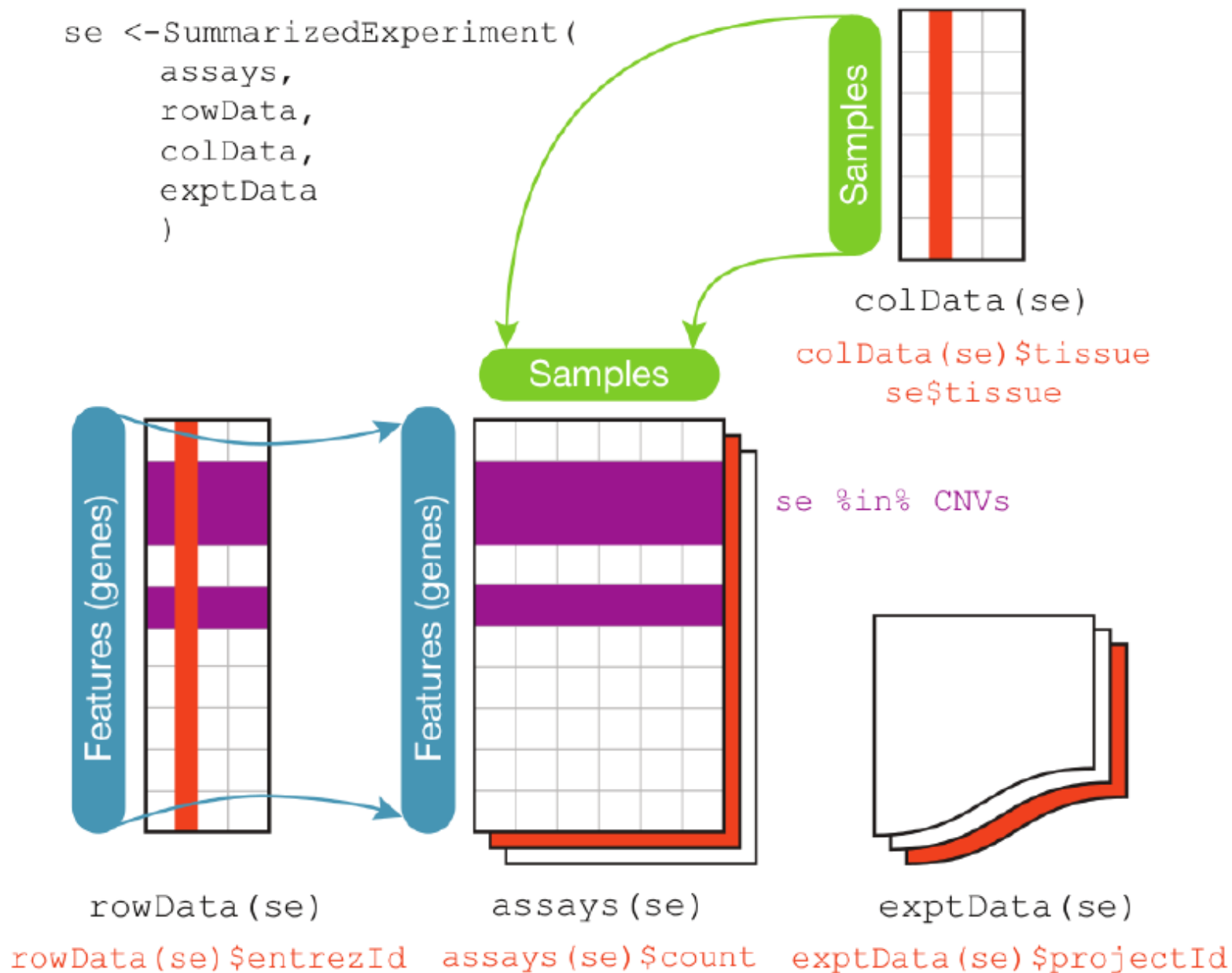
$ECNumber
class: SummarizedExperiment
dim: 2169 59
metadata(4): TotalBasesSequenced TotalBasesSequencedinAnalysis analysis
             ctable
assays(2): BaseCounts GeneCounts
rownames(2169): ECNumber_none EC_3.1.-.- ... EC_3.4.21.66 EC_1.1.1.76
rowData names(2): Accession Description
colnames(59): MR50G1M1D1dna202212 MR50G1M2D1dna202212 ...
              MR50G3M4CECdna202212 MR50G3M5CECdna202212
colData names(15): Sample Well ... GbNAHS PctAss

$Product
class: SummarizedExperiment
dim: 8639 59
metadata(4): TotalBasesSequenced TotalBasesSequencedinAnalysis analysis
             ctable
assays(2): BaseCounts GeneCounts
rownames(8639): hypothetical protein Sensor histidine kinase RcsC ...
                 L-2%2C3-butanediol dehydrogenase Meromycolate extension acyl carrier
                 protein
rowData names(2): Accession Description
colnames(59): MR50G1M1D1dna202212 MR50G1M2D1dna202212 ...
              MR50G3M4CECdna202212 MR50G3M5CECdna202212
colData names(15): Sample Well ... GbNAHS PctAss
```

The JAMSbeta script will
create an R session
containing
SummarizedExperiment
objects for all JAMS
analysis spaces for those
samples.

The SummarizedExperiment object

```
se <- SummarizedExperiment(  
  assays,  
  rowData,  
  colData,  
  exptData  
)
```



From:

Martin, Pascal. (2015). An introduction to R/Bioconductor for the analysis of high-throughput sequencing data.

Beta diversity analysis made easy

There is a dedicated
function (in beta-testing)
for importing
Metaphlan4 results into
a JAMS-compatible .jams
files for using with
JAMSbeta

```
#' mpa2JAMS(mpa_folder = NULL, only_prefixes = NULL, output_folder = NULL,
mpa_file_suffix = "_profile.txt", return_mpa_list.data = TRUE, verbose = TRUE)~
#'-
#'Makes .jams files from Metaphlann output files. This function is experimental, use at
your own risk and peril.~
#'@export~
~
mpa2JAMS <- function(mpa_folder = NULL, only_prefixes = NULL, output_folder = NULL,
mpa_file_suffix = "_profile.txt", return_mpa_list.data = TRUE, verbose = TRUE, asRDS =
TRUE){~
~
  ... flog.warn("This function is experimental, use at your own risk and peril.")~
  ... mpa_list.data <- list()~
  ... list_elem <- 1~
  ... mpa_taxlevel_names <- c("Kingdom", "Phylum", "Class", "Order", "Family", "Genus",
    "Species")~
~
  ... mpa_folder <- fixrelpath(mpa_folder)~
  ... mpa_files_absolute <- list.files(path = mpa_folder, pattern = mpa_file_suffix,
    full.names = TRUE, recursive = TRUE)~
  ... mpa_files <- sapply(1:length(mpa_files_absolute), function(x) {
    tail(unlist(strsplit(mpa_files_absolute[x], split = "/")), n = 1) })~
~
  ... if (length(mpa_files) < 1){~
  ...   ... stop(paste("No MetaPhlAnn output files were found"))~
  ... }~
~
  ... mpa_files_df <- data.frame(MPA_path = mpa_files_absolute, MPA_file = mpa_files,
    stringsAsFactors = FALSE)~
```

Beta diversity analysis made easy

These SummarizedExperiment objects can be used as input for any of the JAMS plotting functions.

```
plot_relabund_heatmap(ExpObj, [filtering], [subsetting], [options])
```

```
plot_Ordination(SEobj, [filtering], [subsetting], [options])
```

```
plot_alpha_diversity(ExpObj, [filtering], [subsetting], [options])
```

```
plot_relabund_features(ExpObj, [filtering], [subsetting], [options])
```

MR50 – a simple mouse microbiome perturbation dataset



vancomycin



vancomycin
+ neomycin



Day0



Day5



Day15



Day56

JAMSBeta tutorial on the JAMS wiki page:

https://github.com/johnmcculloch/JAMS_BW/wiki/Tutorial_JAMSBeta

The MR50 dataset is included within the JAMS package (ver 1.9.8) as example data

	A	B	C	D	E	F	G	H	I	J
1	Sample	Well	Host_species	Group	Timepoint	Timepoint_days	Mouse_number	Treatment	Vancomycin_used	Neomycin_used
2	MR50G1M1D1dna202212	A1	mouse	G1	Day_0	0	Mouse_1G1	none	Not_Used	Not_Used
3	MR50G1M2D1dna202212	B1	mouse	G1	Day_0	0	Mouse_2G1	none	Not_Used	Not_Used
4	MR50G1M3D1dna202212	C1	mouse	G1	Day_0	0	Mouse_3G1	none	Not_Used	Not_Used
5	MR50G1M4D1dna202212	D1	mouse	G1	Day_0	0	Mouse_4G1	none	Not_Used	Not_Used
6	MR50G1M5D1dna202212	E1	mouse	G1	Day_0	0	Mouse_5G1	none	Not_Used	Not_Used
7	MR50G2M1D1dna202212	F1	mouse	G2	Day_0	0	Mouse_1G2	vancomycin	Used	Not_Used
8	MR50G2M2D1dna202212	G1	mouse	G2	Day_0	0	Mouse_2G2	vancomycin	Used	Not_Used
9	MR50G2M3D1dna202212	H1	mouse	G2	Day_0	0	Mouse_3G2	vancomycin	Used	Not_Used
10	MR50G2M4D1dna202212	A2	mouse	G2	Day_0	0	Mouse_4G2	vancomycin	Used	Not_Used
11	MR50G2M5D1dna202212	B2	mouse	G2	Day_0	0	Mouse_5G2	vancomycin	Used	Not_Used
12	MR50G3M1D1dna202212	C2	mouse	G3	Day_0	0	Mouse_1G3	vancomycin+neomycin	Used	Used
13	MR50G3M2D1dna202212	D2	mouse	G3	Day_0	0	Mouse_2G3	vancomycin+neomycin	Used	Used
14	MR50G3M3D1dna202212	E2	mouse	G3	Day_0	0	Mouse_3G3	vancomycin+neomycin	Used	Used
15	MR50G3M4D1dna202212	F2	mouse	G3	Day_0	0	Mouse_4G3	vancomycin+neomycin	Used	Used
16	MR50G3M5D1dna202212	G2	mouse	G3	Day_0	0	Mouse_5G3	vancomycin+neomycin	Used	Used
17	MR50G1M1D5dna202212	H2	mouse	G1	Day_5	5	Mouse_1G1	none	Not_Used	Not_Used
18	MR50G1M2D5dna202212	A3	mouse	G1	Day_5	5	Mouse_2G1	none	Not_Used	Not_Used
19	MR50G1M3D5dna202212	B3	mouse	G1	Day_5	5	Mouse_3G1	none	Not_Used	Not_Used
20	MR50G1M4D5dna202212	C3	mouse	G1	Day_5	5	Mouse_4G1	none	Not_Used	Not_Used
21	MR50G1M5D5dna202212	D3	mouse	G1	Day_5	5	Mouse_5G1	none	Not_Used	Not_Used
22	MR50G2M1D5dna202212	E3	mouse	G2	Day_5	5	Mouse_1G2	vancomycin	Used	Not_Used
23	MR50G2M2D5dna202212	F3	mouse	G2	Day_5	5	Mouse_2G2	vancomycin	Used	Not_Used
24	MR50G2M3D5dna202212	G3	mouse	G2	Day_5	5	Mouse_3G2	vancomycin	Used	Not_Used
25	MR50G2M4D5dna202212	H3	mouse	G2	Day_5	5	Mouse_4G2	vancomycin	Used	Not_Used
26	MR50G2M5D5dna202212	A4	mouse	G2	Day_5	5	Mouse_5G2	vancomycin	Used	Not_Used
27	MR50G3M1D5dna202212	B4	mouse	G3	Day_5	5	Mouse_1G3	vancomycin+neomycin	Used	Used
28	MR50G3M2D5dna202212	C4	mouse	G3	Day_5	5	Mouse_2G3	vancomycin+neomycin	Used	Used
29	MR50G3M3D5dna202212	D4	mouse	G3	Day_5	5	Mouse_3G3	vancomycin+neomycin	Used	Used
30	MR50G3M4D5dna202212	E4	mouse	G3	Day_5	5	Mouse_4G3	vancomycin+neomycin	Used	Used
31	MR50G3M5D5dna202212	F4	mouse	G3	Day_5	5	Mouse_5G3	vancomycin+neomycin	Used	Used
32	MR50G1M1D15dna202212	G4	mouse	G1	Day_15	15	Mouse_1G1	none	Not_Used	Not_Used
33	MR50G1M2D15dna202212	H4	mouse	G1	Day_15	15	Mouse_2G1	none	Not_Used	Not_Used
34	MR50G1M3D15dna202212	A5	mouse	G1	Day_15	15	Mouse_3G1	none	Not_Used	Not_Used
35	MR50G1M4D15dna202212	B5	mouse	G1	Day_15	15	Mouse_4G1	none	Not_Used	Not_Used
36	MR50G1M5D15dna202212	C5	mouse	G1	Day_15	15	Mouse_5G1	none	Not_Used	Not_Used
37	MR50G2M1D15dna202212	D5	mouse	G2	Day_15	15	Mouse_1G2	vancomycin	Used	Not_Used
38	MR50G2M2D15dna202212	E5	mouse	G2	Day_15	15	Mouse_2G2	vancomycin	Used	Not_Used

```

plot_Ordination(ExpObj = expvec_MR50[["LKT"]],
subsetby = NULL, applyfilters = "moderate",
algorithm = "tUMAP", distmethod = "bray", compareby
= "Treatment", connectby = NULL, connection_orderby
= NULL,
colourby = "Treatment", shapeby = "Timepoint",
use_letters_as_shapes = FALSE,
textby = "Timepoint_days", ellipseby = NULL, dotsize =
2.5, permanova = TRUE,
plotcentroids = FALSE, grid = FALSE, forceaspectratio = 1)

```

tUMAP of LKT

Feature must be >250PPM in at least 15% of samples

Taxonomy information must come from >70% contigs in at least 50% of samples

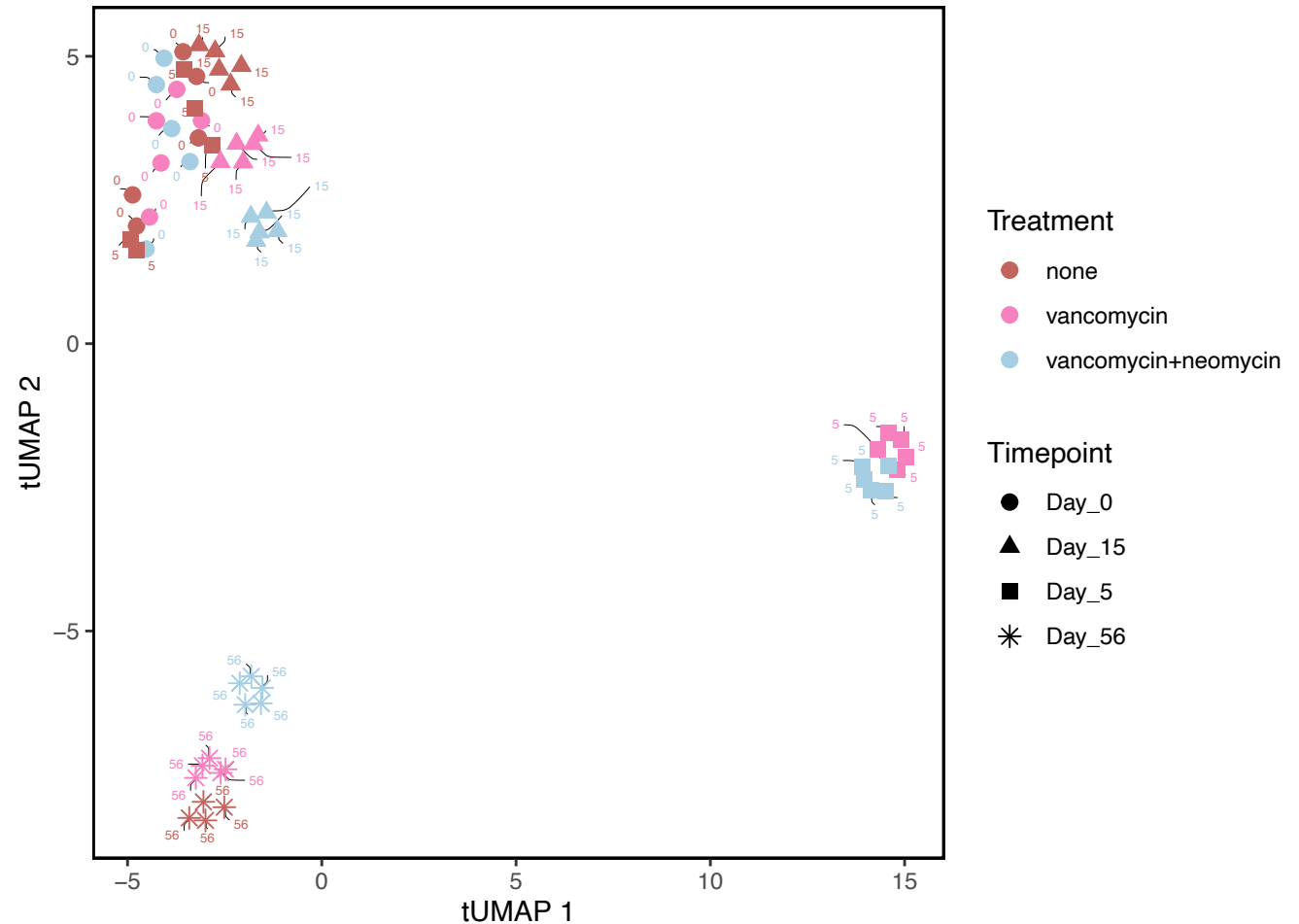
Taxon genome completeness must be >10% in at least 5% of samples

Normalized to number of bases for analysis in sample

Matrix log2 transformed

PERMANOVA $p < 0.0111$; F-Model = 3.6579

Dissimilarity index = bray



```
pdf("Example_Ordination_plots.pdf", paper = "a4r")  
  
for (anal in c("LKT", "MPA4", "Product", "Pfam", "resfinder")){  
  
  for(centroids in c(FALSE, TRUE)){  
    print(plot_Ordination(ExpObj = expvec[[anal]], subsetby = "Timepoint_days",  
      applyfilters = "moderate", algorithm = "tUMAP", distmethod = "bray", compareby =  
      "Treatment", connectby = NULL, connection_orderby = NULL, colourby = "Treatment",  
      shapeby = "Vancomycin_used", use_letters_as_shapes = FALSE, textby = "Mouse_number",  
      ellipseby = NULL, dotsize = 2, permanova = TRUE, plotcentroids = centroids,  
      highlight_centroids = FALSE, show_centroid_distances = FALSE, grid = FALSE,  
      forceaspectratio = 1, addtit = NULL))  
  }  
}  
  
dev.off()
```

tUMAP of LKT within 1

Feature must be >250PPM in at least 15% of samples

Taxonomy information must come from >70% contigs in at least 50% of samples

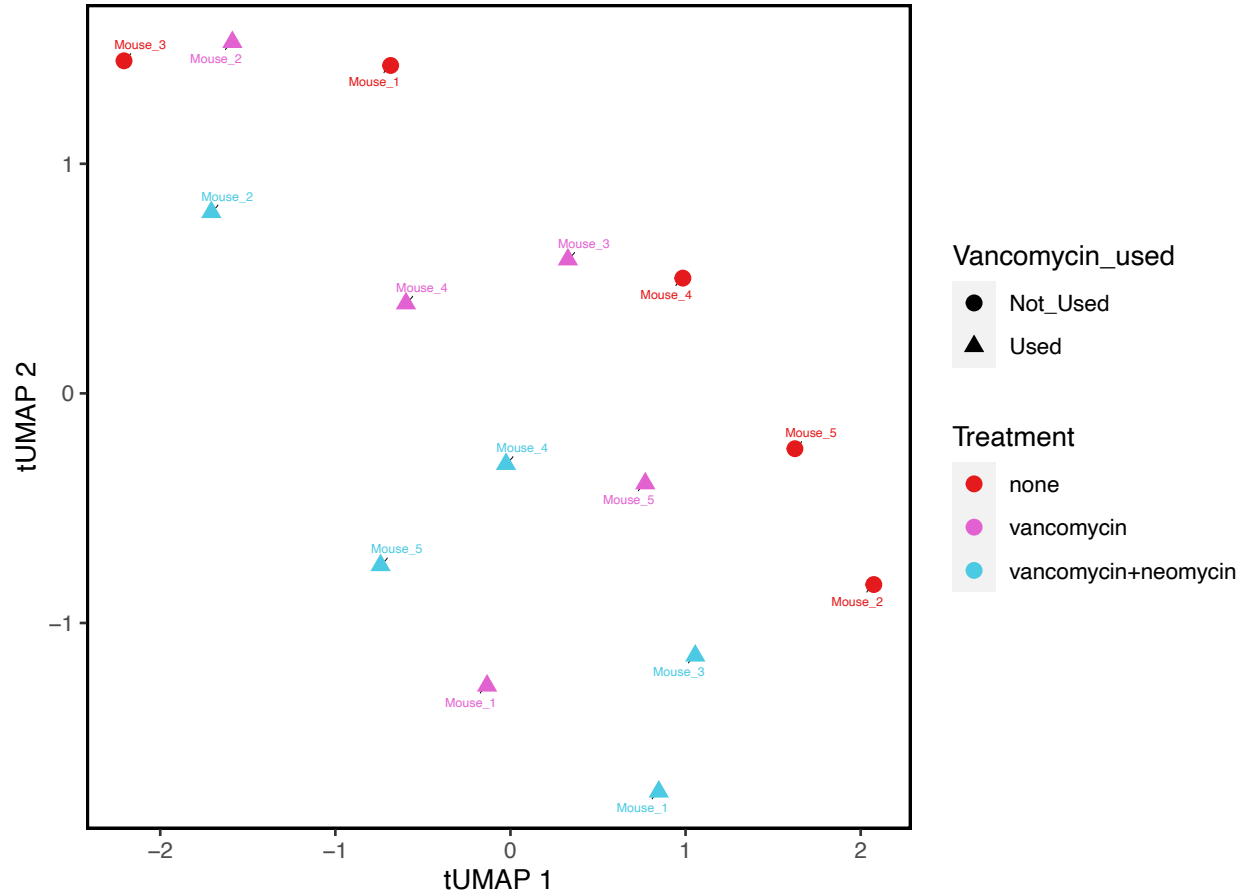
Taxon genome completeness must be >10% in at least 5% of samples

Normalized to number of bases for analysis in sample

Matrix log2 transformed

PERMANOVA $p < 0.5356$; F-Model = 0.8464

Dissimilarity index = bray



tUMAP of LKT within 1

Feature must be >250PPM in at least 15% of samples

Taxonomy information must come from >70% contigs in at least 50% of samples

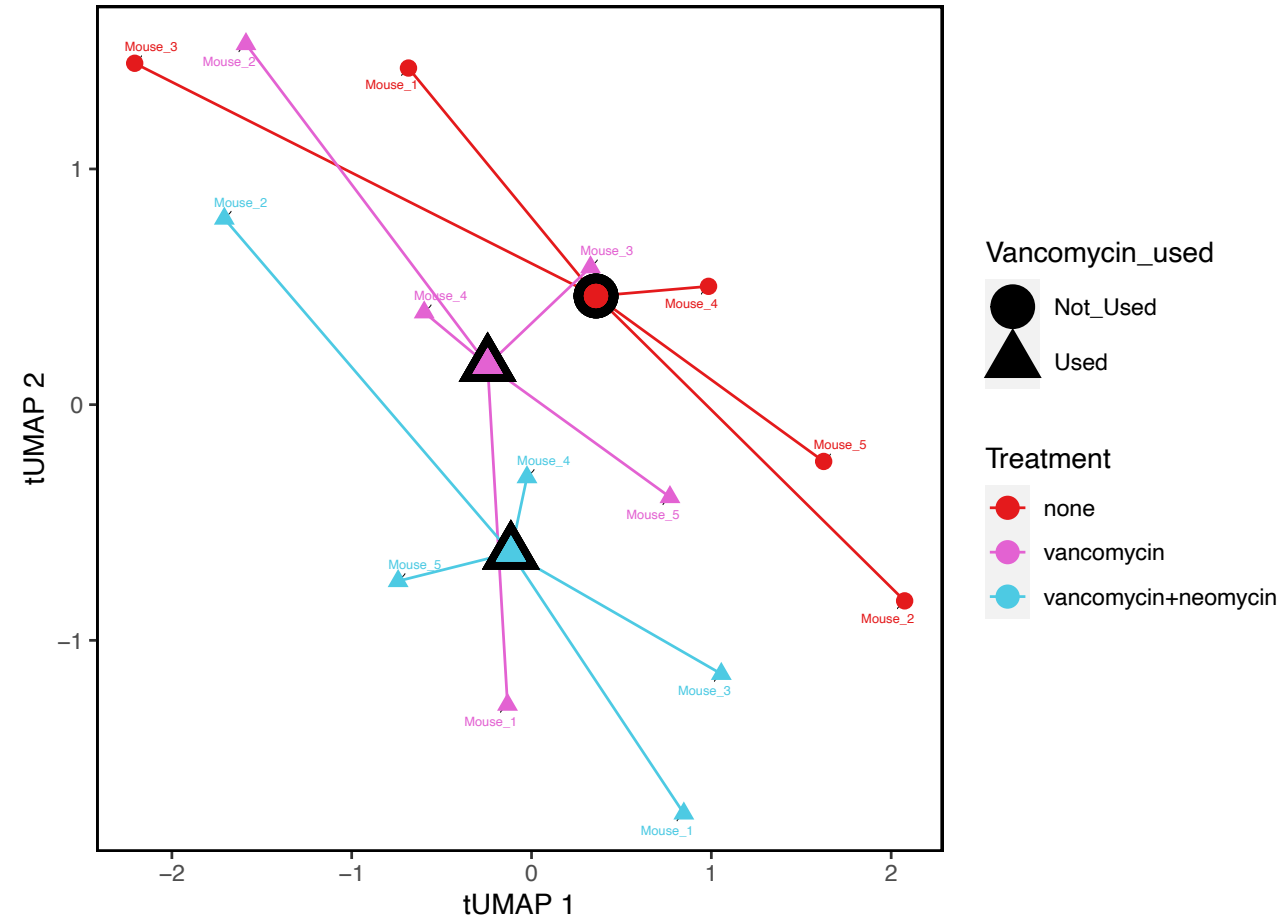
Taxon genome completeness must be >10% in at least 5% of samples

Normalized to number of bases for analysis in sample

Matrix log2 transformed

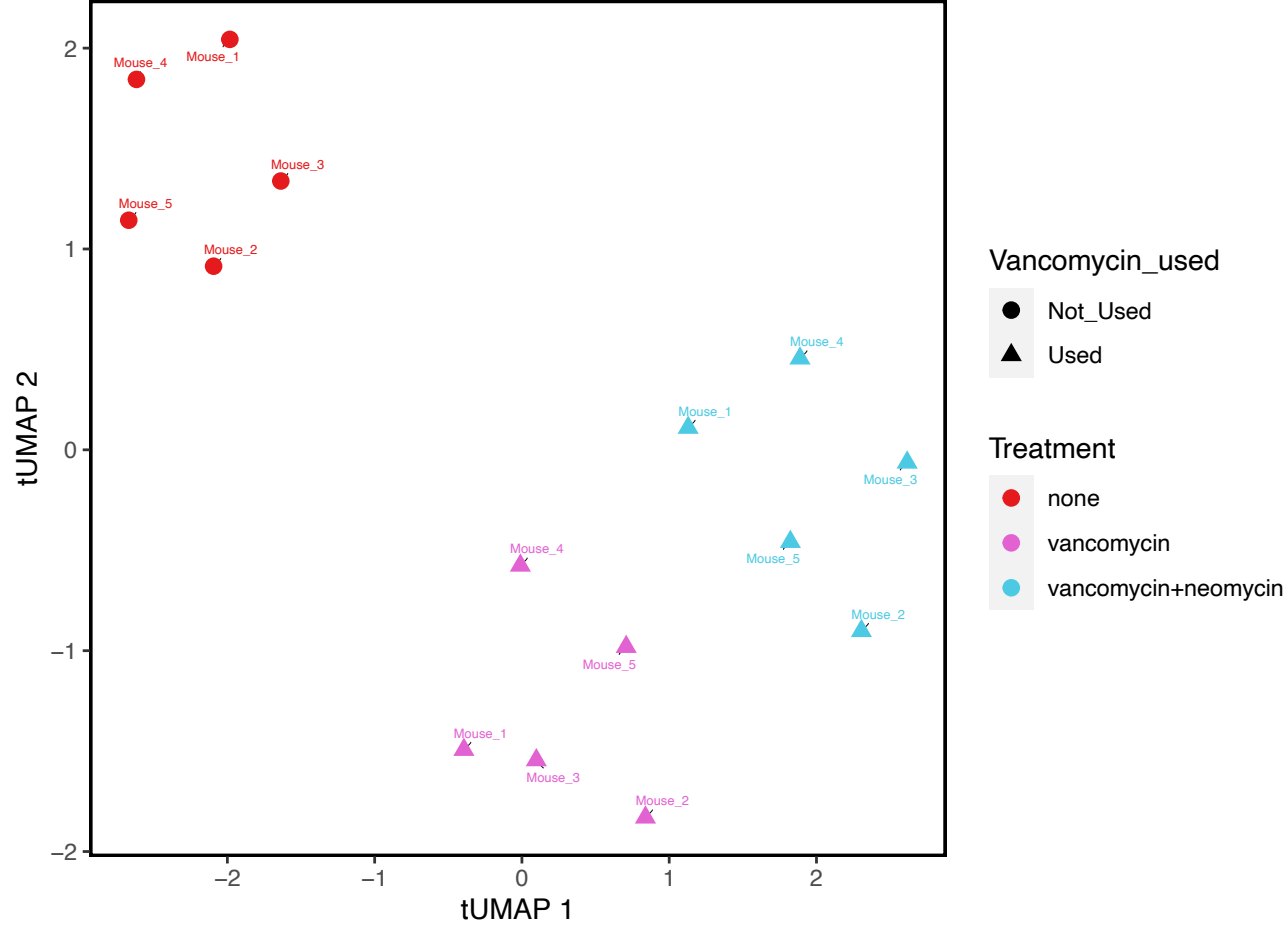
PERMANOVA $p < 0.5356$; F-Model = 0.8464

Dissimilarity index = bray



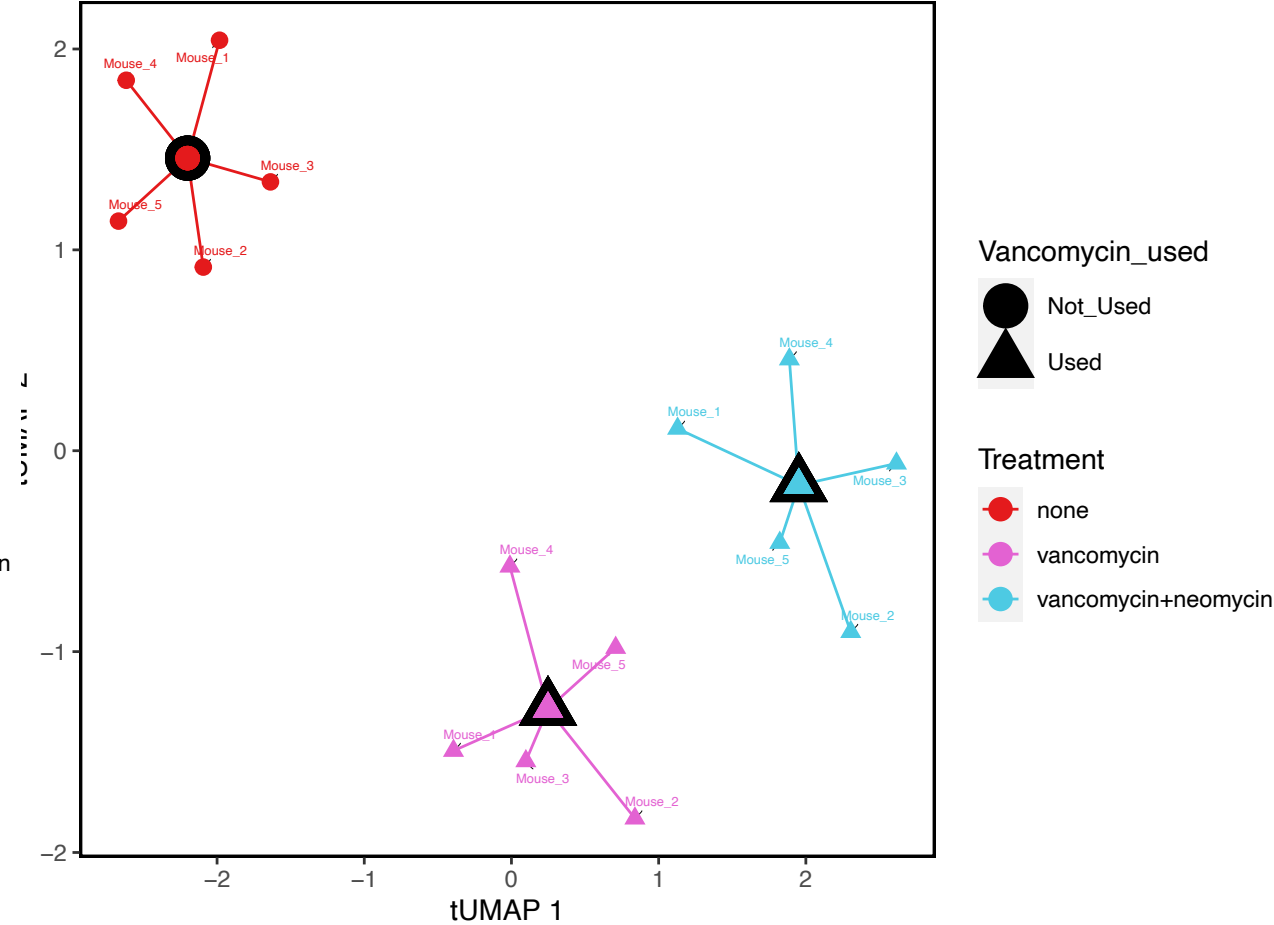
tUMAP of LKT within 5

Feature must be >250PPM in at least 15% of samples
Taxonomy information must come from >70% contigs in at least 50% of samples
Taxon genome completeness must be >10% in at least 5% of samples
Normalized to number of bases for analysis in sample
Matrix log2 transformed
PERMANOVA p <1e-04; F-Model = 50.4804
Dissimilarity index = bray

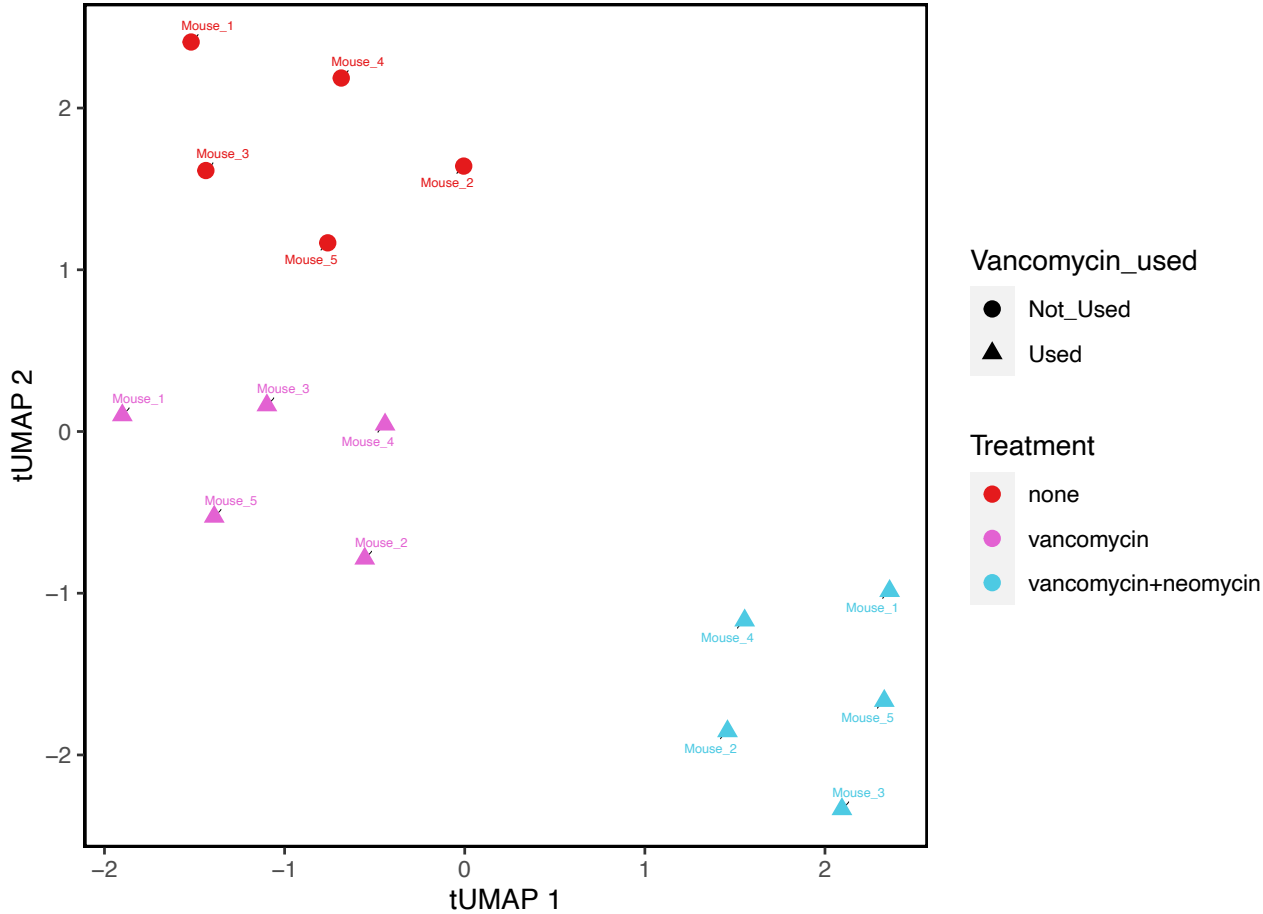


tUMAP of LKT within 5

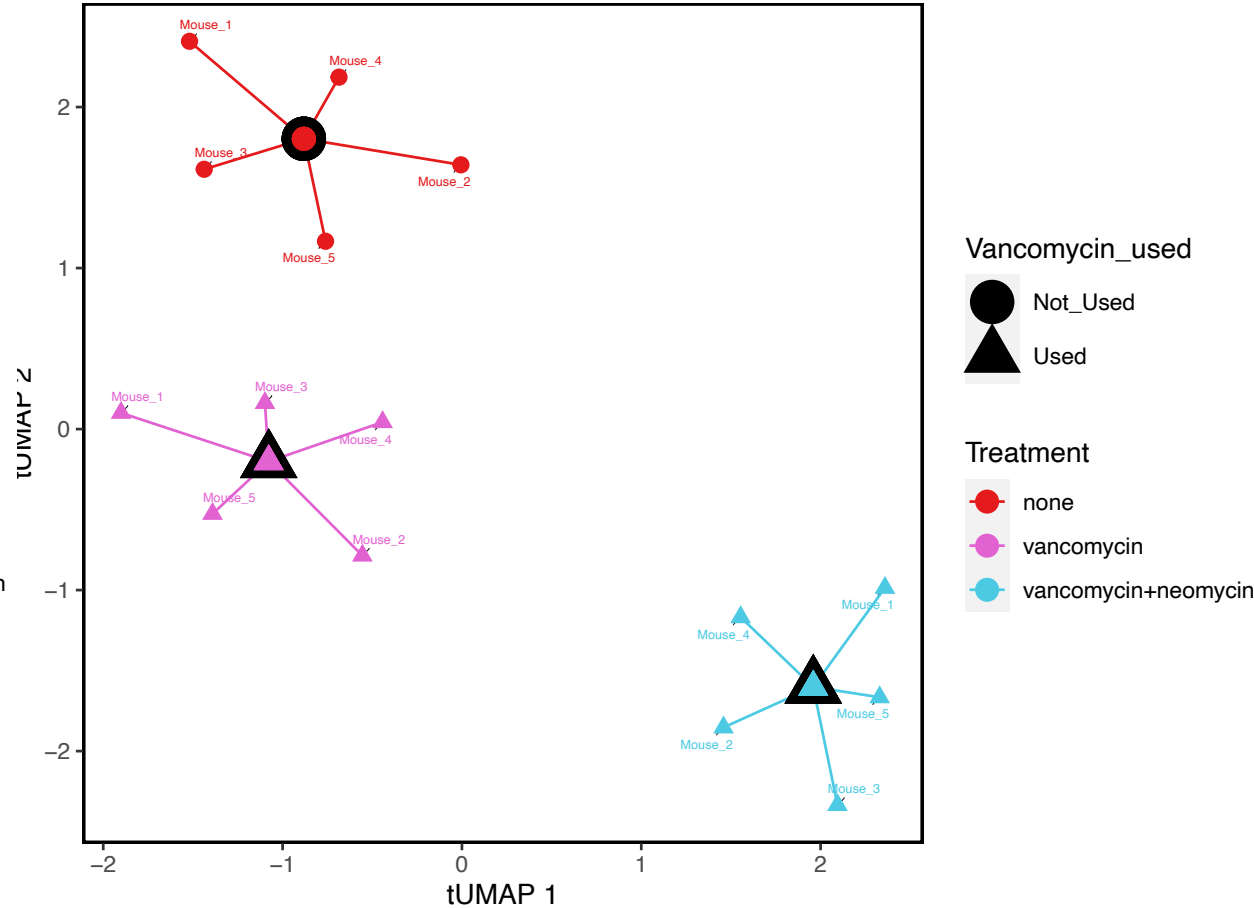
Feature must be >250PPM in at least 15% of samples
Taxonomy information must come from >70% contigs in at least 50% of samples
Taxon genome completeness must be >10% in at least 5% of samples
Normalized to number of bases for analysis in sample
Matrix log2 transformed
PERMANOVA p <1e-04; F-Model = 50.4804
Dissimilarity index = bray



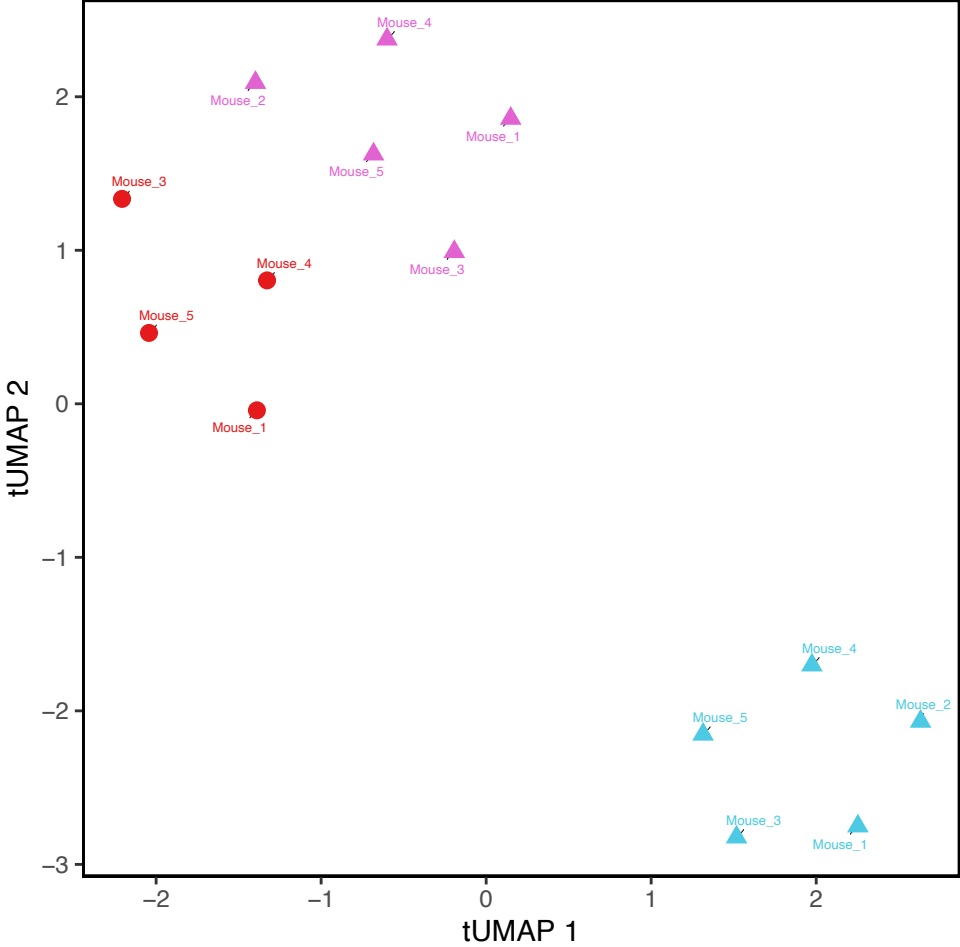
tUMAP of LKT within 15
Feature must be >250PPM in at least 15% of samples
Taxonomy information must come from >70% contigs in at least 50% of samples
Taxon genome completeness must be >10% in at least 5% of samples
Normalized to number of bases for analysis in sample
Matrix log2 transformed
PERMANOVA p <1e-04; F-Model = 21.2438
Dissimilarity index = bray



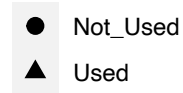
tUMAP of LKT within 15
Feature must be >250PPM in at least 15% of samples
Taxonomy information must come from >70% contigs in at least 50% of samples
Taxon genome completeness must be >10% in at least 5% of samples
Normalized to number of bases for analysis in sample
Matrix log2 transformed
PERMANOVA p <1e-04; F-Model = 21.2438
Dissimilarity index = bray



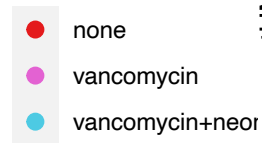
tUMAP of LKT within 56
Feature must be >250PPM in at least 15% of samples
Taxonomy information must come from >70% contigs in at least 50% of samples
Taxon genome completeness must be >10% in at least 5% of samples
Normalized to number of bases for analysis in sample
Matrix log2 transformed
PERMANOVA $p < 1e-04$; F-Model = 13.3718
Dissimilarity index = bray



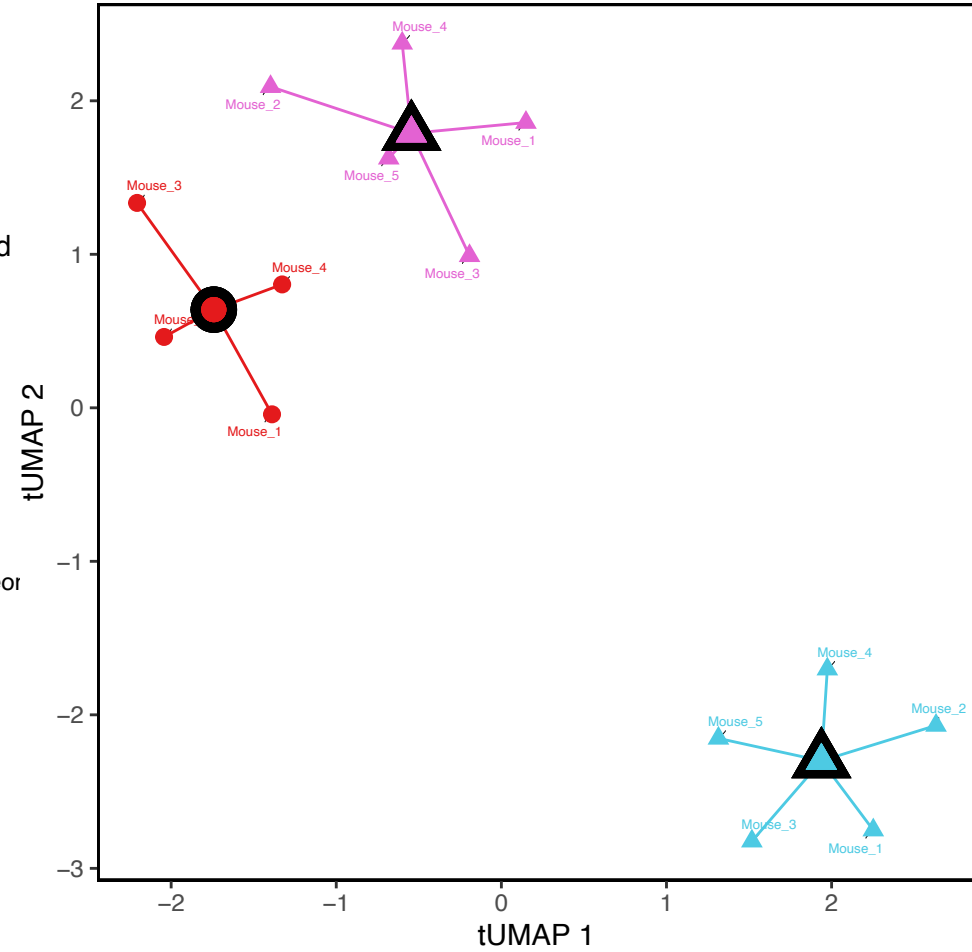
Vancomycin_used



Treatment



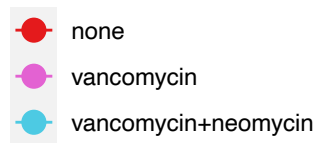
tUMAP of LKT within 56
Feature must be >250PPM in at least 15% of samples
Taxonomy information must come from >70% contigs in at least 50% of samples
Taxon genome completeness must be >10% in at least 5% of samples
Normalized to number of bases for analysis in sample
Matrix log2 transformed
PERMANOVA $p < 1e-04$; F-Model = 13.3718
Dissimilarity index = bray



Vancomycin_used

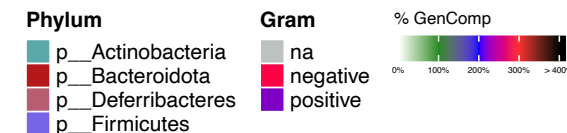
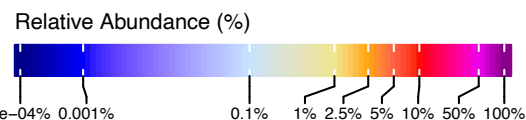
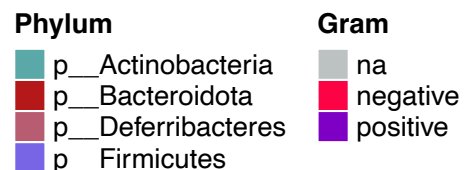
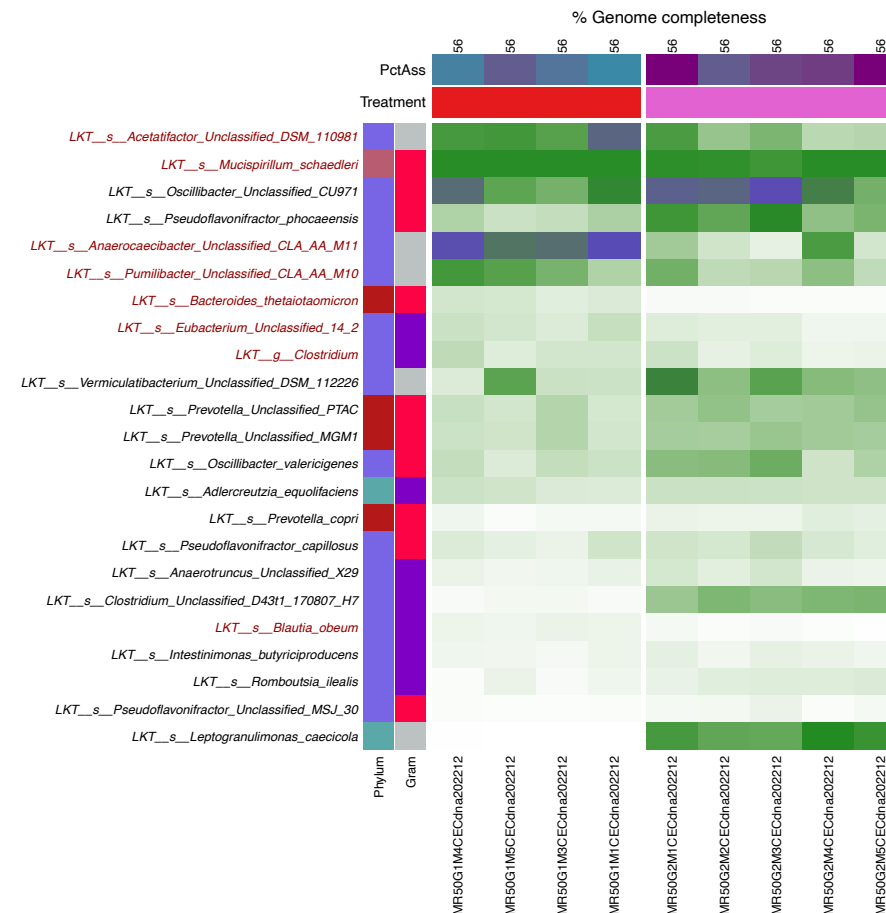
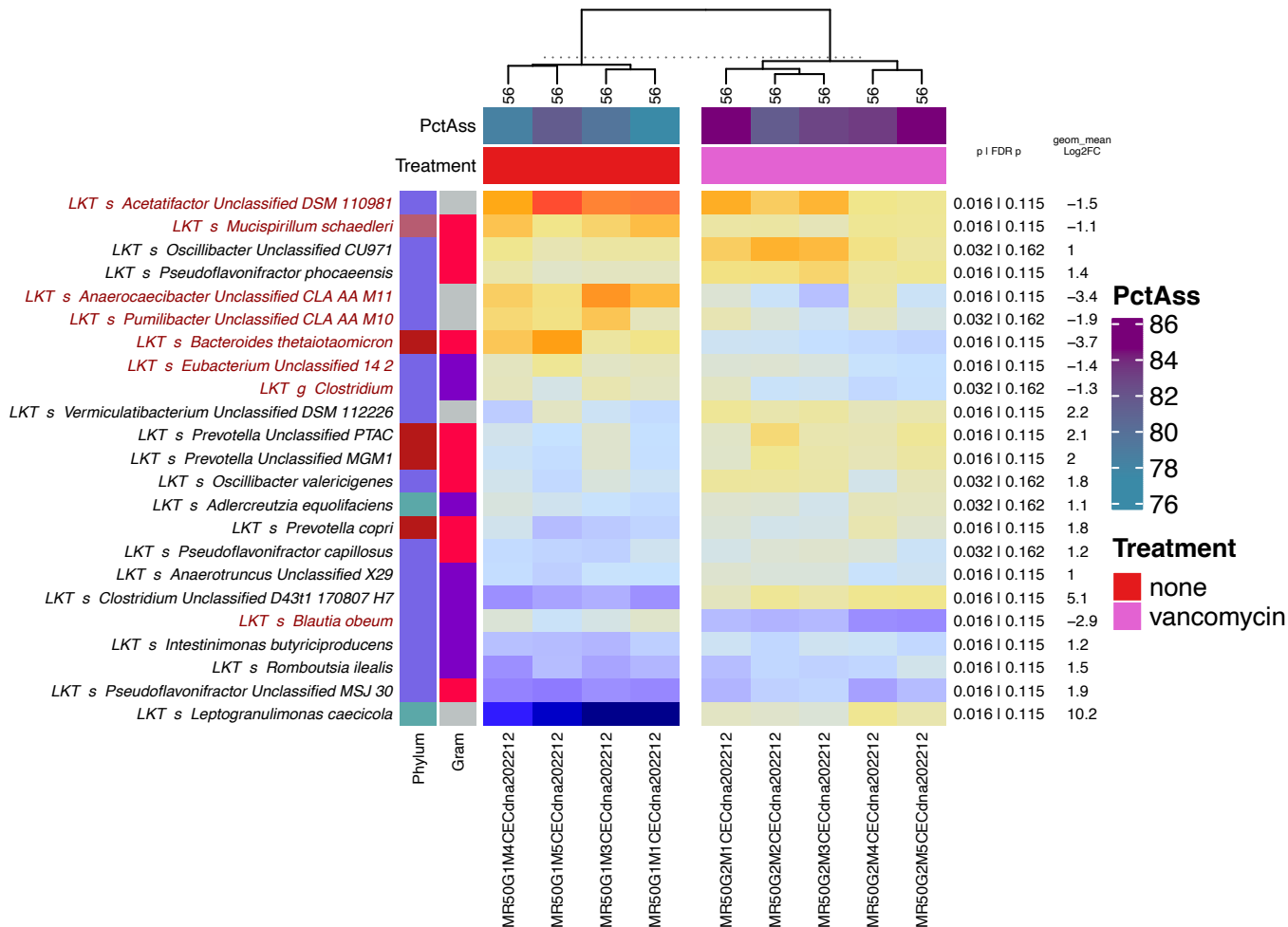
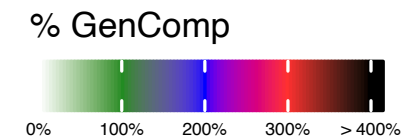


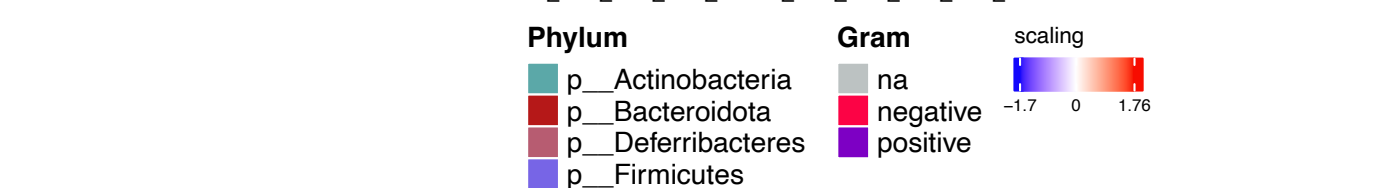
Treatment



```
STK <- subset(pheno, Neomycin_used == "Not_Used")[]$Sample
pdf("Example_Heatmaps.pdf", paper = "a4r")
for (anal in c("LKT", "MPA4", "Product", "Pfam", "resfinder")){
  for(sca in c(FALSE, TRUE)){
    plot_relabund_heatmap(ExpObj = expvec[[anal]], samplesToKeep = STK, glomby = NULL,
      hmtpe = "comparative", applyfilters = "moderate", featcutoff = NULL,
      GenomeCompletenessCutoff = NULL, PPM_normalize_to_bases_sequenced = FALSE, scaled =
      sca,
      subsetby = "Timepoint_days", compareby = "Treatment", invertbinaryorder = TRUE,
      showonlybelow = 0.05, ntop = NULL,
      colcategories = c("PctAss", "Treatment"),
      splitcolsby = "Treatment", ordercolsby = NULL, textby = "Timepoint_days",
      cluster_samples_per_heatmap = TRUE, cluster_features_per_heatmap = TRUE,
      label_samples = TRUE, cluster_rows = TRUE, max_rows_in_heatmap = 50, no_underscores =
      TRUE, showGram = TRUE, show_GenomeCompleteness = TRUE, maxnumheatmaps = 1)
  }
}
dev.off()
```

Relative Abundance Heatmap | LKT | within 56
 pval < 0.05 different between Treatment using MannWhitneyWilcoxon
 Feature must be >250PPM in at least 15% of samples
 Taxonomy information must come from >70% contigs in at least 50% of samples
 Taxon genome completeness must be >10% in at least 5% of samples
 Number of samples in heatmap = 9 | Number of features assessed = 138
 log2foldchange > 1 | log2foldchange > Inf
 Positive l2fc means increased in vancomycin

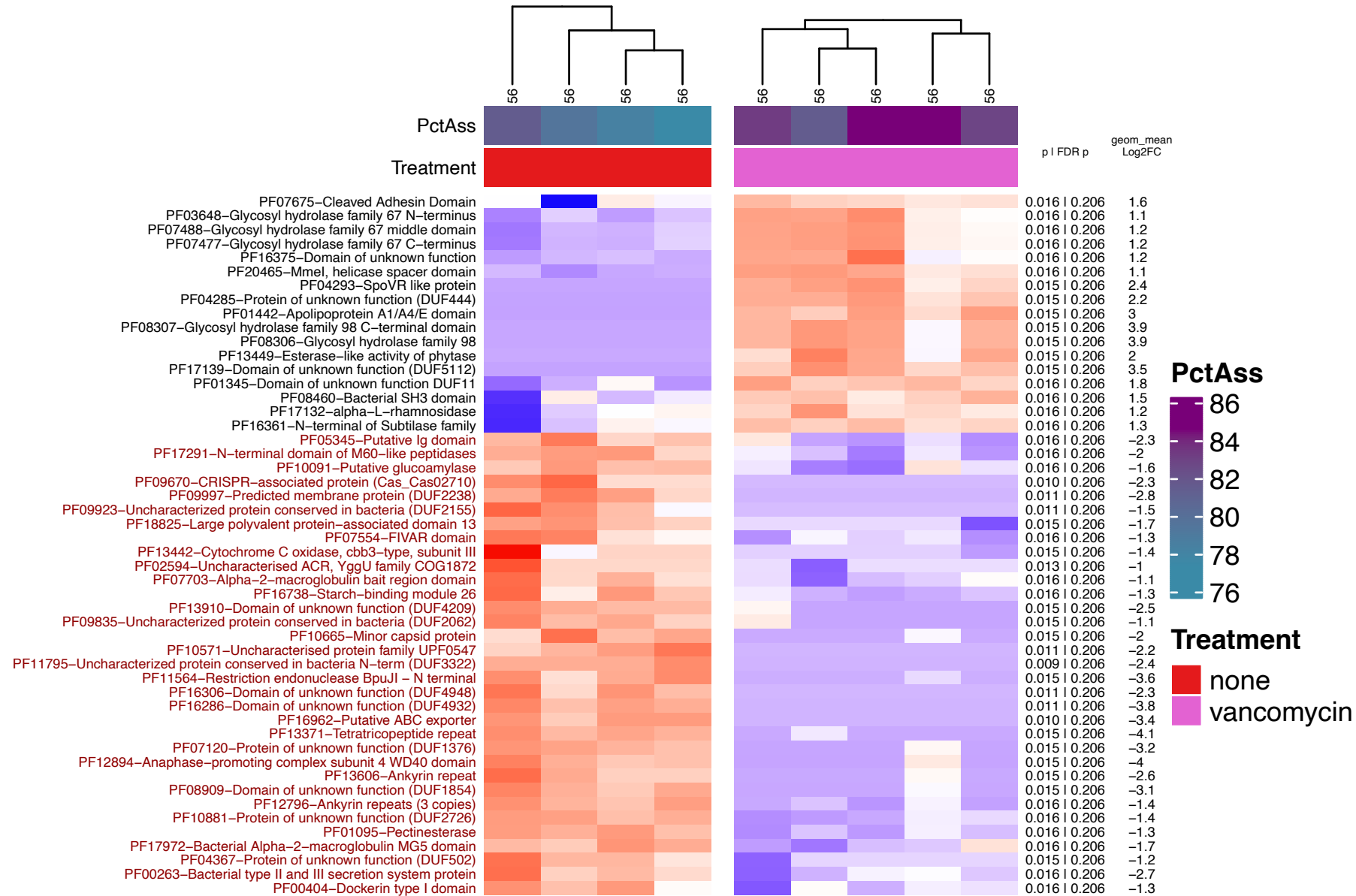




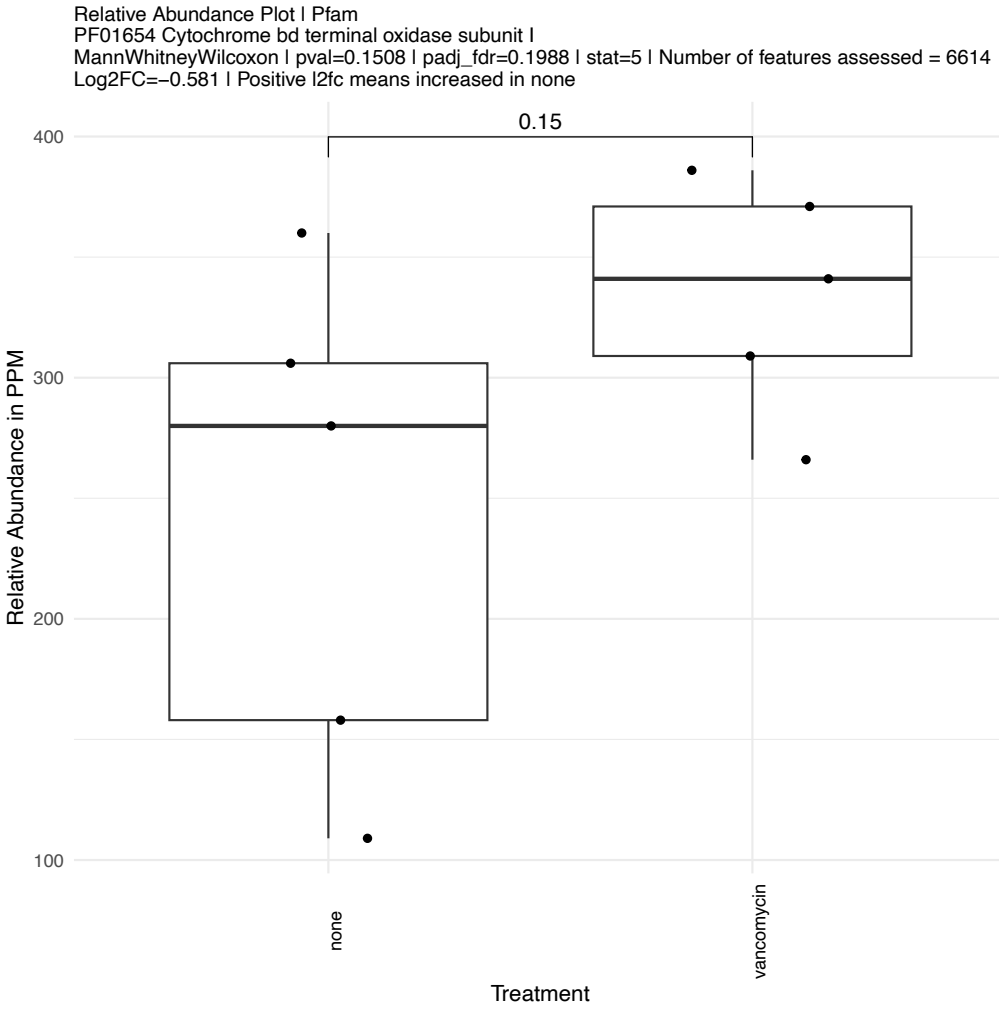
scaling

-2.34 0 2.26

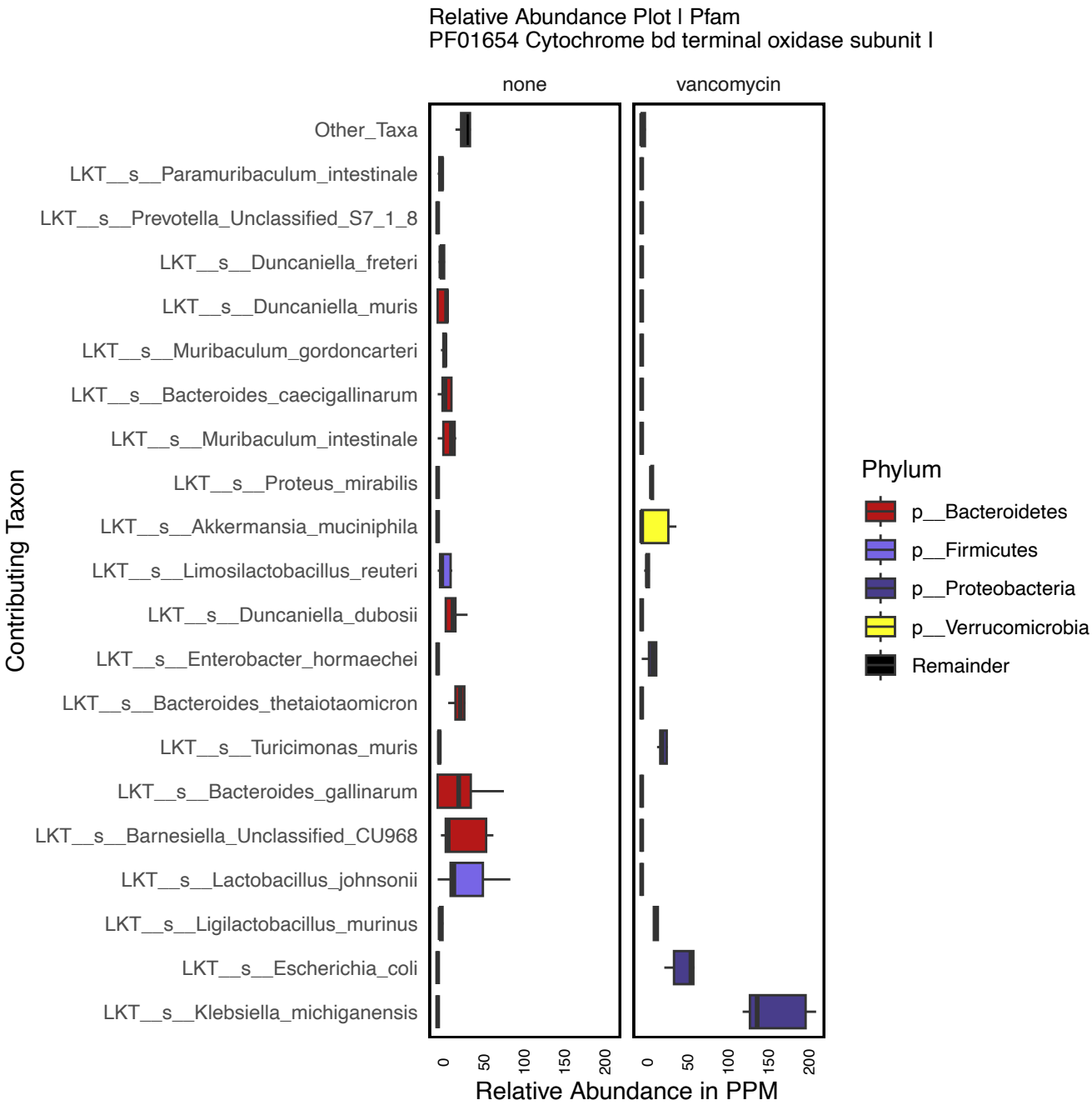
Relative Abundance Heatmap | Pfam | within 56
pval < 0.05 different between Treatment using MannWhitneyWilcoxon
Feature must be >5PPM in at least 5% of samples
Number of samples in heatmap = 9 | Number of features assessed = 4978
log2foldchange > 1 | log2foldchange > Inf
Positive l2fc means increased in vancomycin
Heatmap 1/8



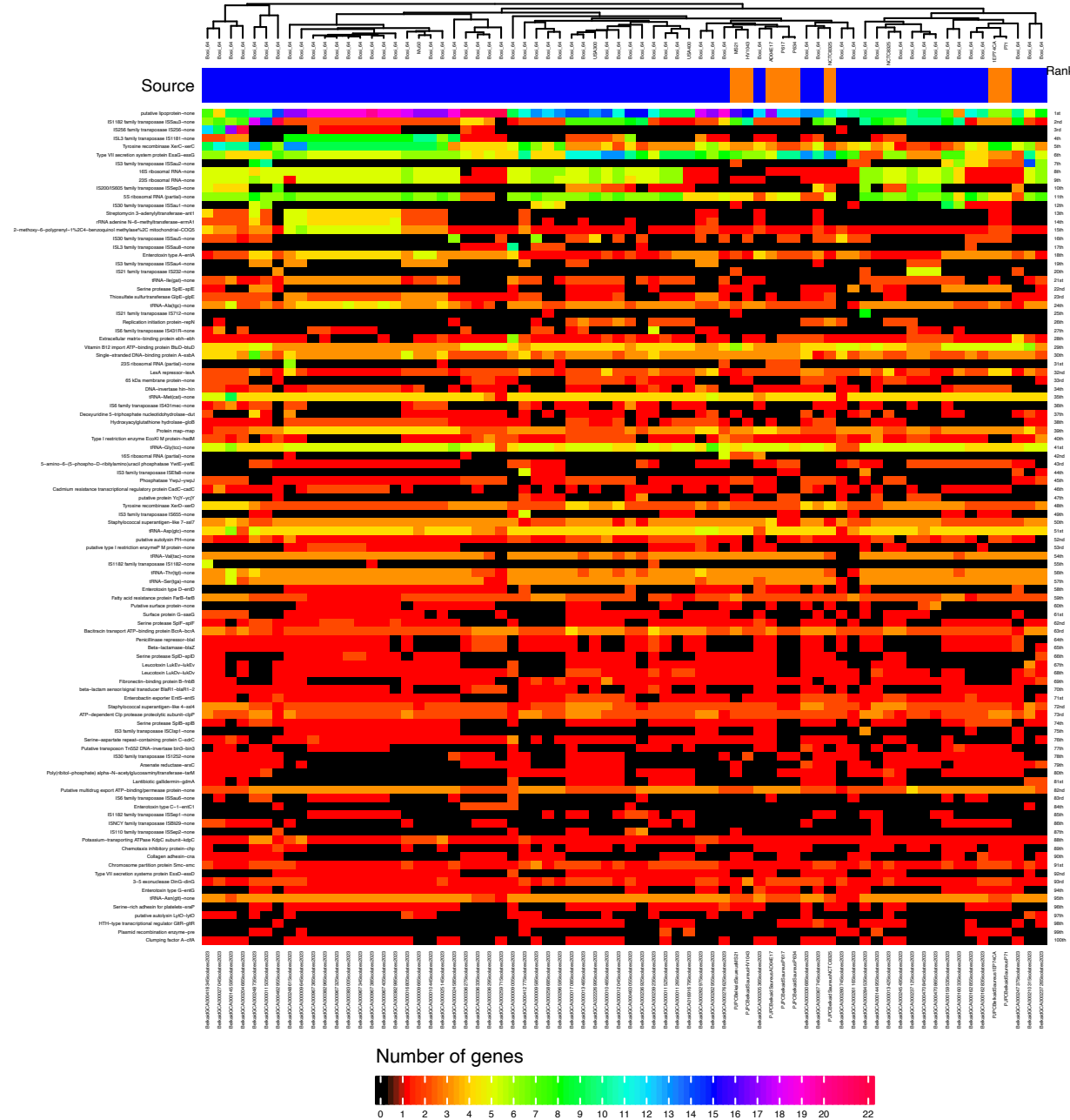
A



B



Relative Abundance Heatmap | Product
 Top 100 most variant features across samples
 Number of samples in heatmap = 72 | Number of features assessed = 1854



JAMS with isolate
 genomic data

Exploration of the
 functional differences
 between bacterial
 isolates

tUMAP of Product

Counts matrix used for ordination represents the number of genes

Taxonomy information must come from >70% contigs in at least 50% of samples

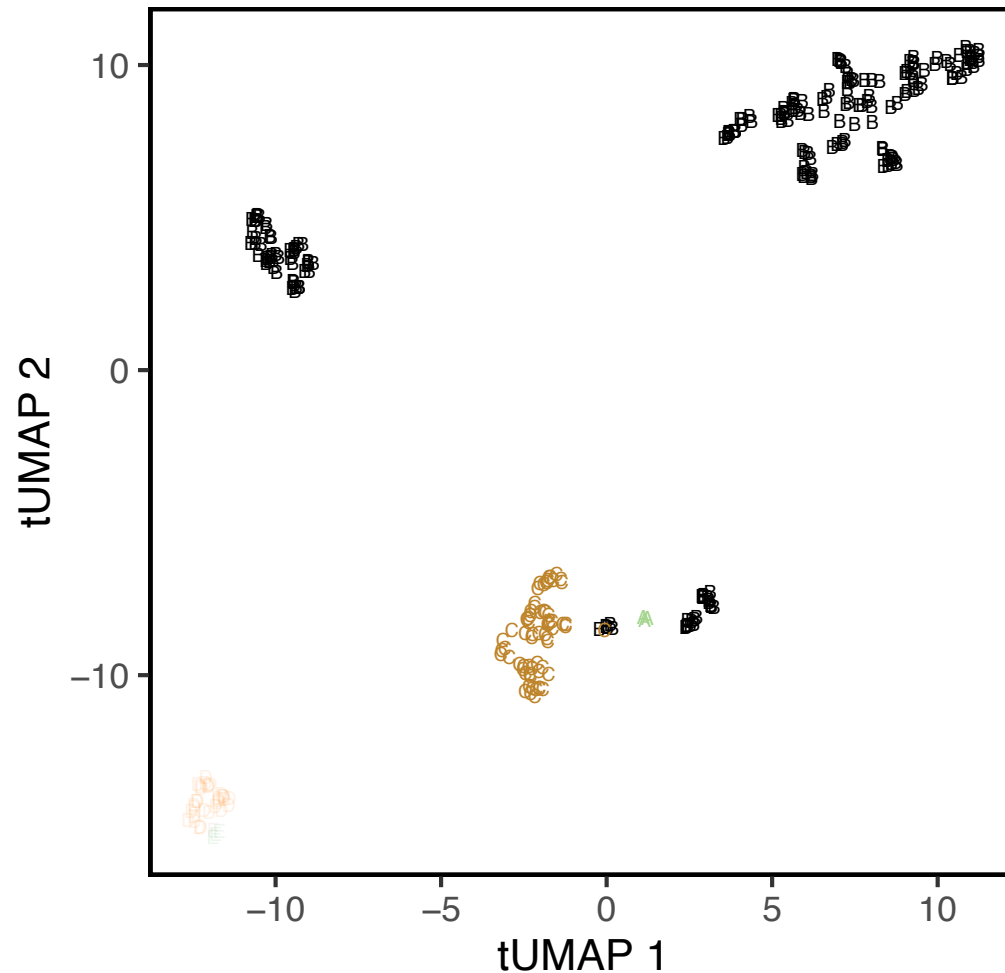
Matrix log2 transformed

Highlighting g__Akkermansia only; Distmethod: jaccard; Counts matrix: GeneCounts

PERMANOVA $p < 1e-04$; F-Model = 284.4547

Dissimilarity index = jaccard

Ordination of
number of
Genes from
ISOLATES
retrieved from
GenBank



NCBI_LKT

- A LKT__s__Akkermansia_glycaniphila
- B LKT__s__Akkermansia_muciniphila
- C LKT__s__Akkermansia_Unclassified
- D LKT__s__Phascolarctobacterium_faecium
- E LKT__s__Phascolarctobacterium_succinatutens
- F LKT__s__Phascolarctobacterium_Unclassified

Ordination of
number of
Genes from
ISOLATES
retrieved from
GenBank

tUMAP of Product

Counts matrix used for ordination represents the number of genes

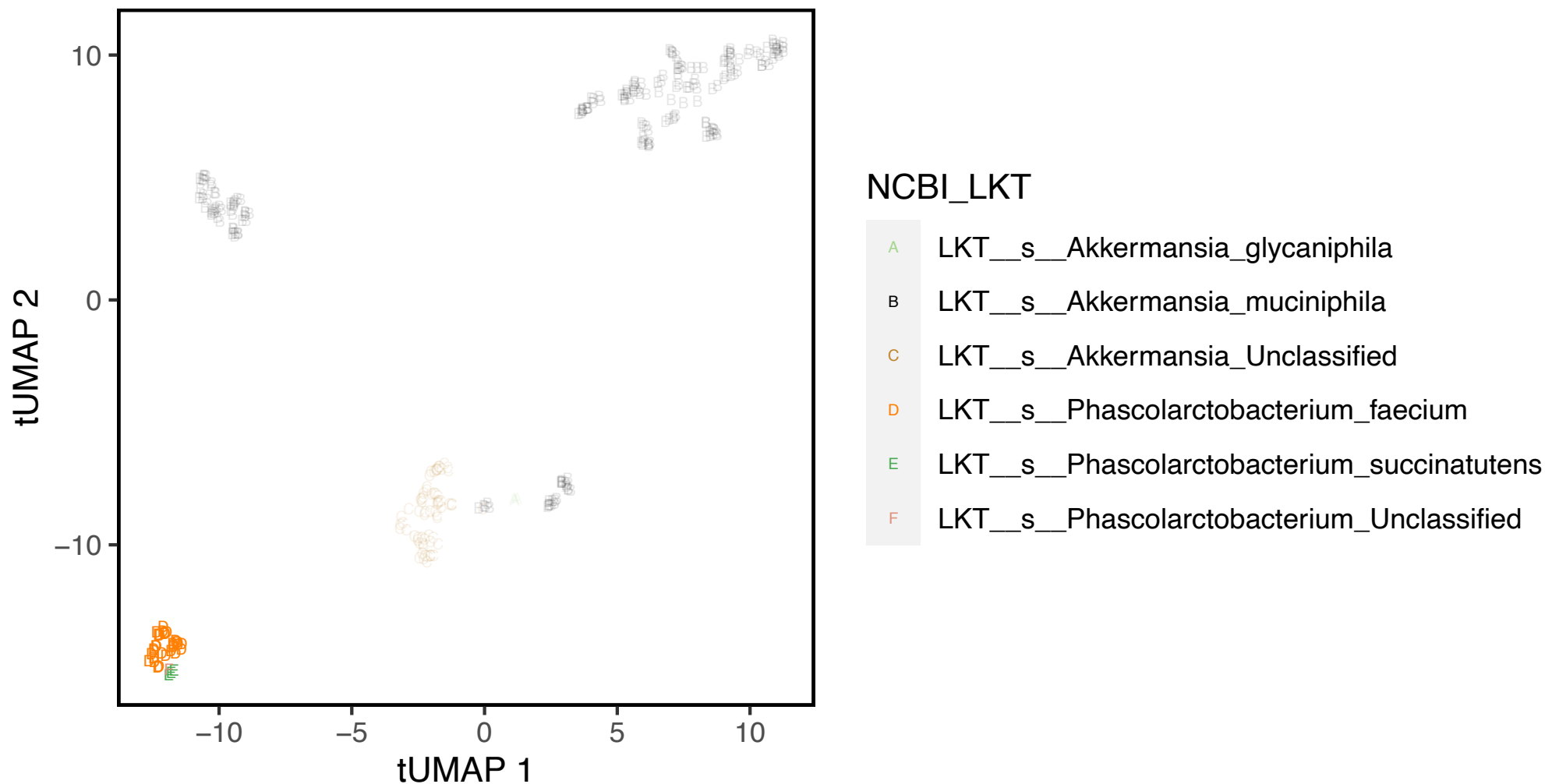
Taxonomy information must come from >70% contigs in at least 50% of samples

Matrix log2 transformed

Highlighting g__Phascolarctobacterium only; Distmethod: jaccard; Counts matrix: Gene

PERMANOVA $p < 1e-04$; F-Model = 20.9881

Dissimilarity index = jaccard



Other JAMS features:

- Importation of Metaphlann4 data from a count table
- JAMS16 is a dada2-based 16S version of JAMS

Acknowledgements

Trinchieri Lab

Dr. Giorgio Trinchieri

Nikki Cannon
Jonathan Badger
Tom Mossington
Amiran Dzutsev
Ascharya Balaji
Lisa Thammavong
Raquel Costa
Maria Clavijo Salomon
Miriam Fernandes
John McCulloch
Stephanie Prescott
Rodrigo Xavier Das Neves
Alice Cole
Rosalba Salcedo
Jenny Fang
Poonam Aggarwal
Sharon Bargo
April Huang
Corinne Salter

NCI Microbiome Core

Colm O'hUigin
Wuxing Yuan (Jane)
Richard Rodrigues
Rashed Shah
Naga Betrapally Padmanabhan

Translational Biobehavioral and Health Disparities Branch NIH

Jennifer J. Barb
Michael Valencia