

Open data science in microbiome research

Grand challenges of data-Intensive science in microbiome & metagenome data analysis and training, October 14, 2021



A family of alchemists at work, an engraving by Philip Galle, after a painting by Pieter Bruegel the Elder, published by Hieronymus Cock, c.1558.

Associate Prof. Leo Lahti | datascience.utu.fi
Department of Computing, University of Turku, Finland



MICROBIOME



@antagomir

The demise of alchemy provides further evidence, if further evidence were needed, that **what marks out modern science is not the conduct of experiments (alchemists conducted plenty of experiments), but the formation of a *critical community capable of assessing discoveries and replicating results*. Alchemy, as a clandestine enterprise, could never develop a community of the right sort. Popper was right to think that science can flourish only in an open society.**

The Invention of Science: A New History of the Scientific Revolution, by David Wootton



A family of alchemists at work, an engraving by Philip Galle, after a painting by Pieter Bruegel the Elder, published by Hieronymus Cock, c.1558.

Open
reporting and communication
were part of academic culture
since the early days

BJHS 45(2): 165–188, June 2012. © British Society for the History of Science 2012
doi:10.1017/S0007087412000064 First published online 20 March 2012

Openness versus secrecy? Historical and
historiographical remarks

KOEN VERMEIR*



Source: Wikimedia Commons / Public domain

Alchemy & algorithms: perspectives on the philosophy and history of open science

Research Ideas and Outcomes 3:e13593, 2017

▼ [Leo Lahti](#), [Filipe da Silva](#), [Markus Petteri Laine](#), [Viivi Lähteenoja](#), [Mikko Tolonen](#)

Beyond Open Access - The Changing Culture of Producing and Disseminating Scientific Knowledge

Heidi Laine

Leo Lahti

Anne Lehto

Proceedings of the 19th International Academic Mindtrek Conference 2015. ACM New York, NY, USA. ISBN: 978-1-4503-3948-3

workflows
community
reproducibility



MICROBIOME

ml4microbiome.eu

(some) elements of microbiome data science

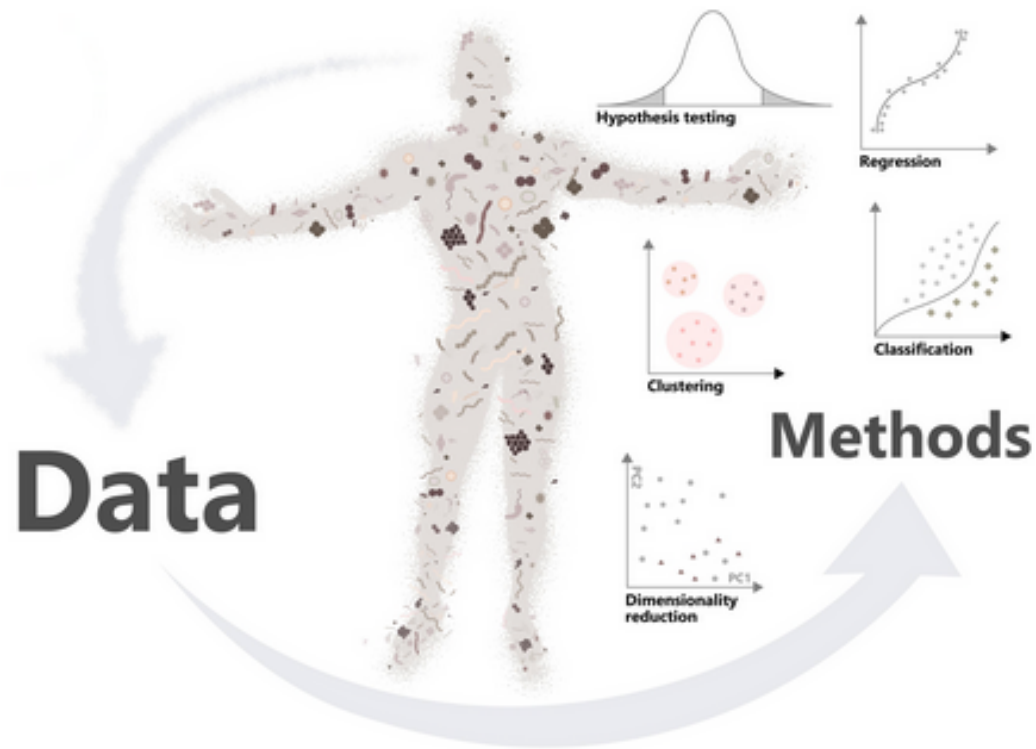
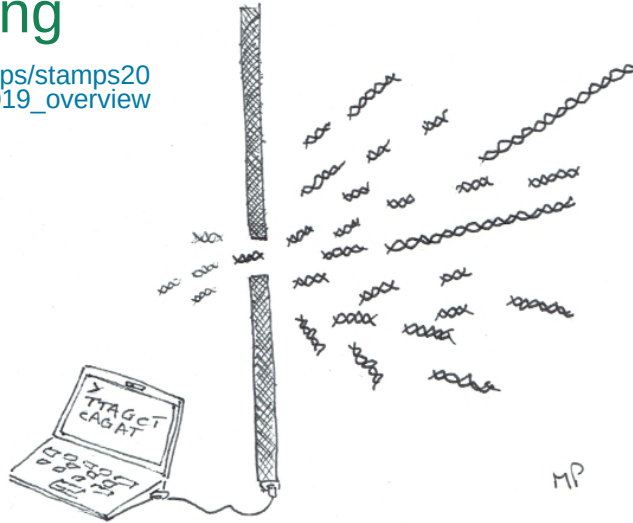


Figure source: Moreno-Indias *et al.* (2021) [Statistical and Machine Learning Techniques in Human Microbiome Studies: Contemporary Challenges and Solutions](#). *Frontiers in Microbiology* 12:11.

https://github.com/mbblstamps/stamps2019/blob/master/STAMPS2019_overview_Pop.pdf



The variety of study types

Data preprocessing

Case-control studies

Interventions

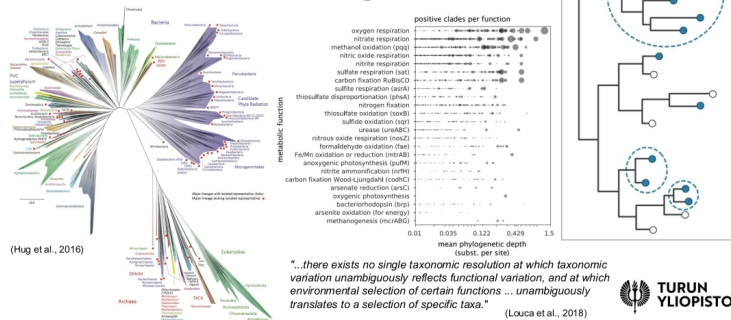
Cross-sectional analysis

Prospective analysis

Longitudinal dynamics

Multi-omics

The use of phylogenetic information in metagenomics

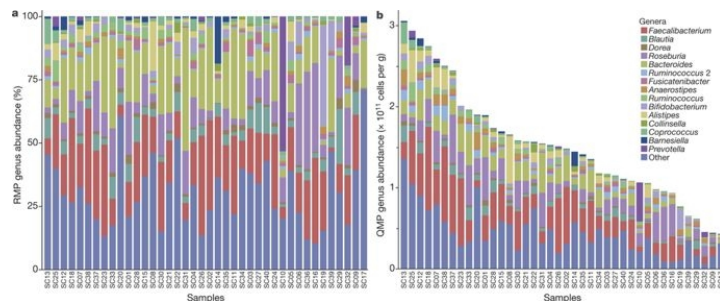


"...there exists no single taxonomic resolution at which taxonomic variation unambiguously reflects functional variation, and at which environmental selection of certain functions ... unambiguously translates to a selection of specific taxa." (Louca et al., 2018)

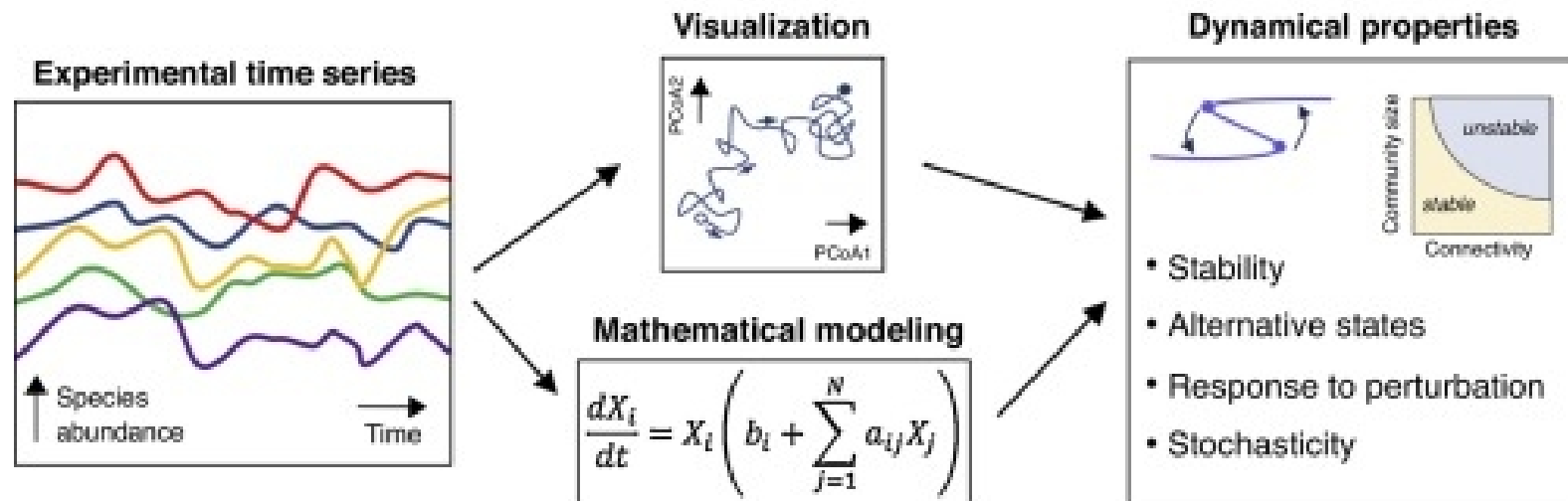


Compositionality

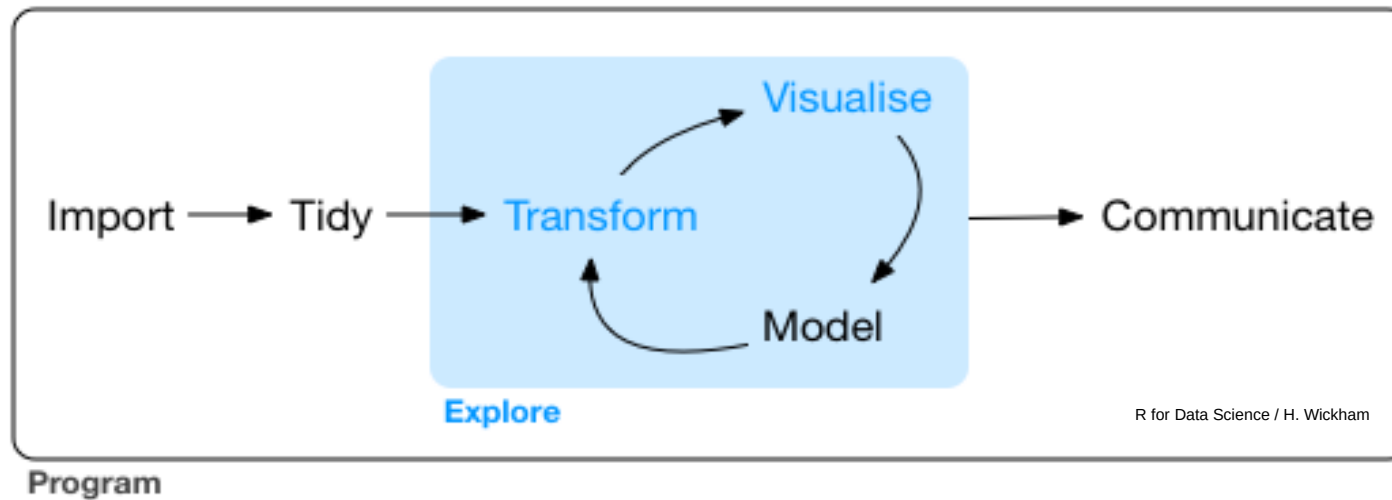
Vandeputte et al. Nature 551:507-511, 2017



Microbiome research is *data-intensive* and relies on a heterogeneous array of *sophisticated computational techniques*



Reproducible workflows improve transparency and robustness



Taxonomic level?

- Phylum
- Family
- Order
- Genus
- Species
- Strain..

Normalization

- None
- TSS
- CSS
- ILR/ALR/CLR
- phILR
- Hellinger

(Dis)similarity?

- Euclidean
- Aitchison
- Bray-Curtis
- Jaccard
- weighted Unifrac
- unweighted Unifrac

Regulation

- Calinski-Harabasz
- Dirichlet Process
- Silhouette Index
- AIC
- BIC
- DIC

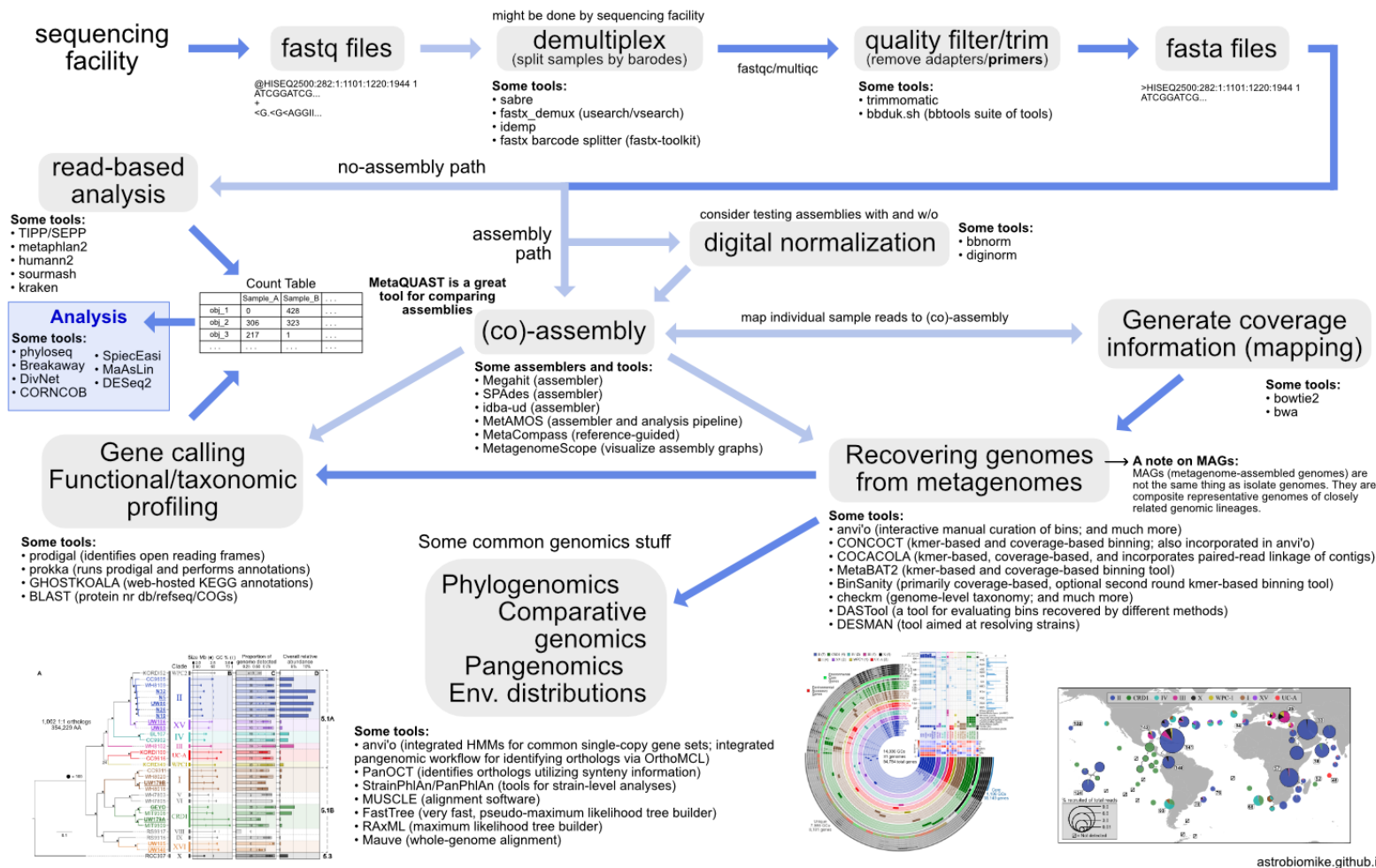
Clustering

- Hierarchical / Ward
- Hierarchical / Complete
- Gaussian mixture
- DMM
- PAMR
- K-means

Overview of generic* metagenomics workflow

*This is generic; specific workflows can vary on the order of steps here and how they are done.

When working with your own data you should never follow any pipeline blindly. There can be critical differences based on your data.



Happy Belly Bioinformatics

JOSE 10.21105/jose.00053

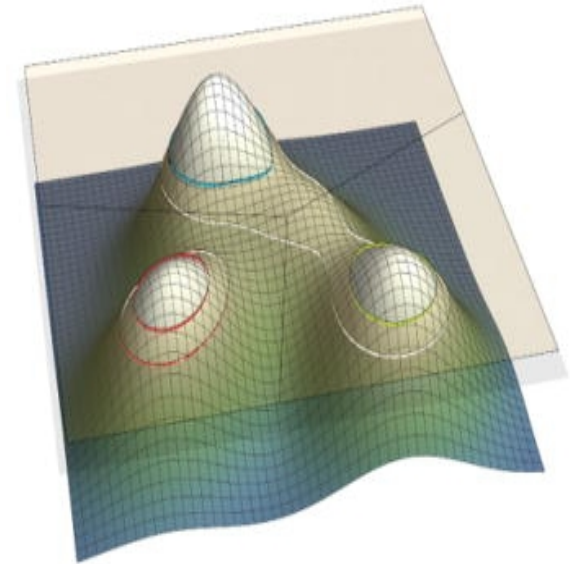
AstroBioMike

Orcid: 0000-0001-7750-9145

Lee, (2019). Happy Belly Bioinformatics: an open-source resource dedicated to helping biologists utilize bioinformatics. Journal of Open Source Education, 4(41), 53, <https://doi.org/10.21105/jose.00053>

How to choose a correct model?

→ a community typing example



Enterotypes in the landscape of gut microbial community composition. Costea *et al.* Nature 2018.

Taxonomic level

- Phylum
- Family
- Order
- Genus
- Species
- Strain..

Filtering

- None
- Prevalent
- Core
- Excl. outliers
- High variance
- Custom

Normalization

- None
- TSS
- CSS
- ILR/ALR/CLR
- phILR
- Hellinger

(Dis)similarity

- Euclidean
- Aitchison
- Bray-Curtis
- Jaccard
- weighted Unifrac
- unweighted Unifrac

Clustering method

- Hierarchical / Ward
- Hierarchical / Complete
- Gaussian mixture
- DMM
- PAMR
- K-means

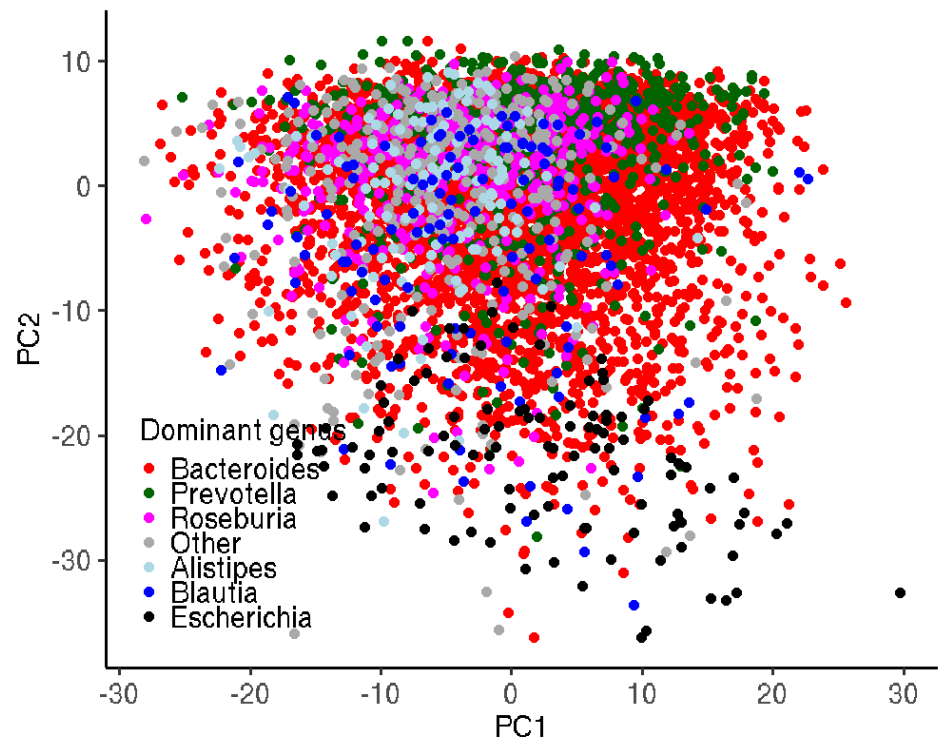
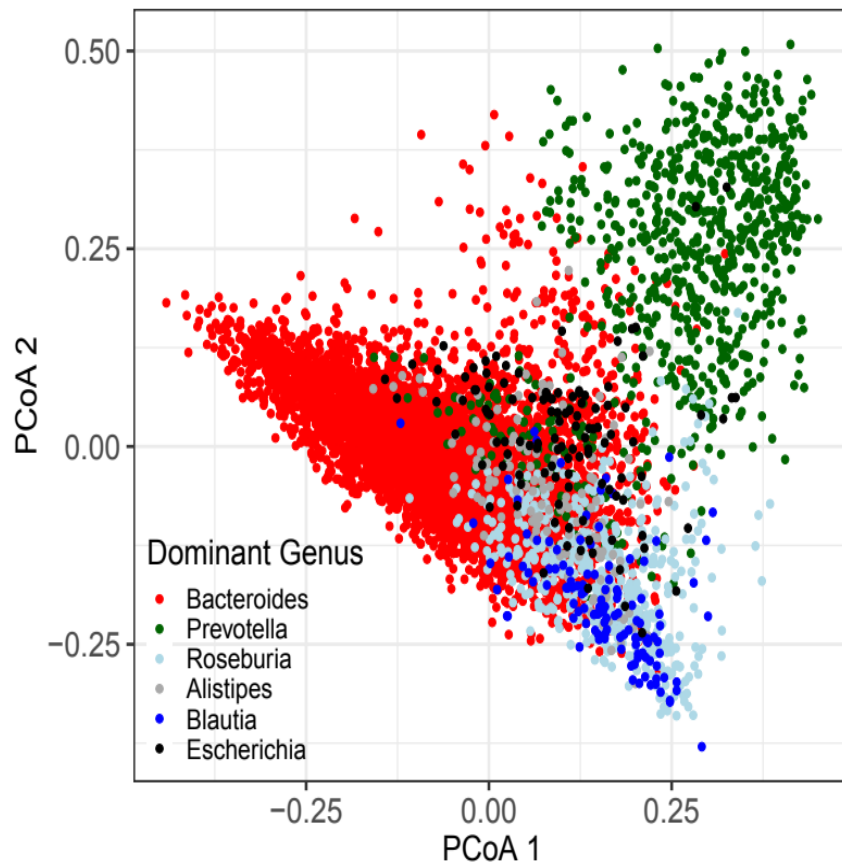
Regulation

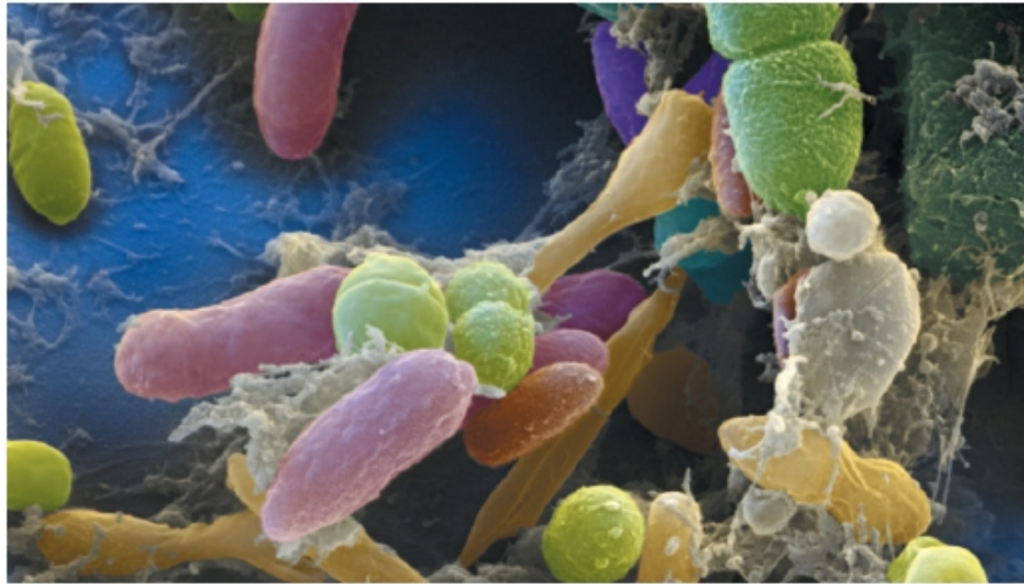
- Calinski-Harabasz
- Dirichlet Process
- Silhouette Index
- AIC
- BIC
- DIC

$$2 \times 6^6 = 93312$$

PCoA or PCA – different methods, different results?

FINRISK – Salosensaari et al. Nat. Comm. 2021






A scanning electron micrograph of bacteria in human faeces, in which 50% of species originate from the gut.

Microbiome science needs a healthy dose of scepticism

To guard against hype, those interpreting research on the body's microscopic communities should ask five questions, says **William P. Hanage**.

Comment August 2014 Nature

The influence of hidden researcher decisions in applied microeconomics

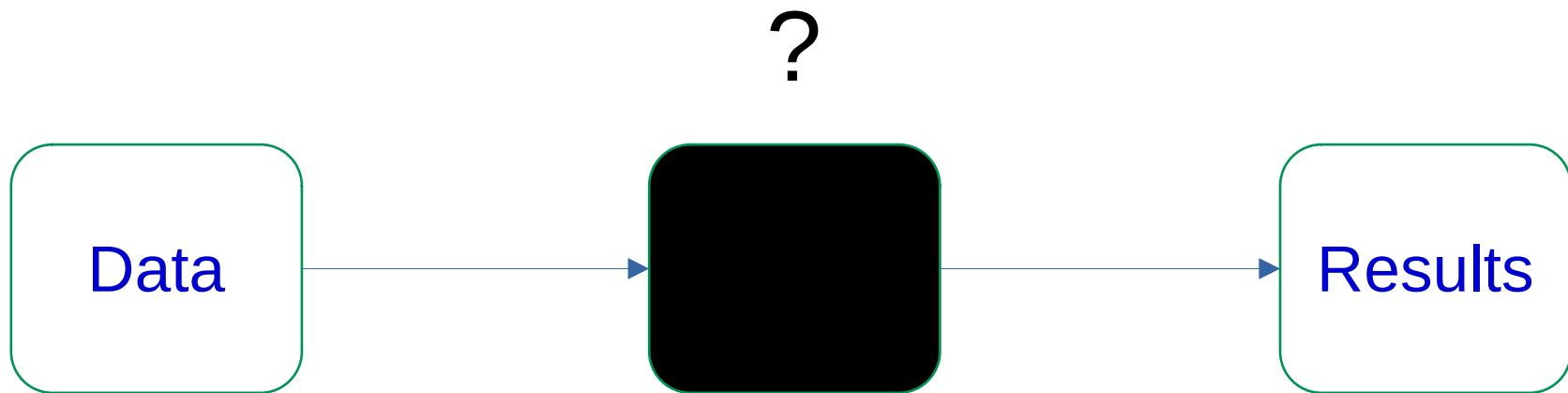
Nick Huntington-Klein , Andreu Arenas, Emily Beam, Marco Bertoni, Jeffrey R. Bloem, Pralhad Burli, Naibin Chen, Paul Grieco, Godwin Ekpe, Todd Pugatch, Martin Saavedra, Yaniv Stopnitzky

First published: 22 March 2021

<https://doi.org/10.1111/ecin.12992>

Researchers make hundreds of decisions about data collection, preparation, and analysis in their research. We use a many-analysts approach to measure the extent and impact of these decisions. Two published causal empirical results are replicated by seven replicators each. We find large differences in data preparation and analysis decisions, many of which would not likely be reported in a publication. No two replicators reported the same sample size. Statistical significance varied across replications, and for one of the studies the effect's sign varied as well. The standard deviation of estimates across replications was 3–4 times the mean reported standard error.






RESEARCH PRIORITIES

Shining Light into Black Boxes

A. Morin¹, J. Urban², P. D. Adams³, I. Foster⁴, A. Sali⁵, D. Baker⁶, P. Sliz^{1,*}

How to Make More Published Research True

John P. A. Ioannidis 

Published: October 21, 2014 • DOI: 10.1371/journal.pmed.1001747

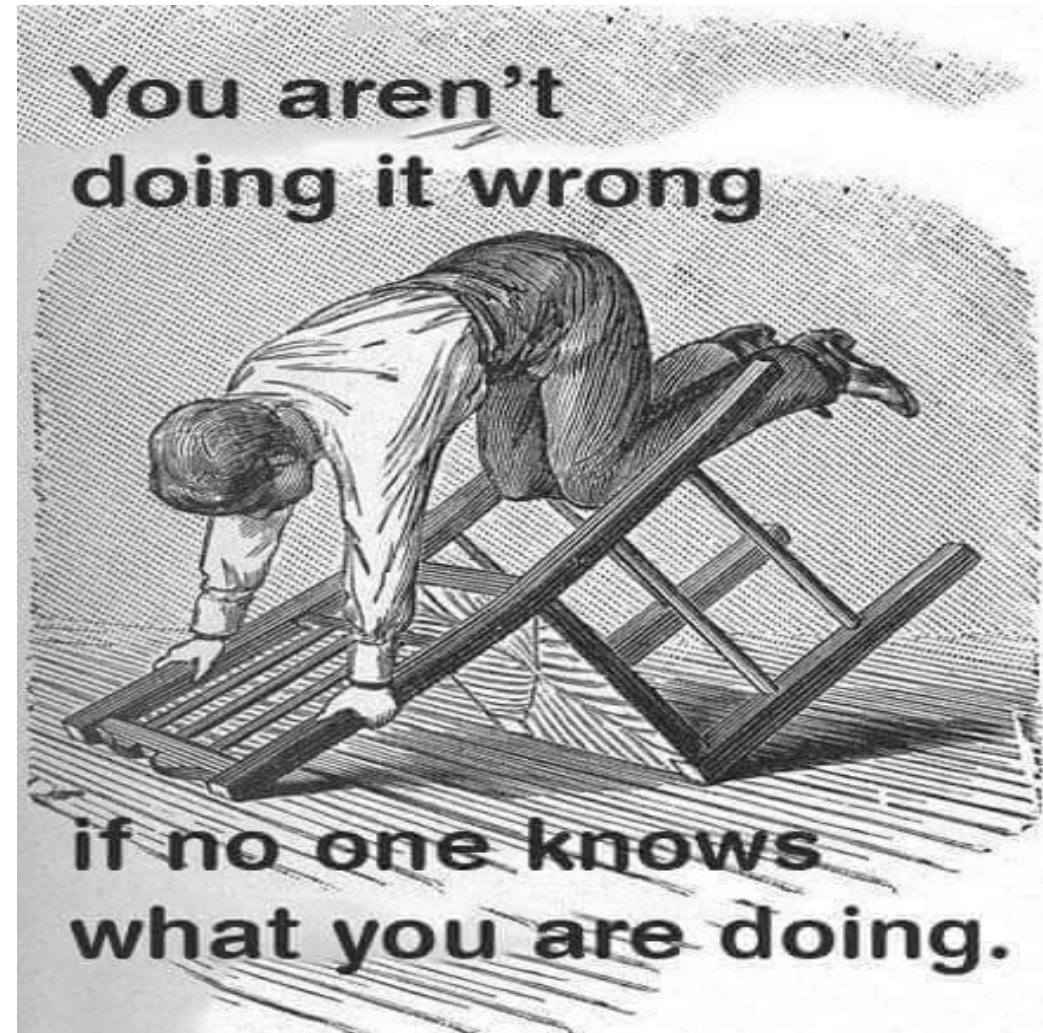
```
int getRandomNumber()  
{  
    return 4; // chosen by fair dice roll.  
              // guaranteed to be random.  
}
```

[http://web.stanford.edu/class/cs109/unrestricted/
images/](http://web.stanford.edu/class/cs109/unrestricted/images/)

RESEARCH PRIORITIES

Shining Light into Black Boxes

A. Morin¹, J. Urban², P. D. Adams³, I. Foster⁴, A. Sali⁵, D. Baker⁶, P. Sliz^{1,*}



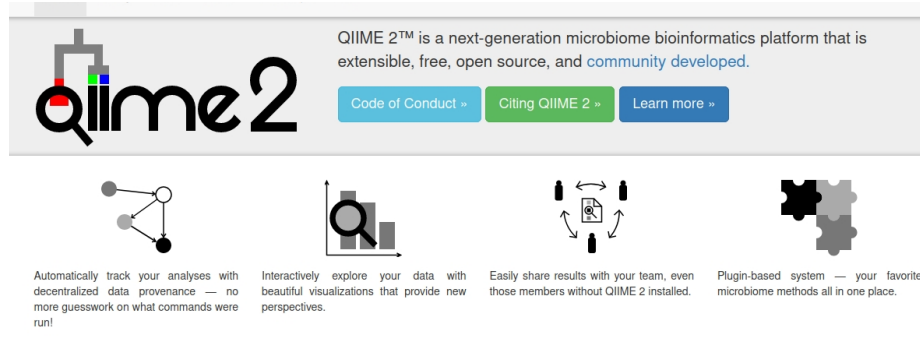
“I have begun to think that no one ought to publish biometric results, without lodging a well arranged and well bound manuscript copy of all his data, in some place whereit should be accessible, under reasonable restrictions, to those who desire to verify his work.”

Francis Galton (1901), *Biometrika* 1:1, pp. 7-10.

Data silo



open data science ecosystems



PeerJ >

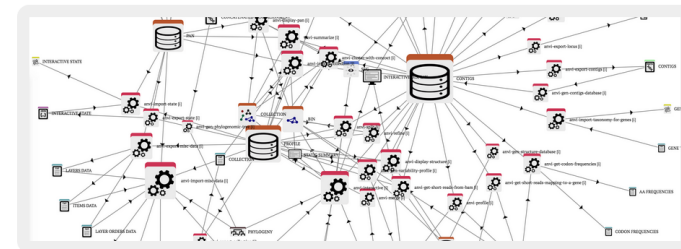
Anvi'o: an advanced analysis and visualization platform for 'omics data

Research article | Bioinformatics | Biotechnology | Computational Biology | Genomics | Microbiology

A. Murat Eren^{✉1,2}, Özcan C. Esen¹, Christopher Quince³, Joseph H. Vineis¹,
Hilary G. Morrison¹, Mitchell L. Sogin¹, Tom O. Delmont¹

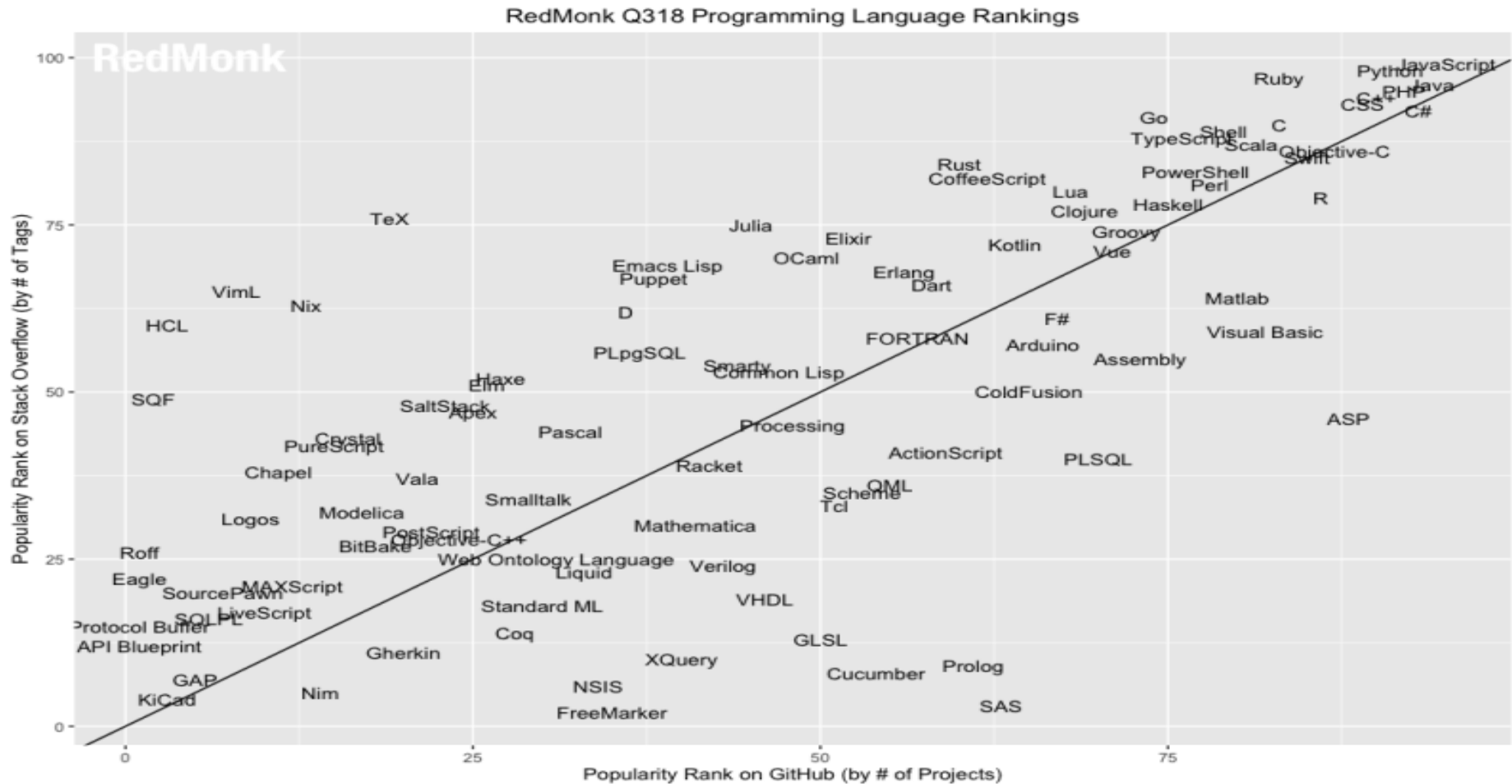
Published October 8, 2015

Anvi'o in a nutshell



Anvi'o is an [open-source, community-driven](#) analysis and visualization platform for 'omics data.

Varying cultures of open collaboration





Bioconductor

OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

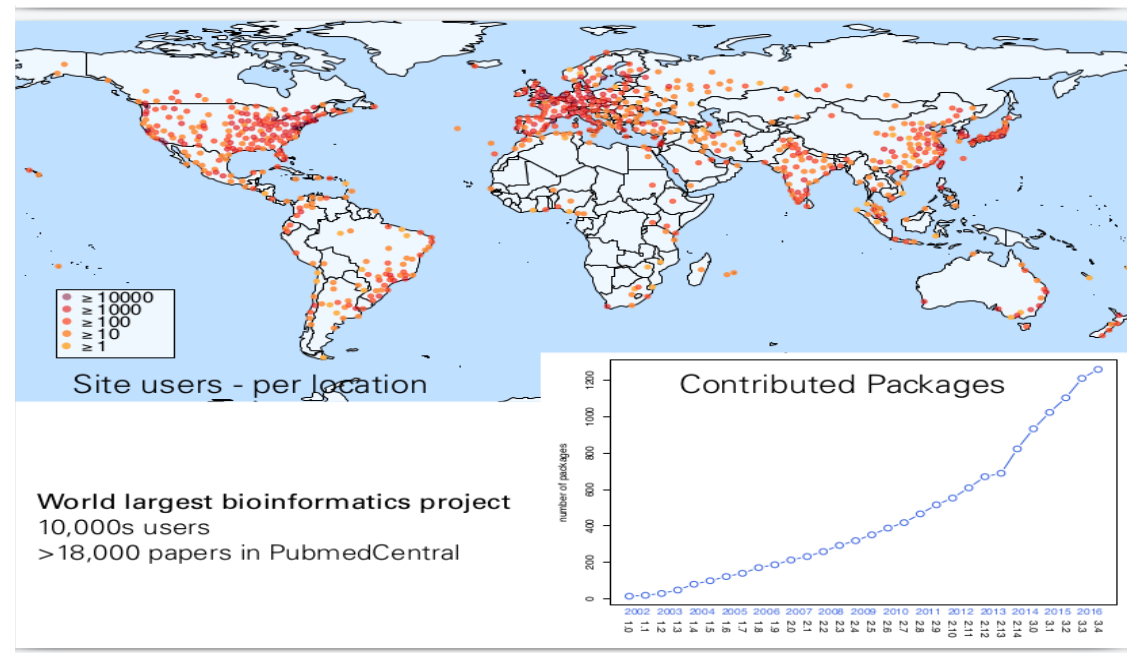
Started 2001 as a platform for analysis & understanding of microarray data

More than 1,600 packages. Domains of expertise:

- Sequencing (RNASeq, ChIPSeq, single-cell, called variants, ...)
- Microarrays (methylation, expression, copy number, ...)
- Flow cytometry
- Proteomics
- Multi-Omics data integration

Important themes

- Reproducible research
- Interoperability between packages & workflows
... even from different authors
- Usability



What is



Bioconductor

OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

?

Principally a collaborative software development project

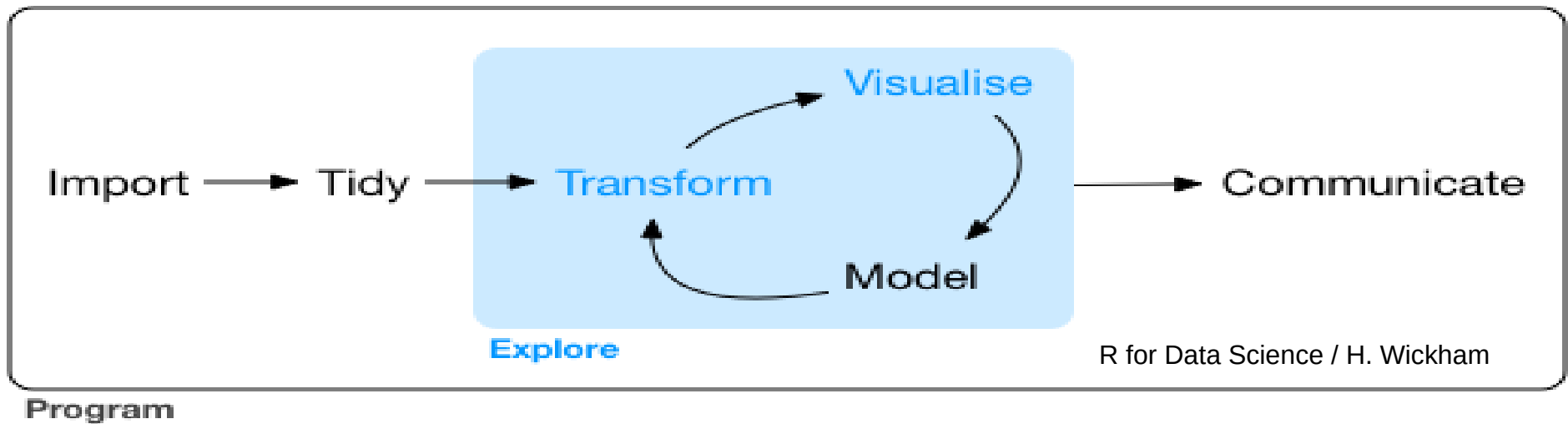
But it is also:

- a software repository
- a bioinformatics support site
- data repository
- publisher for supplementary materials
- source for tutorials and instructional documentation

Managed and maintained by a core team of ~6 people, with contributions coming from all over the world



Data science workflow

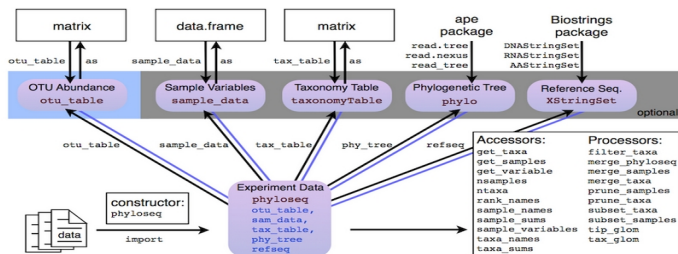


REVISED Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses [version 2; peer review: 3 approved]

Ben J. Callahan¹, Kris Sankaran¹, Julia A. Fukuyama¹, Paul J. McMurdie², Susan P. Holmes

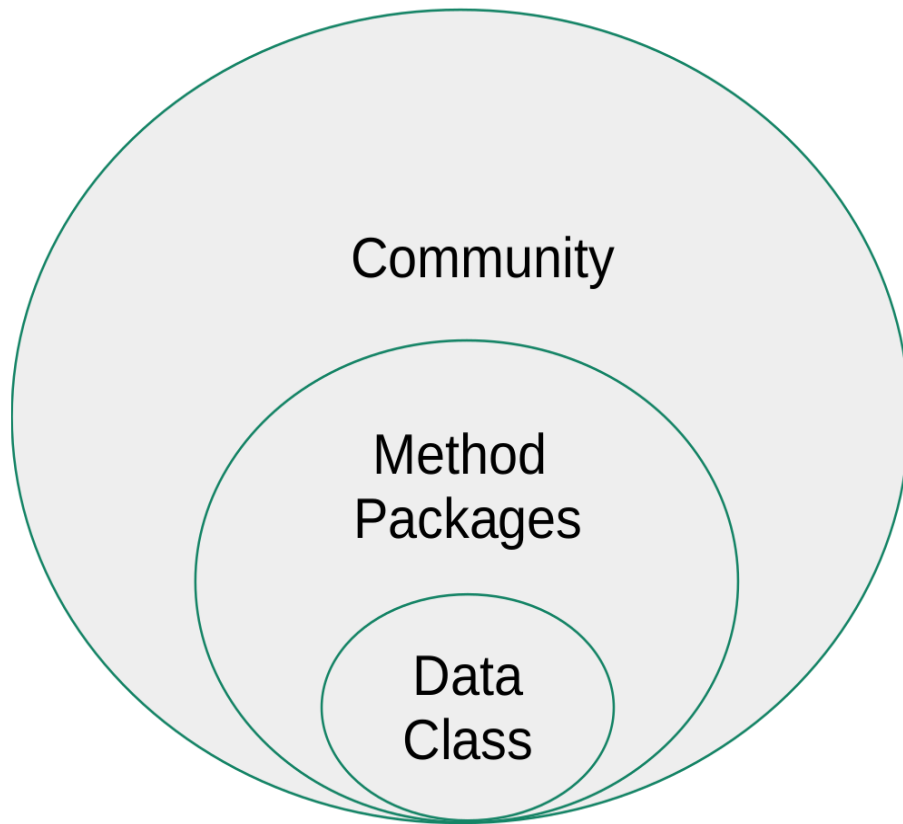


This article is included in the **Bioconductor** gateway.



popular data containers
support collaborative research
and methods development

Reduce overlapping efforts, improve interoperability, ensure sustainability.



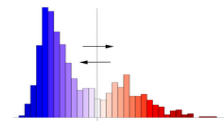
Data packages

ExperimentHub

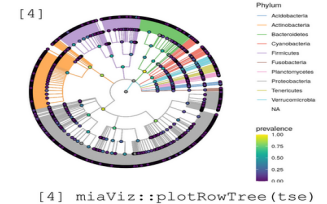
platforms all rank 76 / 1974 posts 2 / 1 / 2e+01 / 1 in Bioc 4 years
build ok updated before release dependencies 72

DOI: [10.18129/B9.bioc.ExperimentHub](https://doi.org/10.18129/B9.bioc.ExperimentHub) [f](#) [t](#)

**mia –
microbiome analysis**
getDiversity(x)
calculateDMM(x)



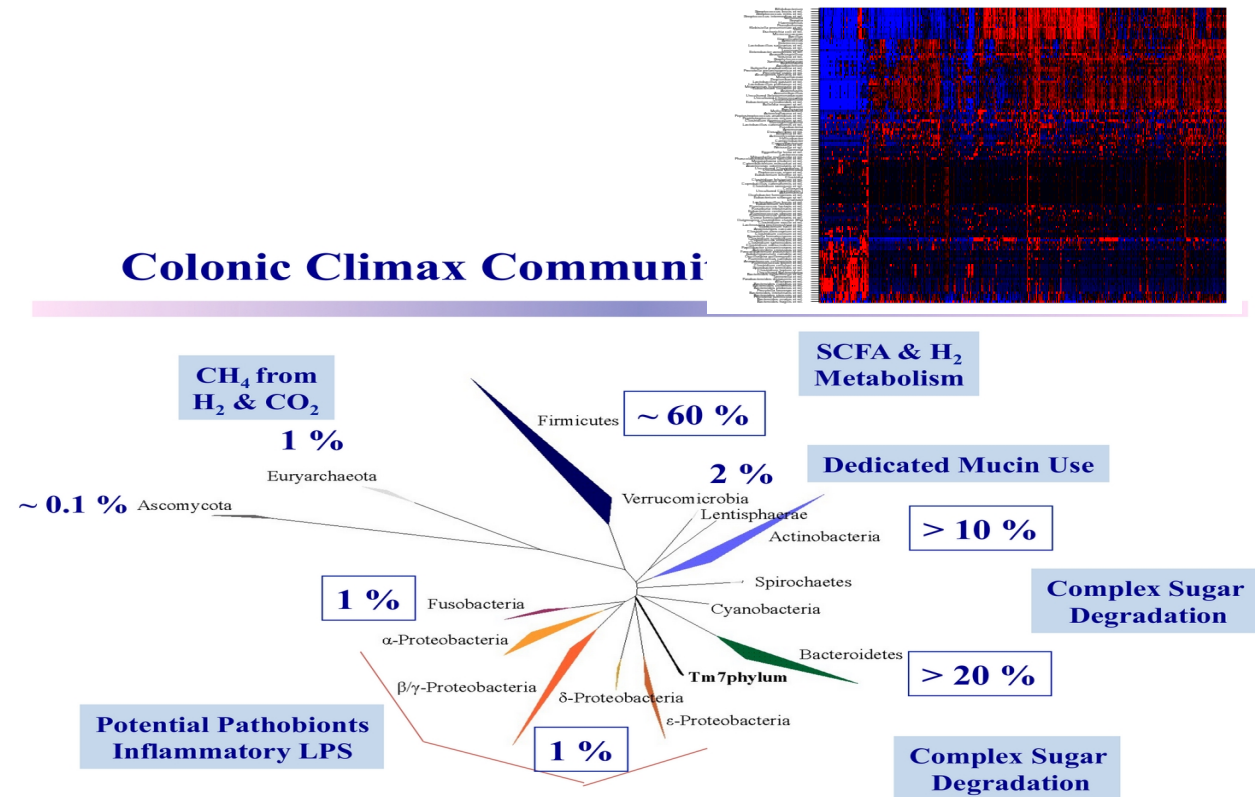
**miaViz -
Visualization**



Package ecosystem

Special properties of microbiome data

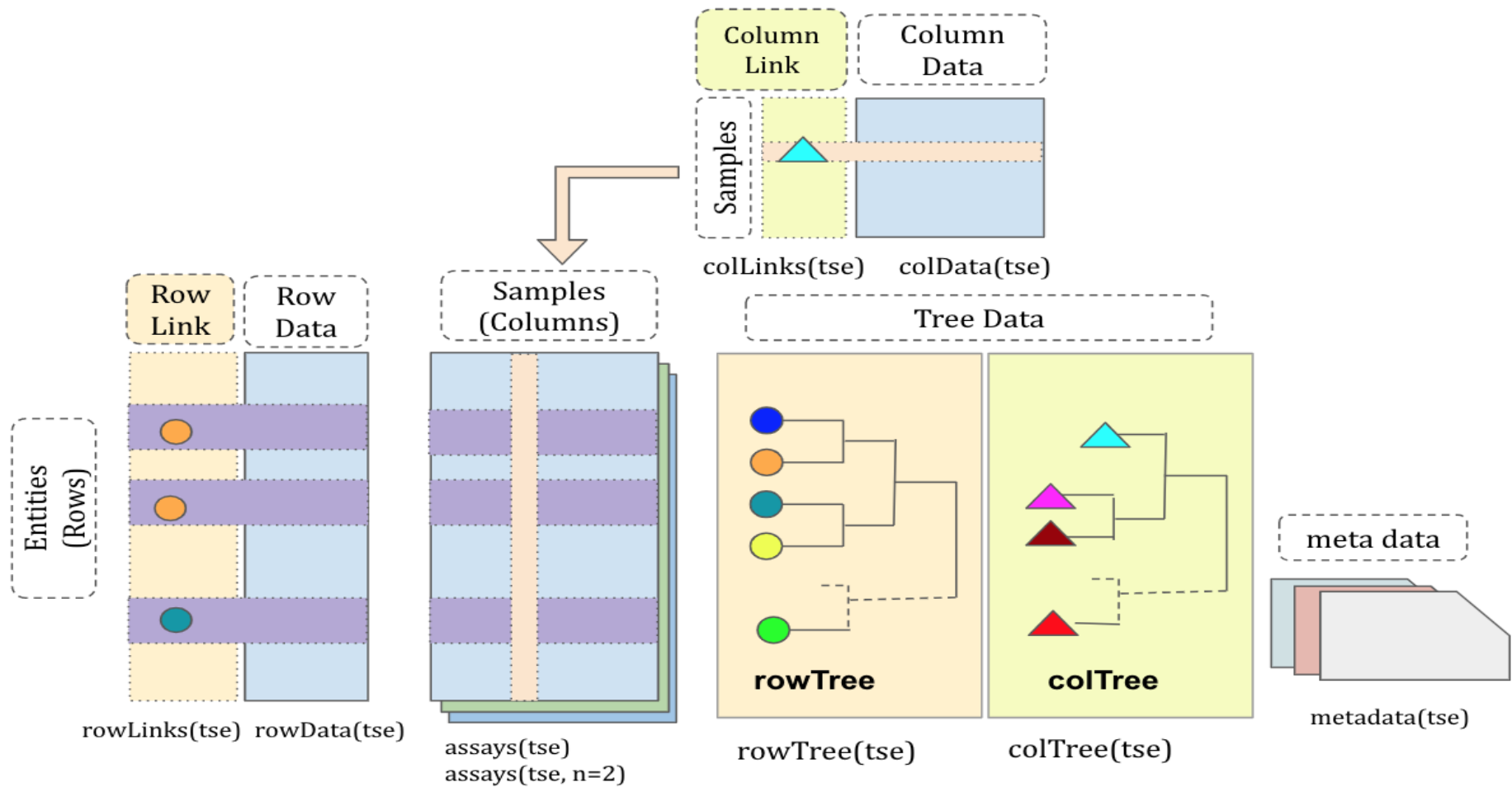
- Sparse
- Compositional
- Non-Gaussian
- Overdispersed
- Discrete
- Complex
- Stochastic
- Multi-level



Zoetendal EG, EE Vaughan & WM de Vos (2006) Mol Microbiol 59: 1639

Lay C, L Rigottier-Gois, K Holmstrom, M Rajilic, EE Vaughan, WM de Vos, MD Collins, R Their, P Namsolleck, M Blaut & J Dore (2005) AEM 71: 4153

Anatomy of TreeSummarizedExperiment



Multitable Methods for Microbiome Data Integration

 Kris Sankaran^{1*} and  Susan P. Holmes²

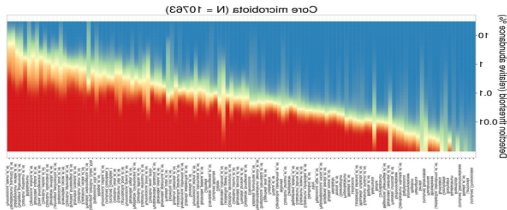
Property	Algorithms	Consequence
Analytical solution	Concat. PCA, CCA, CoIA, MFA, PTA, Statico/Costatis	Methods with analytical solutions generally run much faster than those that require iterative updates, optimization, or Monte Carlo sampling. They tend to be restricted to more classical settings, however.
Require covariance estimate	Concat. PCA, CCA, CoIA, MFA, PTA, Statico/Costatis	Methods that require estimates of covariance matrices cannot be applied to data with more variables than samples, and become unstable in high-dimensional settings.
Sparsity	SPLS, Graph-Fused Lasso, Graph-Fused Lasso	Encouraging sparsity on scores or loadings can result in more interpretable, results for high-dimensional data sets. These methods provide automatic variable selection in the multitable analysis problem.
Tuning parameters	<i>Sparsity</i> : Graph-Fused Lasso, PMD, SPLS <i>Number of Factors</i> : PCA-IV, Red. Rank Regression, Mixed-Membership CCA Prior <i>Parameters</i> : Mixed- Membership CCA, Bayesian Multitask Regression	Methods with many tuning parameters are often more expressive than those without any, since it makes it possible to adapt to different degrees of model complexity. However, in the absence of automatic tuning strategies, these methods are typically more difficult to use effectively.
Probabilistic	Mixed-Membership CCA, Bayesian Multitask Regression	Probabilistic techniques provide estimates of uncertainty, along with representations of cross-table covariation. This comes at the cost of more involved computation and difficulty in assessing convergence.
Not Normal or Nonlinear	CCpNA, Mixed-Membership CCA, Bayesian Multitask Regression	When data are not normal (and are difficult to transform to normality) or there are sources of nonlinear covariation across tables, it can be beneficial to directly model this structure.
>2 Tables	Concat. PCA, CCA, MFA, PMD	Methods that allow more than two tables are applicable in a wider range of multitable problems. Note that these are a subset of the cross-table symmetric methods.
Cross-Table Symmetry	Concat. PCA, CCA, CoIA, Statico/Costatis, MFA, PMD	Cross-table symmetry refers to the idea that some methods don't need a supervised or multitask setup, where one table contains response variable and the other requires predictors. The results of these methods do not change when the two tables are swapped in the method input.

microbiome R package

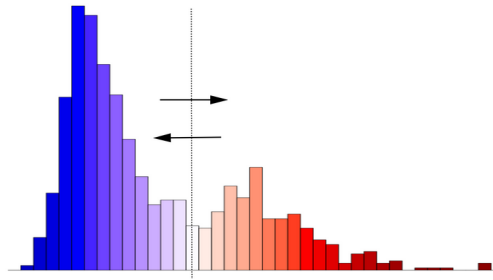
chat on gitter build passing codecov 24% PRs welcome

Core & prevalence

prevalence(x)
core(x)
core_members(x)

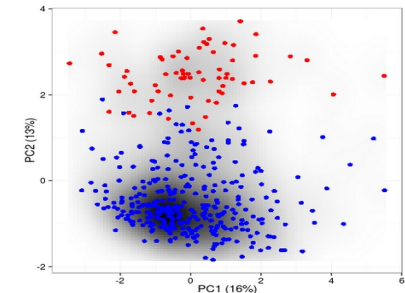


Stability & resilience



Alpha & beta diversity

alpha(x)
diversity(x)
evenness(x)
dominance(x)
rarity(x)
readcount(x)



Transformations

transform(x, "compositional")
transform(x, "clr")
transform(x, "log10p")
transform(x, "hellinger")
transform(x, "identity")

Community

- Online tutorials
- Mailing list
- Gitter chat
- Example data
- Workshops


Quality control


- continuous integration
- unit tests

microbiome.github.io

1. Ampvis2 [Tools for visualising amplicon sequencing data](#)
2. CCREPE [Compositionality Corrected by PErmutation and RENormalization](#)
3. DADA2 [Divisive Amplicon Denoising Algorithm](#)
4. DESeq2 [Differential expression analysis for sequence count data](#)
5. edgeR [empirical analysis of DGE in R](#)
6. mare [Microbiota Analysis in R Easily](#)
7. Metacoder [An R package for visualization and manipulation of community taxonomic diversity data](#)
8. metagenomeSeq [Differential abundance analysis for microbial marker-gene surveys](#)
9. microbiome R package [Tools for microbiome analysis in R](#)
10. MINT [Multivariate INTEgrative method](#)
11. mixDIABLO [Data Integration Analysis for Biomarker discovery using Latent variable approaches for 'Omics studies](#)
12. mixMC [Multivariate Statistical Framework to Gain Insight into Microbial Communities](#)
13. MMinte [Methodology for the large-scale assessment of microbial metabolic interactions \(MMinte\) from 16S rDNA data](#)
14. pathostat [Statistical Microbiome Analysis on metagenomics results from sequencing data samples](#)
15. phylofactor [Phylogenetic factorization of compositional data](#)
16. phylogeo [Geographic analysis and visualization of microbiome data](#)
17. Phyloseq [Import, share, and analyze microbiome census data using R](#)
18. qilmer [R tools compliment qtime](#)
19. RAM [R for Amplicon-Sequencing-Based Microbial-Ecology](#)
20. ShinyPhyloseq [Web-tool with user interface for Phyloseq](#)
21. SigTree [Identify and Visualize Significantly Responsive Branches in a Phylogenetic Tree](#)
22. SPIEC-EASI [Sparse and Compositionally Robust Inference of Microbial Ecological Networks](#)
23. structSSI [Simultaneous and Selective Inference for Grouped or Hierarchically Structured Data](#)
24. Tax4Fun [Predicting functional profiles from metagenomic 16S rRNA gene data](#)
25. taxize [Taxonomic Information from Around the Web](#)
26. labdsv [Ordination and Multivariate Analysis for Ecology](#)
27. Vegan [R package for community ecologists](#)
28. igraph [Network Analysis and Visualization in R](#)
29. MicrobiomeHD [A standardized database of human gut microbiome studies in health and disease *Case-Control*](#)
30. Rhea [A pipeline with modular R scripts](#)
31. microbiomeutilities [Extending and supporting package based on microbiome and phyloseq R package](#)
32. breakaway [Species Richness Estimation and Modeling](#)

[A survey for 16S](https://github.com/microsud/Tools-Microbiome-Analysis) [Github.com/microsud/](https://github.com/microsud/Tools-Microbiome-Analysis) [Tools-Microbiome-Analysis](https://github.com/microsud/Tools-Microbiome-Analysis)



Springer Link



[Journal of Biosciences](#)
 October 2019, 44:115 | [Cite as](#)

Microbiome data science

Authors
Authors and affiliations

Sudarshan A Shetty, Leo Lahti 



HUMAN GUT MICROBIOME ATLAS

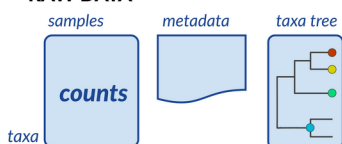
Open access resource for human microbiome. SciLifeLab, King's College London, INRAE

Workflow approach: knitting together open data, methods & application

Import Data

This workflow starts with either raw data directly from relative abundance estimation or taxonomic classification OR pre-existing data objects from widely used software.

RAW DATA

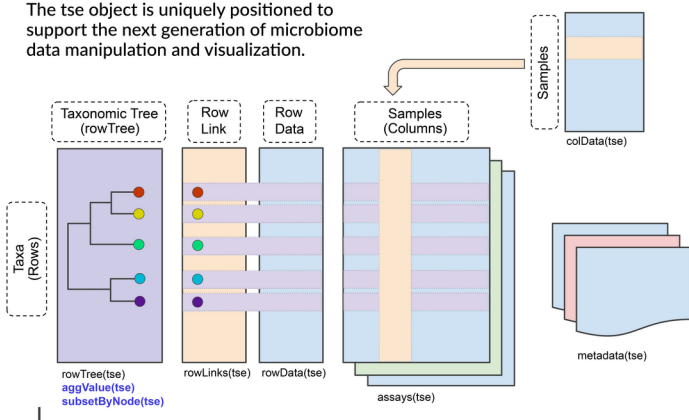


EXISTING DATA



The TreeSE object

The tse object is uniquely positioned to support the next generation of microbiome data manipulation and visualization.

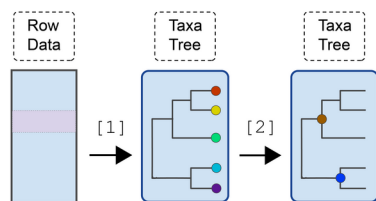


Check the poster
F1000 / EuroBioC!



The mia Pipeline

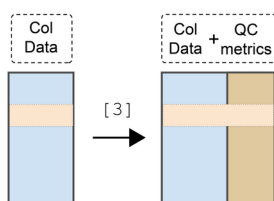
Accessing Taxonomic Info.



```
[1] mia::addTaxonomyTree(tse)
```

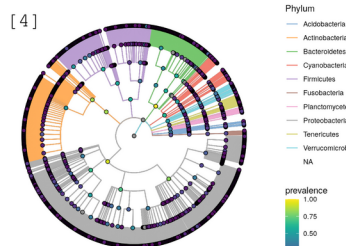
```
[2] TreeSE::aggValue(tse)
```

Quality Control



```
[3] scatter::addPerCellQC(tse)
```

Visualizing with miaViz



```
[4] miaViz::plotRowTree(tse)
```

- comprehensive
- extendable
- reproducible
- collaborative
- transparent

elements of computational workflows

(transparent?) data

(open) algorithms

(reproducible) reporting

A Quick Guide to Software Licensing for the Scientist-Programmer

Andrew Morin, Jennifer Urban, Piotr Sliz

Published: July 26, 2012 • <https://doi.org/10.1371/journal.pcbi.1002598>

Software citation principles

Arfon M. Smith^{1,*}, Daniel S. Katz^{2,*}, Kyle E. Niemeyer^{3,*}
FORCE11 Software Citation Working Group

¹ GitHub, Inc., San Francisco, California, United States

² National Center for Supercomputing Applications & Electrical and Computer Department & School of Information Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, United States

³ School of Mechanical, Industrial, and Manufacturing Engineering, Oregon State University, Corvallis, Oregon, United States

* These authors contributed equally to this work.



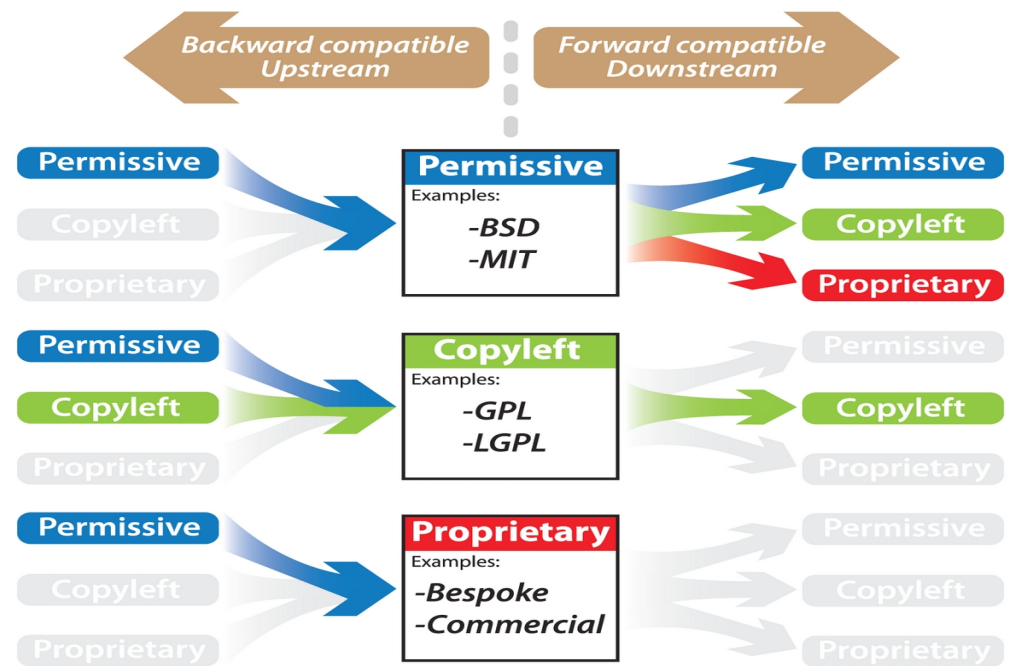
MIT License

Copyright (c) <year> <copyright holders>

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Orchestrating Microbiome Analysis with R/Bioc

microbiome.github.io

Preface

I Introduction

1 Data Infrastructure

1.1 Installation

1.2 Background

1.3 Loading experimental microbi...

1.4 Metadata

1.5 Microbiome and tree data speci...

1.6 Data conversion

1.7 Conclusion

Session Info

Orchestrating Microbiome Analysis

Authors: Leo Lahti [aut], Sudarshan Shetty [aut], Felix GM Ernst [aut, cre]

Version: 0.98.9

Modified: 2021-04-10

Compiled: 2021-07-29

Environment: R version 4.1.0 (2021-05-18), Bioconductor 3.14

License: CC BY-NC-SA 3.0 US

Copyright:

Source: <https://github.com/microbiome/OMA>

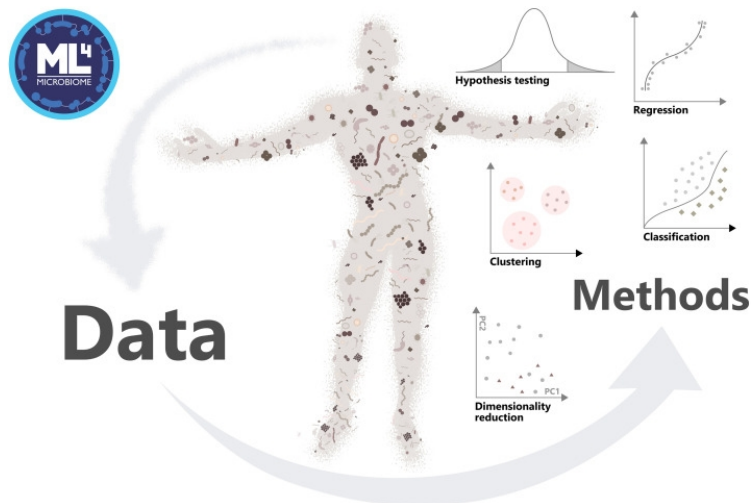


Figure source: Moreno-Indias et al. (2021) Statistical and Machine Learning Techniques in Human Microbiome Studies: Contemporary Challenges and Solutions. *Frontiers in Microbiology* 12:11.

Training events in microbiome data science

ML4Microbiome Symposium: Grand Challenges of Data-Intensive Science in microbiome data analysis and training (Oct 14)

ML4Microbiome Training School, Sep/Oct 2021

Workshop on modeling microbial community time series. Leuven, **Belgium**, November, 2021

Brain, Bacteria and Behaviour: Understanding the Gut-Brain Axis online summer school, The **Netherlands**, July 2021

NORBIS Summer School; National research school in bioinformatics, biostatistics and systems biology, **Norway**, Aug 2021

Microbiome Data Analysis Workshop - Hasselt University, Limburg, **Belgium**, Apr 2021

Techniques for skin microbiome research - Savitribai Phule Pune University, Pune, **India**, Jan 2021

Modern statistics for microbiome bioinformatics - Pune, **India**, Dec 2019

Intestinal microbiome of humans and animals. Wageningen University and Research Center, The **Netherlands**, Oct 2019

Microbiome data science. **Singapore** Centre for Environmental and Life Science Engineering Sep, 2019

Statistical techniques in microbiome bioinformatics - Sep 2019 Radboud University Nijmegen, The **Netherlands**

International summer school on microbial community modeling - Sep 2019 KU Leuven, **Belgium**

International spring school on open microbiome data analysis - 2018 Wageningen, The **Netherlands**

International summer school on microbial network analysis - 2017 KU Leuven, **Belgium**

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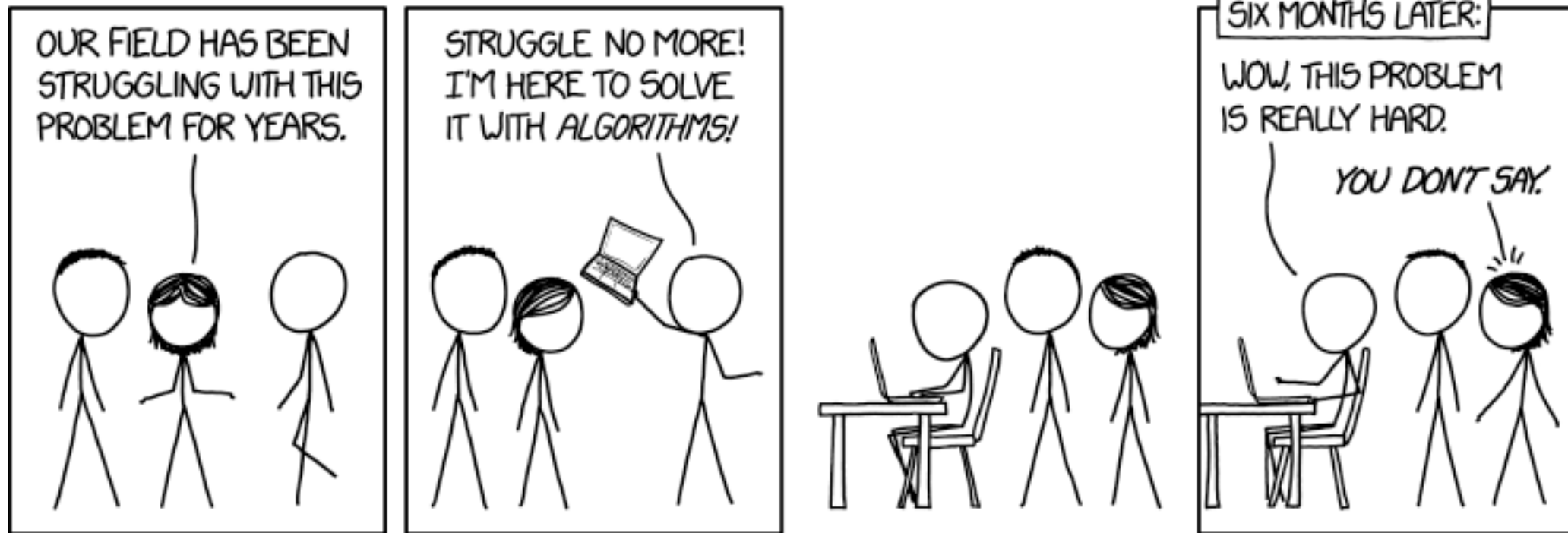


Statistical and Machine Learning Techniques in Human Microbiome Studies: Contemporary Challenges and Solutions



MICROBIOME

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Open workflows

transparency & reproducibility

collaborative research & training

quality & efficiency



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