Core Bioinformatics
Workshops 2024

Metagenomic profiling with BioBakery

MetaPhlAn and HuMANN

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Session overview

- Introduction
- Biobakery Tools
- Taxonomic profiling using MetaPhlAn 4
- Functional Profiling using HuMANN3

- Questions!

Alise Ponsero

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Introduction to short reads profiling

github.com/quadram-institute-bioscience/biobakery-2024

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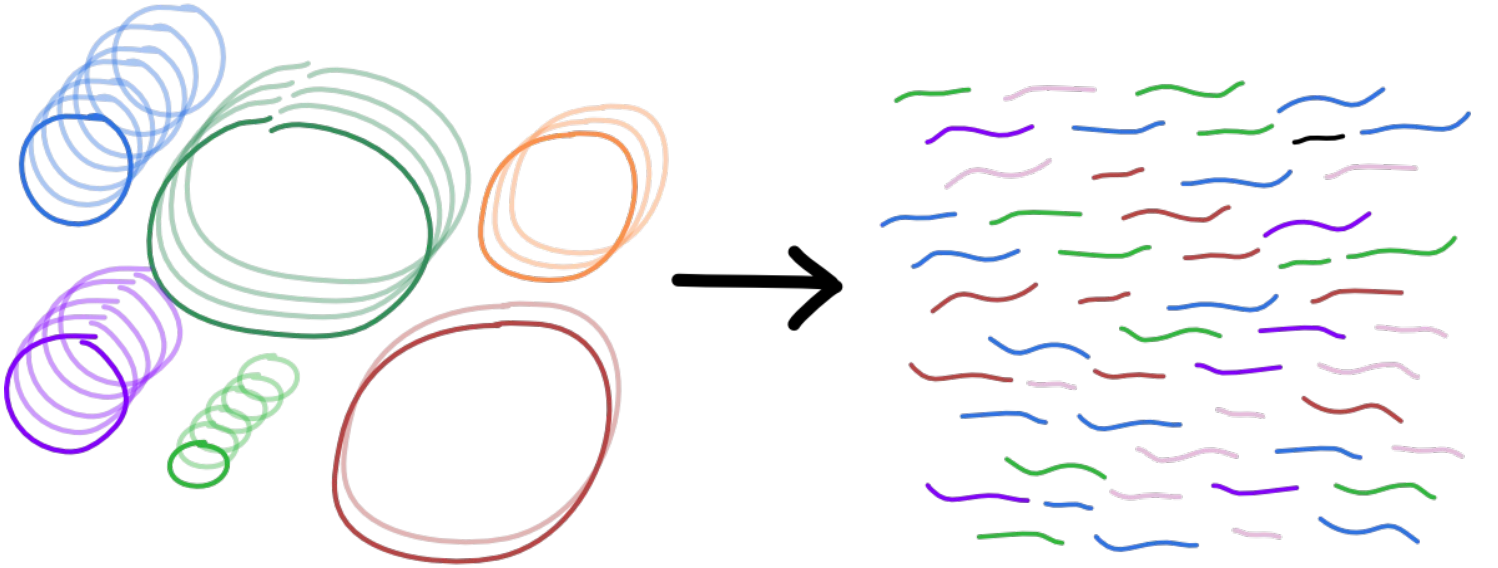

A "Metagenome"

Whole Metagenome Shotgun (WMS)

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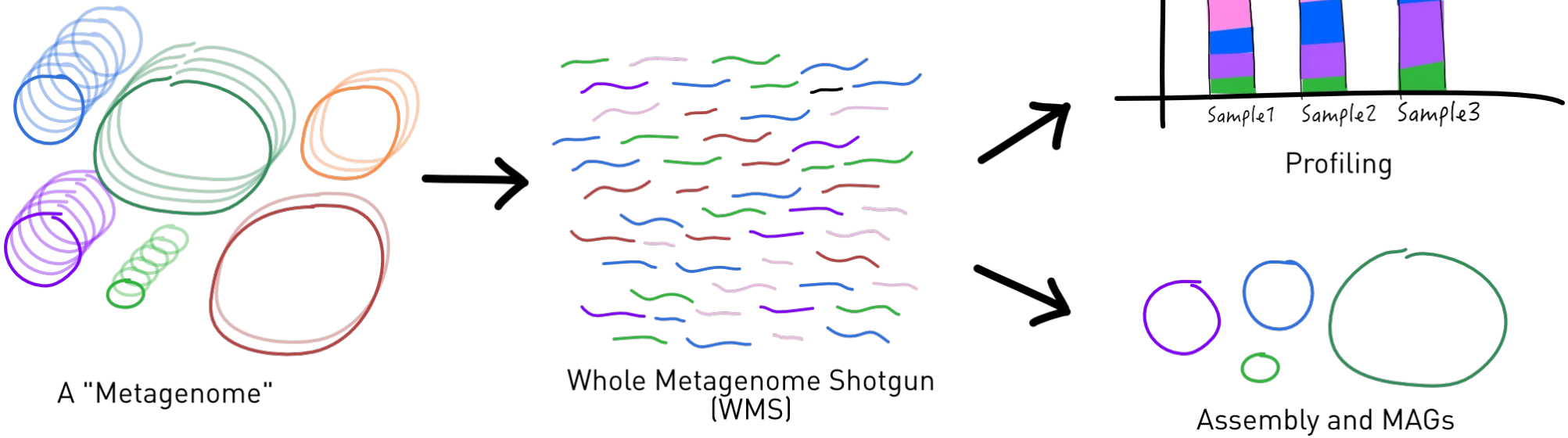

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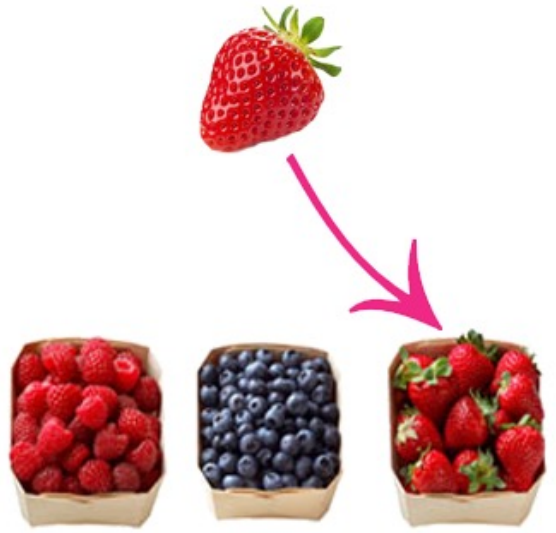
Classifying reads

- Mapping against known genomes?

Alternatives

- DNA *k*-mer based approaches (notably: **Kraken2**)
- Marker based approaches (notably: **MetaPhlAn**)
- Protein sequence alignment (e.g.: Kaiju)
- Pseudomapping (e.g. KMCP)



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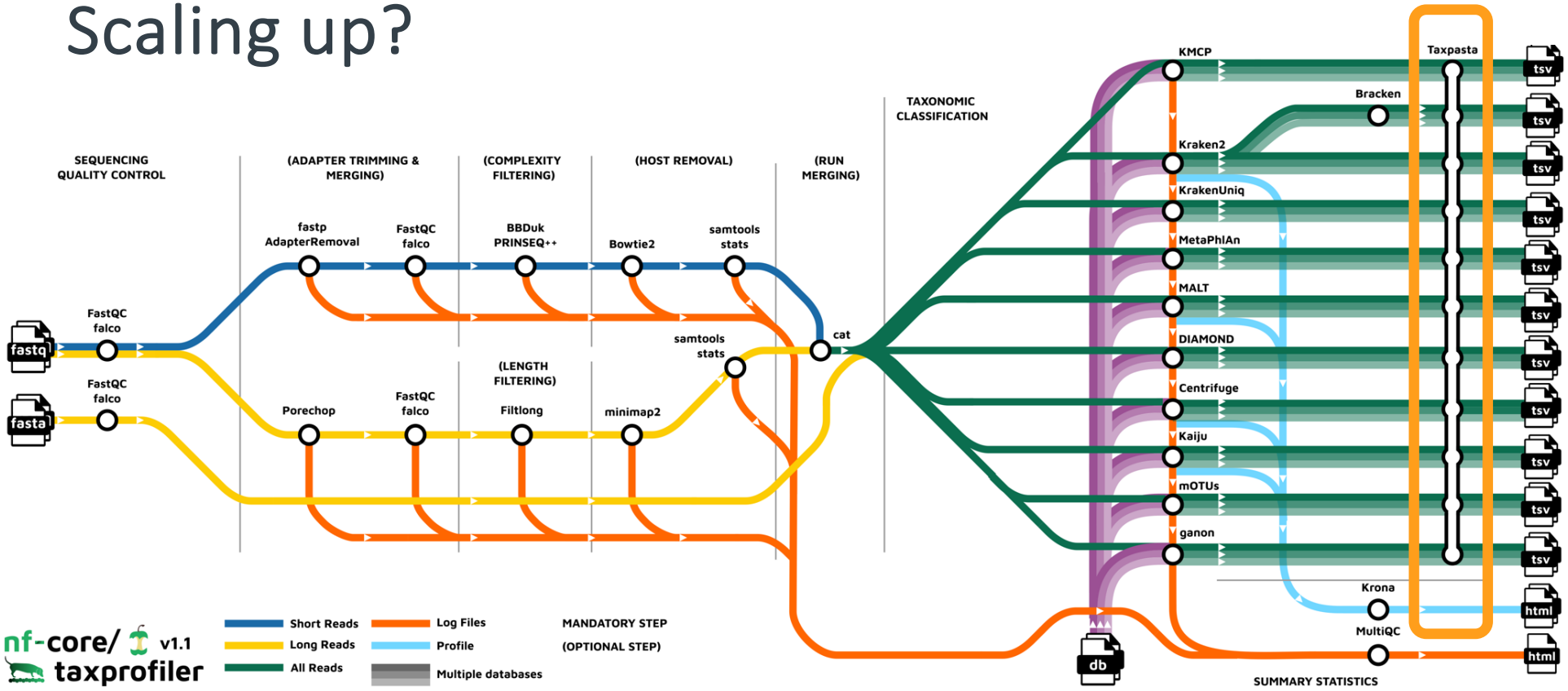
Scaling up?



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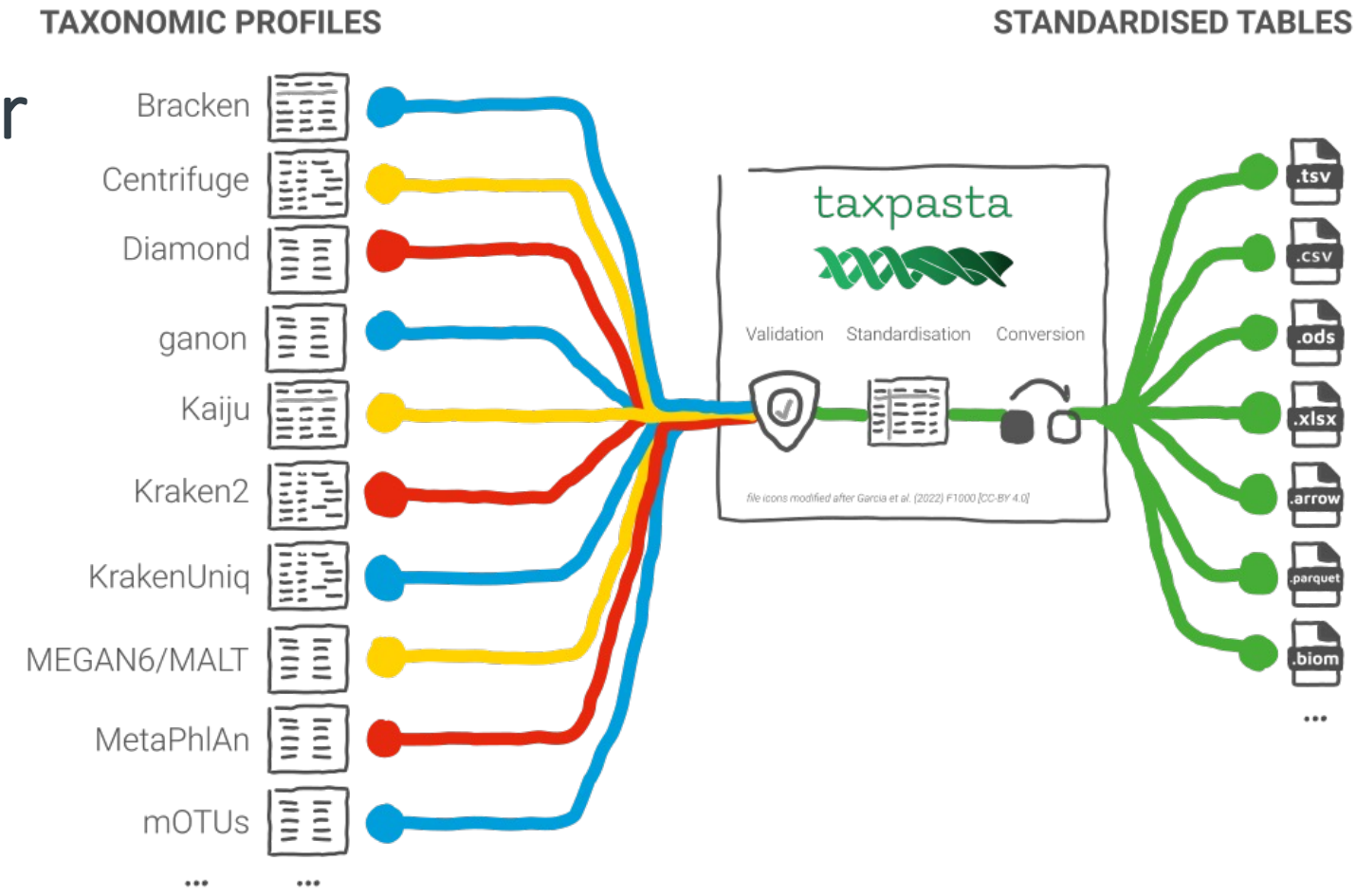
Taxprofiler USP



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What is the Biobakery?

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The Biobakery

The bioBakery tools methods for microbial community profiling developed by the Huttenhower lab.

- Most tools are supported both as individual software packages or as workflows

- The tools cover methods for microbial community profiling but also downstream analysis and statistical methods

- The community is supported by a forum where users can ask questions or requests

The Biobakery

Microbial community profiling tools

Note: not all tools of the Biobakery can work together...

They are mostly meant to be run independent of each other!

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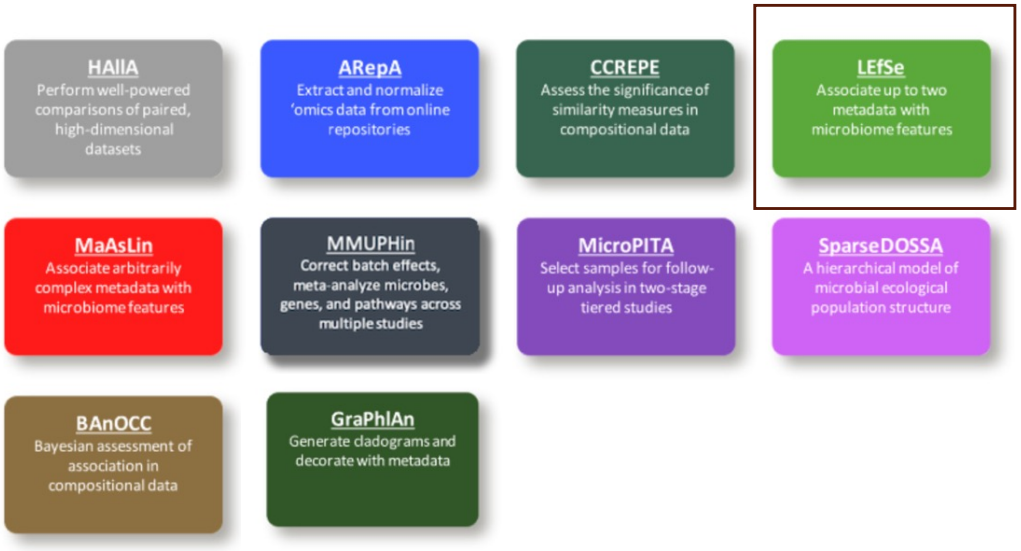
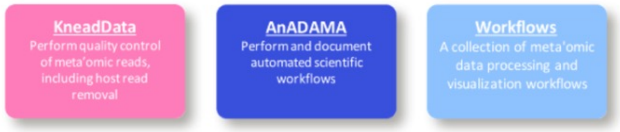
The Biobakery

Visualization and statistical analysis tools



Utilities



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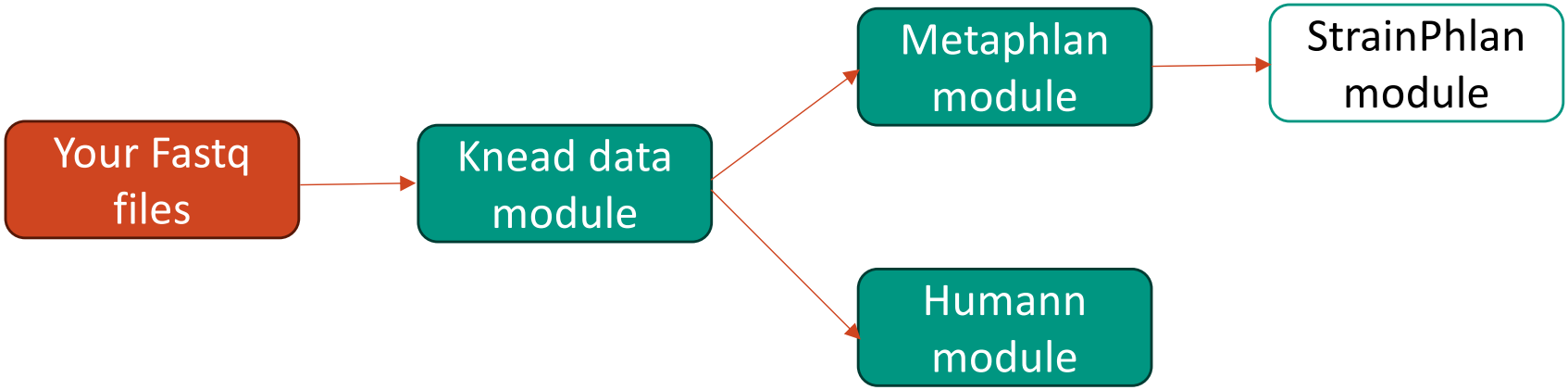
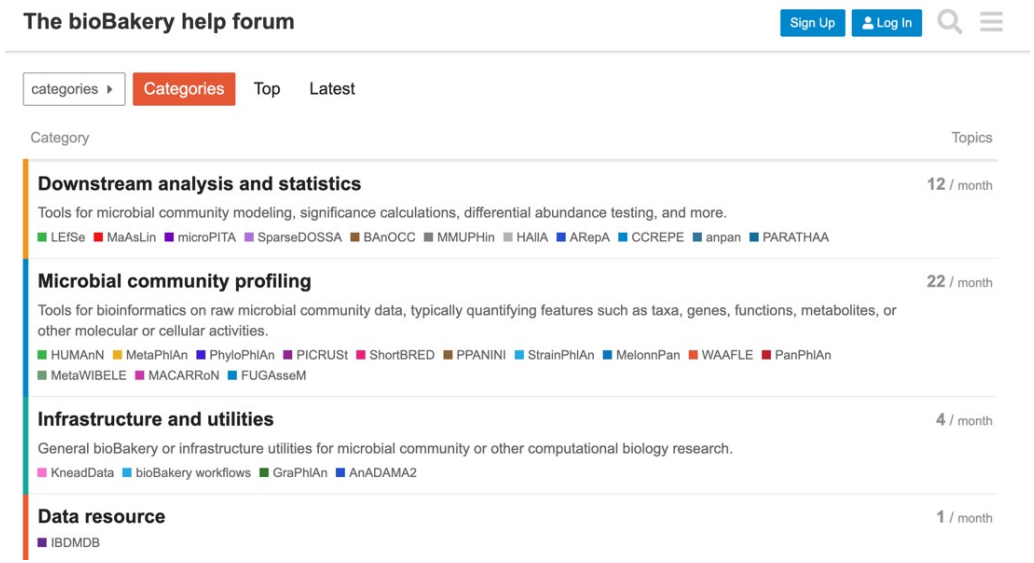
The Biobakery workflows

Several workflows are proposed to link the main Biobakery tools together:



The Biobakery help forum



Bug, requests, and suggestions are handled in the Biobakery help forum (not through Github)

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Taxonomic profiling with Metaphlan4

concepts and quirks

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Taxonomic profiler or classifier?

Taxonomic Classifiers (Kraken2):

- Aim to classify each sequencing reads independently from each other
- Do not account for genome size
- Do not aim to give you the relative abundance of taxa in the community

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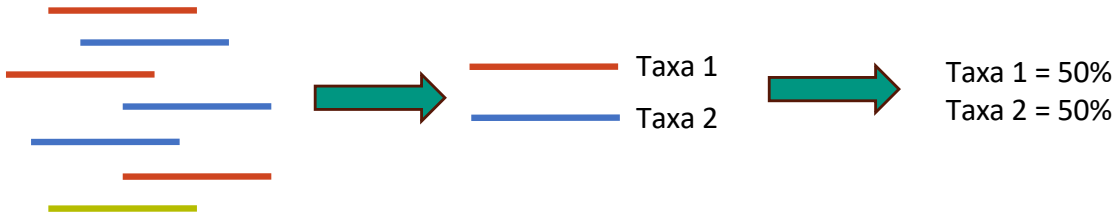
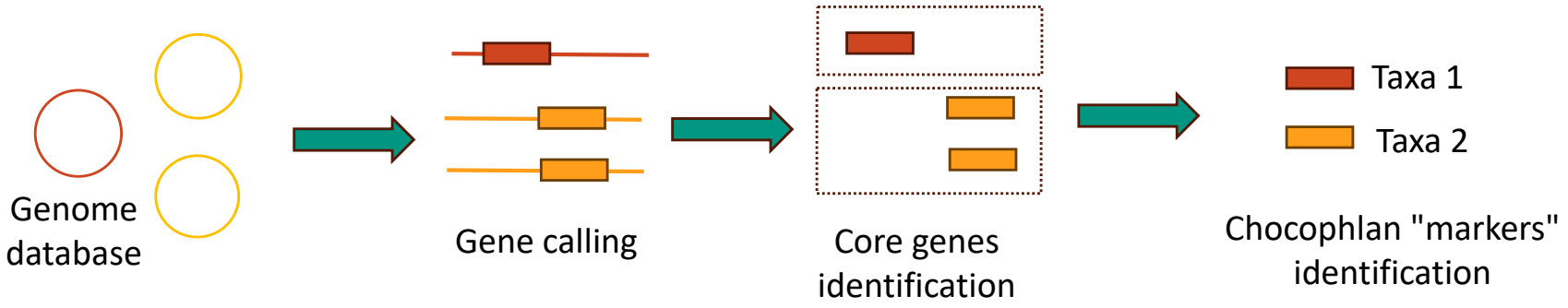
Taxonomic profiler or classifier?

Taxonomic profiler (MetaPhlan):

- Takes into account genome size
- Aims to give you the relative abundance of the taxa in the community
- Can overlook the unclassified/unknowns in the community



Chocophlan3 database



Chocophlan is a pangenome database used in Metaphlan and Humann

--> Most of the Chocophlan databases do not include viral/eukaryotic sequences

--> Does not include a human reference

Chocophlan3 database

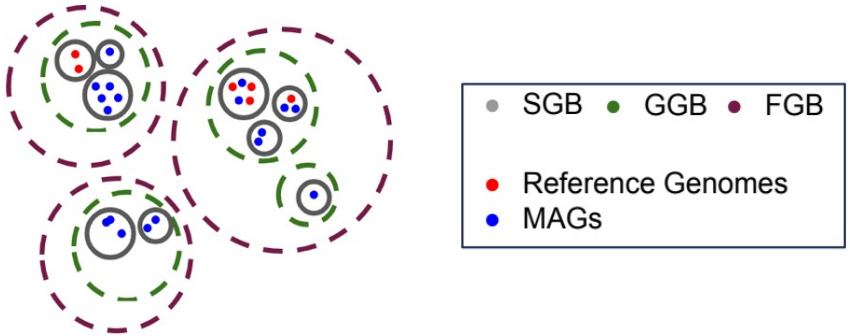
Chocophlan3 database

Chocophlan1 = 400,141 species markers from 2,834 NCBI/IMG genomes

Chocophlan3 = ~7.3M unique clade-specific marker genes
from 36,822 species-level genome bins (SGB)
(11,062 of them taxonomically unidentified at the species level)

Cell
Volume 176, Issue 3, 24 January 2019, Pages 649-662.e20

Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle



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Estimation of the unknown in Metaphlan4

$$\%\text{uncl.reads} = \frac{\text{Total reads} - \left(\sum_{sp=0}^{n} \left(\text{avg nonzero markers coverage}_{sp} \times \text{avg genome length}_{sp}\right)\right) / \text{avg read length}}{\text{Total reads}}$$

$$sp = \text{indices of all the SGBs reported in the MetaPhlAn profile}$$

MetaPhlAn 4 includes a feature for estimating the fraction of input reads that **cannot be assigned** to taxa in the database.

Calculated by subtracting from the total number of input reads the average read depth of each reported SGB normalized by its SGB-specific average genome length

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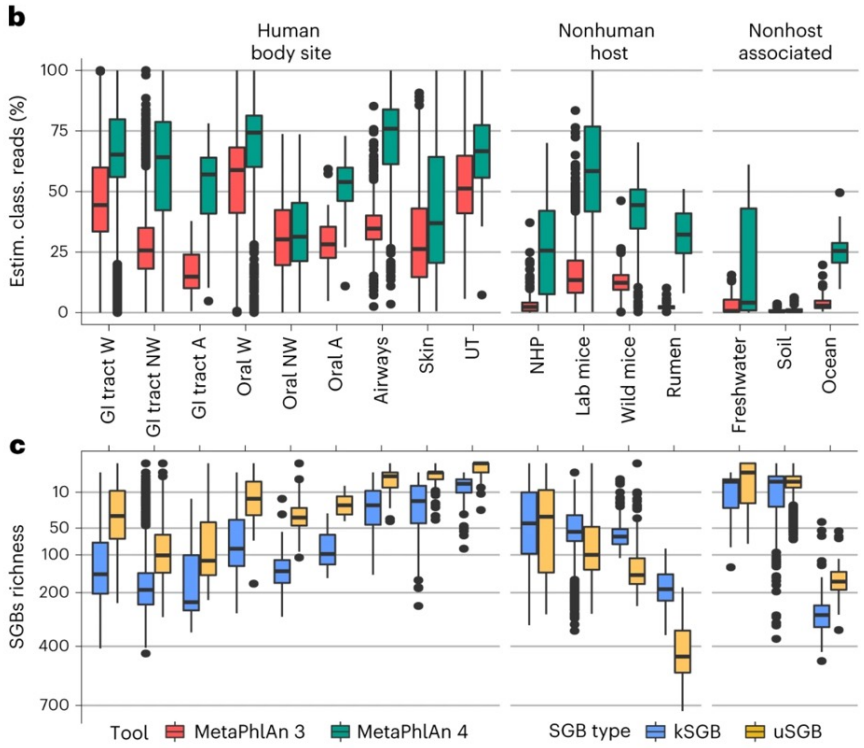
Metaphlan3 vs Metaphlan4



Metaphlan4 new database significantly improves the % of classified reads

in particular for human associated ecosystems

Blanco-Míguez, A., Beghini, F., Cumbo, F. *et al.* Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nat Biotechnol* **41**, 1633–1644 (2023). https://doi.org/10.1038/s41587-023-01688-w

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Conclusions & take-home messages

- Metaphlan4 is a metagenomic profiler
- Relies on the Chocophlan pangenome database for its predictions
- Chocophlan3 includes a large proportion of MAGs clustered *de-novo*
- Metaphlan4 is able to give an estimate of the proportion of unknowns in the metagenome (but not by default)

Functional profiling with HuMaNN3

concepts and quirks

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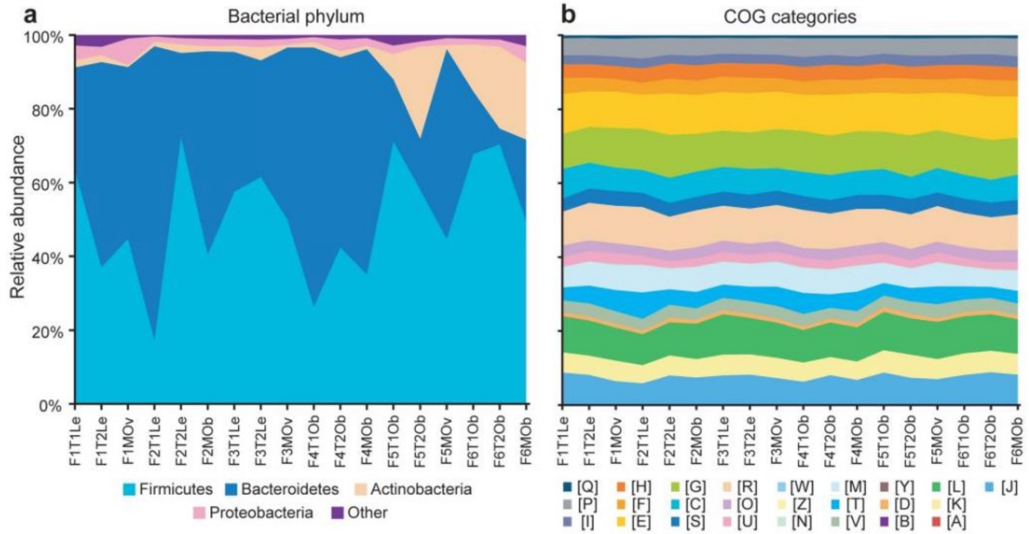
Functional profiling principles

While taxonomic composition and functional potential of the microbial communities are linked...



A change in the microbial taxonomic composition does not mean a change in the functional potential!

10.1038/nature07540

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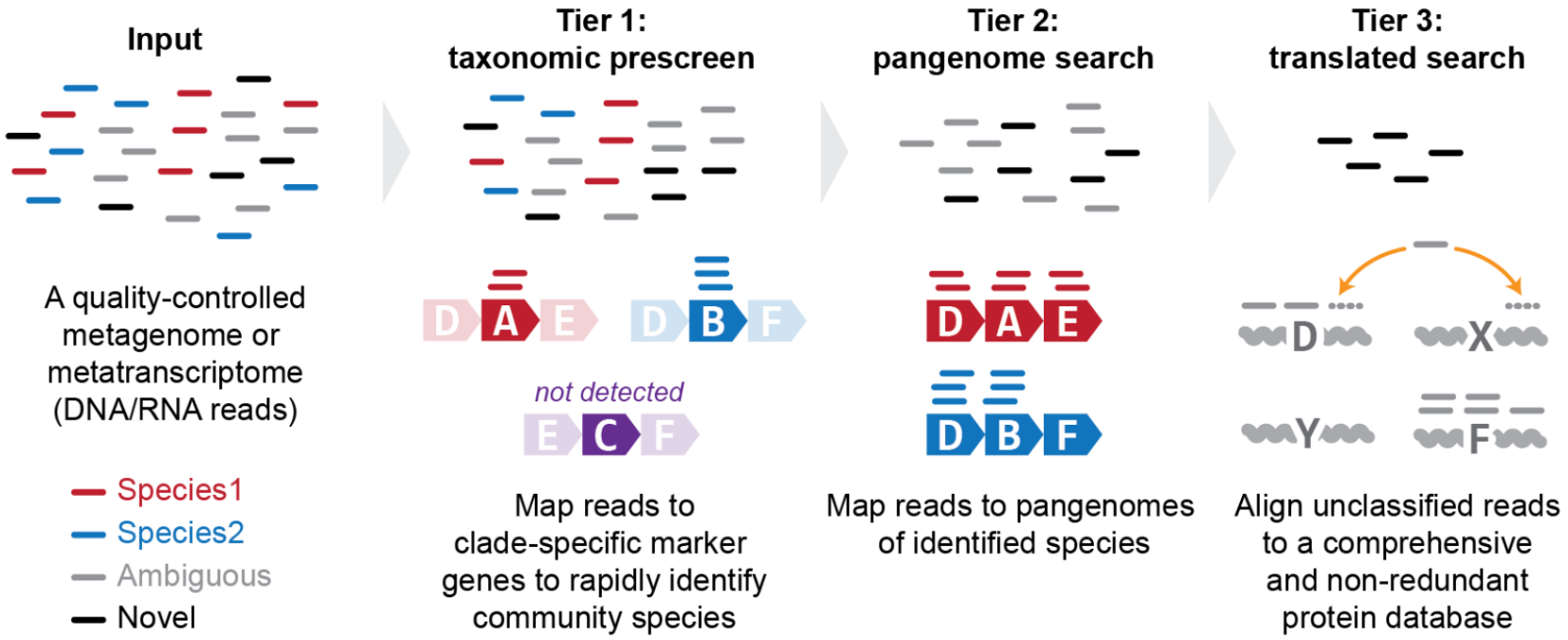
Humann3 tiered search approach



https://github.com/biobakery/biobakery/wiki/humann3

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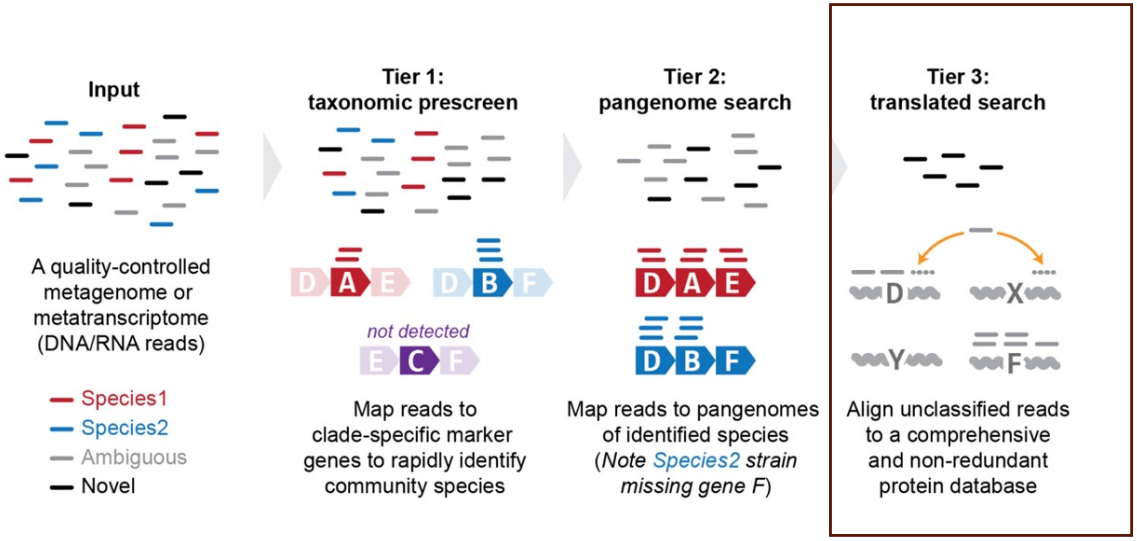
Using UniRef90 vs UniRef50



Humann3 uses UniRef protein clusters as a gene family system.

Clustering proteins from UniProt :

- Uniref90: clustering non-redundant proteins at 90% identity and selecting representative sequences
- UniRef50: clustering UniRef90 representative sequences at 50% identity to produce broader clusters.

The representative of a UniRef cluster is the best-annotated member of the cluster.

https://github.com/biobakery/biobakery/wiki/humann3

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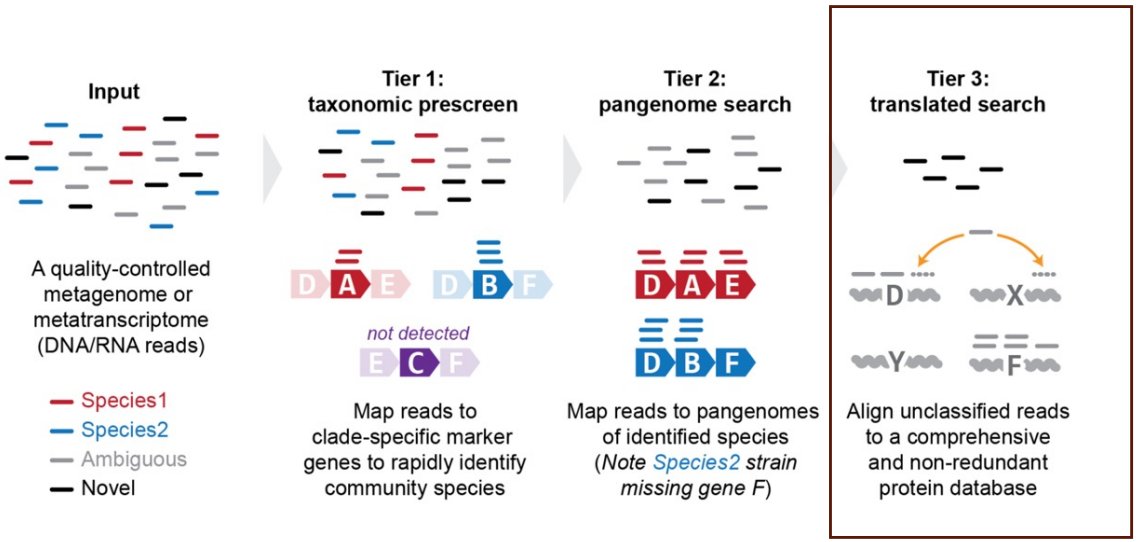
Using UniRef90 vs UniRef50



You can choose to use either UniRef90 or UniRef50 in Humann3:

- Default : UniRef90
- Poorly characterized environments: UniRef50

expected to explain a larger portion of sample read
but reduced functional resolution

https://github.com/biobakery/biobakery/wiki/humann3

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Humann3 gene and pathways abundance

Feature	RPK
GeneA	2
GeneA|Species1	2
GeneB	3
GeneB|Species2	3
∑ GeneD	8
GeneD|Species1	2
GeneD|Species2	3
GeneD|unclassified	3
GeneE	2
GeneE|Species1	2
GeneF	5
GeneF|unclassified	5

Process mapping results to estimate per-species and community total gene family abundance, weighting by 1) alignment quality, 2) gene length, and 3) gene coverage

RPK : Reads per kilobases

Normalize count per gene length

--> Utilities to convert the counts to relative abundances (RPKM)

RPKM : RPK per million reads

Normalize your RPK by the total number of reads in your sample

Note : in Humann3, RPKM is refered as "CPM"

https://github.com/biobakery/biobakery/wiki/humann3

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Humann3 gene and pathways abundance

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GeneE	2
GeneE|Species1	2
GeneF	5
GeneF|unclassified	5

Process mapping results to estimate per-species and community total gene family abundance, weighting by 1) alignment quality, 2) gene length, and 3) gene coverage

Mappings are available for both UniRef90 and UniRef50 gene families to :

- MetaCyc Reactions
- **KEGG Orthogroups (KOs)**
- Pfam domains
- Level-4 enzyme commission (EC) categories
- EggNOG (including COGs)
- Gene Ontology (GO)
- Informative GO

Importantly: some genes will not be mapped to the new gene families (and will be labeled as "unmapped")

https://github.com/biobakery/biobakery/wiki/humann3

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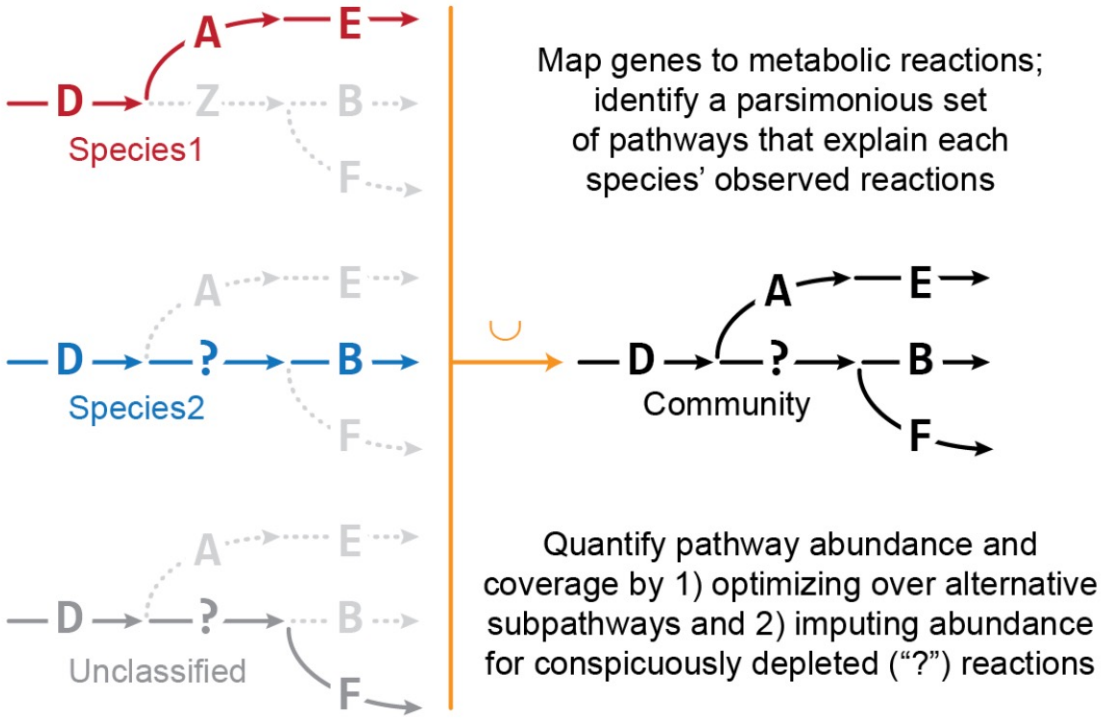
Humann3 gene and pathways abundance



HUMAnN3 uses MetaCyc pathway definitions and MinPath by default.

--> The user can provide a custom pathways database

https://github.com/biobakery/biobakery/wiki/humann3

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Humann3 quirks

- Humann3 (just like Metaphlan4) does not account for the information of paired-end reads:
    - The authors suggest to concatenate your two files
    - In my opinion, this inflates the computational runtime and I suggest using only the forward pair

- Human genome decontamination is important to do before running Humann3

- Can be used on meta-transcriptomes

https://github.com/biobakery/biobakery/wiki/humann3

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Thank you

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