

Microbial communities through the lens of high throughput sequencing, data integration and metabolic networks analysis

developing computational approaches to better understand microbial assemblages

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PhD candidate



1. Microbial ecology from a computational point-of-view
2. PEMA: a metabarcoding pipeline
3. PREG0: a knowledge-base for organisms - environments - processes associations
4. dingo: a Python library for metabolic flux sampling
5. Take home messages & future work

Microbial ecology & biogeochemical cycles

a corner-stone for life on earth

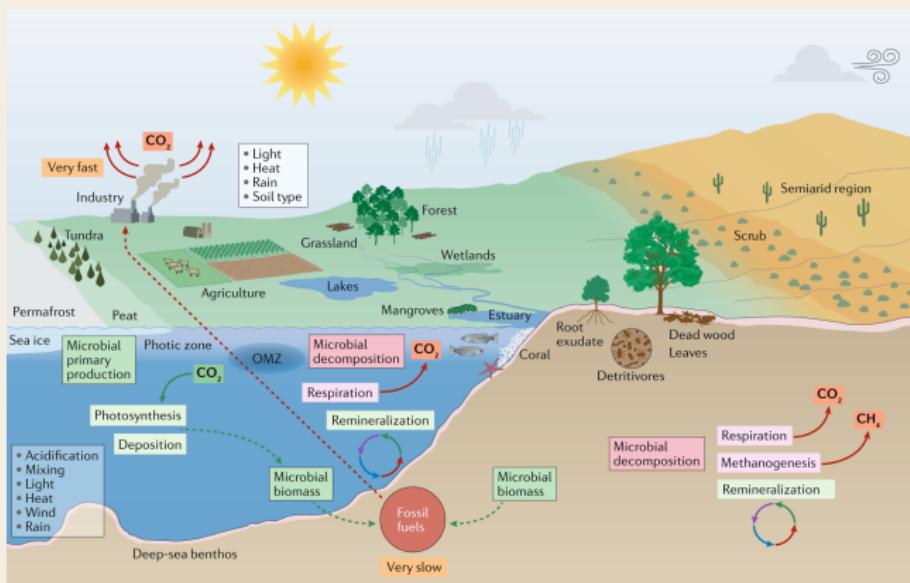


Figure from: Cavicchioli et al. Nature Reviews Microbiology 17.9 (2019): 569-586.

Questions to address

for a deeper understanding of microbial assemblages

Community
structure
who

Functional
potential
what

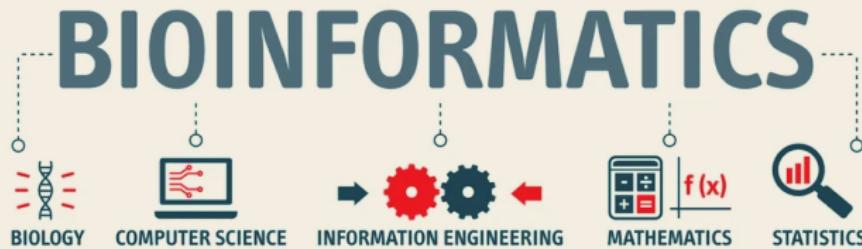
Microbial
interactions
why / how

everyone is everywhere

zero-sum game

the entangled bank

We are living in a computational era
both a challenge & an opportunity



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PEMA

a pipeline for eDNA metabarcoding analysis

<https://github.com/hariszaf/pema>

pema.hcmr.gr

eDNA metabarcoding for biodiversity assessment

Marker genes

1. **16S rRNA:** Bacteria, Archaea
2. **12S rRNA:** Vertebrates
3. **18S rRNA:** Small eukaryotes, Metazoa
4. **ITS:** Fungi
5. **COI:** Eukaryotes
6. ***rbcl:*** Plants
7. ***dsrb:*** Bacteria, Archaea
8. ...

Methodology

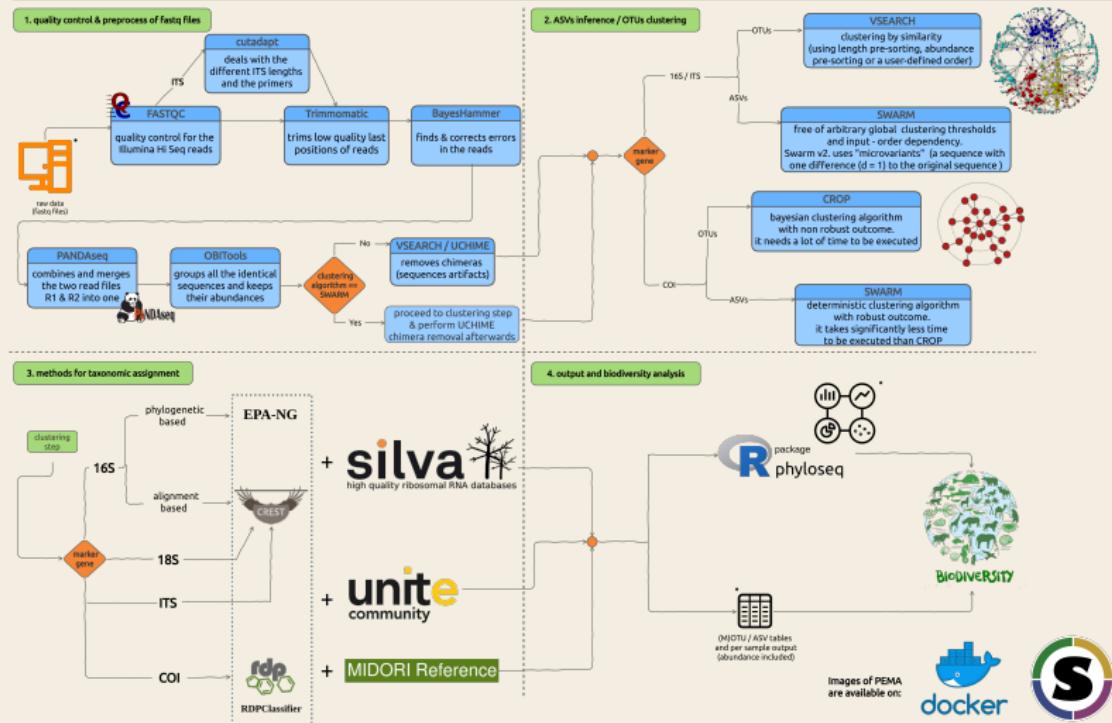


- Sampling
- Extraction
- Bioinformatics
- Biodiversity analysis

PEMA features

one step at a time!

PEMA in a nutshell



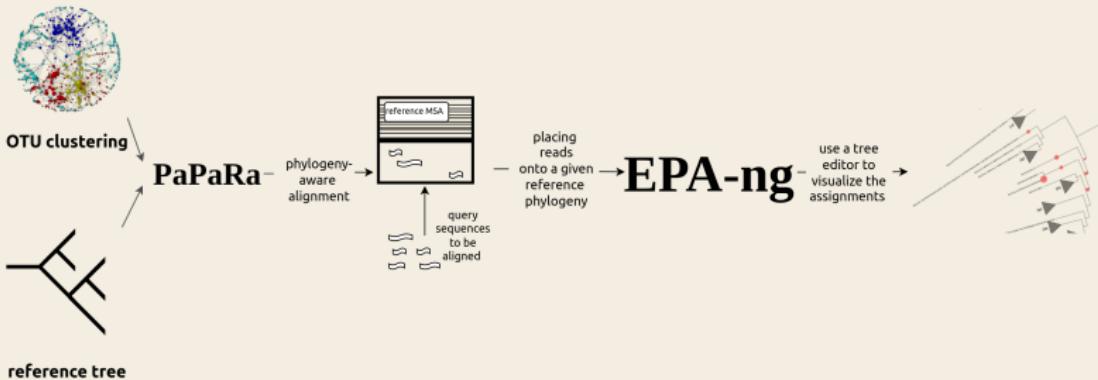
PEMA features

one step at a time!

A. create reference tree



B. phylogeny-based taxonomy assignment

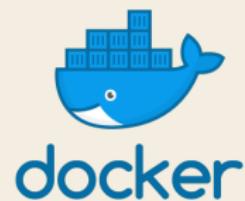


PEMA coding insights

Being a geek just for a bit !

```
for(int i : range(1,  
    in := "in_$i.tx  
    sys date > $in  
  
    out := "out_$i.t  
    task( out <- in  
        sys echo Tas  
    }  
}
```

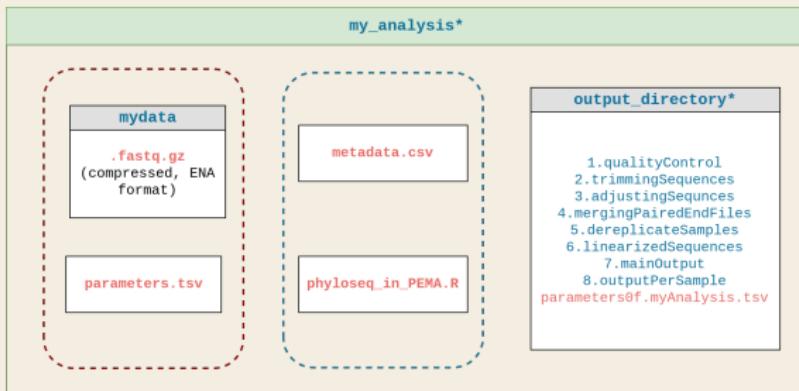
BigDataScript
programming language



Containerization

Mount your I/O

give & take

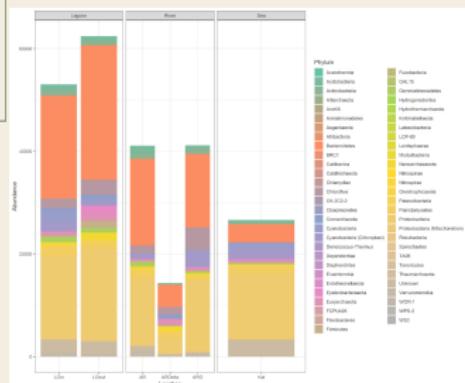


text file directory

*user can edit the name
(the rest **need** or will have the exact names shown)

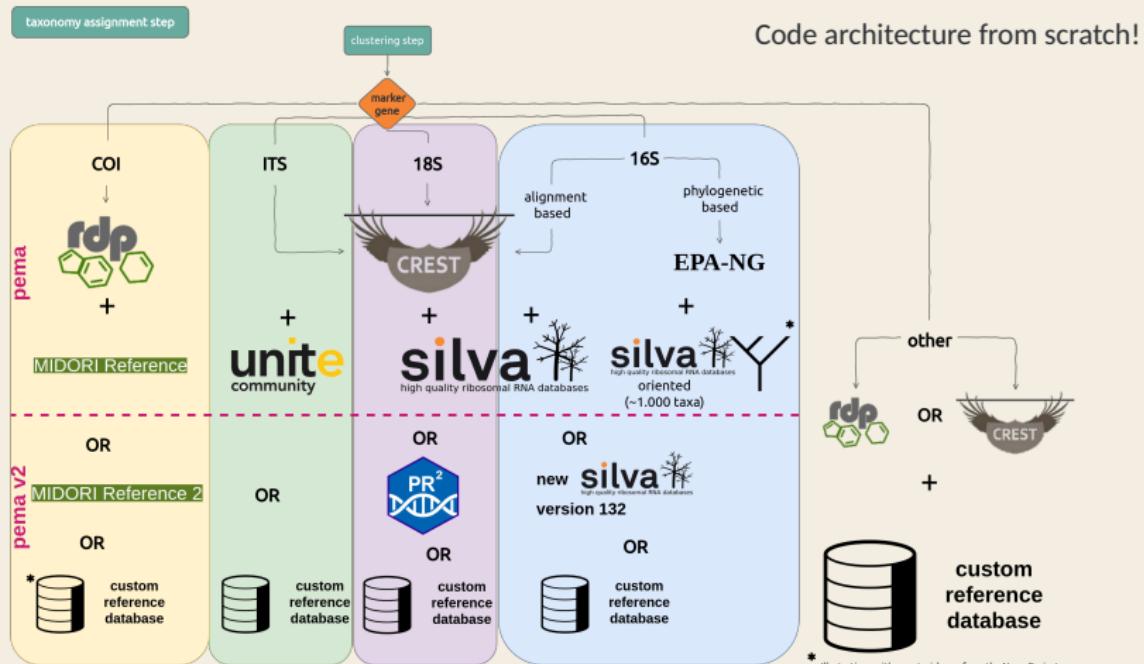
— mandatory input files
— optional input files

	Sample 1	Sample 2	Sample 3	Sample 4
Taxon 1	1	0	1	2
Taxon 2	0	1	0	2
Taxon 3	1	1	0	4



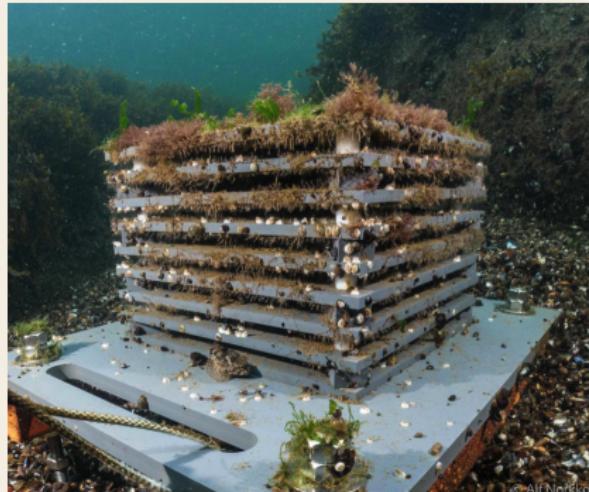
PEMA v.2

addressing some of the challenges



* Illustrations with an asterisk are from the Noun Project

Latest PEMa version *addressing the challenges of the community*



ASSEMBLE 
ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED

MBON
Marine Biodiversity
Observation Network

pema:v.2.1.4 includes:

1. analysis of 12S rRNA data now supported ([12S Vertebrate Classifier v2.0.0-ref database](#))
2. PR2 as an alternative reference database for the case of 18S rRNA
3. the ncbi-taxonomist tool was added to return the NCBI Taxonomy Id of the taxonomies found

How to and further documentation at pema.hcmr.gr

A place for sharing news as well as thoughts and remarks on how to run metabarcoding analyses using the PEMA containers or other software. Made by Haris Zafeiropoulos.

- Home
- Get PEMA
- Basics for running PEMA
- PEMA output
- Running on HPC
- Running on personal computer
- 16/18S analysis
- COI analysis
- ITS analysis
- Training the CREST classifier
- Training the RDPClassifier
- Tuning tuning tuning!
- GitHub repo
- Blog

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PEMA investigating metabarcoding

Welcome

Hey there!

This is the PEMA main site for *how to use* and further metabarcoding tips and hints!

You may find PEMA as a Docker and as a Singularity container.

Here is the [PEMA GitHub repository](#) if you want to have a look on the source code and why not, to contribute to!

For any running issues you may have, or for any further features you would like to see included on PEMA, you can reach us through the [PEMA Gitter community](#) or at pema@hcmr.gr.

Thanks for your interest on PEMA! Keep metabarcoding!

The PEMA team



PEMA
a pipeline for eDNA metabarcoding analysis

Metagenome go deeper than amplicon studies yet come with vast computational challenges

A workflow for marine Genomic Observatories data analysis

Digital Life Sciences Open Call
Funded Projects
eosc-life.eu/opencall

EOSC-Life

COMBINE

EMBL

IMBIEC

Work in progress
To be available in the months to come.

You may find more about this project through its [GitHub repository](#)

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PROCESS ENVIRONMENT ORGANISM

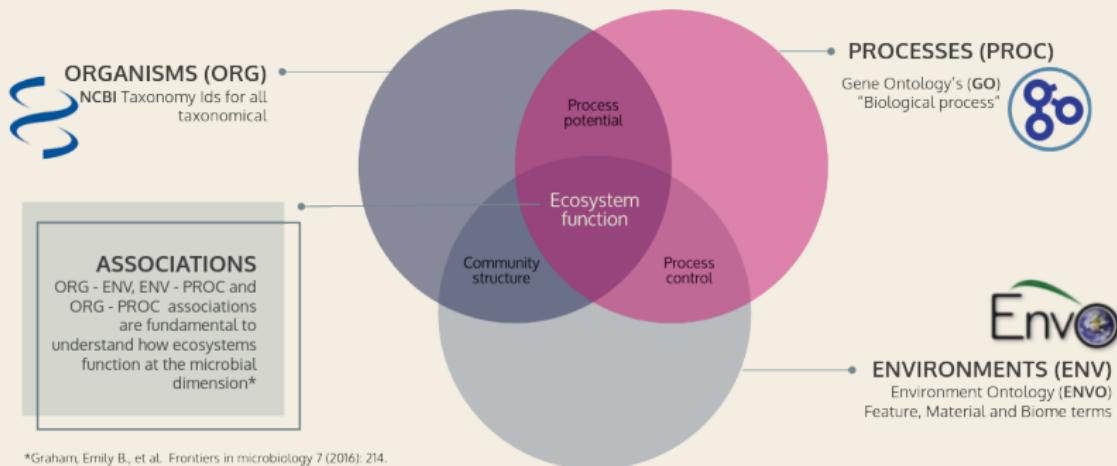
related repositories under

<https://github.com/orgs/lab42open-team>

web-app under

<http://prego.hcmr.gr/>

PREGO as processes - environments - organisms and how to link them



*Graham, Emily B, et al. Frontiers in microbiology 7 (2016): 214.

Metadata example

from various metagenome repositories

Sample metadata [-]



Collection date:	11/1/11
Elevation:	200
Environment (biome):	soil
Environment (feature):	nosZ
Environment (material):	soil DNA
Environmental package:	MIGS/MIMS/MIMARKS.soil
Geographic location (depth):	15-20cm
Instrument model:	454 GS FLX Titanium
Investigation type:	metres-survey
NCBI sample classification:	410658
Project name:	EcoFINDERS



Project Information

Cultured	No
Ecosystem	Environmental
Ecosystem Category	Aquatic
Ecosystem Subtype	Oceanic
Ecosystem Type	Marine

MG-RAST ID	name	biome	feature	material	sample	library	location	country	coordinates	download
mgm4702467.3	06032015b_S2_L001_R2_001	Large lake biome	lake	water	mgs485560	mg485562	Cincinnati	USA	39.11, -84.5	
mgm4702469.3	06052015a_S3_L001_R2_001	Large lake biome	lake	water	mgs485566	mg485568	Cincinnati	USA	39.11, -84.5	
mgm4702471.3	06032015a_S1_L001_R2_001	Large lake biome	lake	water	mgs485554	mg485556	Cincinnati	USA	39.11, -84.5	

MG-RAST
metagenomics analysis server

Named Entity Recognition

tagging the literature

Identification of potentially important pathways missing from the model

EXTRACT	X
Protein	
Chemical compound	
Organism	
Environment	
Tissue	
Disease/phenotype	
Gene Ontology term	

From the metagenomic bins, we were able to identify two **metabolic processes** that were not previously included in the model. A number of MAGs (bin.59, bin.15, bin.73) clustered to the KEGG genomes of **freshwater sulfur**-oxidizing autotrophs capable of denitrification, *Sulfuritalea hydrogentivorans* [41], and *Sulfuricella denitrificans* [42]. These MAGs contained the diagnostic genes for **carbon fixation** (*rbcLS*), **sulfur** cycling (*dsrAB*), and denitrification (*nosZ*). One MAG (bin.59) also clustered with **iron** oxidizing autotroph *Sideroxydans lithotrophicus* **ES-1**. Bin.59 is the most relatively abundant bin from 17 to 21 m depth. Thus, if this MAG is associated with **iron** oxidation, it also contains **sulfur**-cycling genes that add to metabolic flexibility, which was previously observed [40]. The model did not include **sulfide oxidation** with **nitrate**, so it is unclear from the current model predictions where this process is expected to occur within the water column to compare to the MAG distributions.

Example text from Arora-Williams et al. Microbiome 6.1 (2018): 1-16.

PREGO methodology

co-occurrence again!

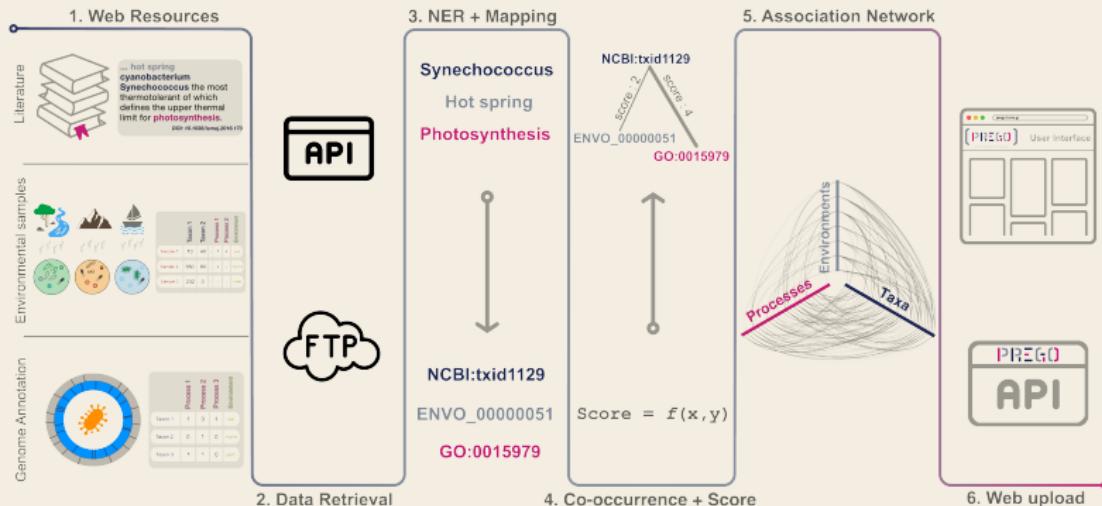
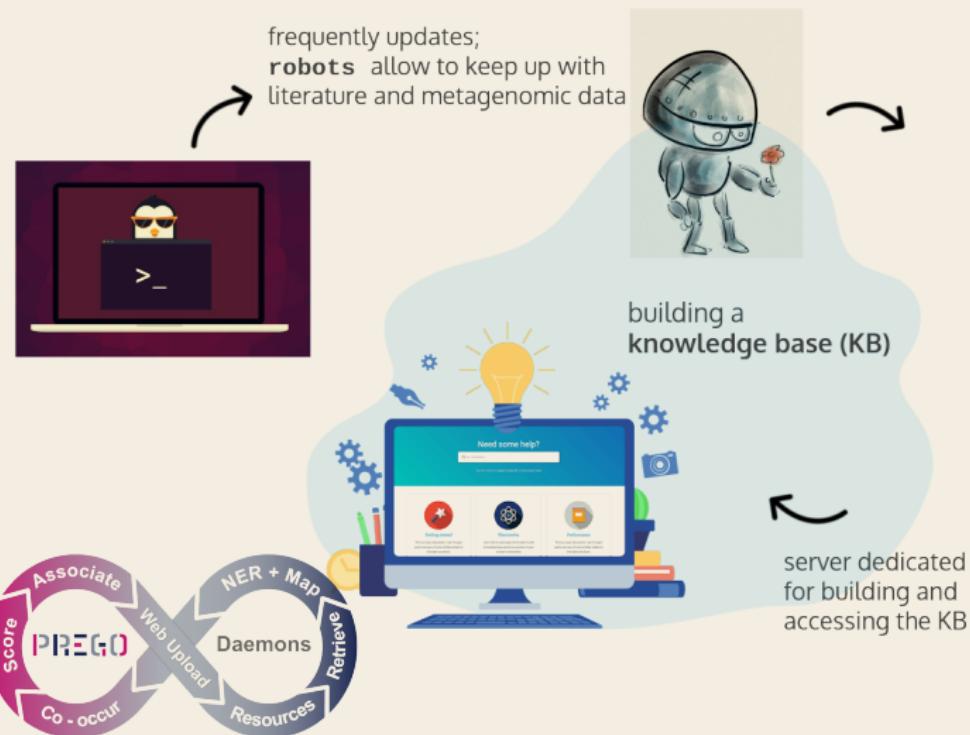


Figure from the PREGO publication that is now under review.

Building a knowledge-base

development and information technology operations



PREGO in action

looking for environments a taxon is present

Desulfatiglans anilini DSM 4660 [1121399]

Synonyms: Desulfatiglans anilini DSM 4660, D. anilini DSM 4660, D anilini DSM 4660, Desulfatiglans DSM 4660, Desulfatiglans str. DSM 4660 ...

Environments Biological Processes Molecular Function Documents Downloads

Literature

Search:

Name	Z-score	Confidence
Oil seep	3.0	★★★★★
Marine mud	3.0	★★★★★
Marine sediment	2.8	★★★★★
Brackish water	2.4	★★★★★
Oil reservoir	2.2	★★★★★
Anaerobic sediment	2.0	★★★★★
Cold seep	1.8	★★★★★
Contaminated sediment	1.7	★★★★★
Neritic sub-litoral zone	1.4	★★★★★
Oil spill	1.4	★★★★★
Petroleum	1.1	★★★★★
Sea floor	1.1	★★★★★

Showing 1 to 12 of 12 entries

Environments Biological Processes Molecular Function Documents Downloads

Literature

Search:

Name	Z-score	Confidence
benzoyl-CoA catabolic process	4.3	★★★★★
Benzene catabolic process	3.6	★★★★★
Acetone metabolic process	3.5	★★★★★
Phenanthrene catabolic process	3.5	★★★★★
Sulfate reduction	3.3	★★★★★
Ketone body catabolic process	3.2	★★★★★
Naphthalene catabolic process	3.2	★★★★★
Alkane catabolic process	3.1	★★★★★
Benzoate catabolic process	3.0	★★★★★
Denitrification pathway	2.8	★★★★★
Sulfide ion homeostasis	2.6	★★★★★
Ketone catabolic process	2.6	★★★★★
Methanogenesis	1.8	★★★★★
Electron transport chain	1.0	★★★★★

Showing 1 to 14 of 14 entries

The PREGO knowledge-base is available at
<http://prego.hcmr.gr/>.

What about taxa or functions of your interest? or even environments!

Uranium bioremediation
by adding high
concentrations of acetate

What taxa support
"Response to acetate"

Have a look!

Geobacter is the first hit !

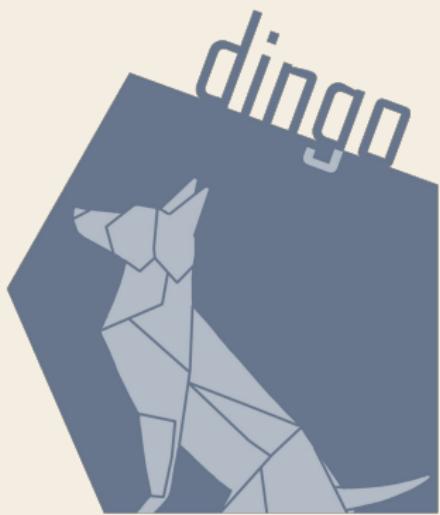
What about ***Posidonia*** ?

Literature suggests that Planctomycetes
and especially *Blastopirellula*
and *Rhodopirellula* are commonly
found in its microbiome.

Why so ?

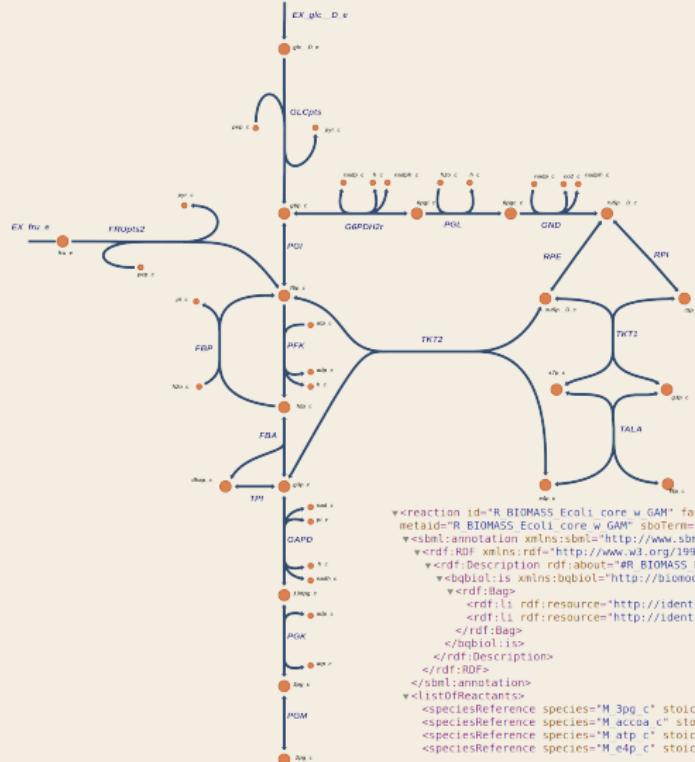
You may have a look [here](#)

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<https://github.com/GeomScale/dingo>

Metabolic modelling and the biomass function



Metabolic models allow us to move from a metabolic map to mathematical structures the study of which may provide fundamental biological insight

Genome-scale metabolic reconstruction approaches, pros and cons

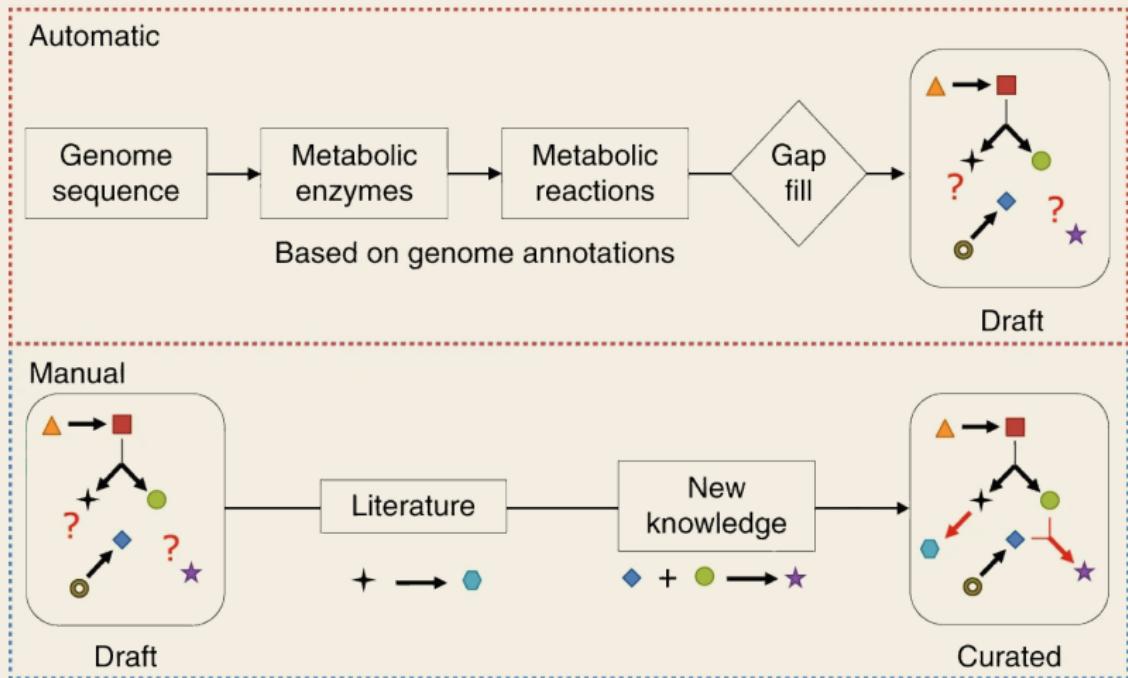


Figure from: Heirendt et al. Nature protocols 14.3 (2019): 639-702.

From a stoichiometric matrix

to a constraint-based model

In a **steady state**
the production rate
of each metabolite
equals its consumption rate

Reactions

	R ₁	R ₂	R ₃	R ₄	R ₅
Metabolites	-1	0	0	0	0
▲	1	-1	0	0	0
■	0	1	-1	0	0
●	0	1	0	0	-1
◆	0	0	1	0	0
○	0	0	0	-1	0
◆	0	0	0	1	-1
★	0	0	0	0	1

S-matrix

$\times \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$

Flux vector

The **flux vector** is a vector with
the value of
each reaction flux
in a certain steady
state.

The steady state assumption
is ensured by
the **zero-vector**.

The region of steady states

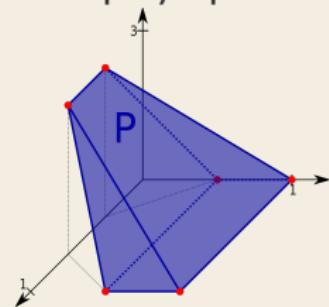
moving to full dimensional polytope

The *constraints* on the reactions fluxes.

$$Sv = 0, \quad (1) \quad v = Nx$$
$$v_{lb} \leq v \leq v_{ub}$$

$$S \in \mathbb{R}^{m \times n}, v \in \mathbb{R}^n$$

As a *full dimensional* polytope



$$P := \{x \in \mathbb{R}^d | Ax \leq b\}$$

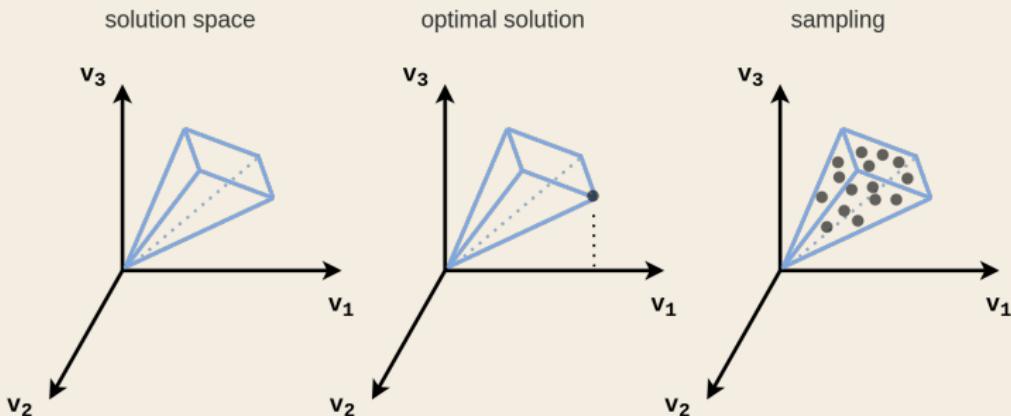
$N \in \mathbb{R}^{n \times d}$ denotes the matrix of the null space of S , i.e. $SN = 0_{m \times d}$.

By replacing v with Nx in Equation 1, we get the full dimensional polytope P , where

$$A = \begin{pmatrix} I_n N \\ -I_n N \end{pmatrix} \text{ and } b = \begin{pmatrix} v_{ub} \\ v_{lb} \end{pmatrix} N, \text{ (in } \mathbb{R}^d\text{).}$$

Flux sampling

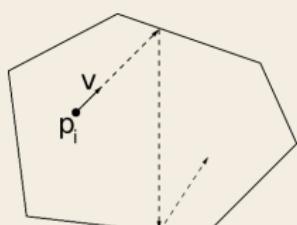
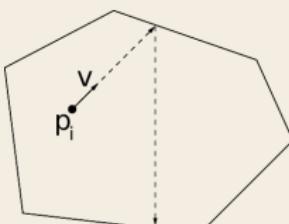
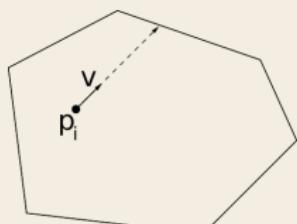
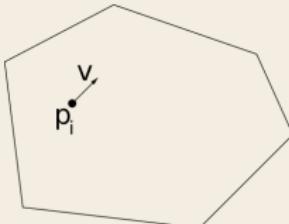
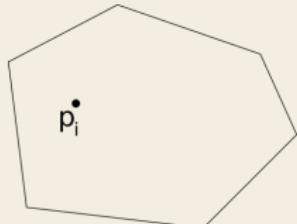
an alternative approach



- enables the analysis of GEMs without the need of an objective function
- determines the feasible solution spaces for fluxes in a network based on a set of conditions as well as the probability of obtaining a solution

Billiard walk

for random sampling



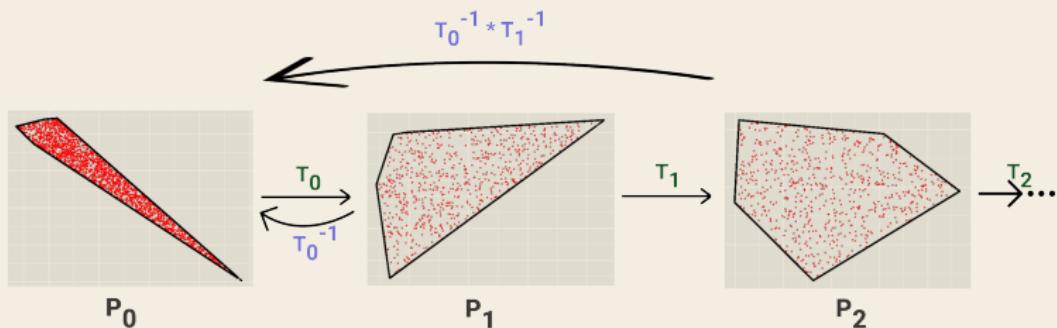
Generate the length of the trajectory $L \sim D$.

Pick a uniform direction v to define the trajectory.

The trajectory reflects on the boundary if necessary.

Return the end of the trajectory as $p_i + 1$.

Our Markov Chain Monte Carlo (MCMC) algorithm for flux sampling



Steps of an MMCS phase

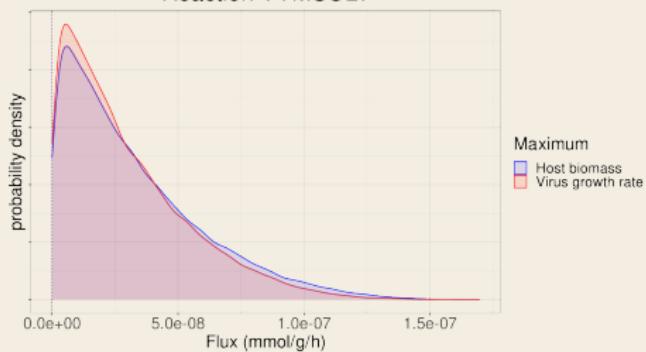
- **sampling step:** using a variant of the **Billiard walk**
- **rounding step:** calculate a linear transformation T_i that puts the sample into isotropic position and then apply it on P_i to obtain the polytope of the next phase
- check several statistic tests

Find possible targets against SARS-CoV-2

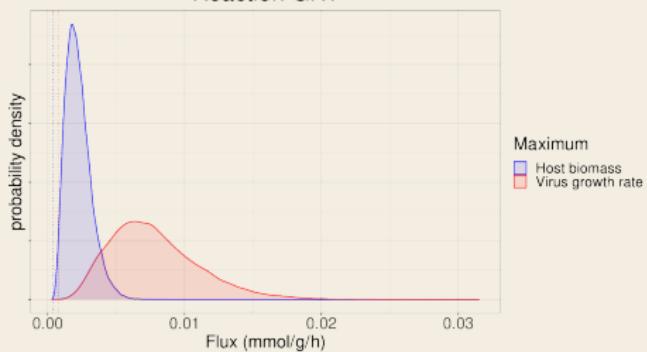
a flux sampling application



Reaction TYMSULT

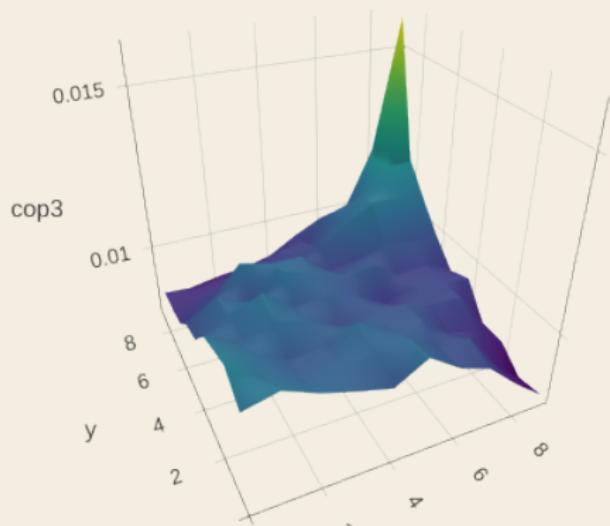


Reaction GK1



- Check if the flux distribution of a reaction changes.
- Find possible anti-viral targets and study further.

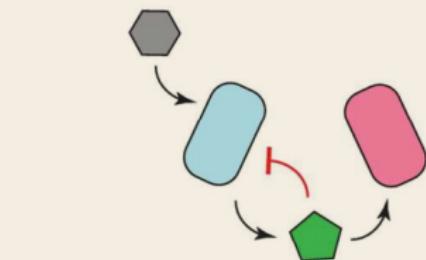
What is the probability of a flux value of a reaction
with respect to the flux value of another reaction



Further applications of metabolic flux sampling



Scott, William T., et al. "Metabolic flux sampling predicts strain-dependent differences related to aroma production among commercial wine yeasts." Microbial cell factories 20.1 (2021): 1-15.



What about microbial interactions ?

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Bioinformatics allow us to address several challenges in microbial ecology

yet, it is more than a means to an end

- Bioinformatics approaches enhance microbial diversity assessment based on HTS data
- Containerization technologies and e-infrastructures provide the means for computational capacity and reproducibility
- High quality metadata enable efficient exploitation of sequencing data in a meta-analysis level
- Markov Chain Monte Carlo approaches enable flux sampling in high-dimensional polytopes

Perspective for future work

More holistic approaches are essential to uncover the underlying mechanisms governing microbial communities

Thank you for your attention
and your patience ;)

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Mr. Potirakis Ant.

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