FishMed-PhD

Hologenomics

applied to host-microbiota interactions in marine organisms

Antton Alberdi

University of Copenhagen

antton.alberdi@sund.ku.dk

alberdilab.dk

CEH
CENTER FOR
EVOLUTIONARY
HOLOGENOMICS

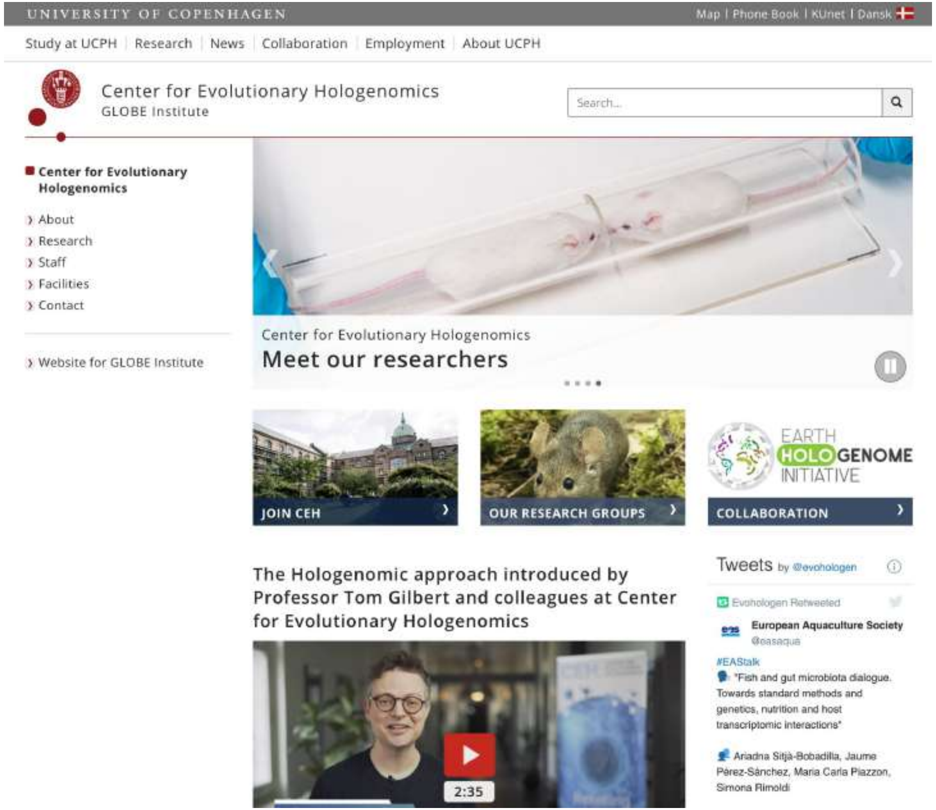
UNIVERSITY OF
COPENHAGEN

Hologenomics

FishMed-PhD

CENTER FOR EVOLUTIONARY HOLOGENOMICS

UNIVERSITY OF COPENHAGEN



Center for Evolutionary Hologenomics
GLOBE Institute

Center for Evolutionary Hologenomics

Center for Evolutionary Hologenomics
Meet our researchers

The Hologenomic approach introduced by Professor Tom Gilbert and colleagues at Center for Evolutionary Hologenomics

Tweets by @evohologen

Evohologen Retweeted

European Aquaculture Society
@easaqua

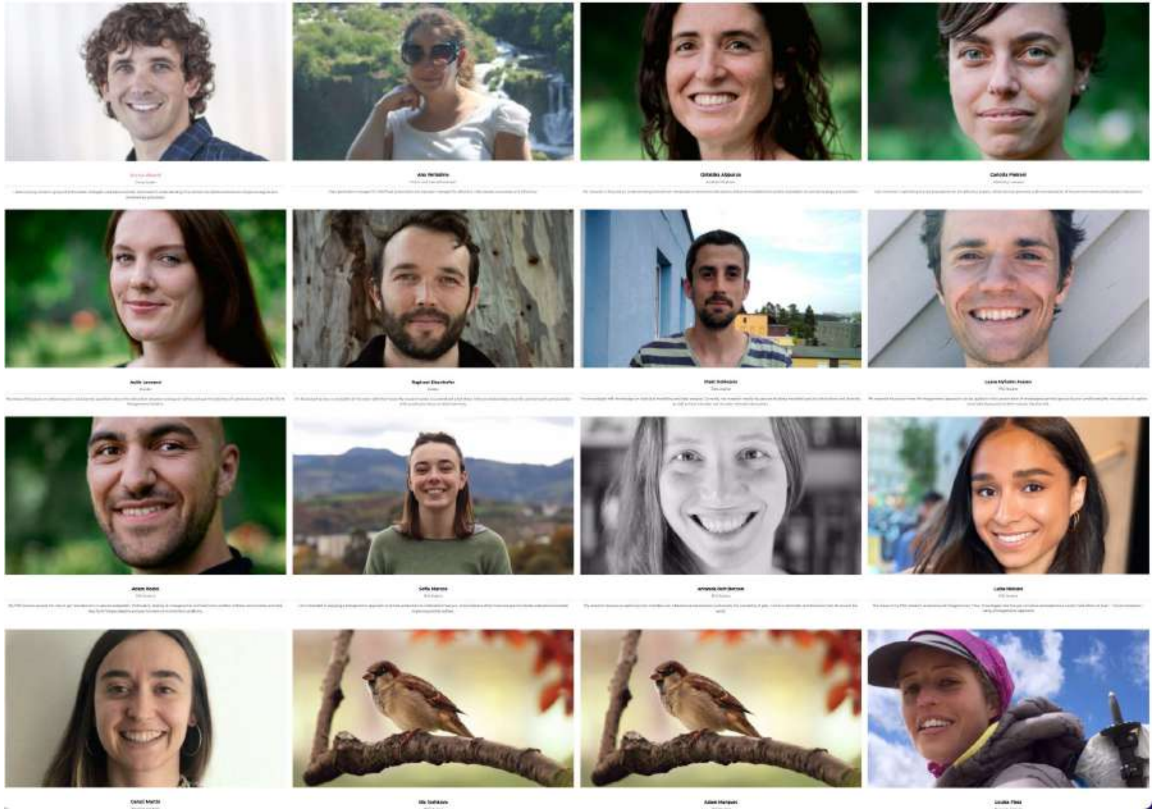
#EAStalk
"Fish and gut microbiota dialogue. Towards standard methods and genetics, nutrition and host transcriptomic interactions"

Ariadna Sitjà-Bobadilla, Jaume Pérez-Sánchez, Maria Carla Piazzon, Simona Rimoldi

Hologenomics

FishMed-PhD

alberdilab.dk



Hologenomics

FishMed-PhD

Antton Alberdi

University of Copenhagen

antton.alberdi@sund.ku.dk

Hologenomics

FishMed-PhD

55’ **14.30 - 15.25**	**Session 1** Animal-microbiota interactions
50’ **15.30 - 16.20**	**Session 2** Drivers of animal-microbiota interaction variation
75’ **16.30 - 17.45**	**Session 3** Hologenomics to study animal-microbiota interactions
30’ **18.00 - 18.30**	**Session 4** Applications of hologenomics: opportunities and feasibility

Questions

Many

Questions

Hologenomics

Hologenomics

Silence

Long

Silence

Hologenomics

Session 1

Animal-microbiota

INTERACTIONS

Hologenomics

FishMed-PhD

WHAT IS AN ANIMAL FOR A MICROORGANISM?

Hologenomics

Habitat

Shelter

Source of nutrients

...

WHAT IS A MICROBE FOR AN ANIMAL?

Hologenomics

Source of nutrients

Pathogen

Stimulus

...

FishMed-PhD

Session 1

Animal-microbiota

INTERACTIONS

Hologenomics

HOW DO ANIMALS AND MICROORGANISMS INTERACT?

Hologenomics

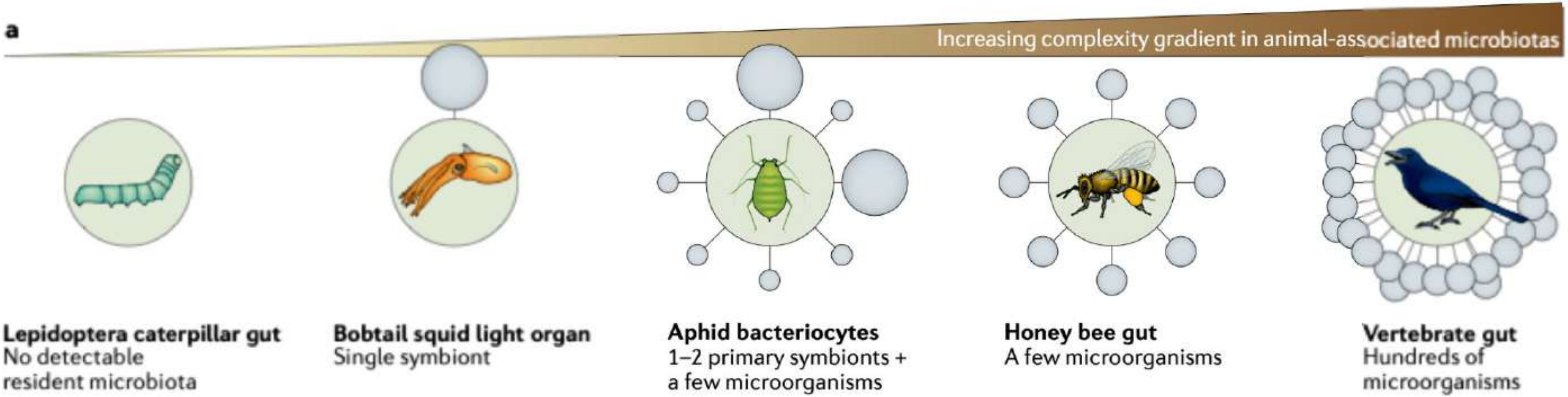
FishMed-PhD

Evolutionary interactions

Ecological interactions

Molecular interactions

Hologenomics



Alberdi et al. 2021

Nature Reviews Genetics

RESOLUTION

Photo: Todd Bretl

Hologenomics

RESOLUTION

Light organ

Gut

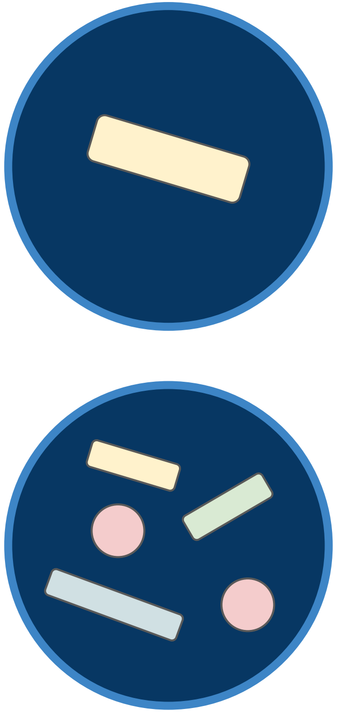
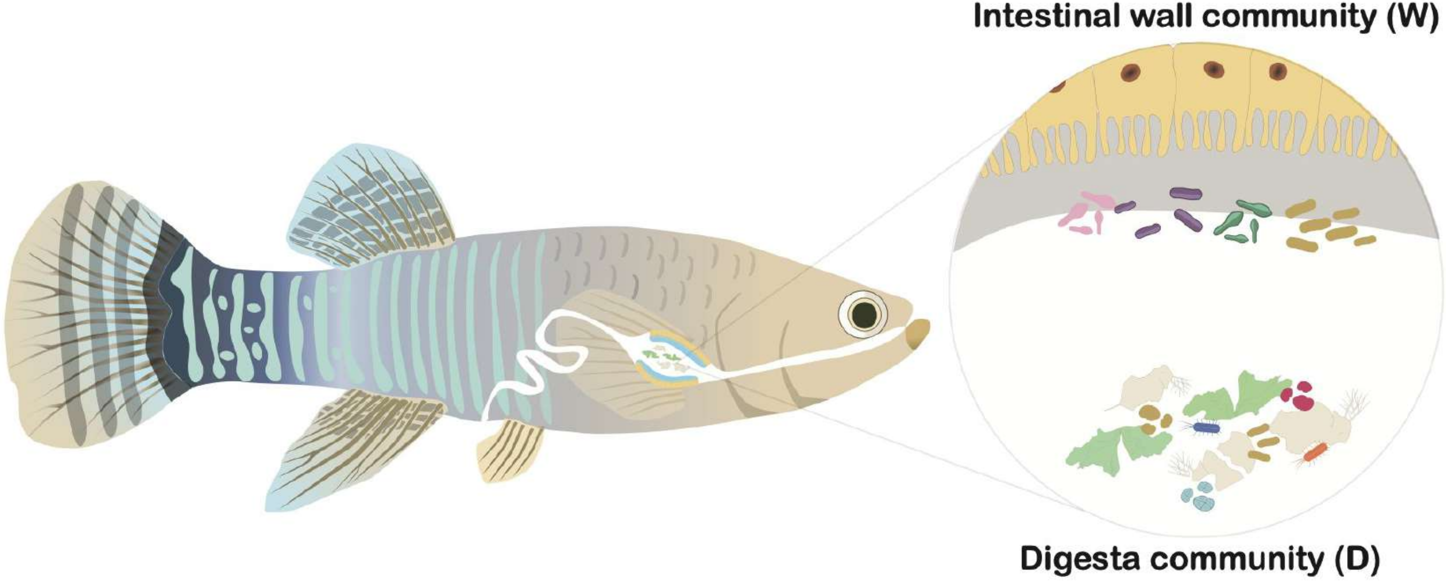


Photo: Todd Bretl

Hologenomics

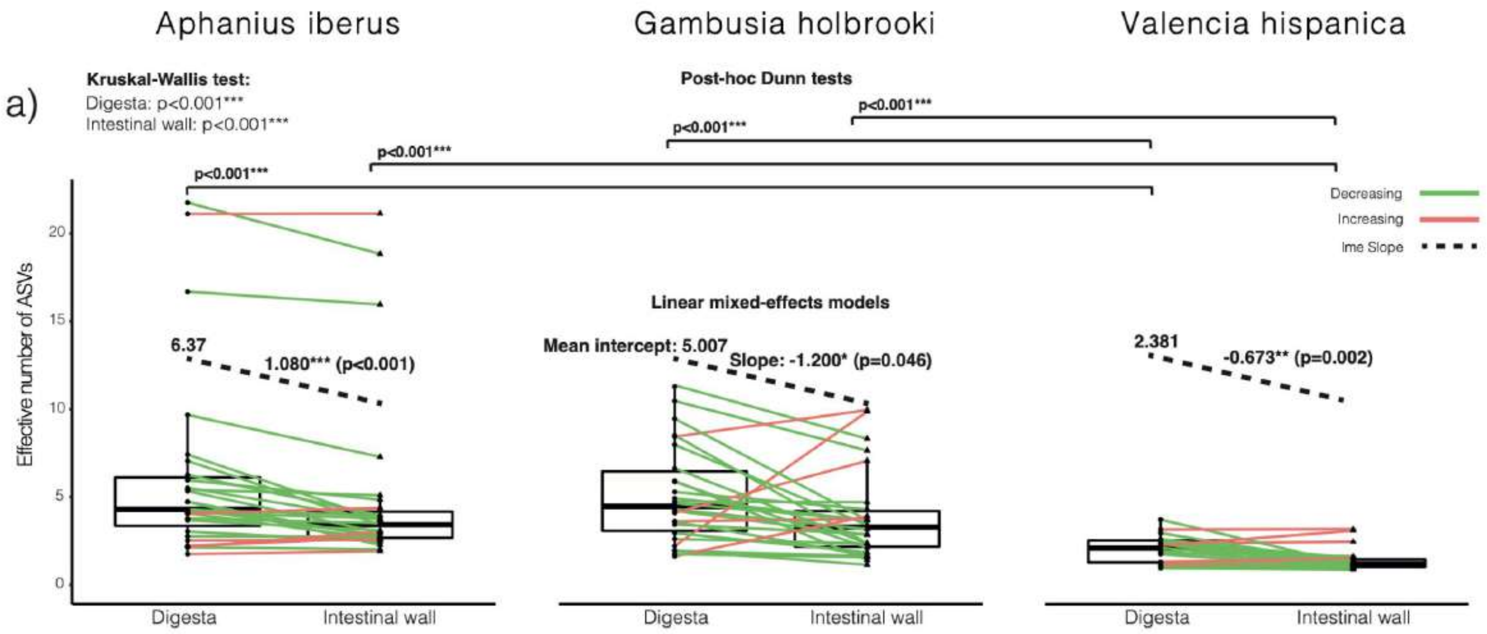
RESOLUTION



Nyholm et al. 2022

PeerJ

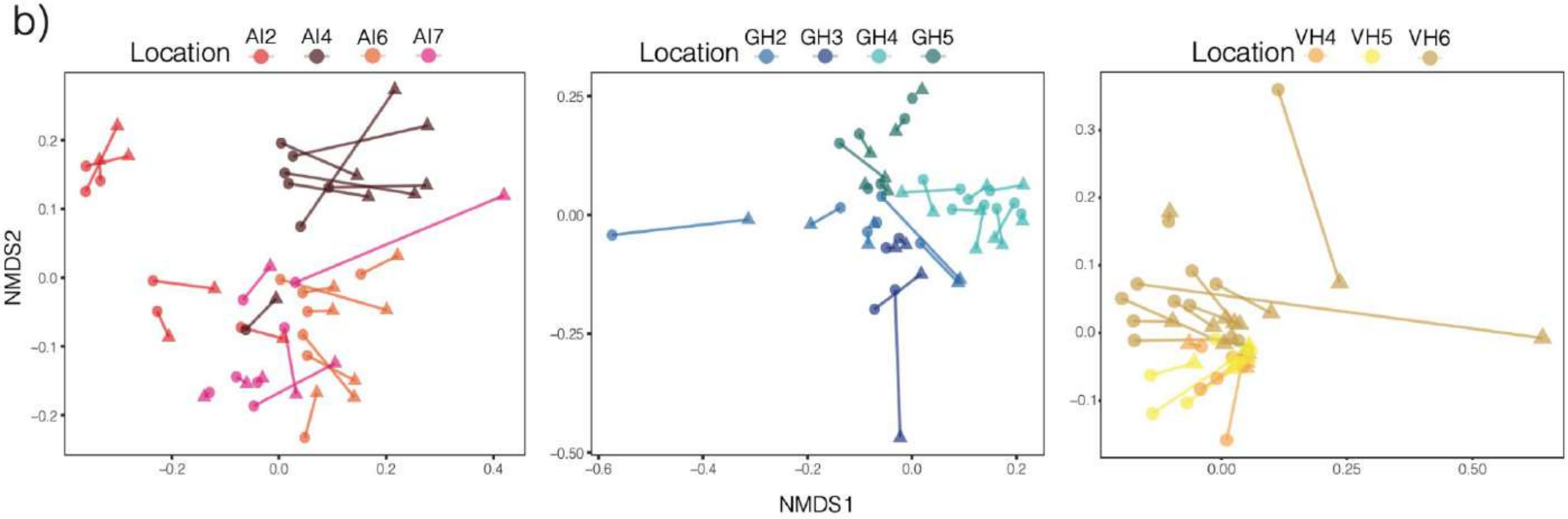
Hologenomics



Nyholm et al. 2022

PeerJ

Hologenomics



Nyholm et al. 2022

PeerJ

FishMed-PhD

WHAT DOES IT MEAN TO BE ASSOCIATED WITH A HOST?

Group discussion

FishMed-PhD

WHAT DOES IT MEAN TO BE ASSOCIATED WITH A HOST?

Is every microbe we detect in the skin
or the intestine of animals
“associated” with it?

Hologenomics

FishMed-PhD

Occur?

Occur for a long time?

Occur in multiple individuals?

Proliferate?

Influence the host?

Hologenomics

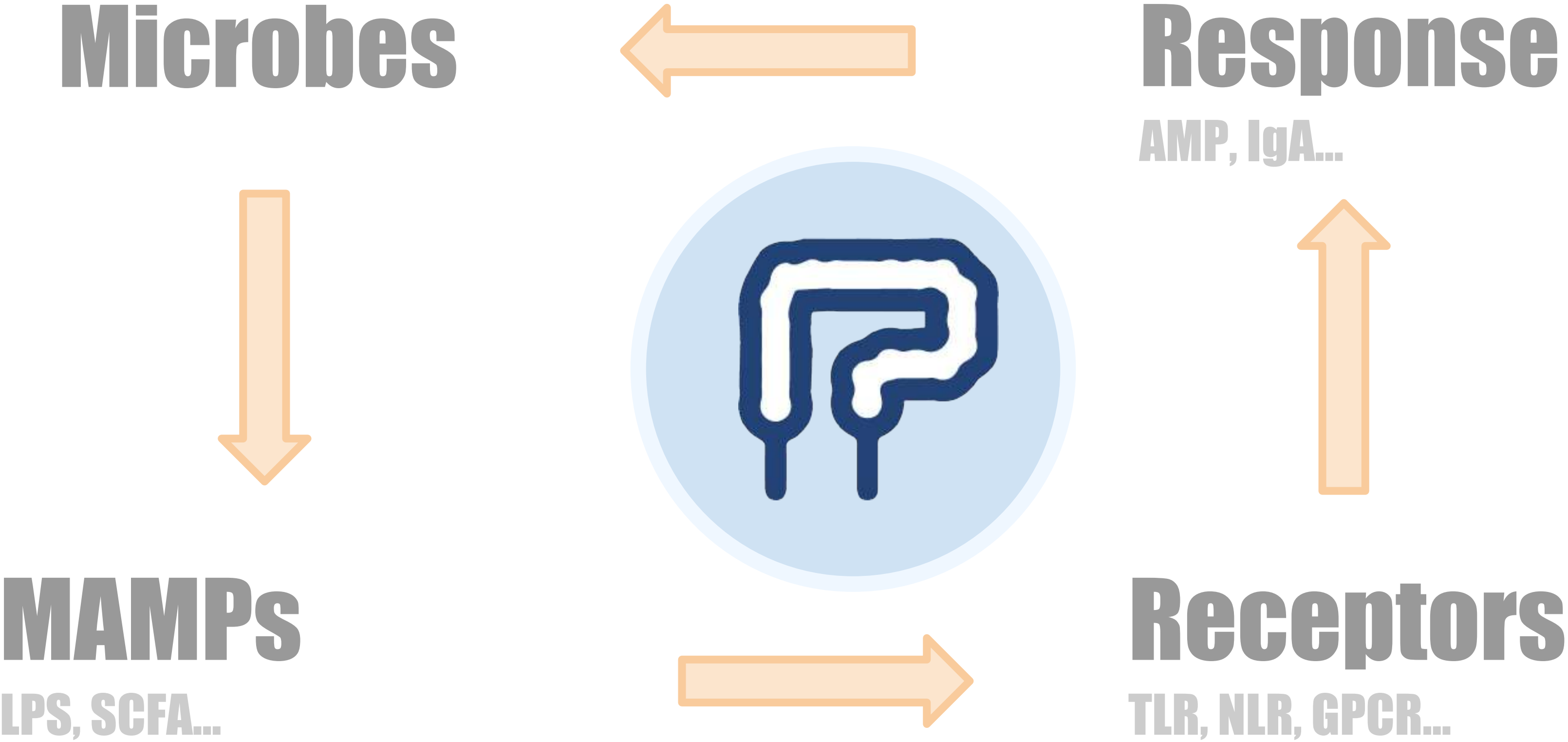
INTERESTING READINGS

Hologenomics

HOW DO ANIMALS AND MICROORGANISMS INTERACT?

MOLECULAR LEVEL

Hologenomics



Microbes

Response

AMP, IgA...

MAMPs

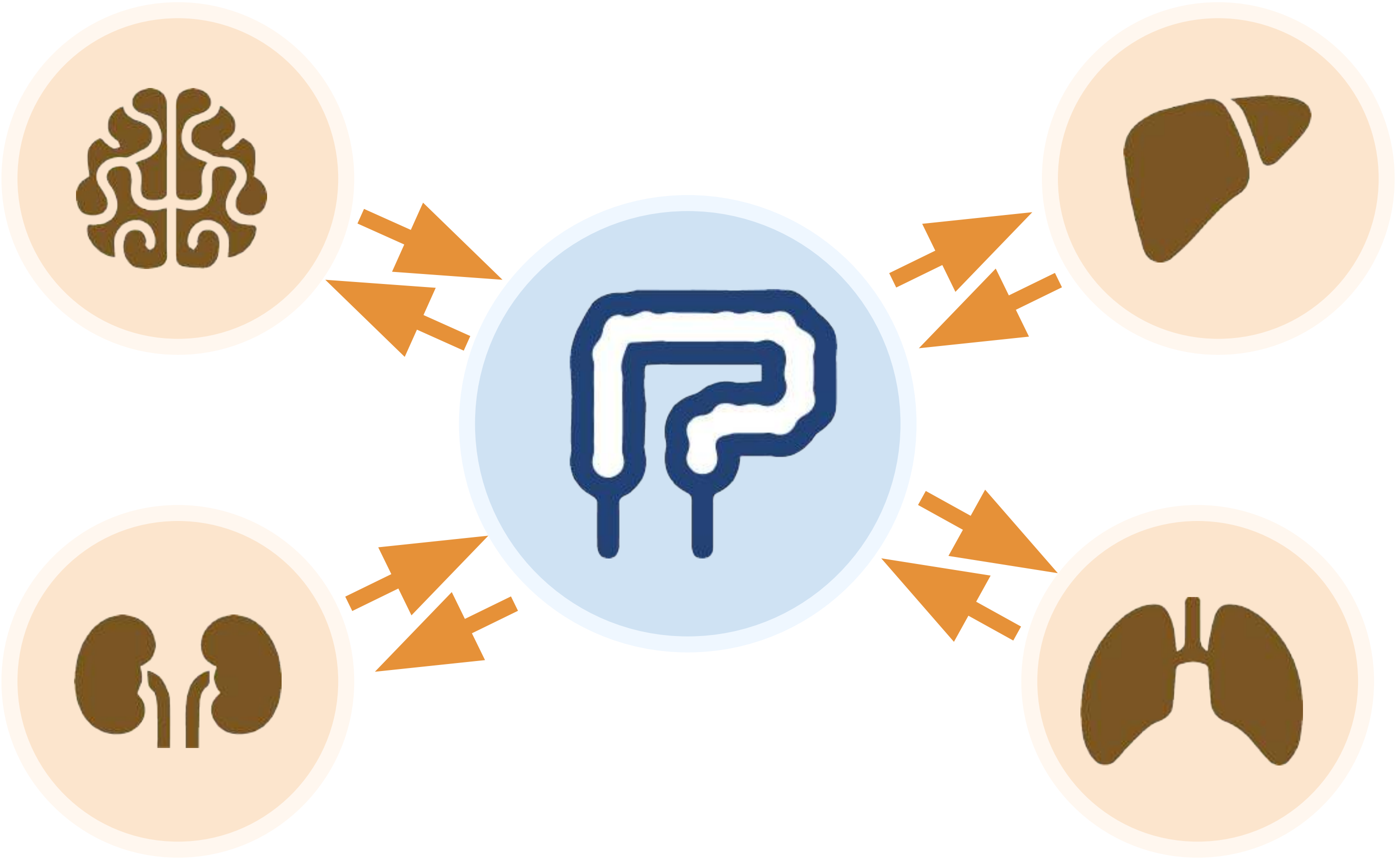
LPS, SCFA...

Receptors

TLR, NLR, GPCR...

Hologenomics

FishMed-PhD



Gut-brain axis

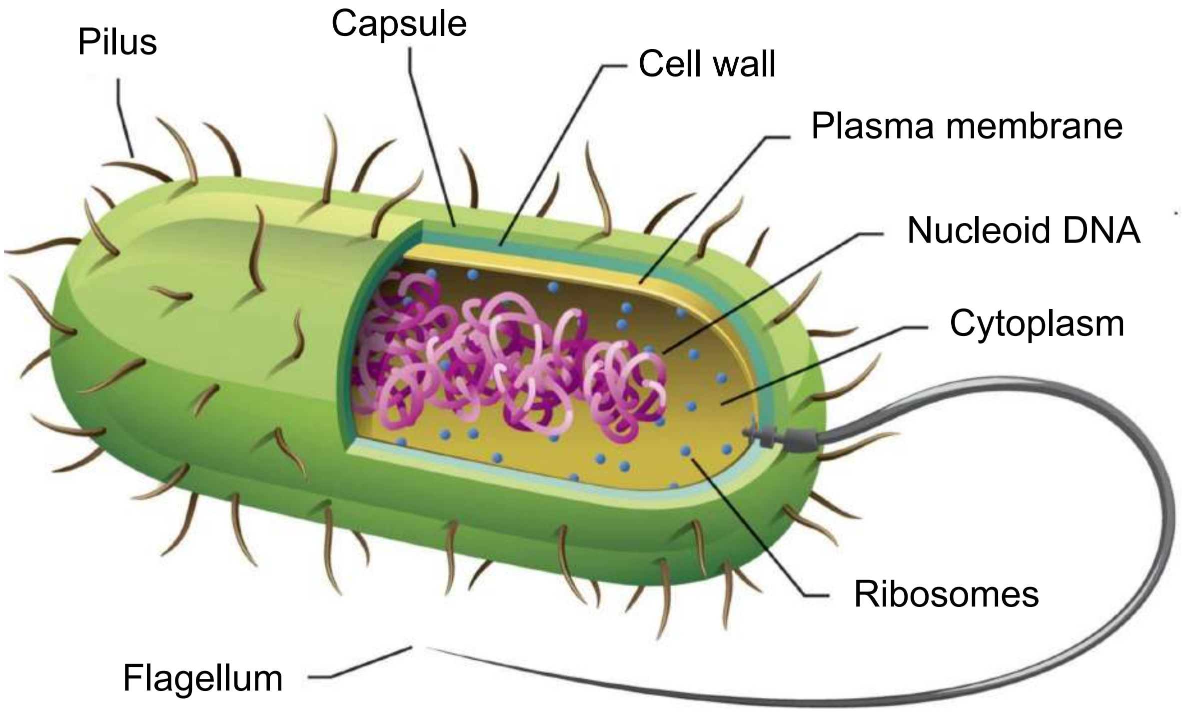
Gut-liver axis

Gut-kidney axis

Gut-lung axis

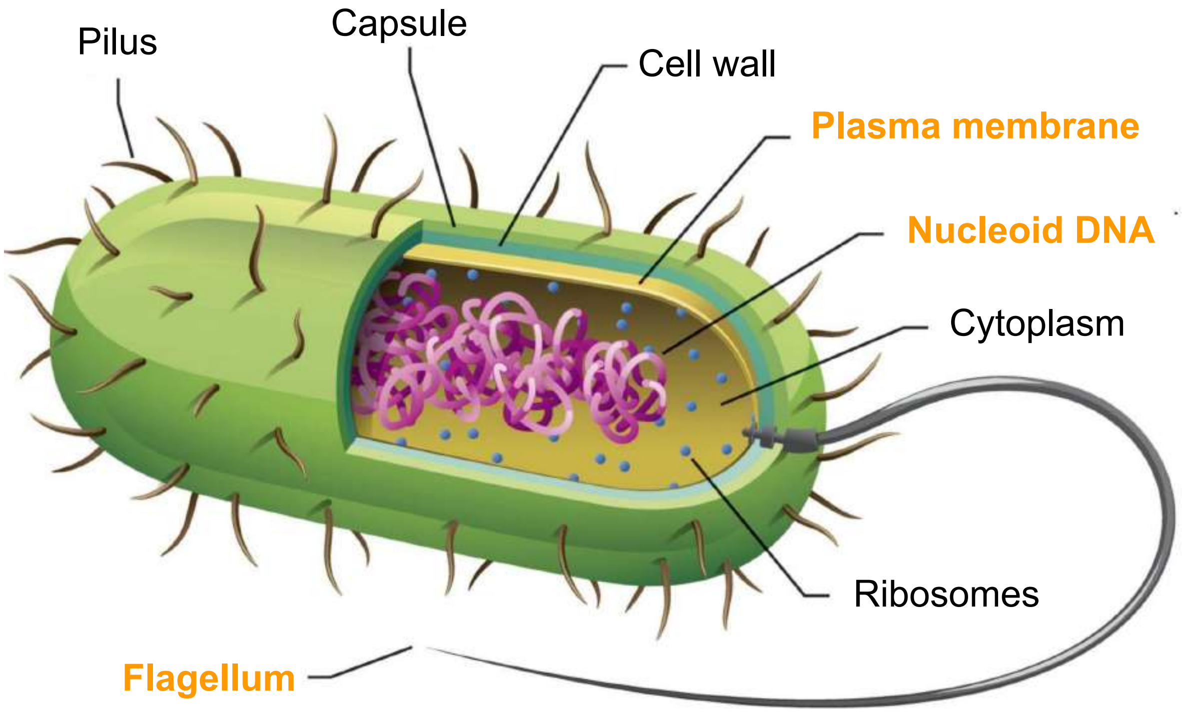
Hologenomics

FishMed-PhD



Hologenomics

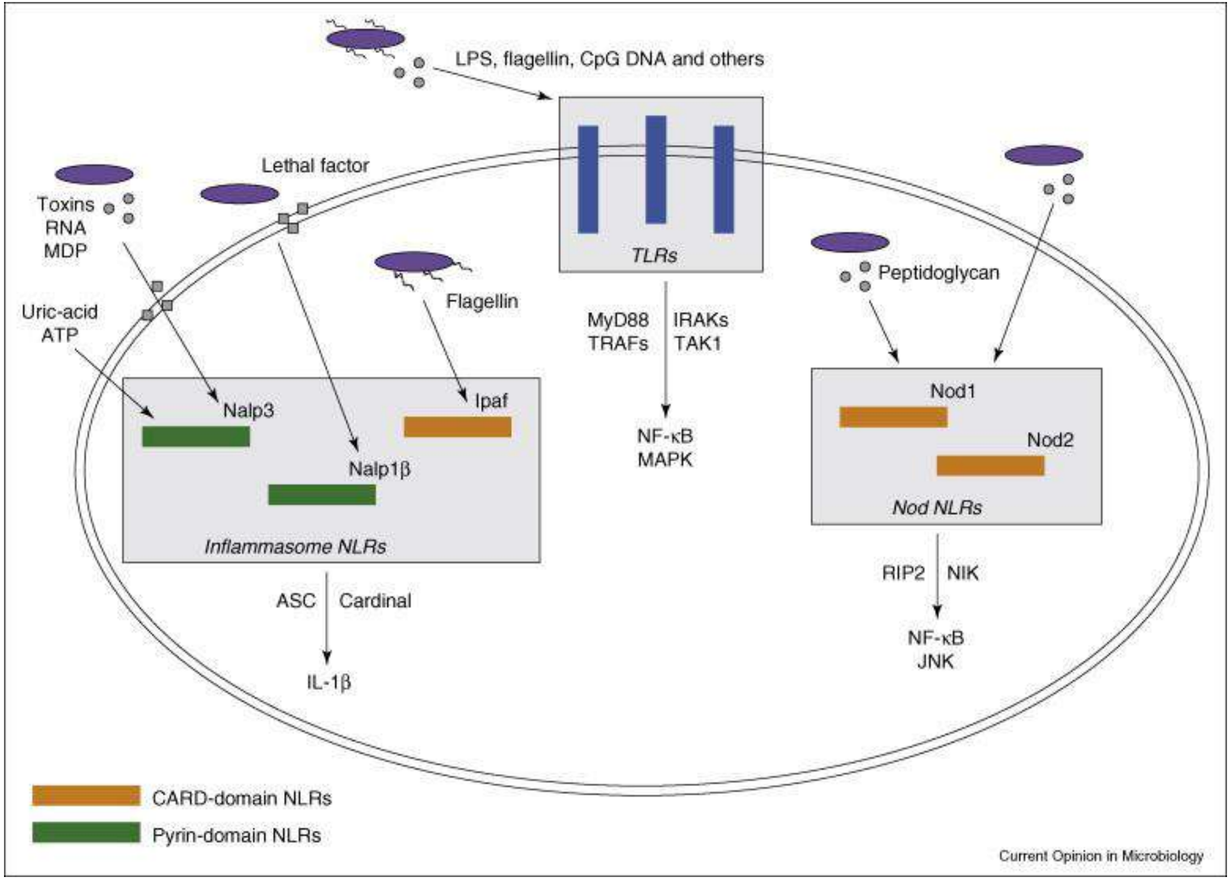
FishMed-PhD



Hologenomics

- Lipopolysaccharides (LPS)
- Di- and triacylated lipopeptides
- Lipoteichoic acids (LTA)
- Flagellin
- CpG DNA
- Peptidoglycan
- Polysaccharide A (PSA)

Hologenomics



Toll-like receptors (TLR)

Nod-like Receptors (NLR)

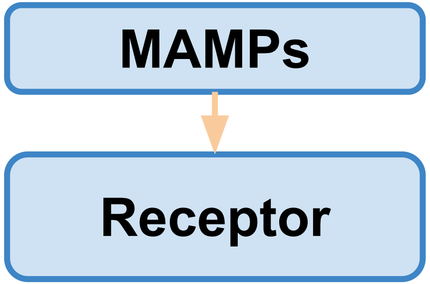
Kufer et al. 2007

Current Opinion in Microbiology

Hologenomics

MAMPs

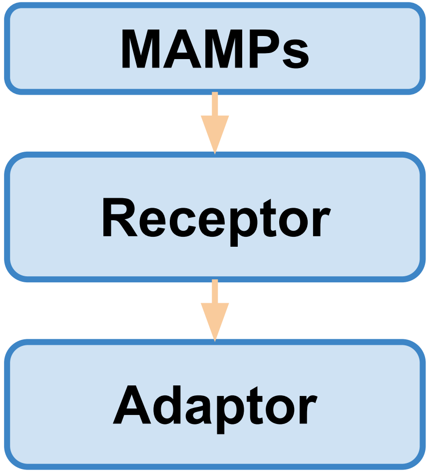
Hologenomics



MAMPs

Receptor

Hologenomics



MAMPs

Receptor

Adaptor

Hologenomics

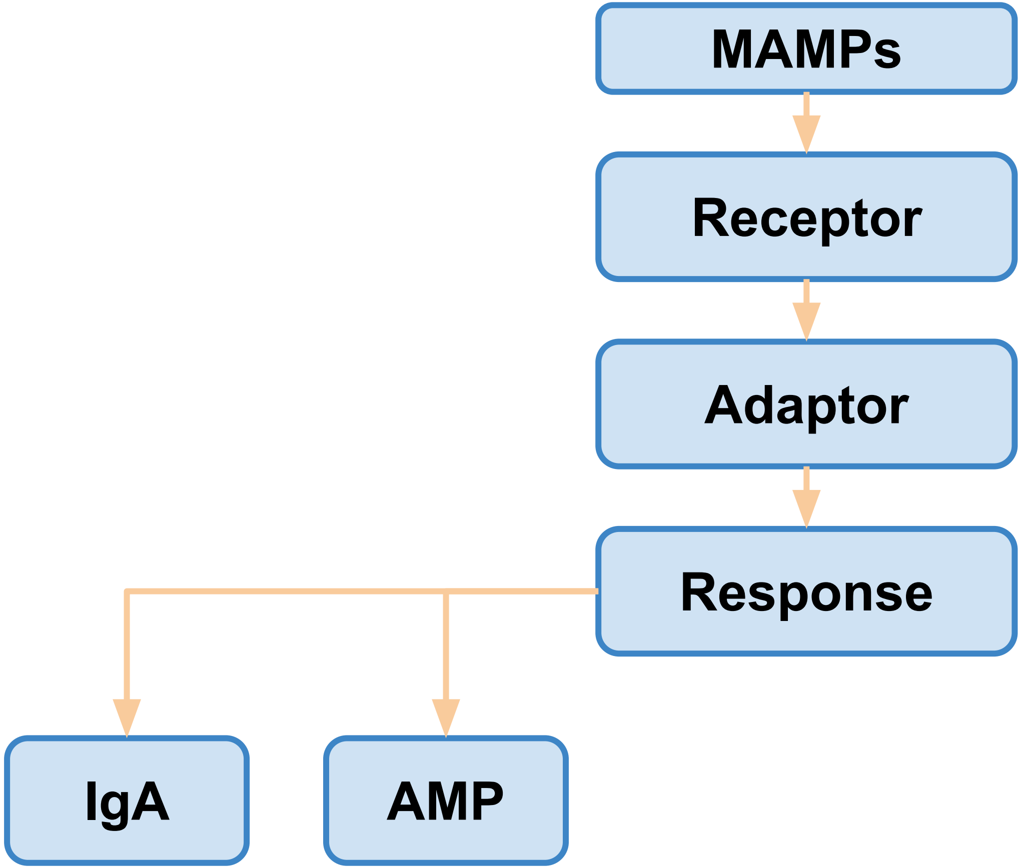
MAMPs

Receptor

Adaptor

Response

Hologenomics



MAMPs

Receptor

Adaptor

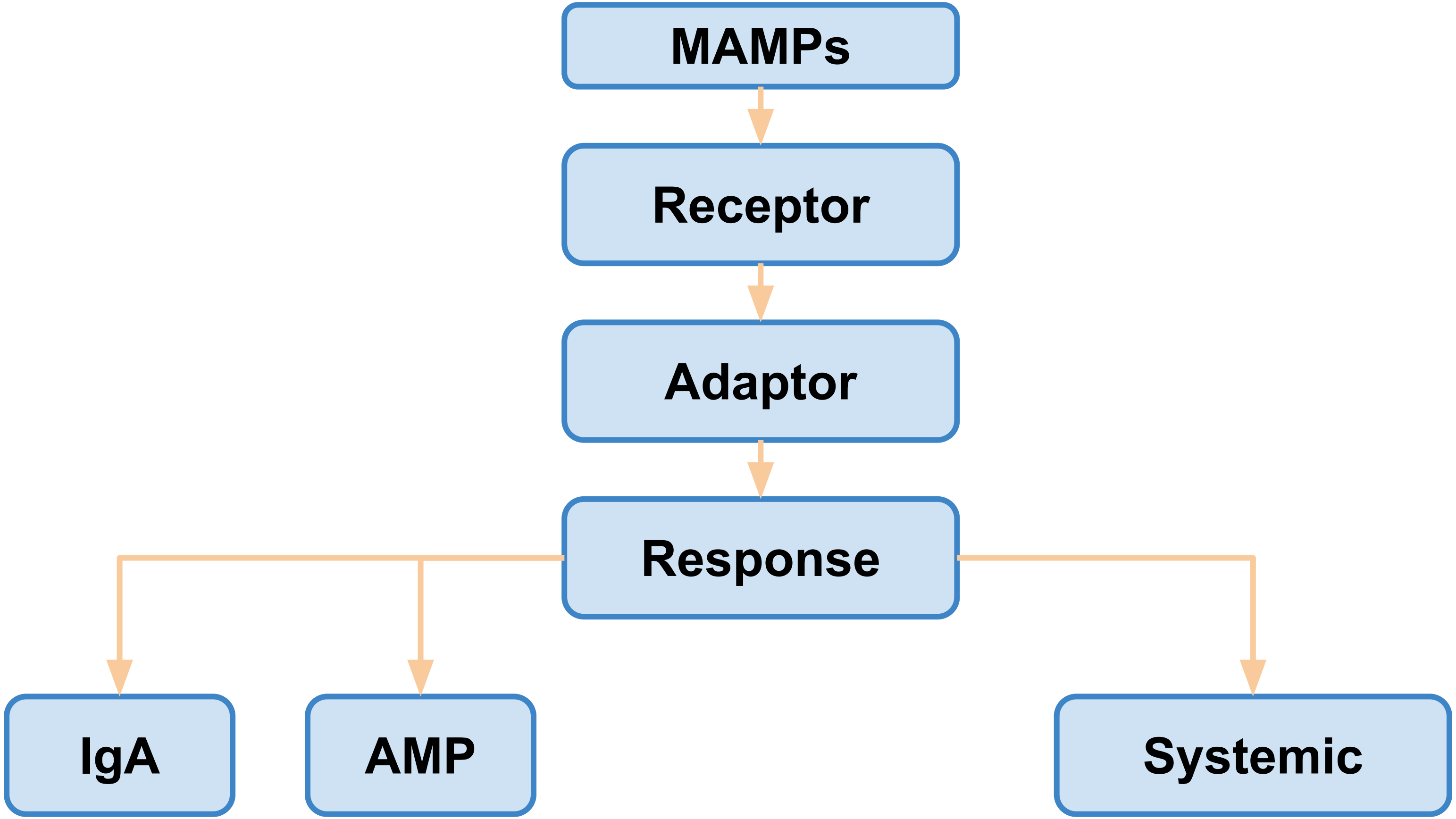
Response

IgA

AMP

FishMed-PhD

Hologenomics



MAMPs

Receptor

Adaptor

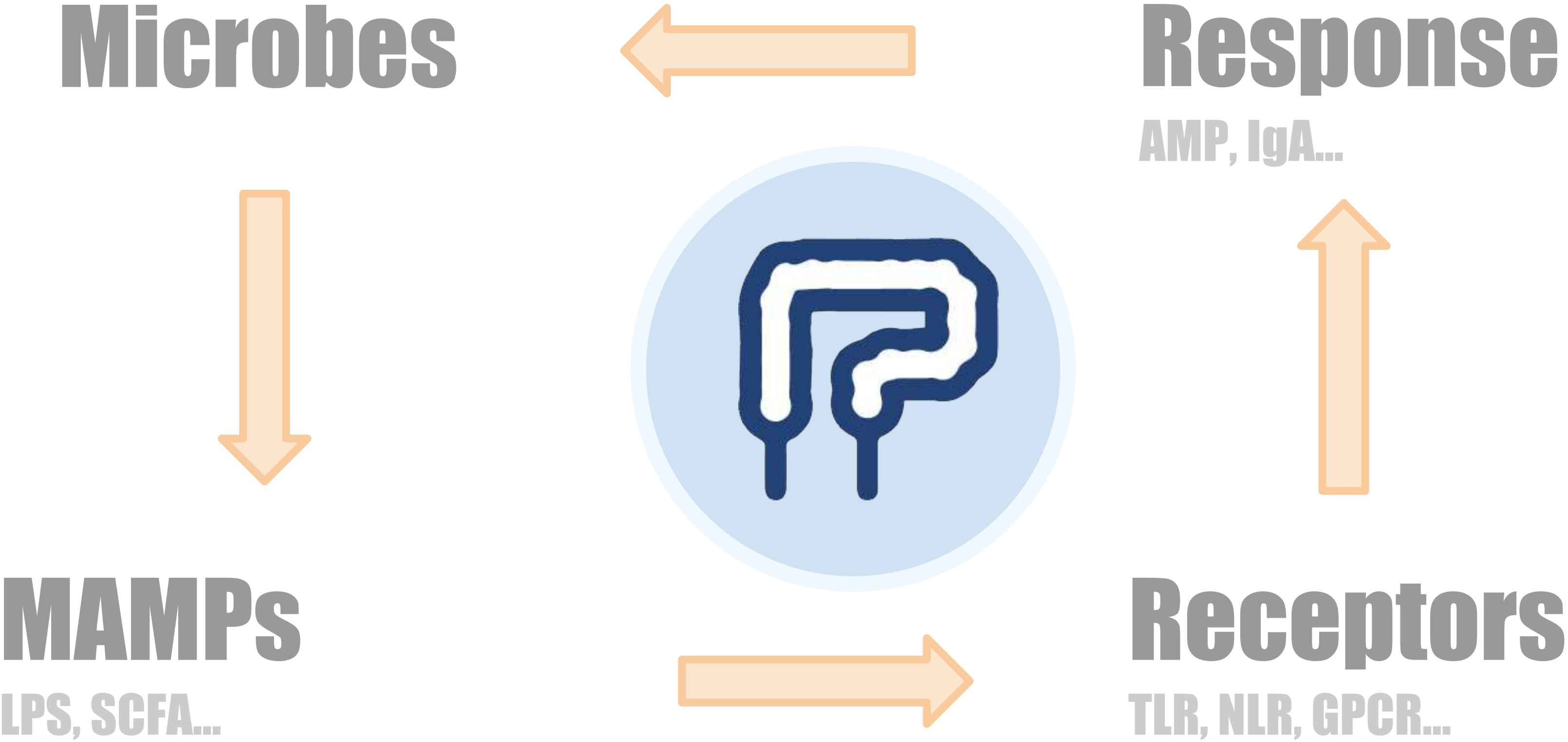
Response

IgA

AMP

Systemic

Hologenomics



Microbes

MAMPs

LPS, SCFA...

Receptors

TLR, NLR, GPCR...

Response

AMP, IgA...

Hologenomics

INTERESTING READINGS

Hologenomics

FishMed-PhD

HOW SELECTIVE IS THE HOST RESPONSE?

What are the implications of being more or less selective?

HOW DO ANIMALS AND MICROORGANISMS INTERACT?

EVOLUTIONARY LEVEL

CAN HOSTS AND BACTERIA CO-EVOLVE?

Hologenomics

CAN HOSTS AND BACTERIA
CO-EVOLVE?

CAN HOSTS AND MICROBIOMES
CO-EVOLVE?

Hologenomics

INTERESTING READINGS

FishMed-PhD

Hologenomics

applied to host-microbiota interactions in marine organisms

Antton Alberdi
University of Copenhagen
antton.alberdi@sund.ku.dk

alberdilab.dk

CEH
CENTER FOR
EVOLUTIONARY
HOLOGENOMICS

UNIVERSITY OF
COPENHAGEN

FishMed-PhD

Session 2

DRIVERS AND DYNAMICS of animal-microbiota interactions

Hologenomics

FishMed-PhD

WHAT FACTORS DRIVE ANIMAL-MICROBIOTA INTERACTIONS?

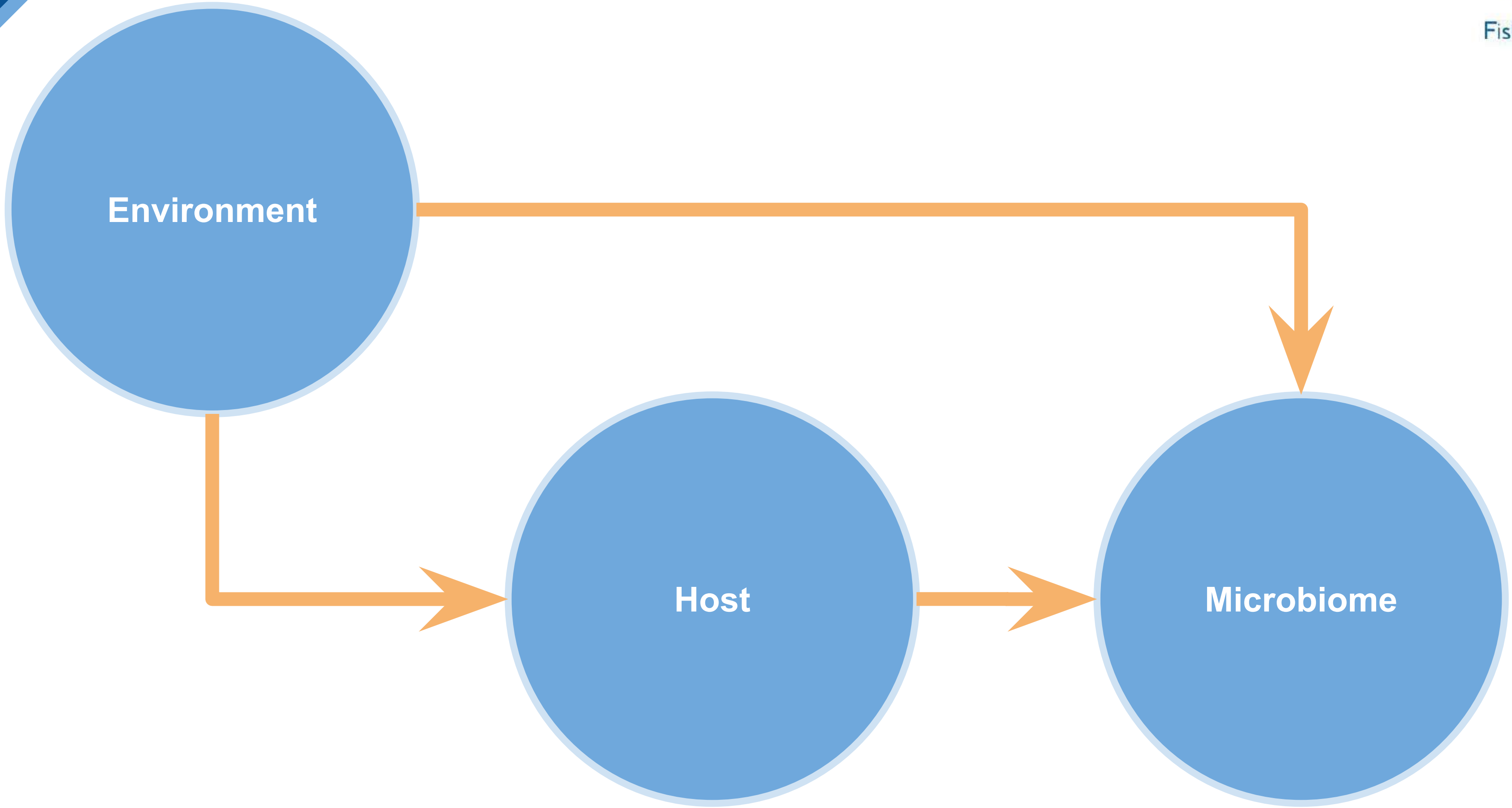
Hologenomics

Environment

Microbial genetics

Host genetics

Hologenomics


Environment

Host

Microbiome

FishMed-PhD

WHAT IS AN ENVIRONMENTAL FACTOR?

Hologenomics

Any factor not encoded in the genetic material of microbes and hosts

Hologenomics

FishMed-PhD

WHICH ENVIRONMENTAL FACTORS DO AFFECT ANIMAL-MICROBIOTA INTERACTIONS?

Hologenomics

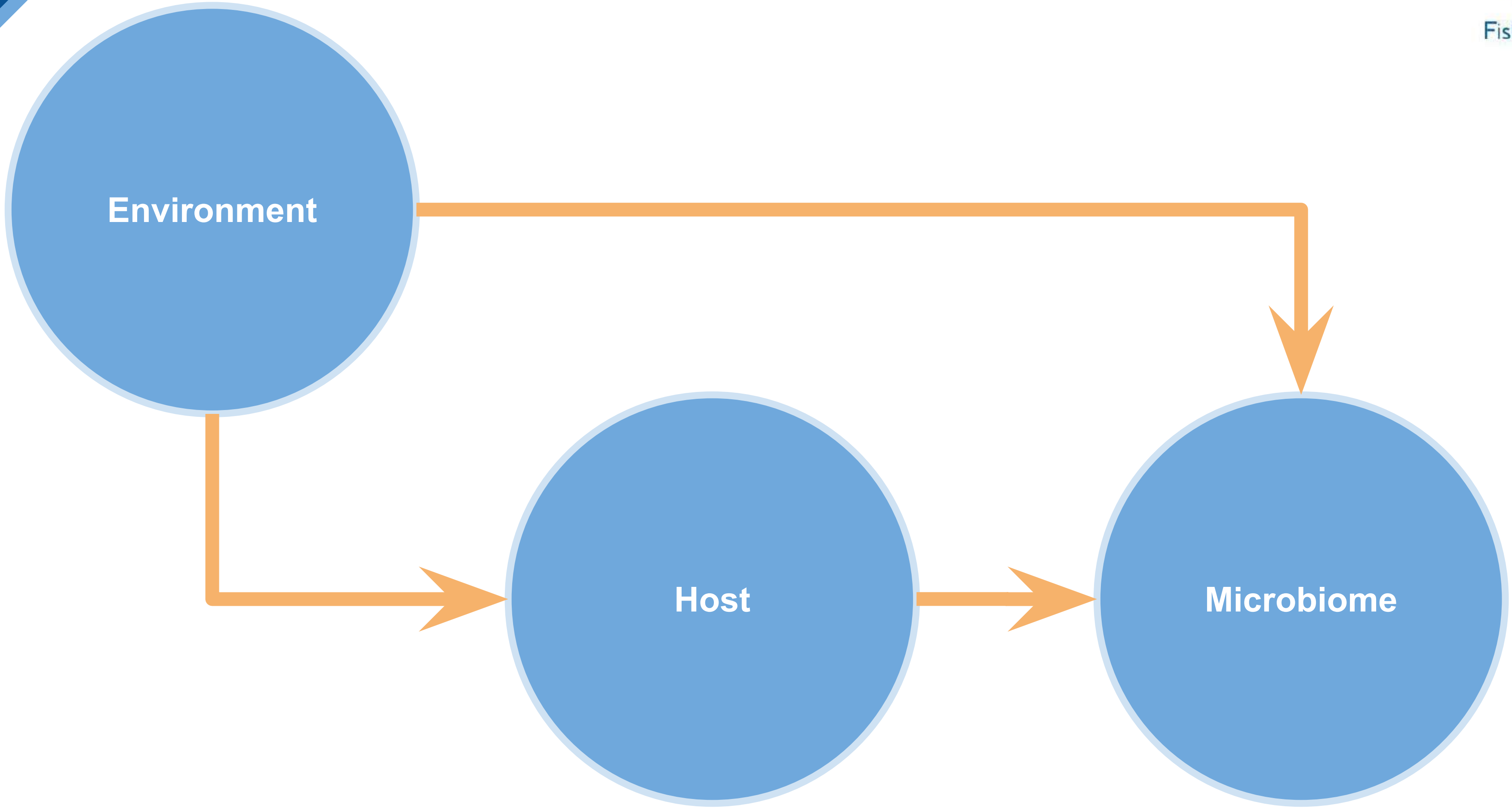
Diet

Temperature

Humidity

Physiological state
of the host

Hologenomics

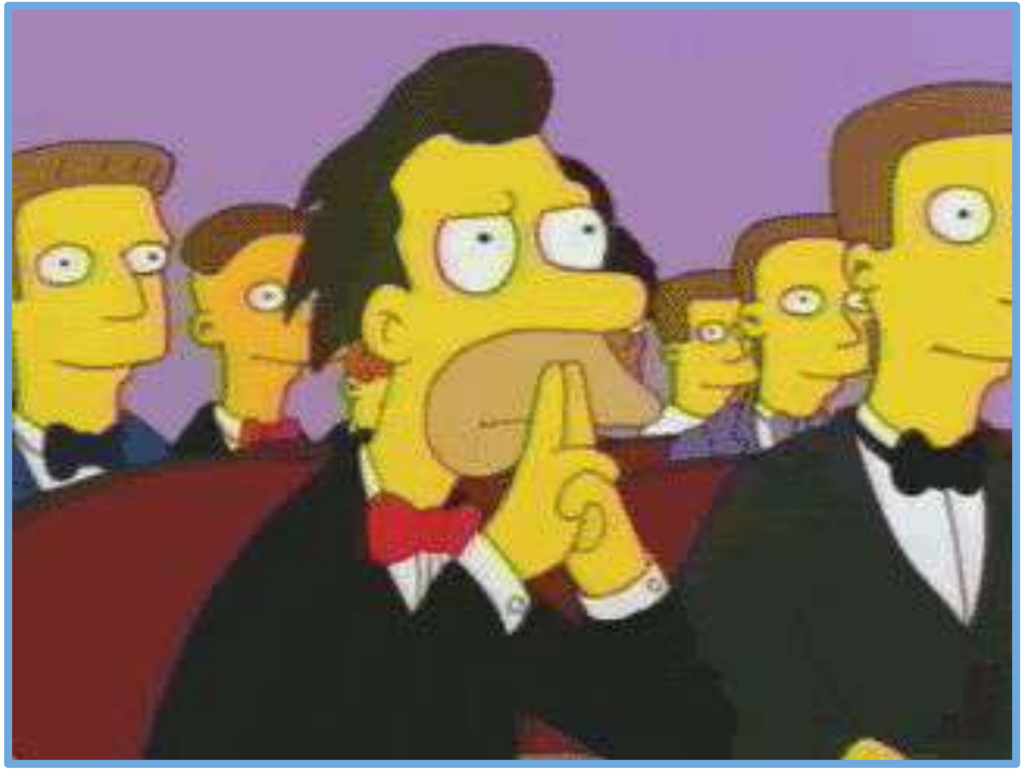


Hologenomics

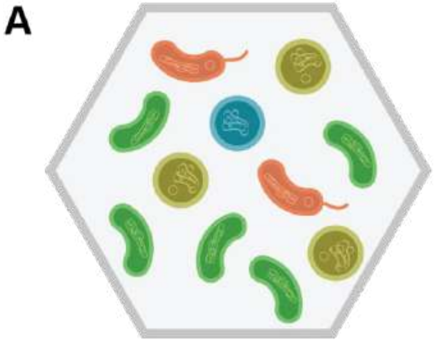
INTERESTING READINGS

METAGENOMIC SPACE

Hologenomics

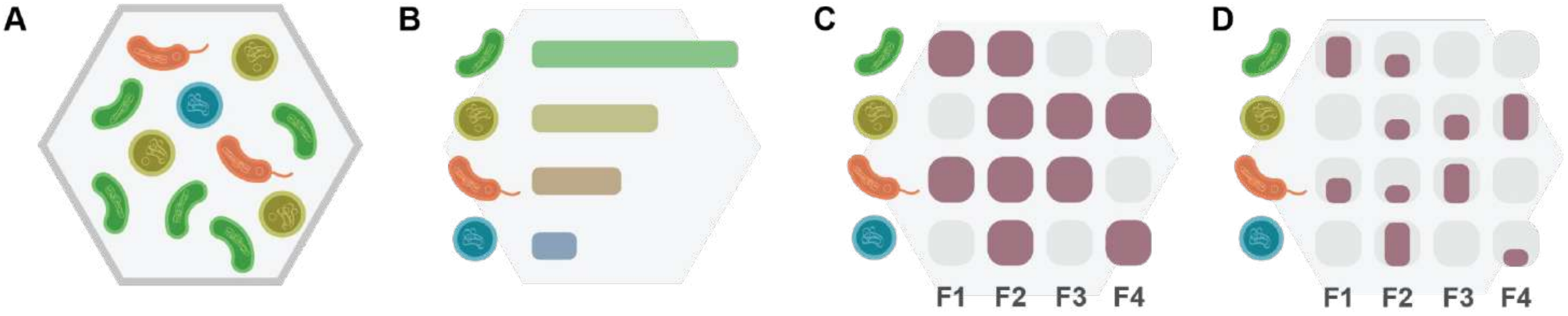


Hologenomics



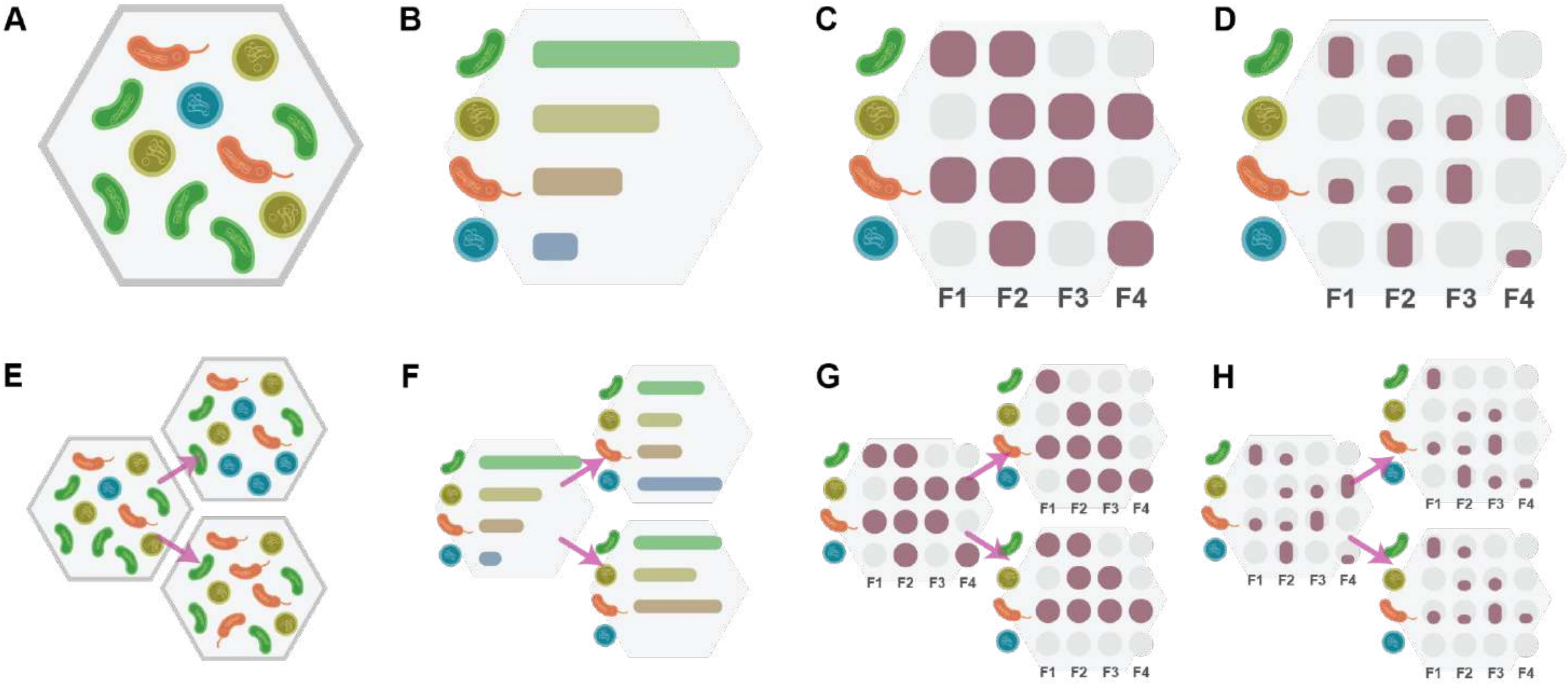
METAGENOMIC STATE

Hologenomics

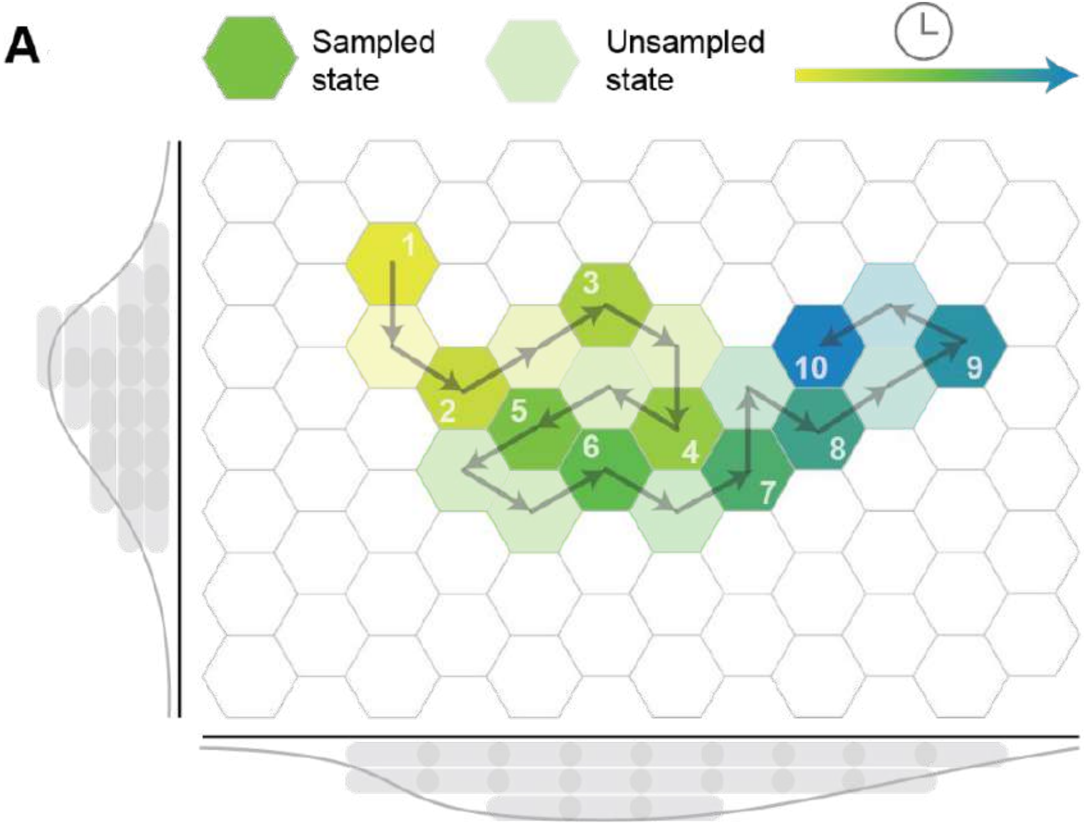


METAGENOMIC STATE

Hologenomics

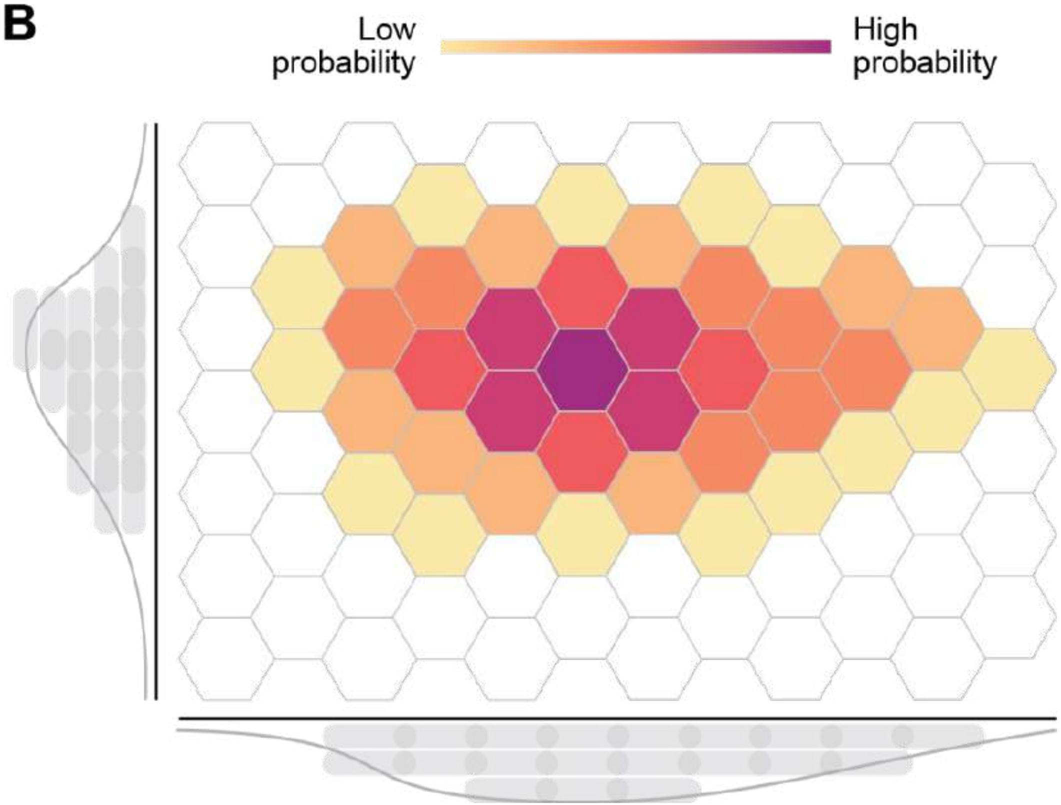


Hologenomics



METAGENOMIC SPACE

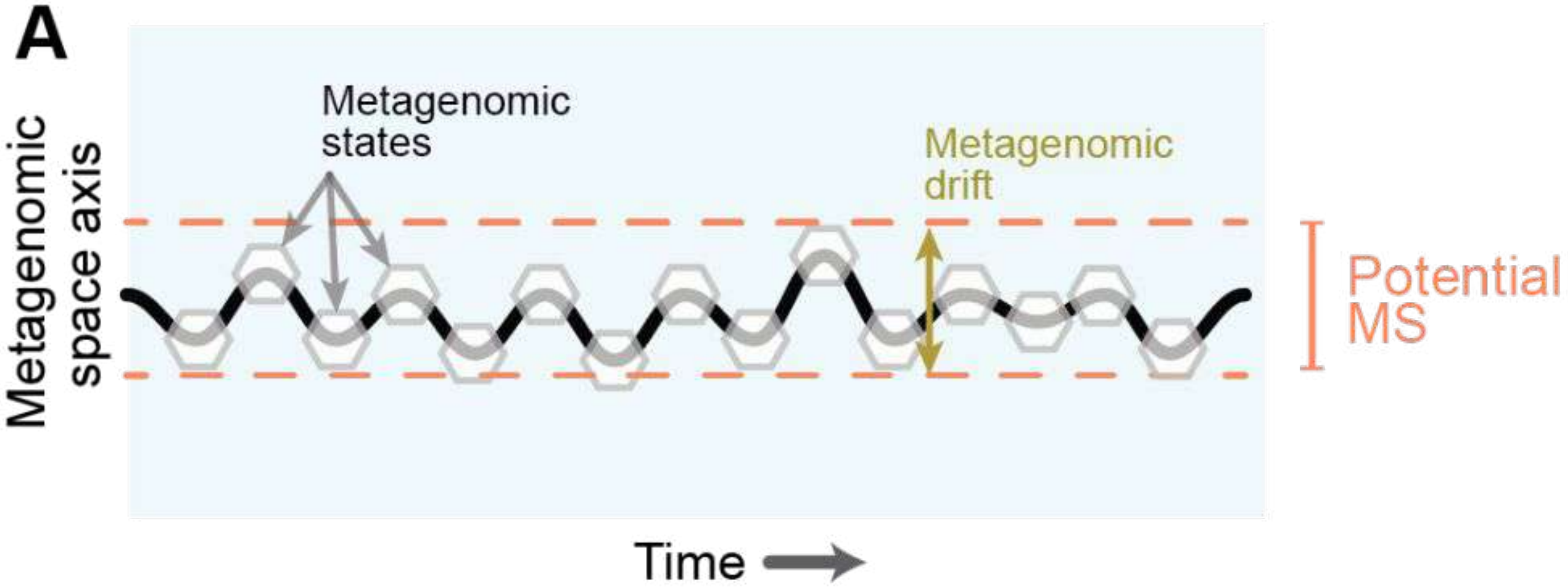
Hologenomics



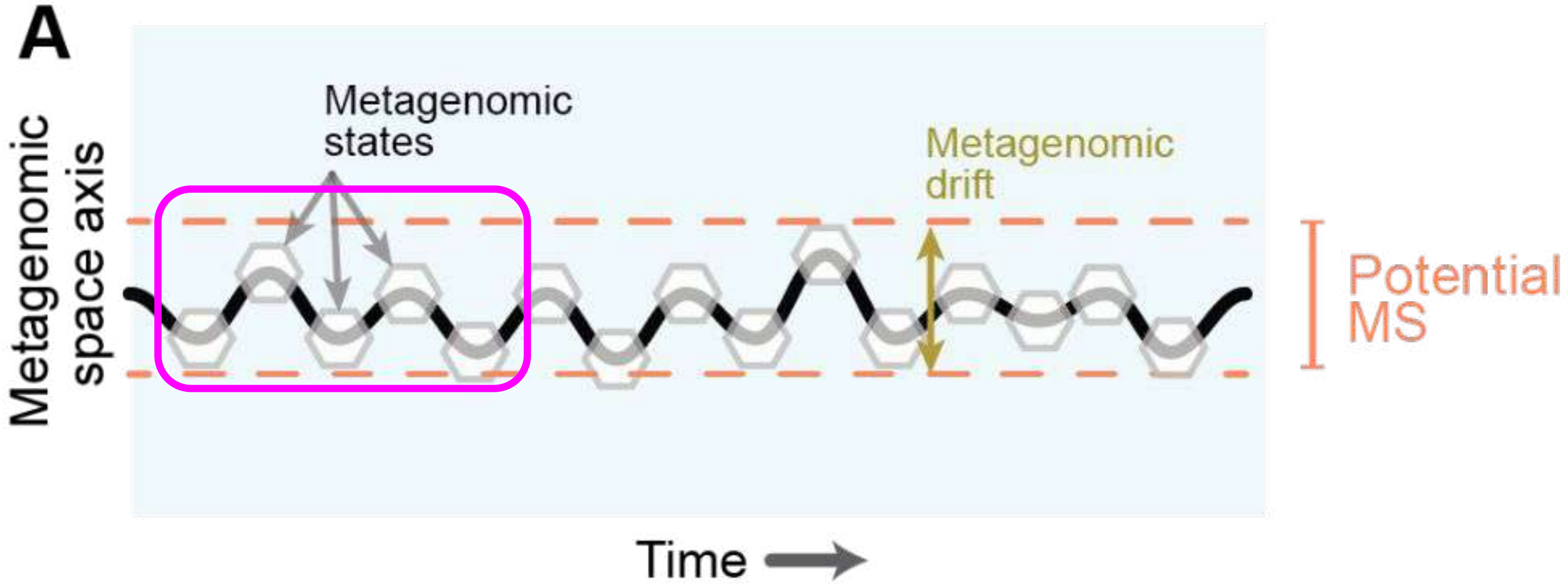
METAGENOMIC SPACE MODEL

FishMed-PhD

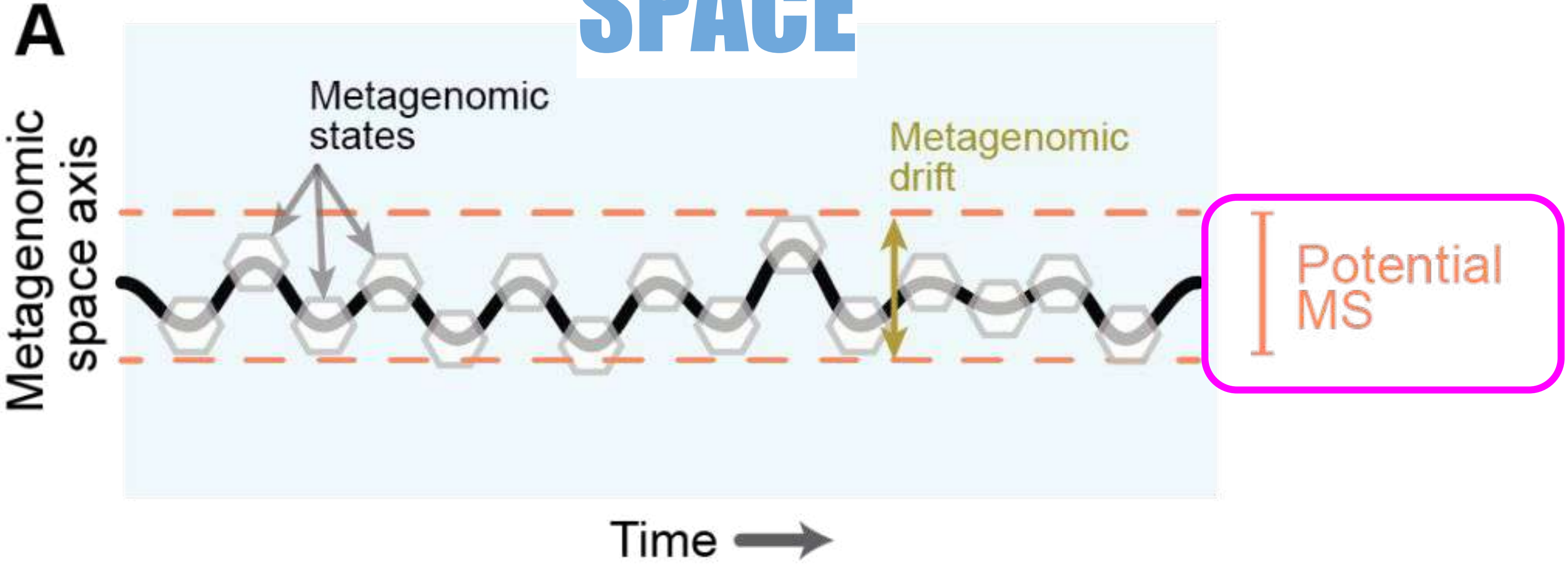
METAGENOMIC STATE DYNAMICS



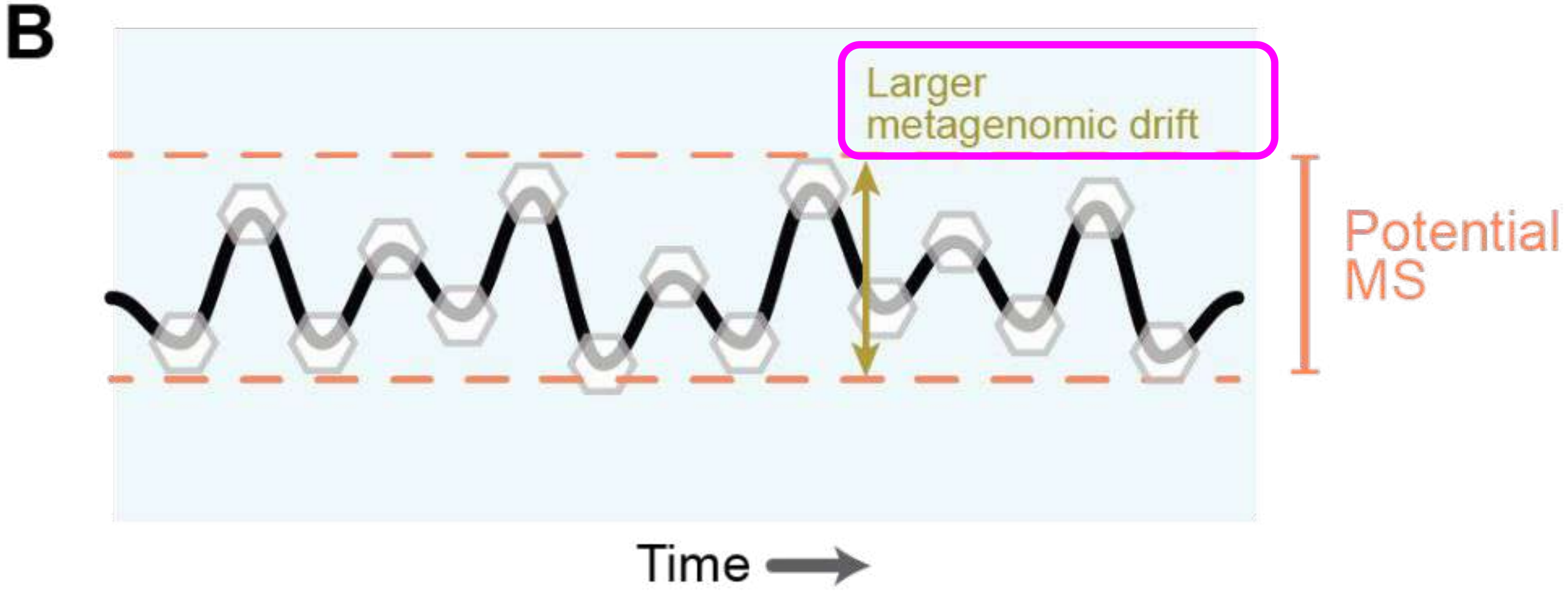
METAGENOMIC STATE DYNAMICS



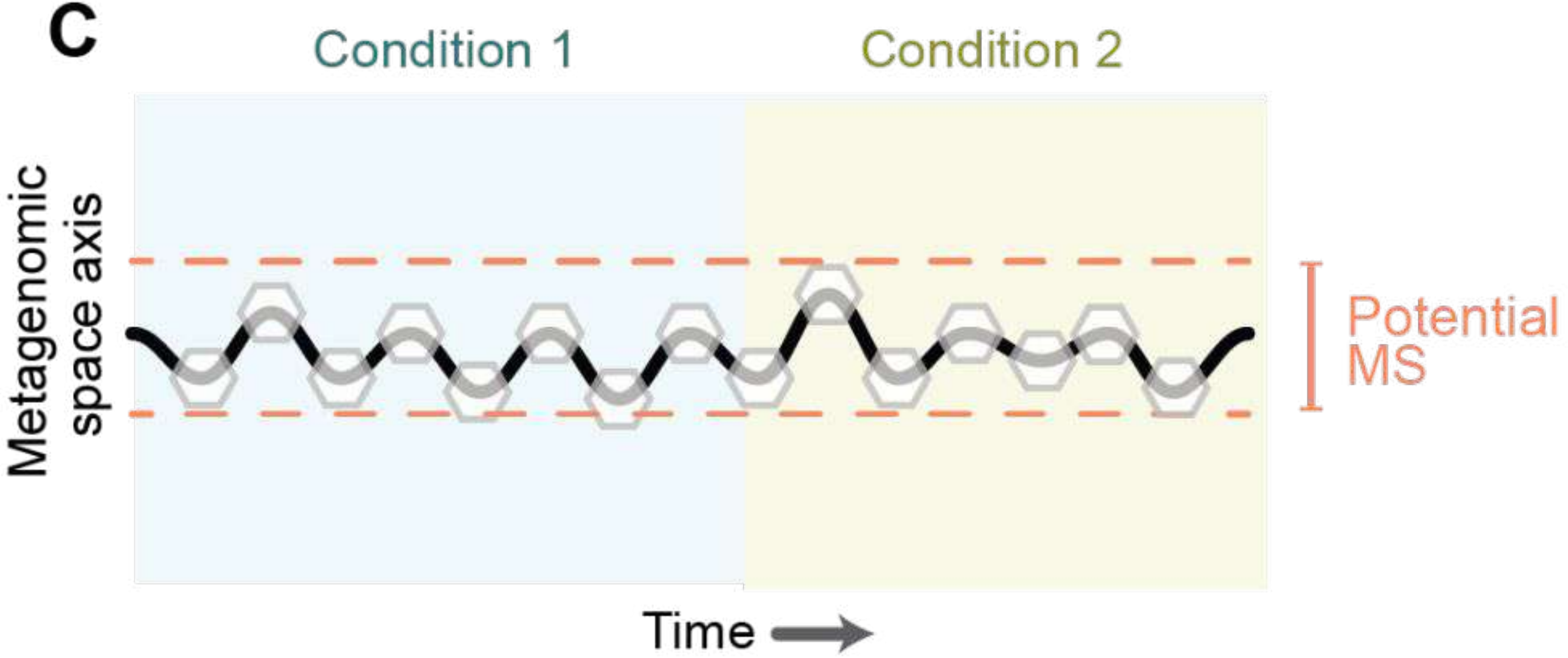
POTENTIAL METAGENOMIC SPACE



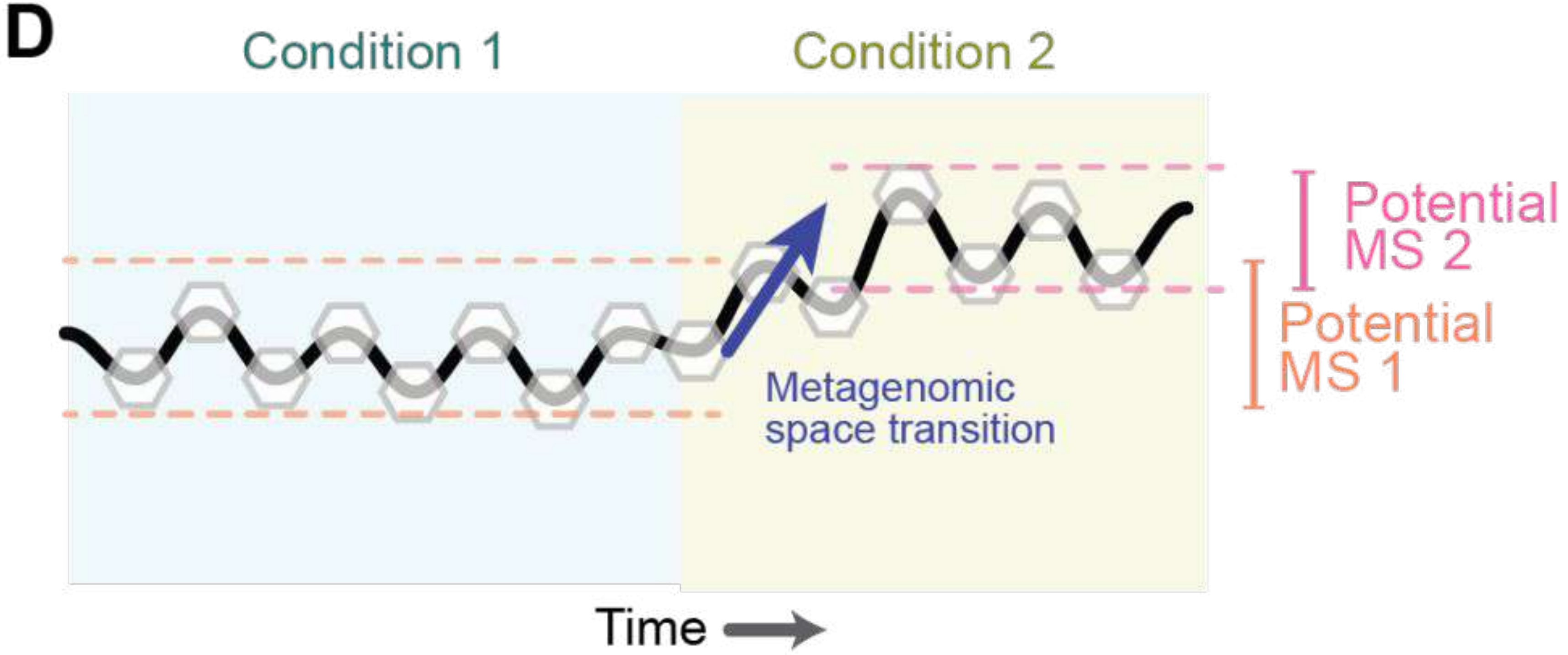
Hologenomics



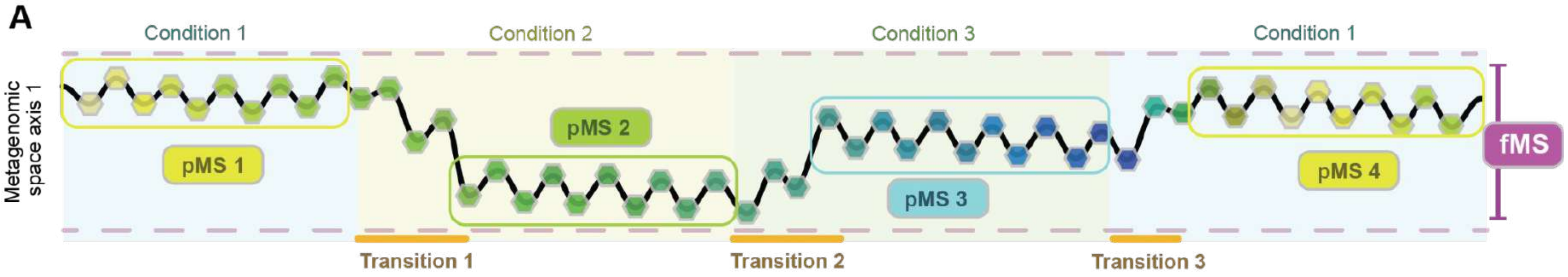
Hologenomics



Hologenomics

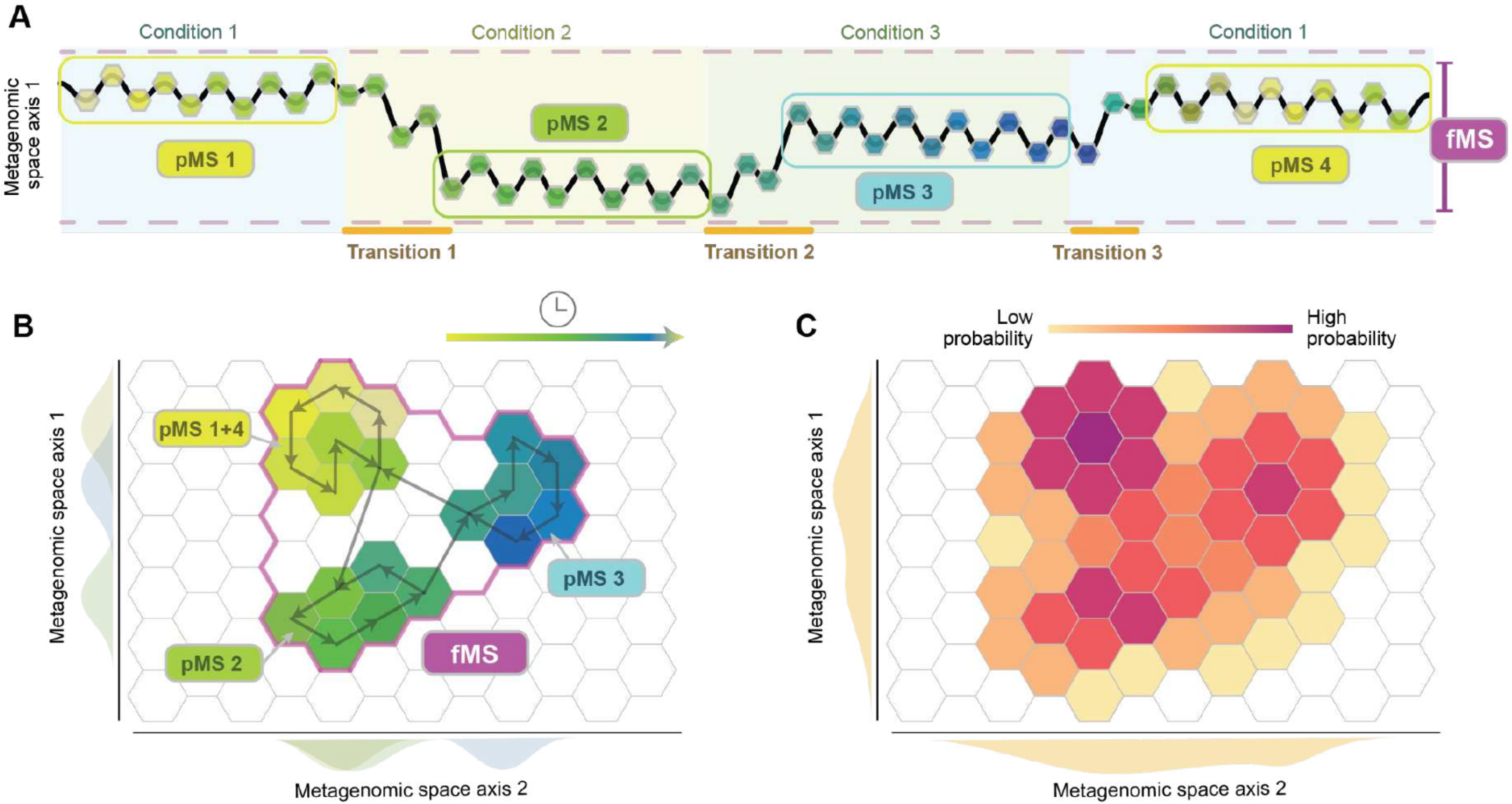


Hologenomics



FUNDAMENTAL METAGENOMIC SPACE

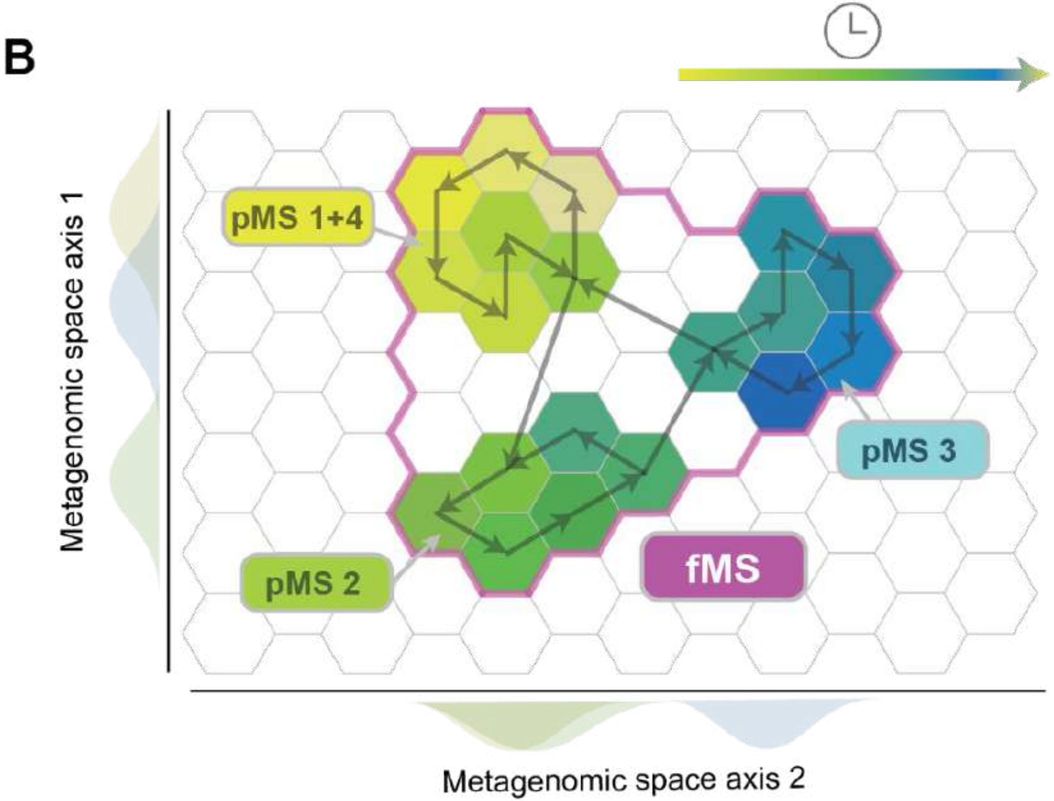
Hologenomics



FishMed-PhD

WHAT FACTORS DO DETERMINE POTENTIAL AND FUNDAMENTAL METAGENOMIC SPACES?

Hologenomics



FishMed-PhD

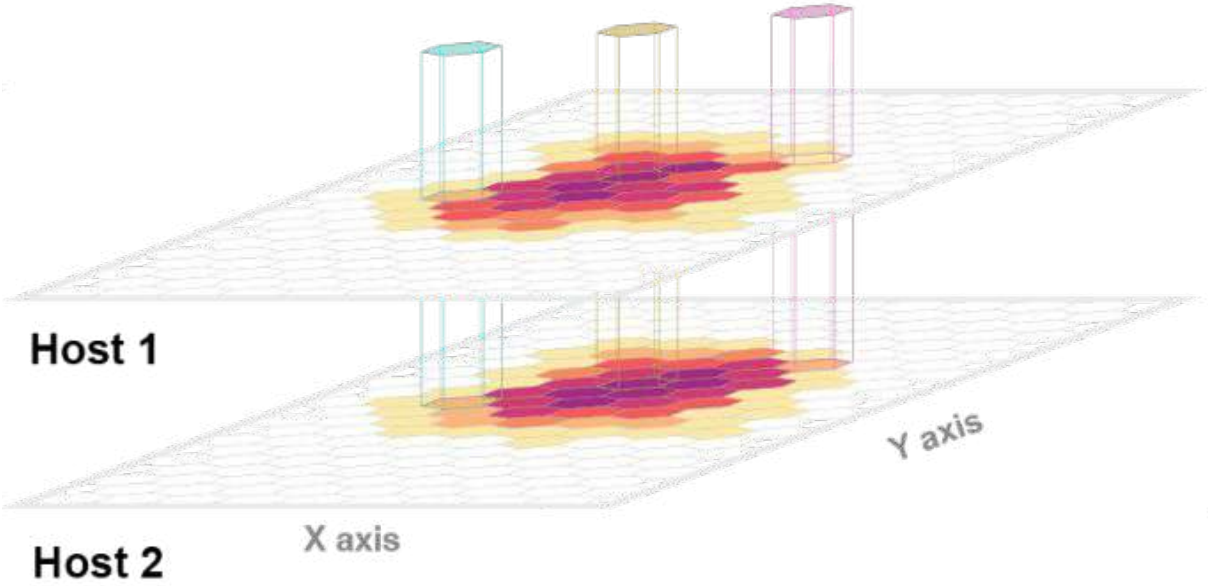
HOW CAN METAGENOMIC SPACES BE ESTIMATED?

Hologenomics

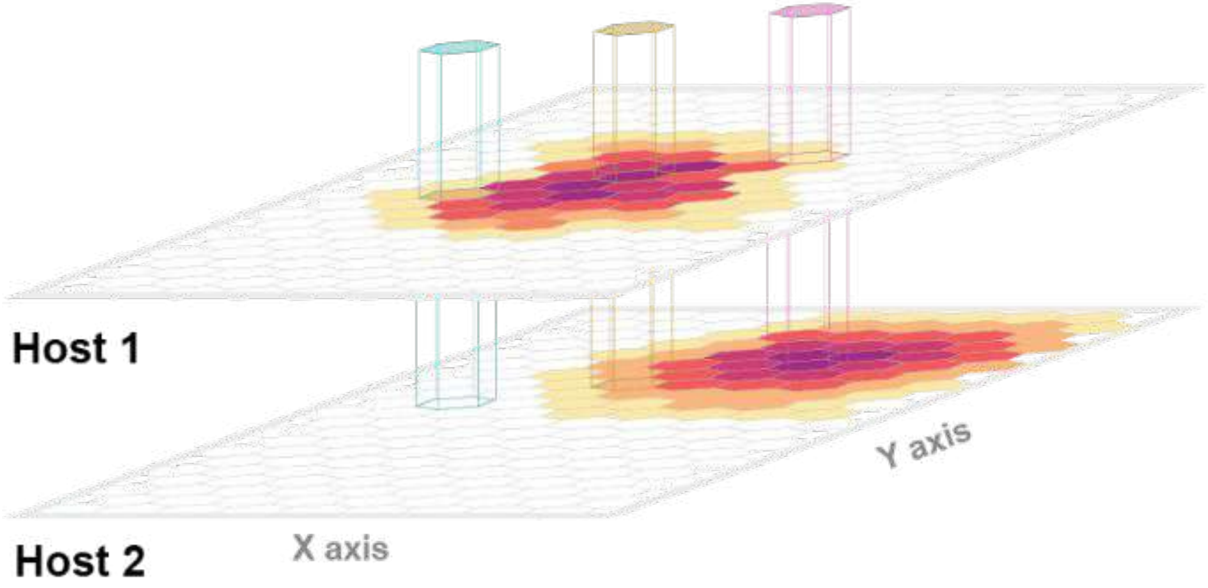
FishMed-PhD

WHAT ARE THE IMPLICATIONS OF METAGENOMIC SPACES?

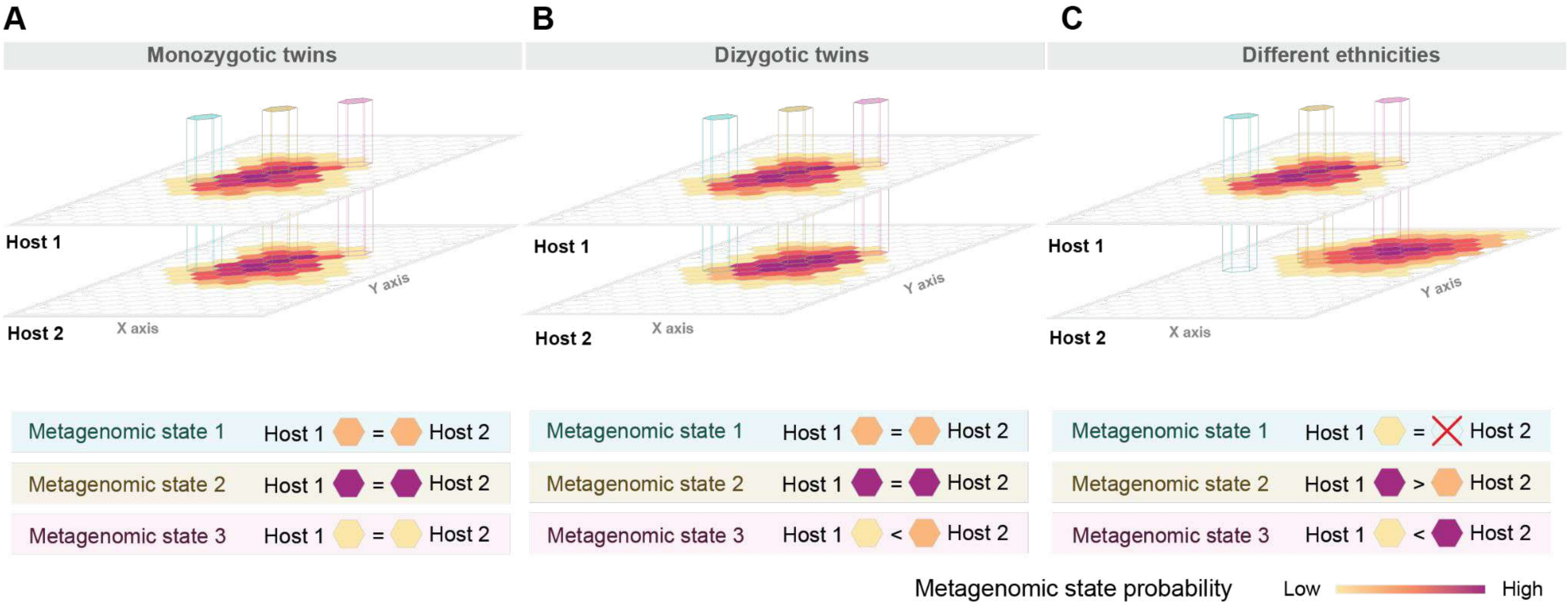
Hologenomics



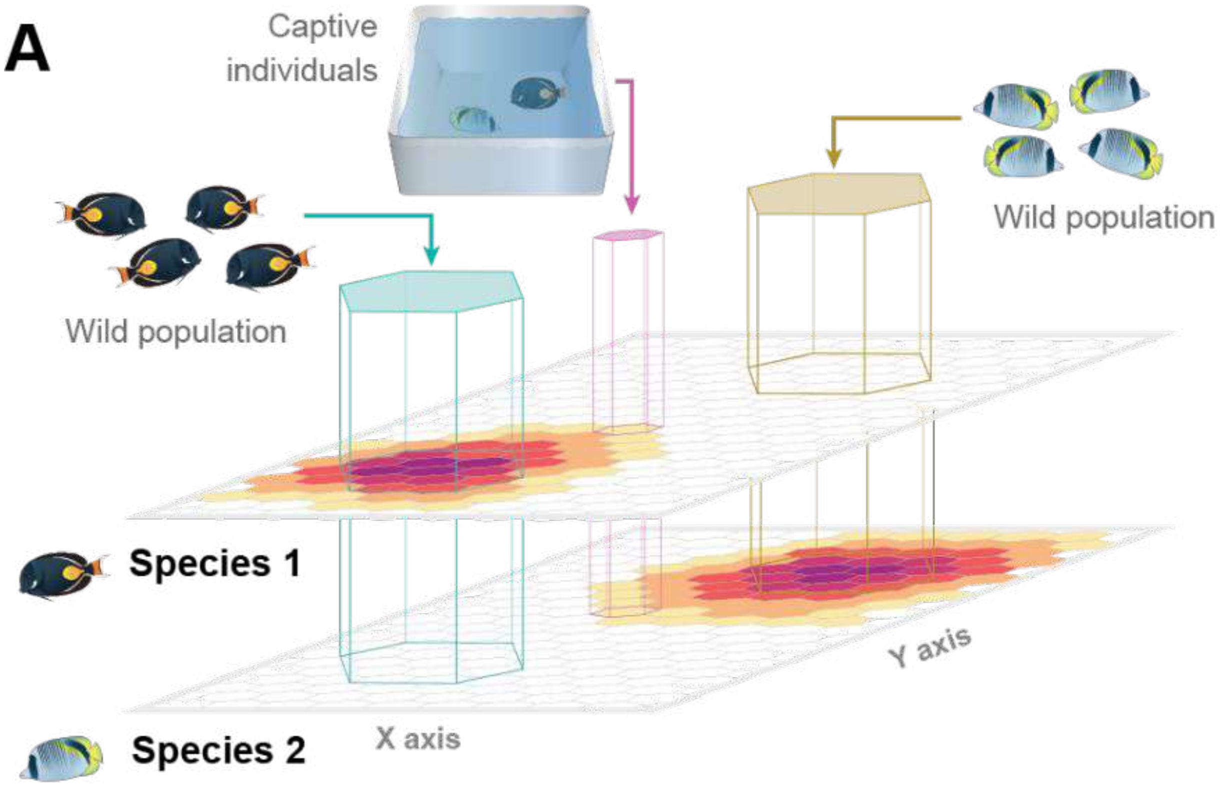
Hologenomics



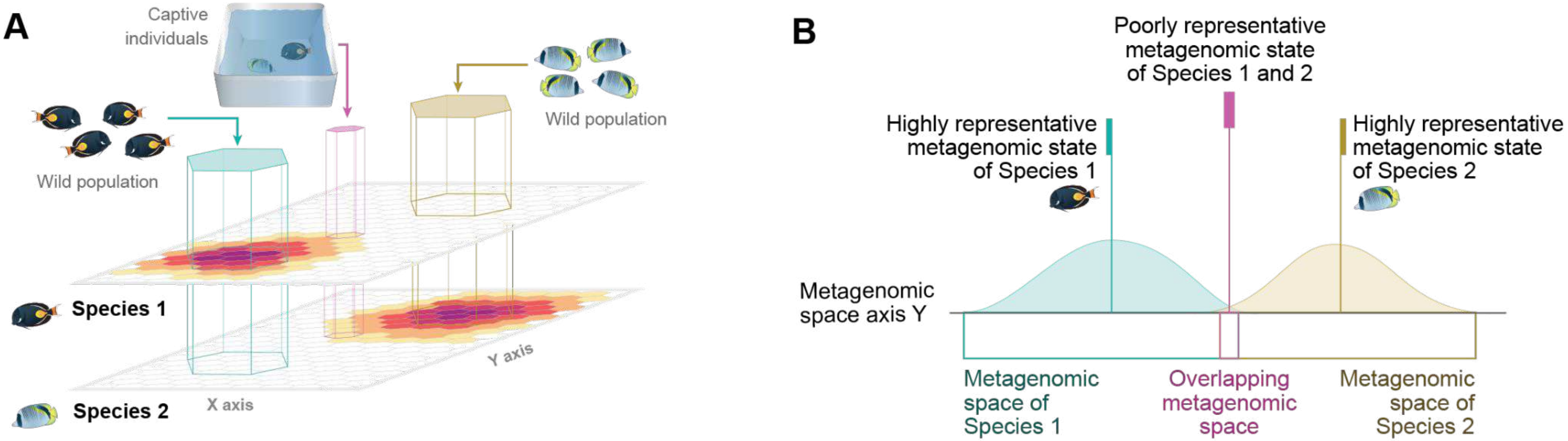
Hologenomics



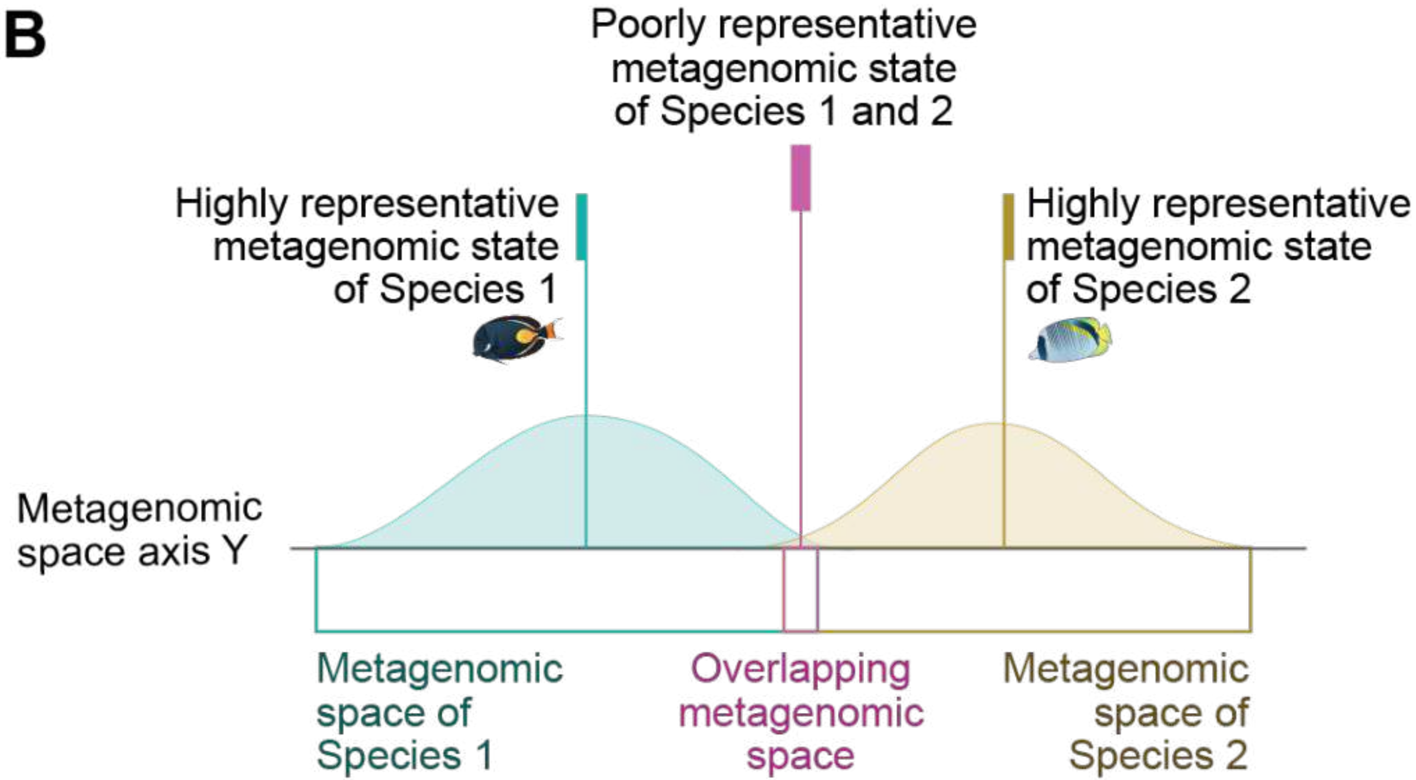
Hologenomics



Hologenomics



Hologenomics



FishMed-PhD

Hologenomics

applied to host-microbiota interactions in marine organisms

Antton Alberdi

University of Copenhagen

antton.alberdi@sund.ku.dk

alberdilab.dk

CEH | CENTER FOR EVOLUTIONARY HOLOGENOMICS

UNIVERSITY OF COPENHAGEN

FishMed-PhD

Session 3

HOLOGENOMICS to study animal-microbiota interactions

Hologenomics

How would you define hologenomics?

Hologenomics

Hologenomics

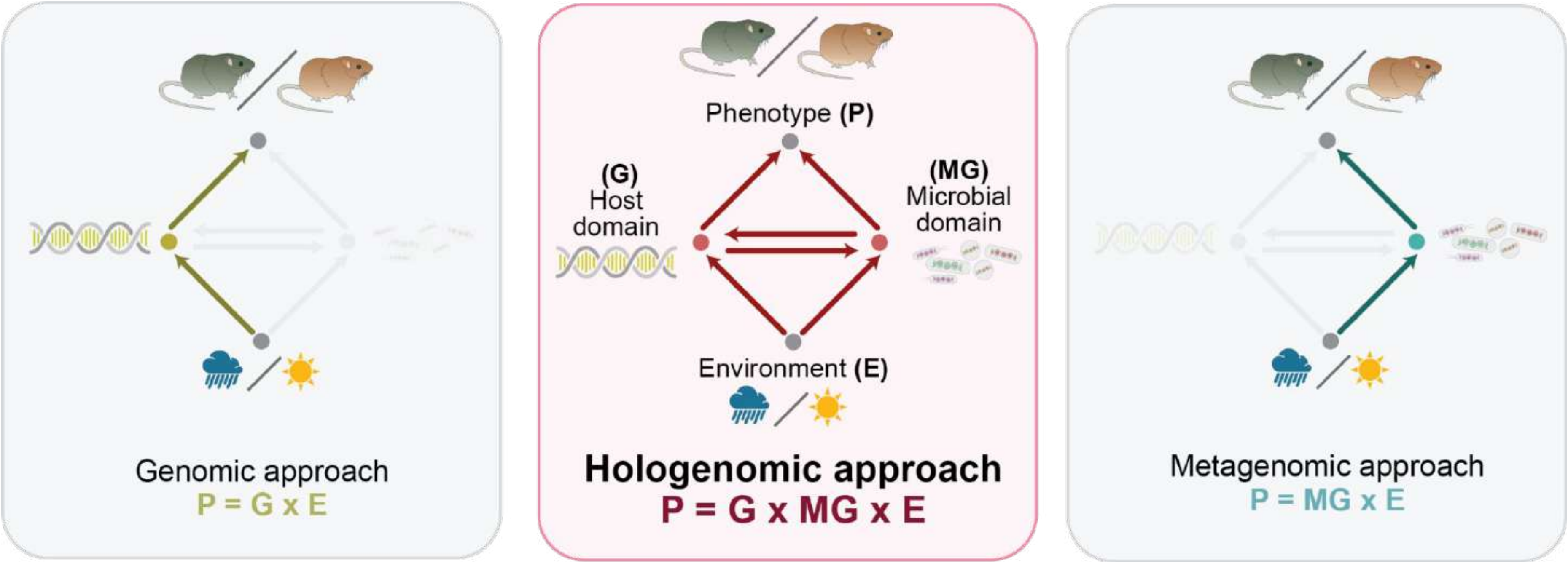
The integrated study of the genetic features of a eukaryotic host alongside that of its associated microbes

Hologenomics

Hologenome

The entirety of the genetic information contained by a eukaryotic host and the microbial community associated with that host.

Hologenomics



Phenotype **(P)**

(G)
Host
domain

(MG)
Microbial
domain

Environment **(E)**

Genomic approach
P = G x E

Hologenomic approach
P = G x MG x E

Metagenomic approach
P = MG x E

Hologenomics

$$P = G \times E$$

Hologenomics

$$P = MG \times E$$

Hologenomics

$$P = G \times MG \times E$$

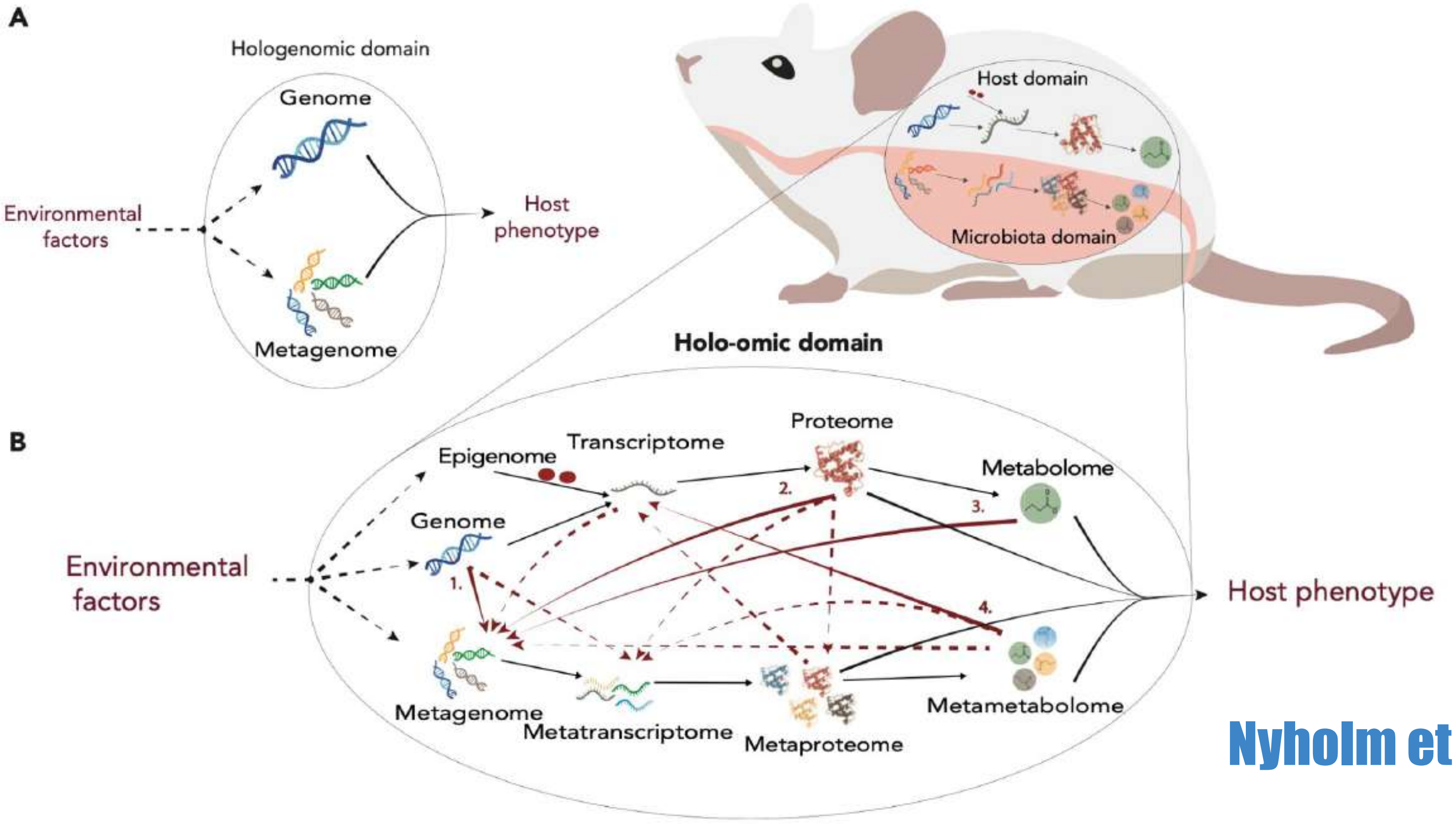
Hologenomics

FishMed-PhD

$$P = G \times MG \times E$$

Interactions

Hologenomics



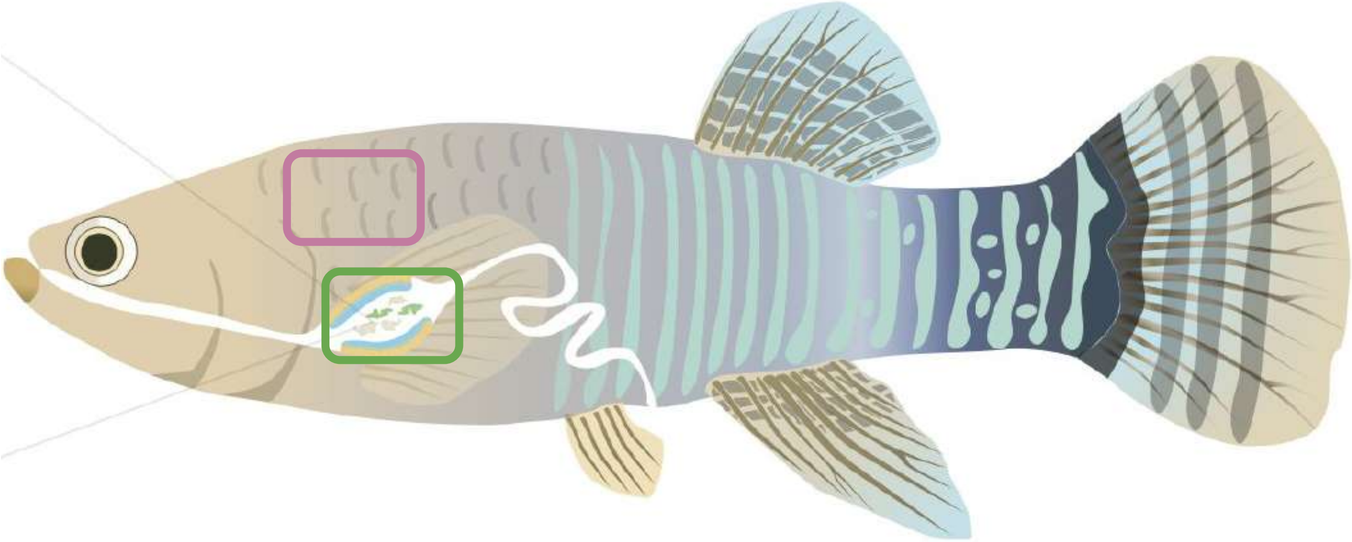
FishMed-PhD

Nyholm et al. 2020

iScience

Sample collection preservation and extraction

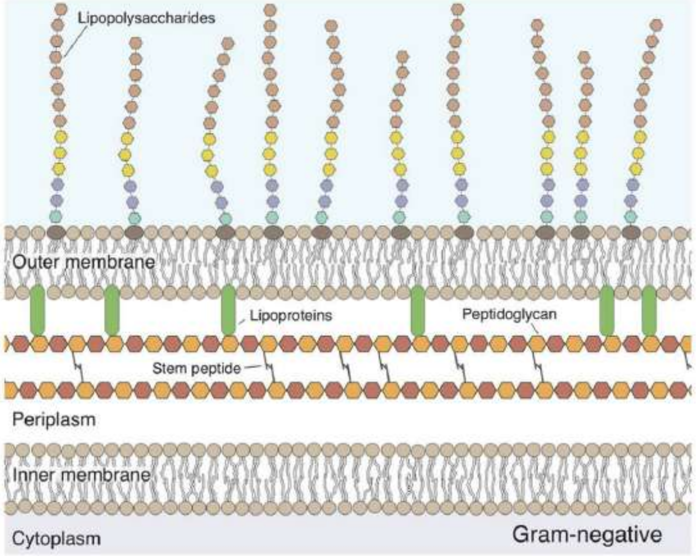
Hologenomics

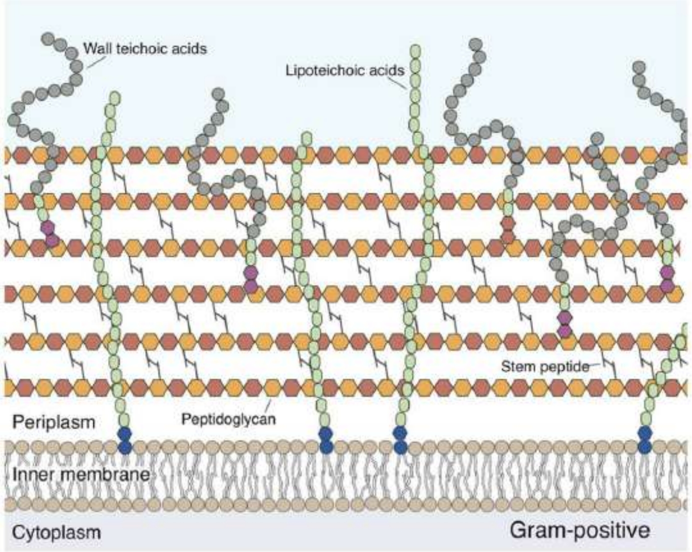


Microbial DNA

Host DNA

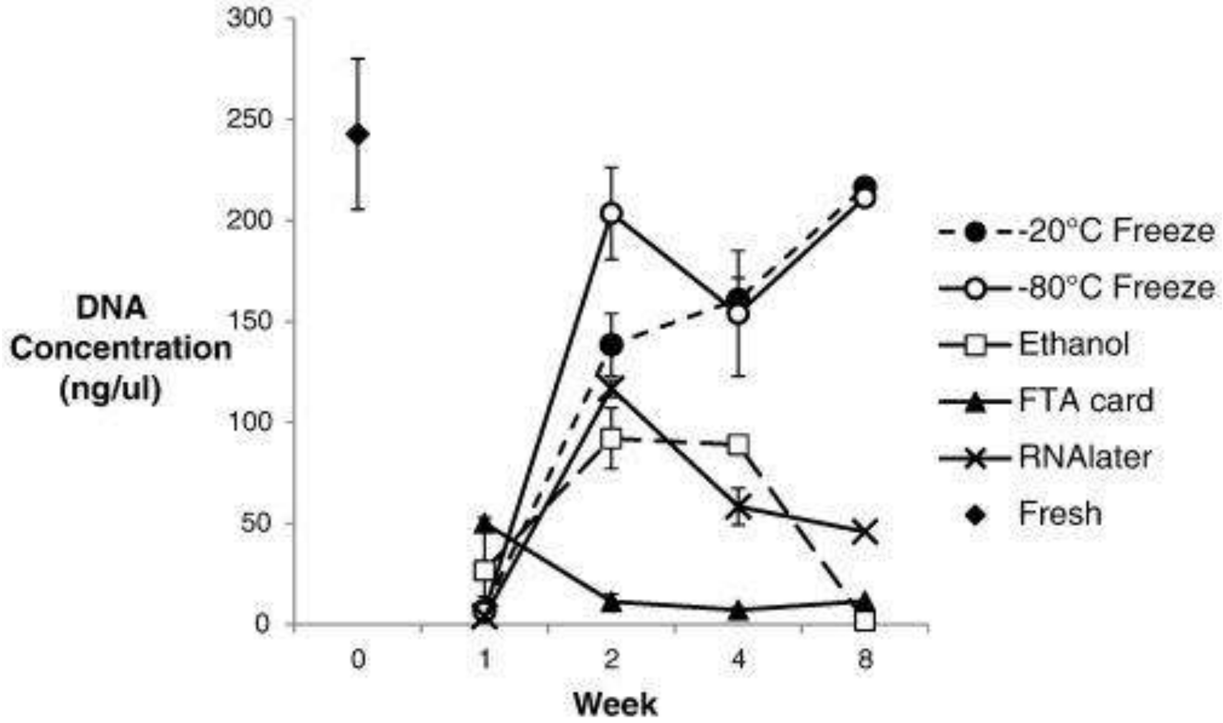
Hologenomics





Auer and Weibel 2017
Biochemistry

Hologenomics

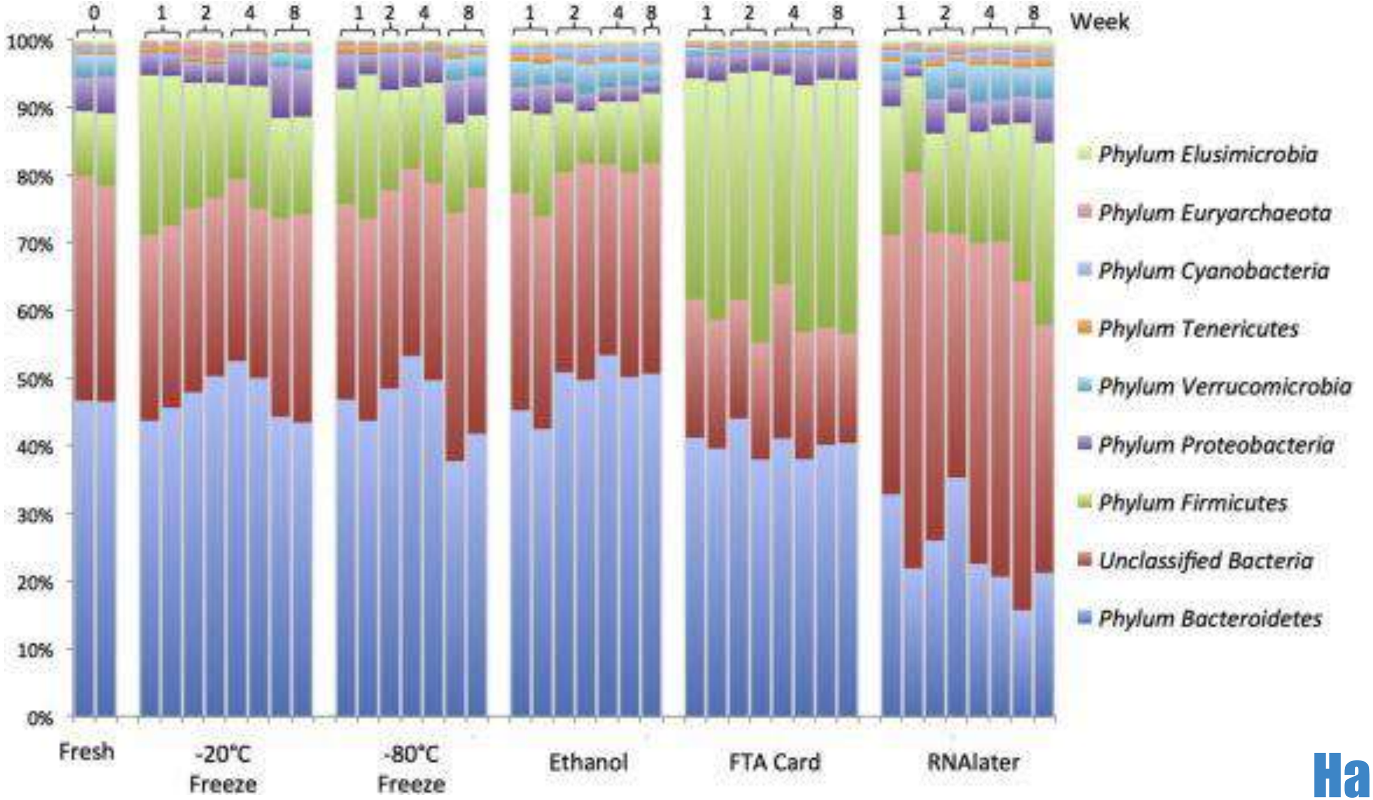


Hale et al. 2015

Journal of Microbiological Methods

Hologenomics

FishMed-PhD



Hale et al. 2015

Journal of Microbiological Methods

Hologenomics

FishMed-PhD

Ready-to-use tubes

Up to 162 sample collection tubes pre-labelled and pre-filled with preservation buffer.

Different-sized swabs

For sampling the oral cavity and anus of animals. Adjusted to the size of the targeted animals.

Sampling guidelines

Simple guidelines to ensure standardised sample collection and preservation.

Complementary material

Collaborators with limited resources can request complementary materials such as gloves, tweers or face masks.

EARTH HOLOGENOME INITIATIVE

www.earthhologenome.org

Hologenomics

How much sequencing?

Hologenomics

Lactobacillus	2 MB	2,000,000 bp
Enterococcus	3 MB	3,000,000 bp
Bacteroides	7 MB	7,000,000 bp

Hologenomics

Illumina NovaSeq S4

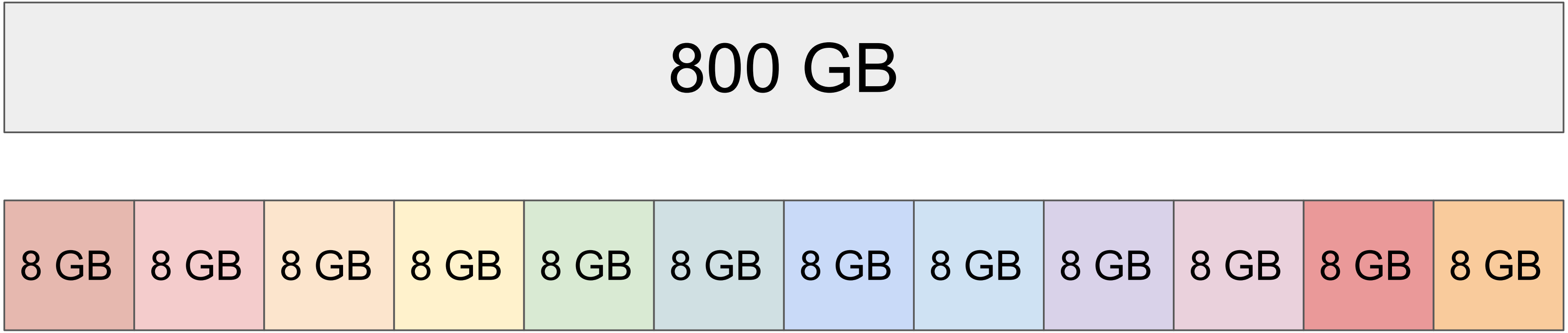
800 GB
800,000 MB

Lactobacillus	*Enterococcus*	*Bacteroides*
2 MB	3 MB	7 MB
x400,000	**x260,000**	**x114,000**

Multiplexing

800 GB

Multiplexing



800 GB

8 GB | 8 GB | 8 GB | 8 GB | 8 GB | 8 GB | 8 GB | 8 GB | 8 GB | 8 GB | 8 GB | 8 GB

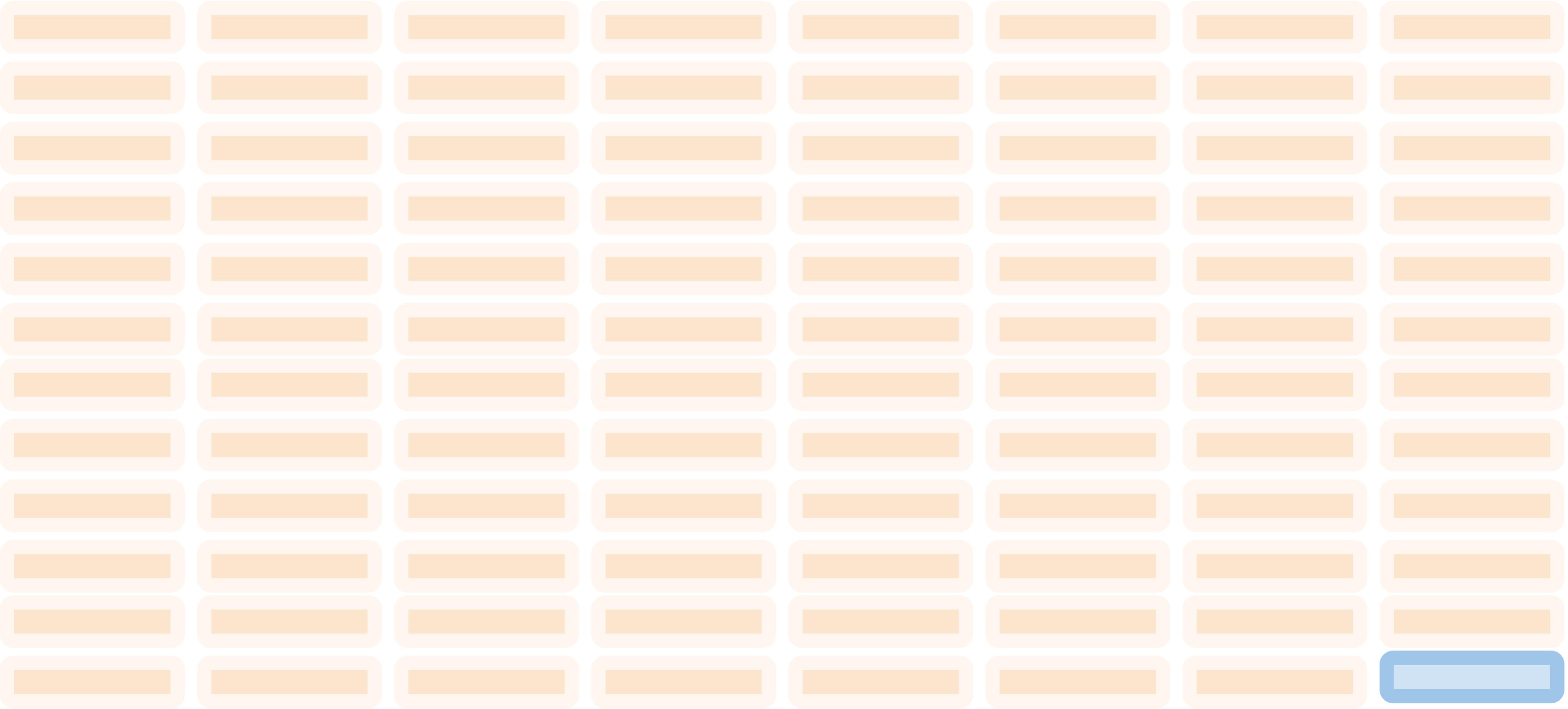
288 libraries

Hologenomics

8 GB
8,000 MB

Lactobacillus	*Enterococcus*	*Bacteroides*
2 MB	3 MB	7 MB
x4,000	x2,600	x1,140

Hologenomics



Host-bacterial fractions

8 GB

Host-bacterial fractions

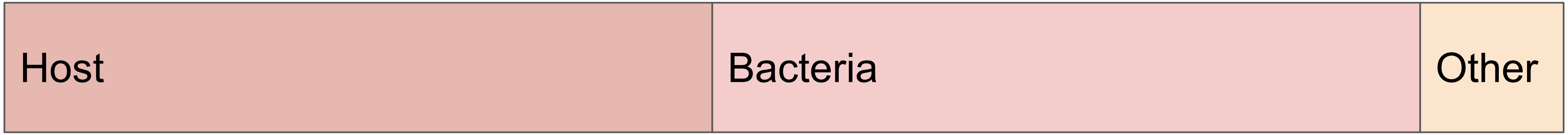
8 GB

Host

Bacteria

Other

Host-bacterial fractions

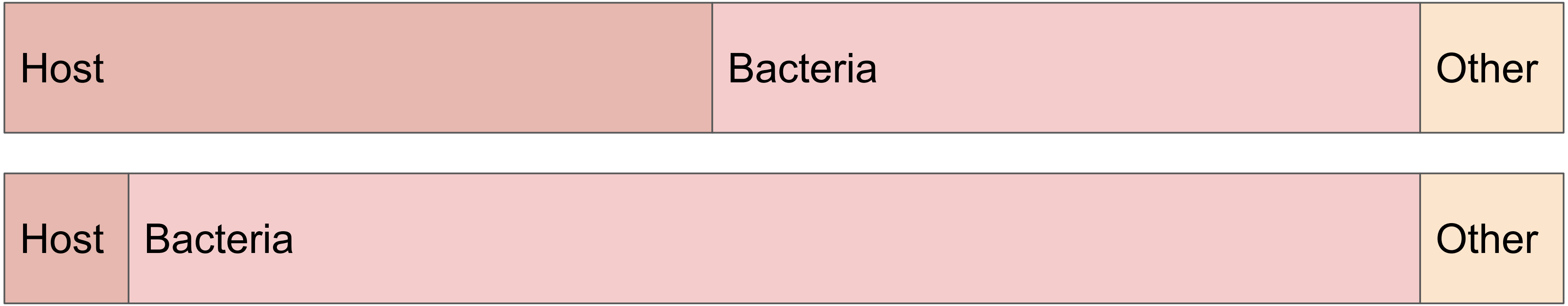


Host

Bacteria

Other

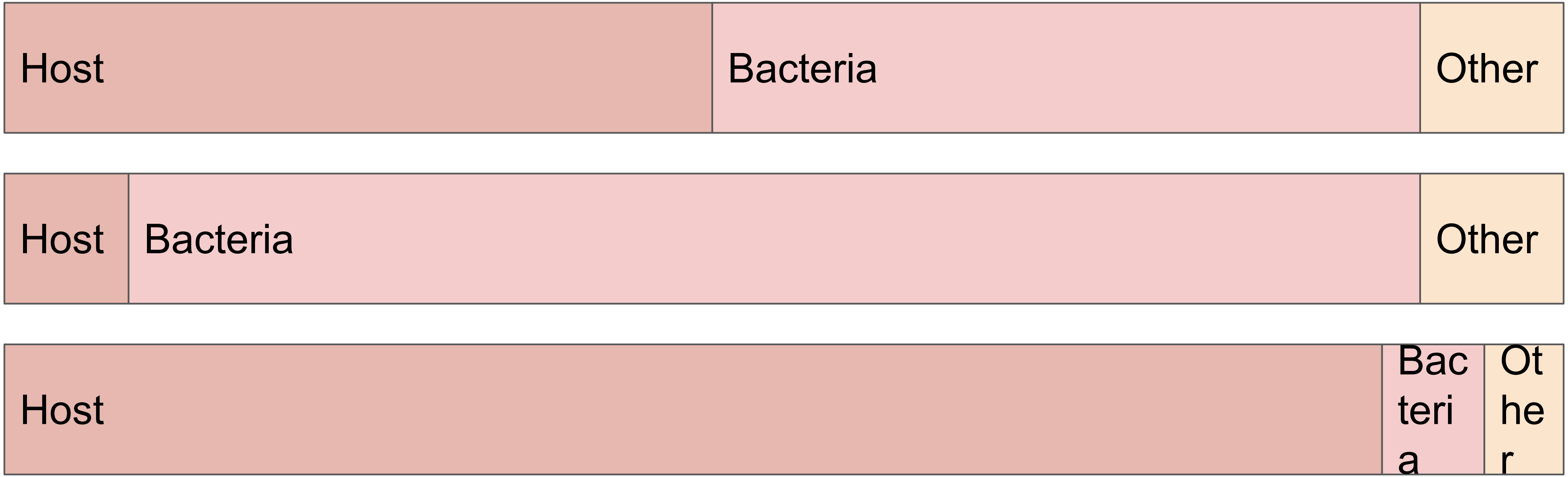
Host-bacterial fractions



Host Bacteria Other

Host Bacteria Other

Host-bacterial fractions

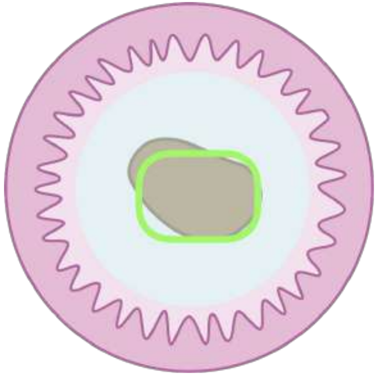


Host | Bacteria | Other

Host | Bacteria | Other

Host | Bacteria | Other

Hologenomics

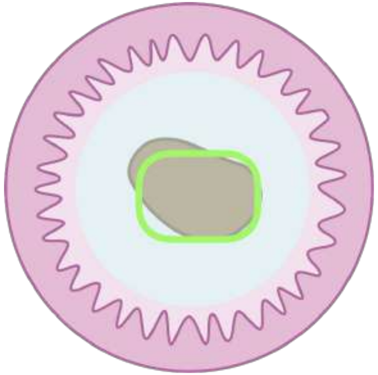


Ileum

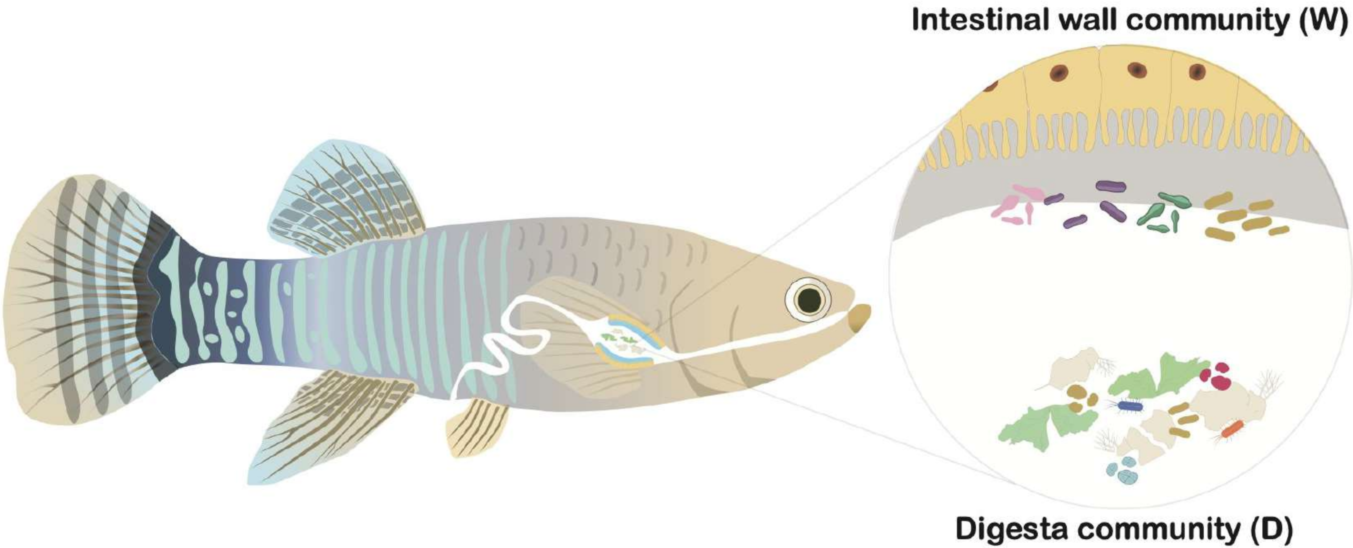
95% Host DNA

95% Microbial DNA

Caecum



Hologenomics



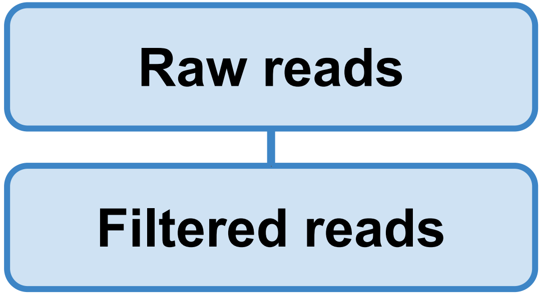
>99% host

90-95% host

FishMed-PhD

Bioinformatic approach

Hologenomics



Raw reads

Filtered reads

Hologenomics

Raw reads

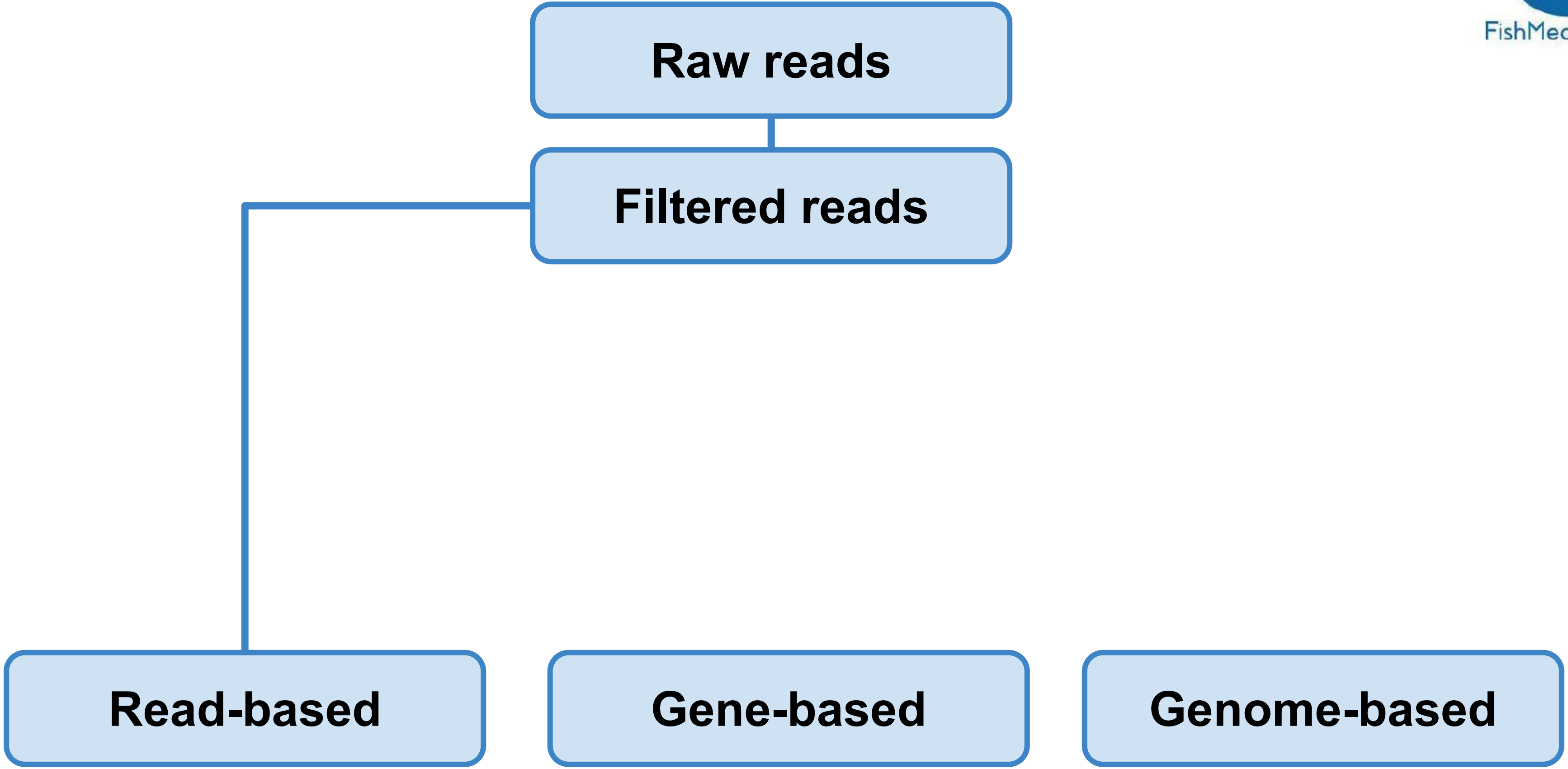
Filtered reads

Read-based

Gene-based

Genome-based

Hologenomics



Raw reads

Filtered reads

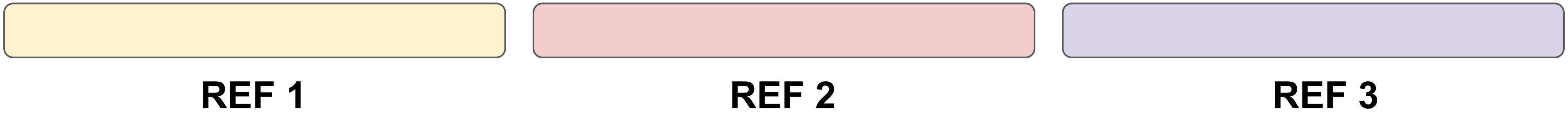
Read-based

Gene-based

Genome-based

Hologenomics

Read-based

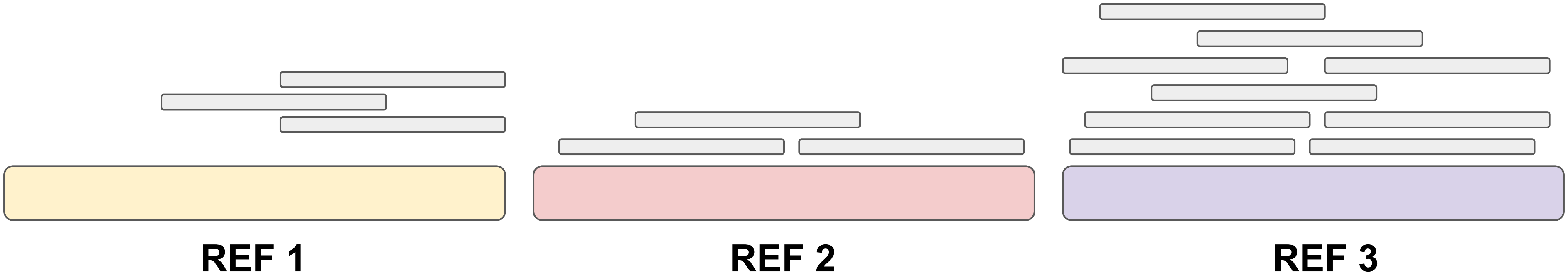


REF 1

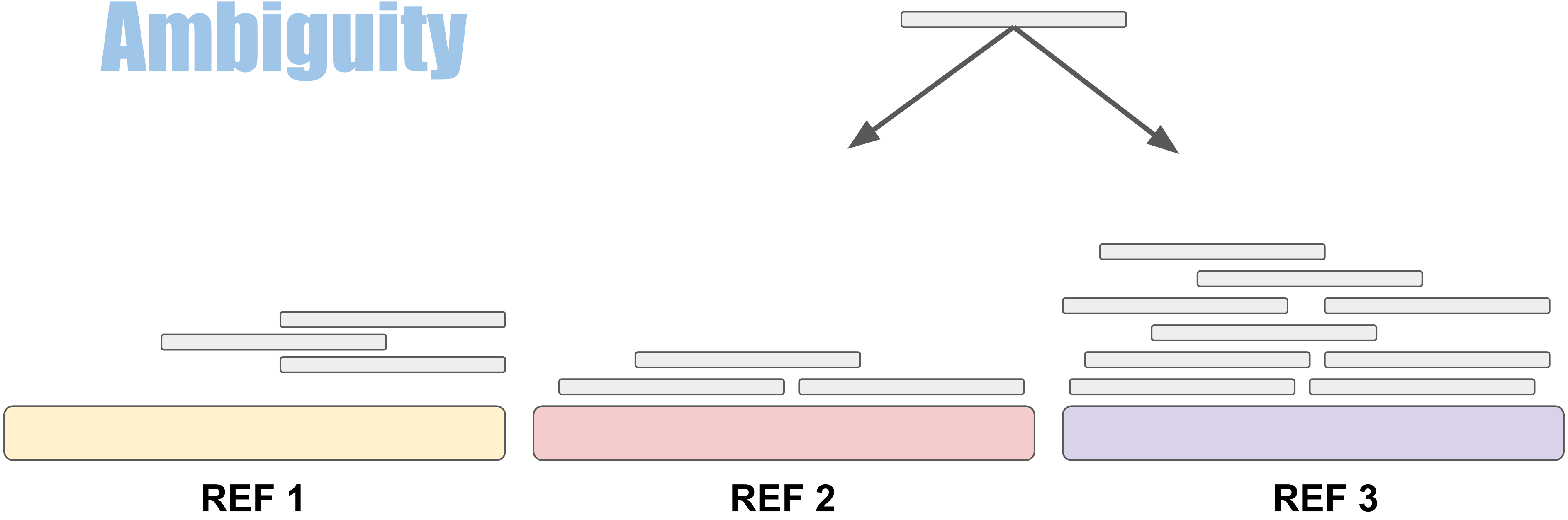
REF 2

REF 3

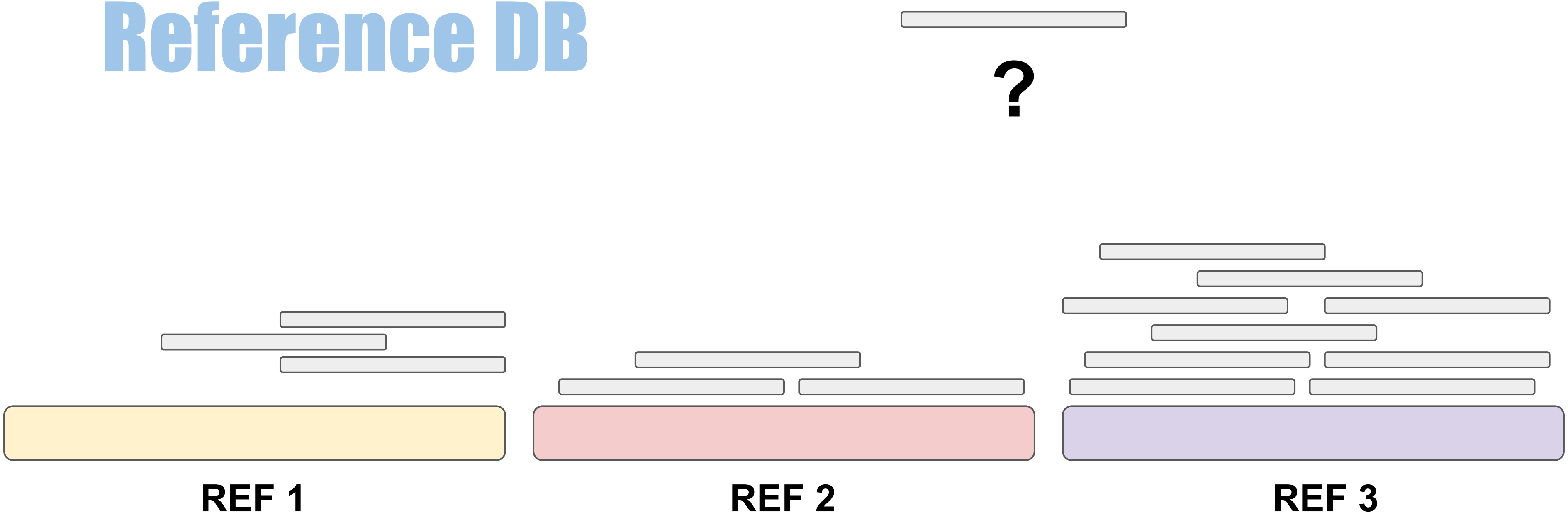
Read-based



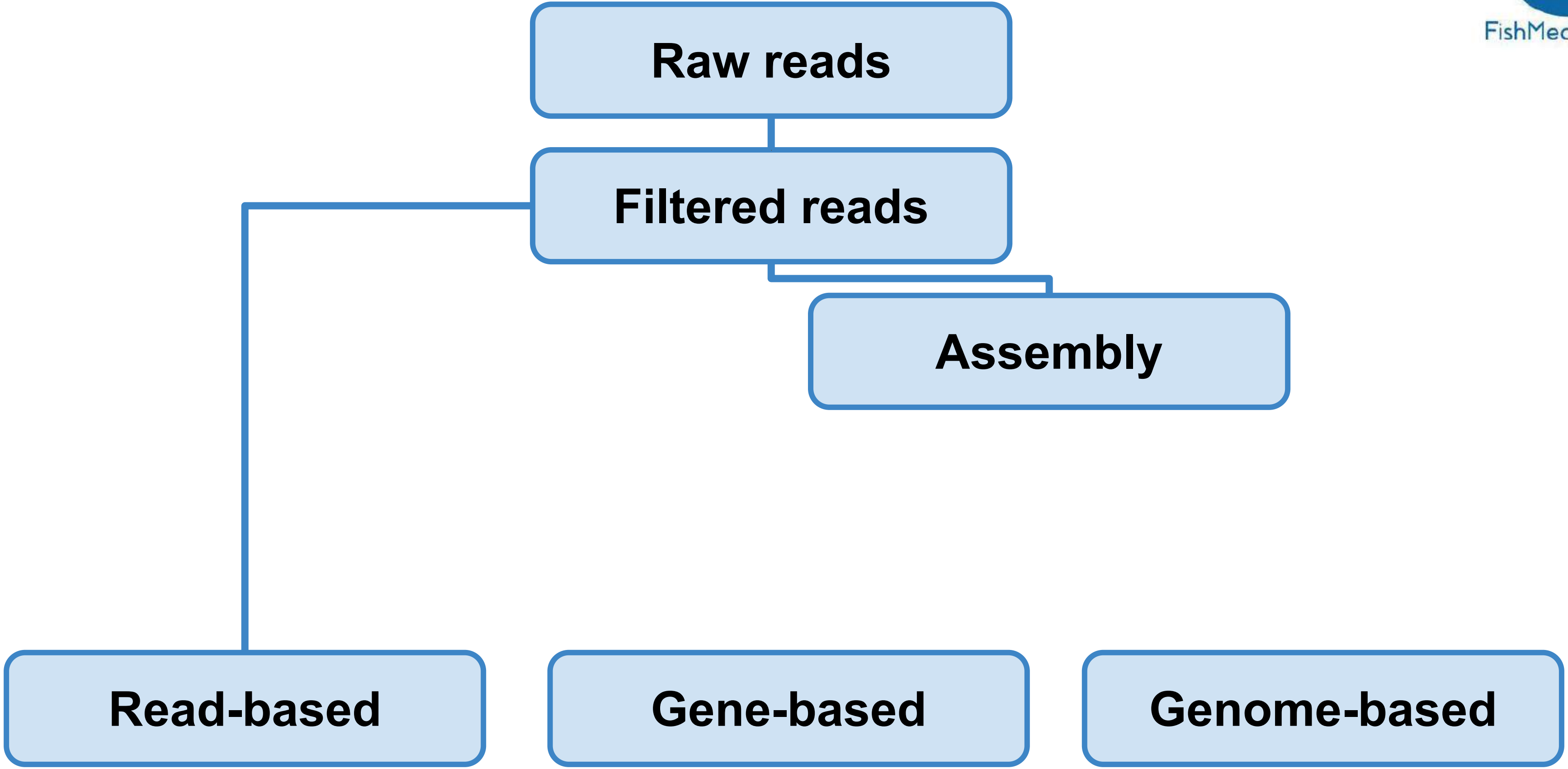
Read-based Ambiguity



Read-based
Reference DB



Hologenomics



Raw reads

Filtered reads

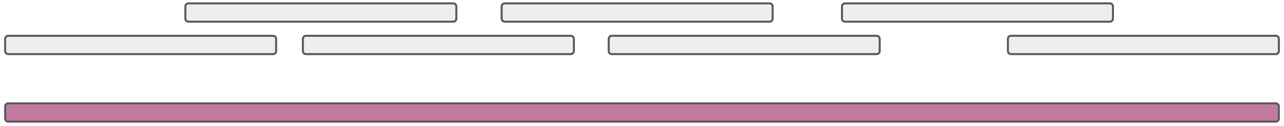
Assembly

Read-based

Gene-based

Genome-based

Assembly

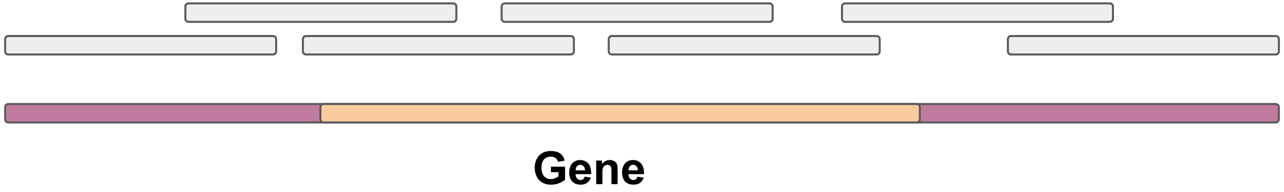


Contig

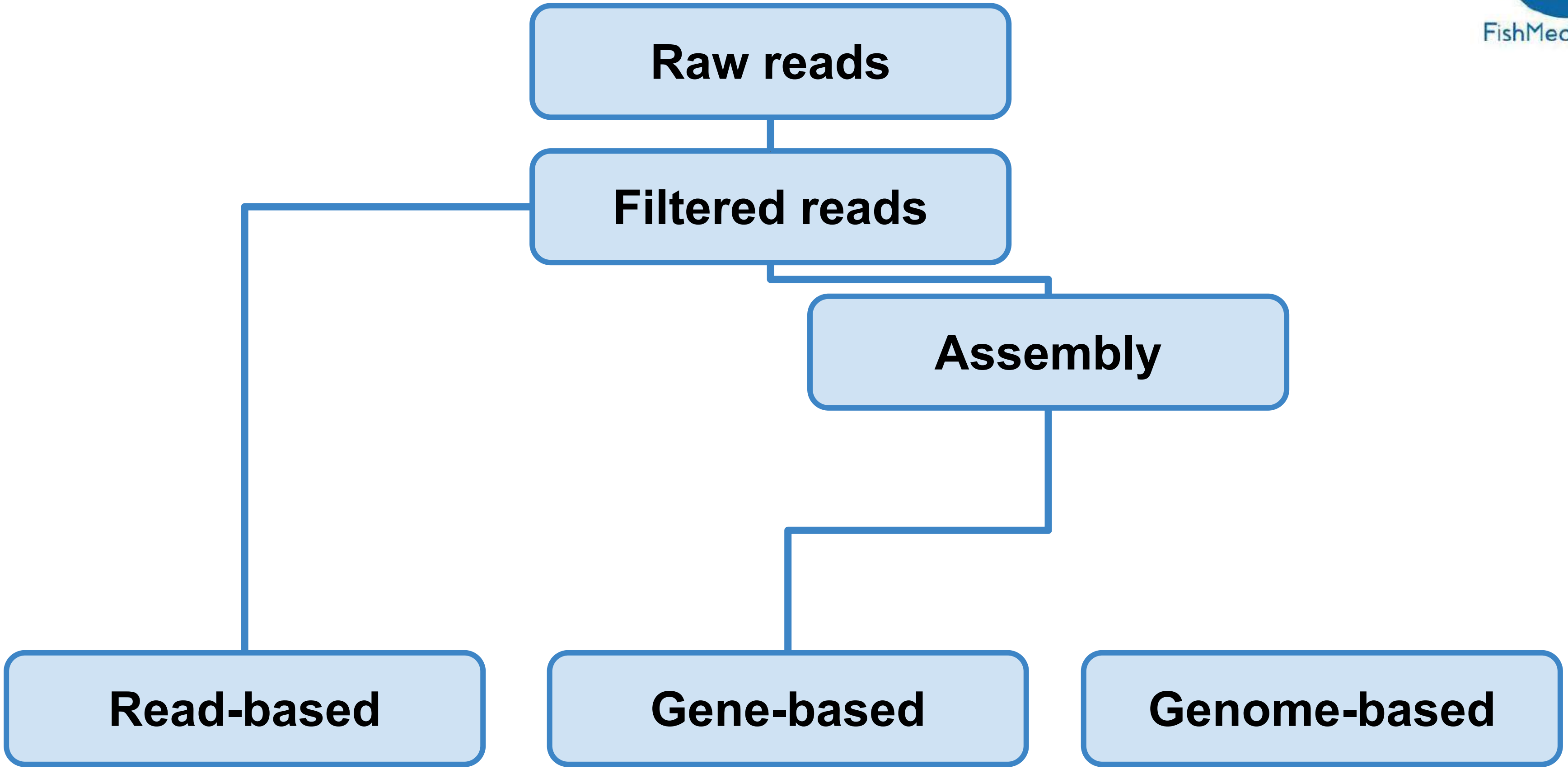
Hologenomics

Assembly

Gene prediction



Hologenomics



Raw reads

Filtered reads

Assembly

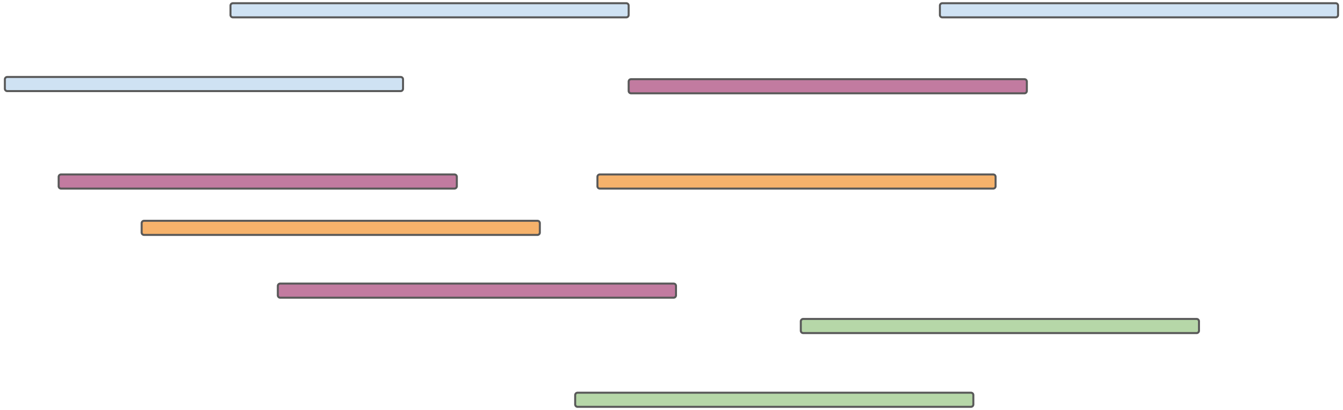
Read-based

Gene-based

Genome-based

Hologenomics

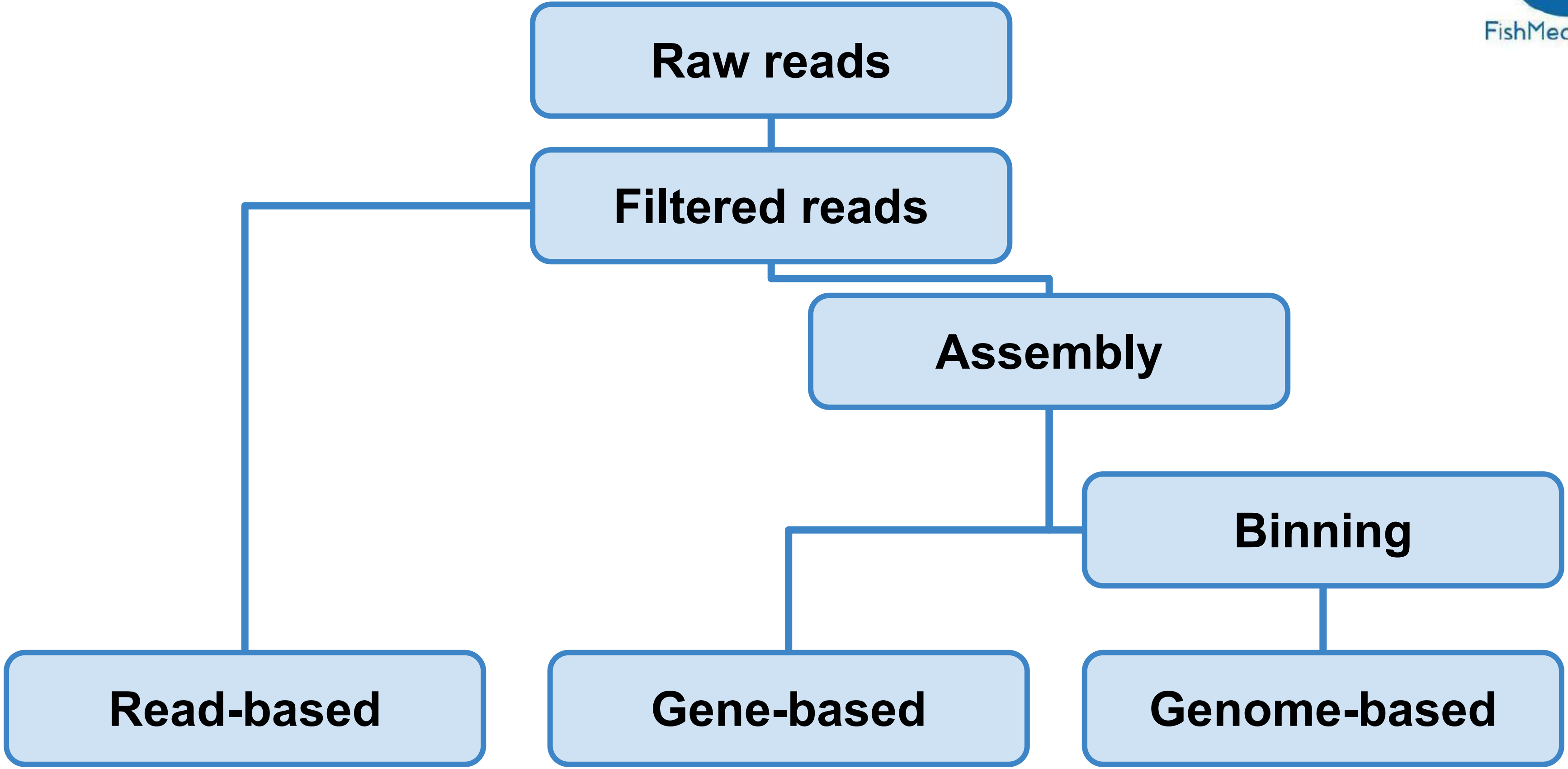
Gene-based



Hologenomics

Gene-based

Hologenomics


Raw reads

Filtered reads

Assembly

Binning

Read-based

Gene-based

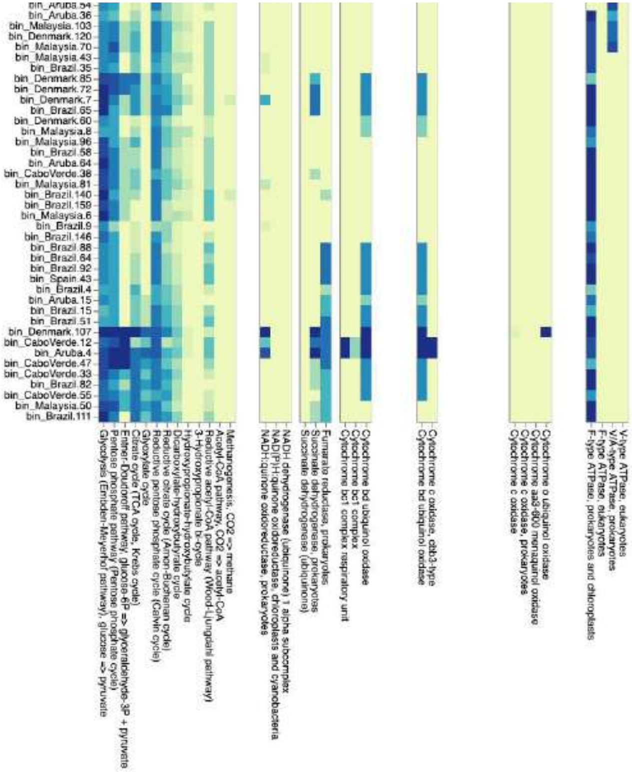
Genome-based

Binning

Draft genomes

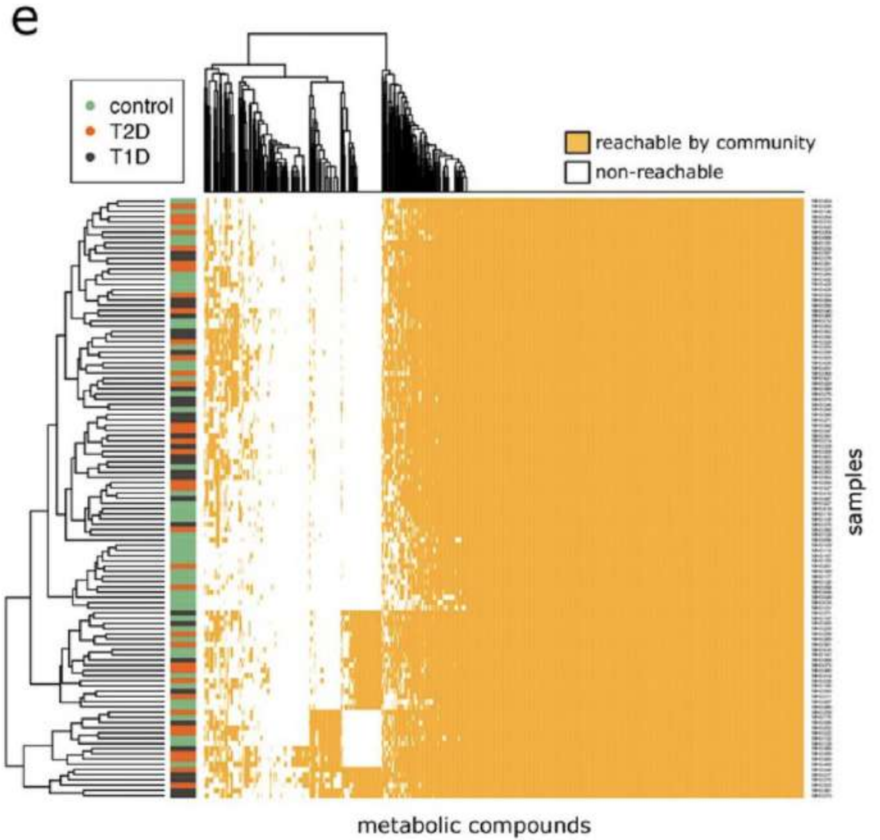
Hologenomics

Annotations



Hologenomics

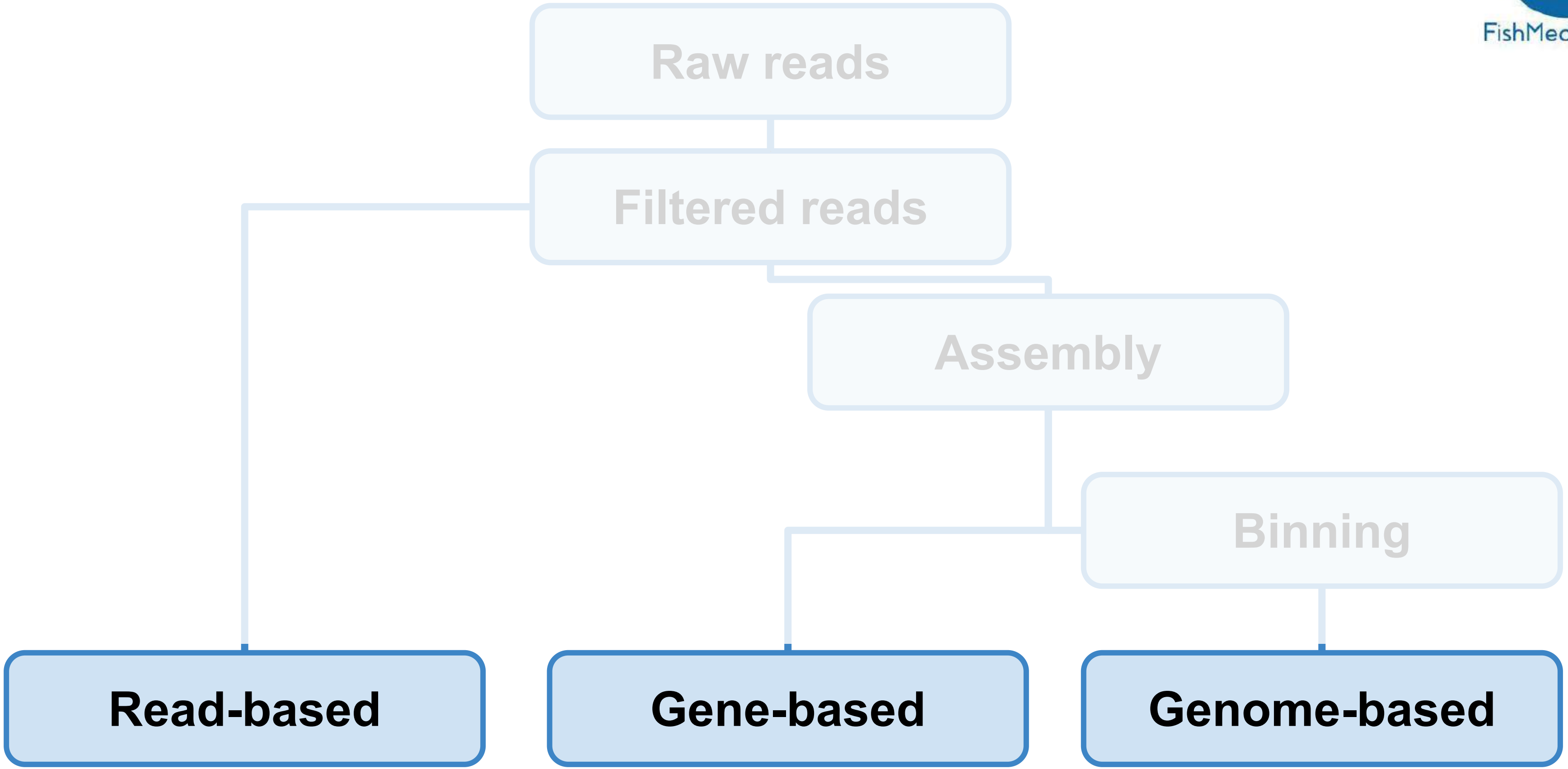
Genome-scale metabolic networks



Belcour et al. 2021

ELife

Hologenomics



Raw reads

Filtered reads

Assembly

Binning

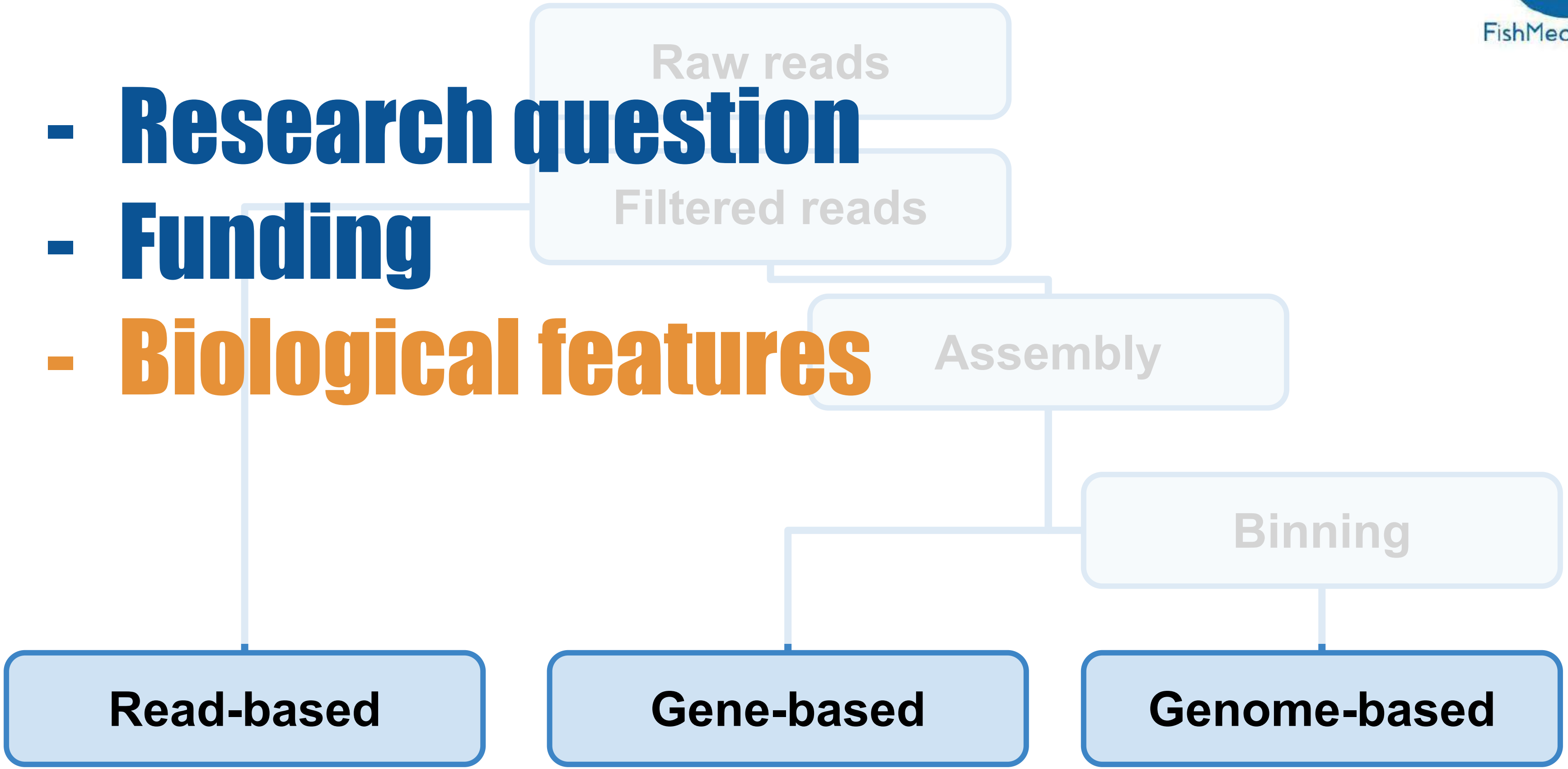
Read-based

Gene-based

Genome-based

Hologenomics

- Research question
- Funding
- Biological features



Raw reads

Filtered reads

Assembly

Binning

Read-based

Gene-based

Genome-based

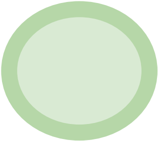
Hologenomics

INTERESTING READINGS

Bacteria vs. host

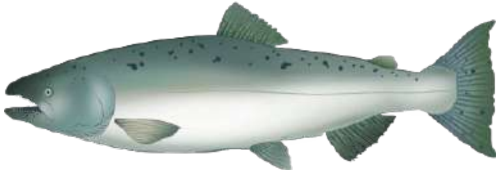
Enterococcus

3 MB



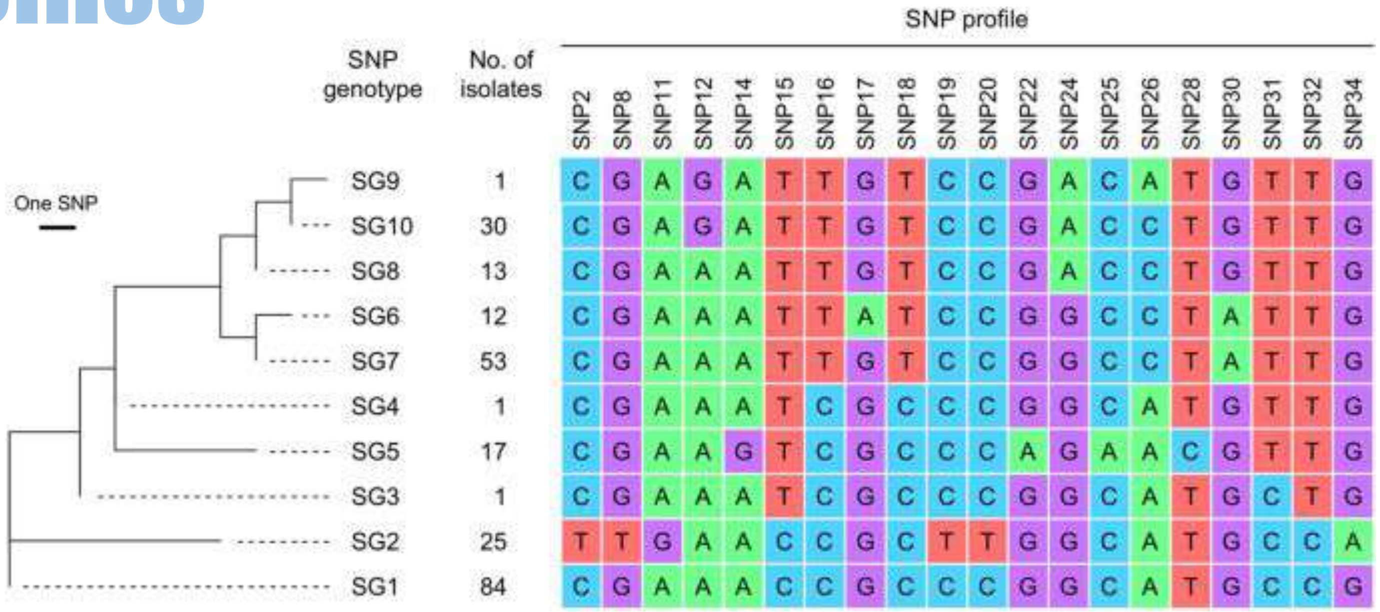
Salmo salar

3000 MB



Hologenomics

Host
SNP profiles

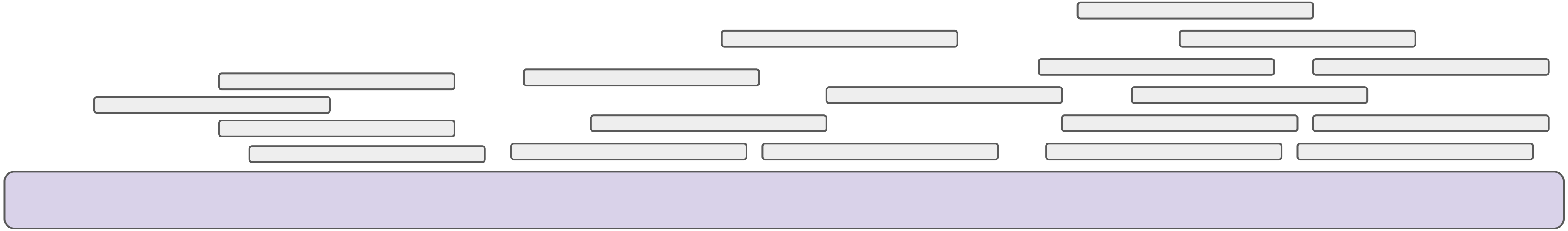


SNP genotype	No. of isolates	SNP2	SNP8	SNP11	SNP12	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19	SNP20	SNP22	SNP24	SNP25	SNP26	SNP28	SNP30	SNP31	SNP32	SNP34
SG9	1	C	G	A	G	A	T	T	G	T	C	C	G	A	C	A	T	G	T	T	G
SG10	30	C	G	A	G	A	T	T	G	T	C	C	G	A	C	C	T	G	T	T	G
SG8	13	C	G	A	A	A	T	T	G	T	C	C	G	A	C	C	T	G	T	T	G
SG6	12	C	G	A	A	A	T	T	A	T	C	C	G	G	C	C	T	A	T	T	G
SG7	53	C	G	A	A	A	T	T	G	T	C	C	G	G	C	C	T	A	T	T	G
SG4	1	C	G	A	A	A	T	C	G	C	C	C	G	G	C	A	T	G	T	T	G
SG5	17	C	G	A	A	G	T	C	G	C	C	C	A	G	A	A	C	G	T	T	G
SG3	1	C	G	A	A	A	T	C	G	C	C	C	G	G	C	A	T	G	C	T	G
SG2	25	T	T	G	A	A	C	C	G	C	T	T	G	G	C	A	T	G	C	C	A
SG1	84	C	G	A	A	A	C	C	G	C	C	C	G	G	C	A	T	G	C	C	G

Hologenomics

Host

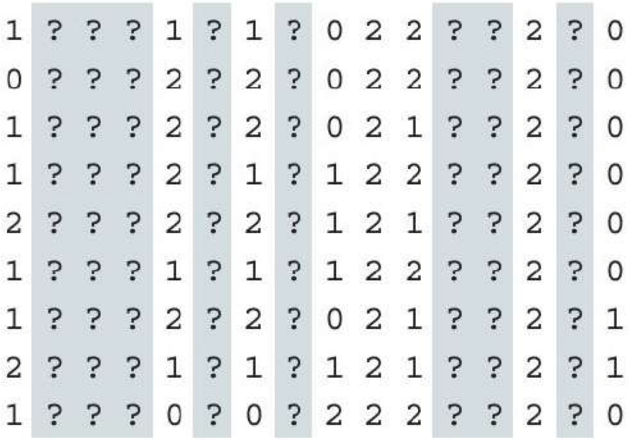
SNP profiles



Depth

Host

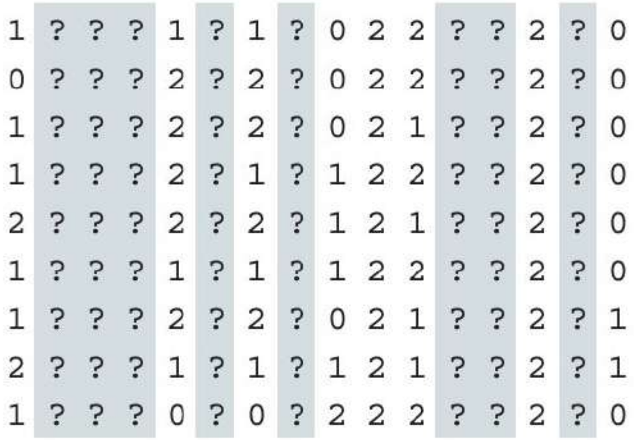
Genotype inputation



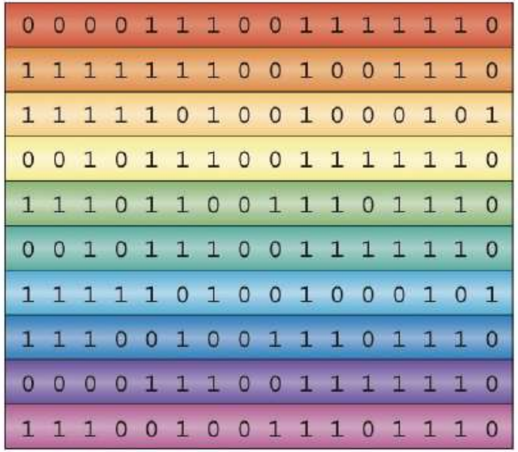
1	?	?	?	1	?	1	?	0	2	2	?	?	2	?	0
0	?	?	?	2	?	2	?	0	2	2	?	?	2	?	0
1	?	?	?	2	?	2	?	0	2	1	?	?	2	?	0
1	?	?	?	2	?	1	?	1	2	2	?	?	2	?	0
2	?	?	?	2	?	2	?	1	2	1	?	?	2	?	0
1	?	?	?	1	?	1	?	1	2	2	?	?	2	?	0
1	?	?	?	2	?	2	?	0	2	1	?	?	2	?	1
2	?	?	?	1	?	1	?	1	2	1	?	?	2	?	1
1	?	?	?	0	?	0	?	2	2	2	?	?	2	?	0

Hologenomics

Host
Genotype inputation



1	?	?	?	1	?	1	?	0	2	2	?	?	2	?	0
0	?	?	?	2	?	2	?	0	2	2	?	?	2	?	0
1	?	?	?	2	?	2	?	0	2	1	?	?	2	?	0
1	?	?	?	2	?	1	?	1	2	2	?	?	2	?	0
2	?	?	?	2	?	2	?	1	2	1	?	?	2	?	0
1	?	?	?	1	?	1	?	1	2	2	?	?	2	?	0
1	?	?	?	2	?	2	?	0	2	1	?	?	2	?	1
2	?	?	?	1	?	1	?	1	2	1	?	?	2	?	1
1	?	?	?	0	?	0	?	2	2	2	?	?	2	?	0

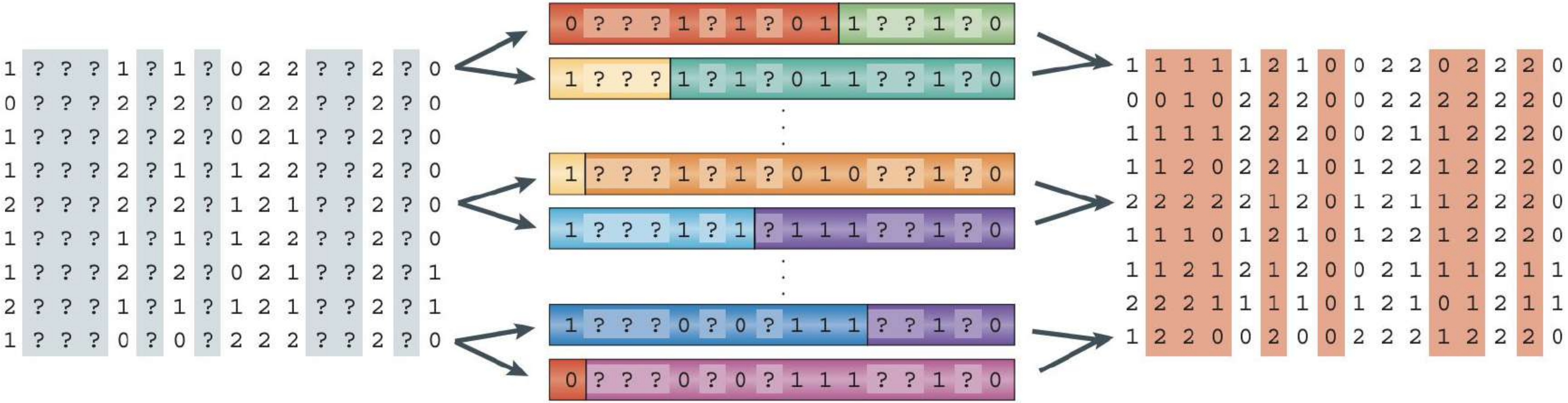


0	0	0	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	0
1	1	1	1	1	0	1	0	0	1	0	0	0	1	0	1
0	0	1	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	0	1	1	0	0	1	1	1	0	1	1	1	0
0	0	1	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	1	1	0	1	0	0	1	0	0	0	1	0	1
1	1	1	0	0	1	0	0	1	1	1	0	1	1	1	0
0	0	0	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	0	0	1	0	0	1	1	1	0	1	1	1	0

Hologenomics

Host
Genotype inputation

Marchini & Howie 2010
Nature Reviews Genetics



1 ? ? ? 1 ? 1 ? 0 2 2 ? ? 2 ? 0
0 ? ? ? 2 ? 2 ? 0 2 2 ? ? 2 ? 0
1 ? ? ? 2 ? 2 ? 0 2 1 ? ? 2 ? 0
1 ? ? ? 2 ? 1 ? 1 2 2 ? ? 2 ? 0
2 ? ? ? 2 ? 2 ? 1 2 1 ? ? 2 ? 0
1 ? ? ? 1 ? 1 ? 1 2 2 ? ? 2 ? 0
1 ? ? ? 2 ? 2 ? 0 2 1 ? ? 2 ? 1
2 ? ? ? 1 ? 1 ? 1 2 1 ? ? 2 ? 1
1 ? ? ? 0 ? 0 ? 2 2 2 ? ? 2 ? 0

0 ? ? ? 1 ? 1 ? 0 1 1 ? ? 1 ? 0
1 ? ? ? 1 ? 1 ? 0 1 1 ? ? 1 ? 0
⋮
1 ? ? ? 1 ? 1 ? 0 1 0 ? ? 1 ? 0
1 ? ? ? 1 ? 1 ? 1 1 1 ? ? 1 ? 0
⋮
1 ? ? ? 0 ? 0 ? 1 1 1 ? ? 1 ? 0
0 ? ? ? 0 ? 0 ? 1 1 1 ? ? 1 ? 0

1 1 1 1 1 2 1 0 0 2 2 0 2 2 2 0
0 0 1 0 2 2 2 0 0 2 2 2 2 2 2 0
1 1 1 1 2 2 2 0 0 2 1 1 2 2 2 0
1 1 2 0 2 2 1 0 1 2 2 1 2 2 2 0
2 2 2 2 2 1 2 0 1 2 1 1 2 2 2 0
1 1 1 0 1 2 1 0 1 2 2 1 2 2 2 0
1 1 2 1 2 1 2 0 0 2 1 1 1 2 1 1
2 2 2 1 1 1 1 0 1 2 1 0 1 2 1 1
1 2 2 0 0 2 0 0 2 2 2 1 2 2 2 0

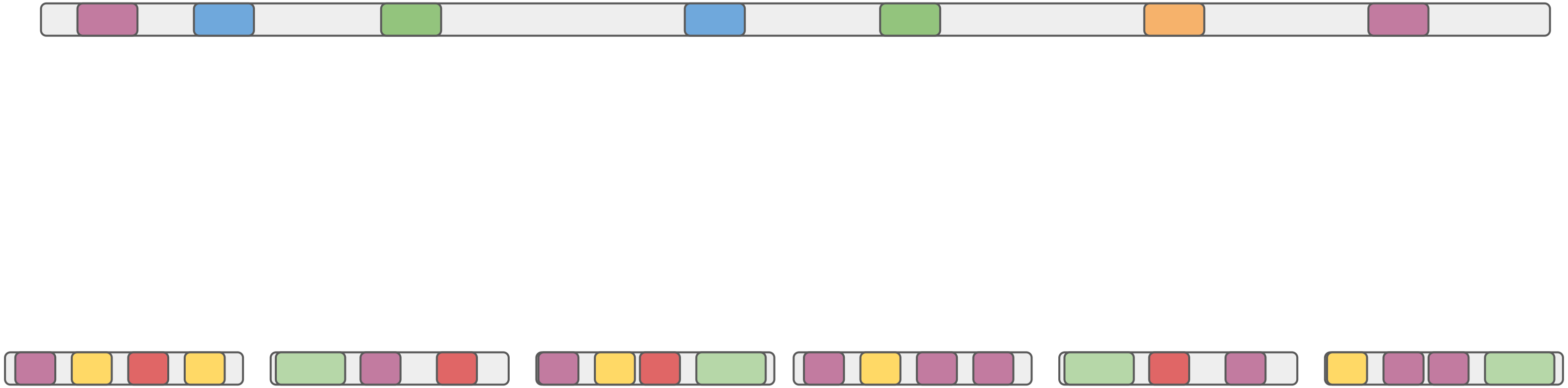
Hologenomics

FishMed-PhD

INTERESTING READINGS

Hologenomics

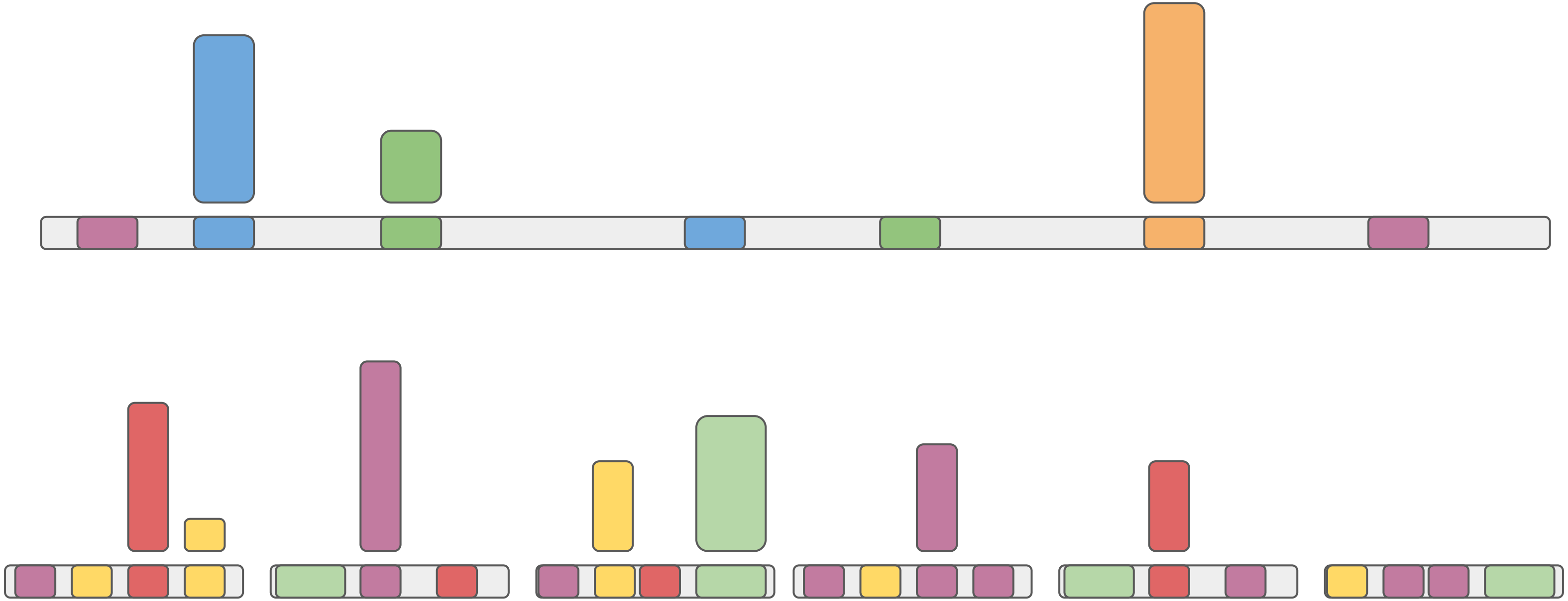
Hologenomics



FishMed-PhD

Is there any other relevant information we are missing?

Gene expression



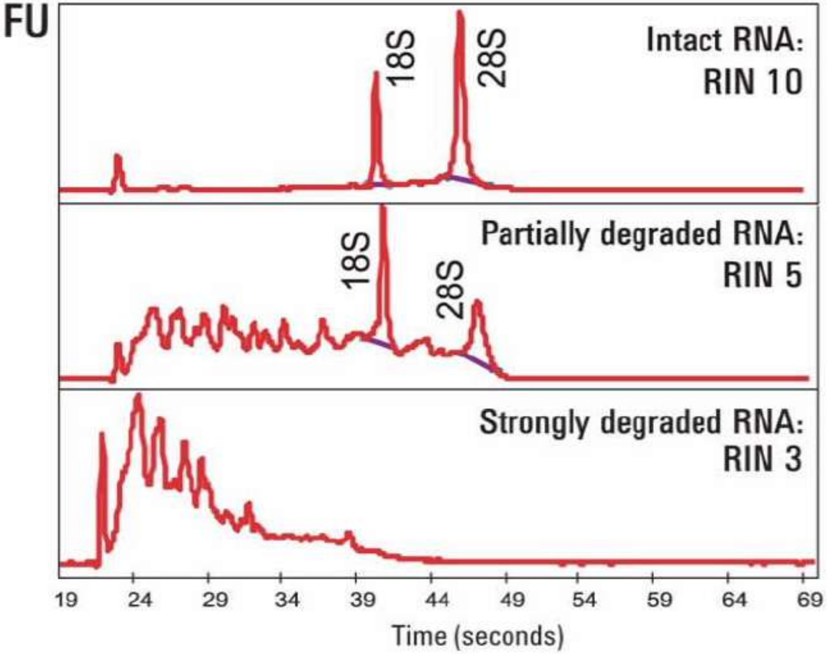
Hologenomics

Gene expression

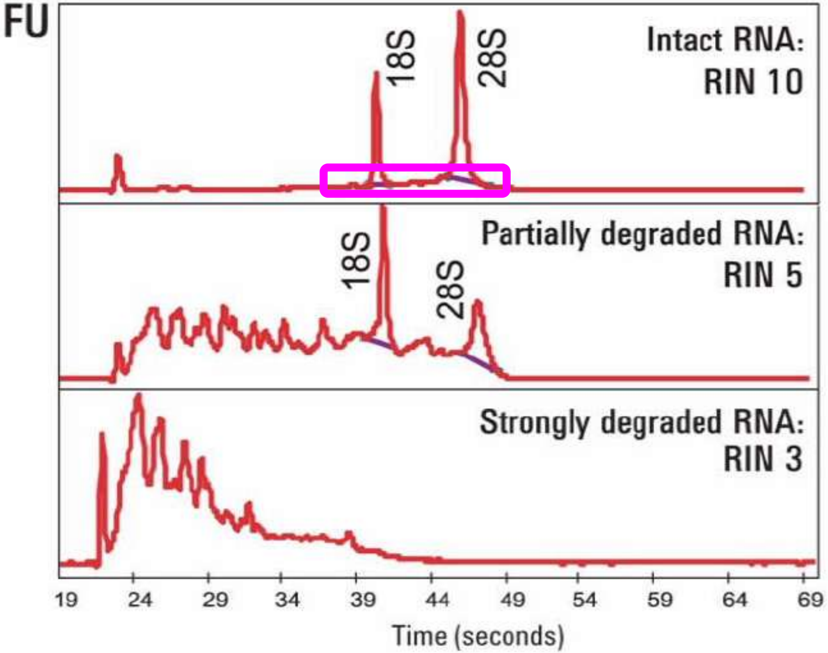
Challenges

- RNA preservation
- rRNA depletion

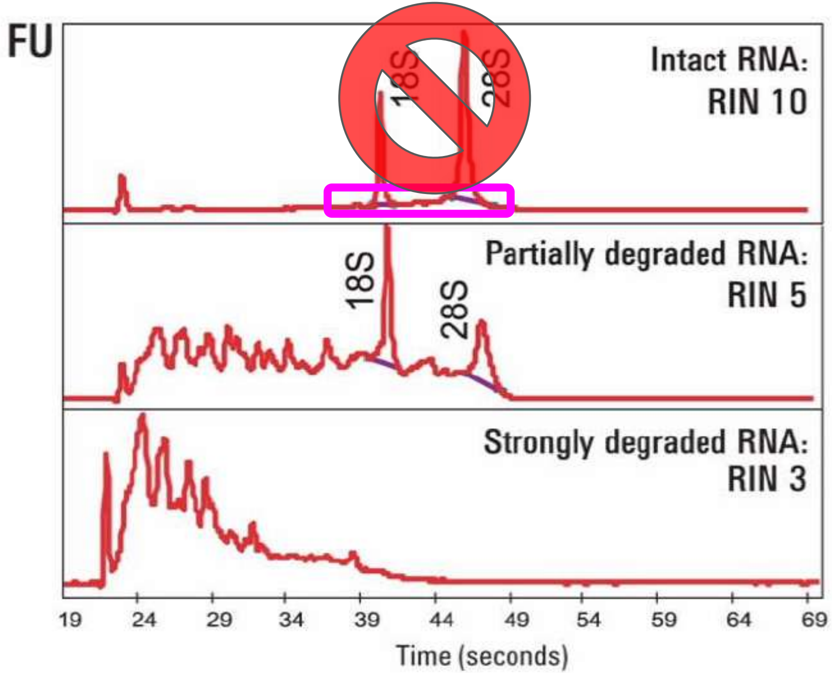
Gene expression



Gene expression



Gene expression



Gene expression

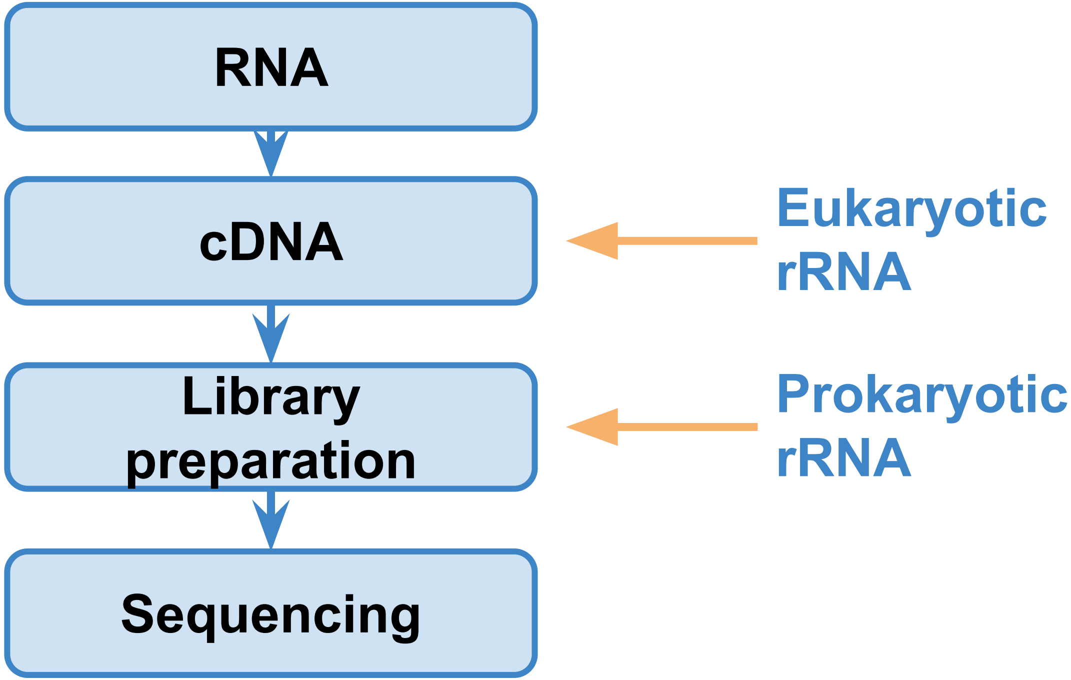
RNA

cDNA

Library preparation

Sequencing

Gene expression



RNA

cDNA

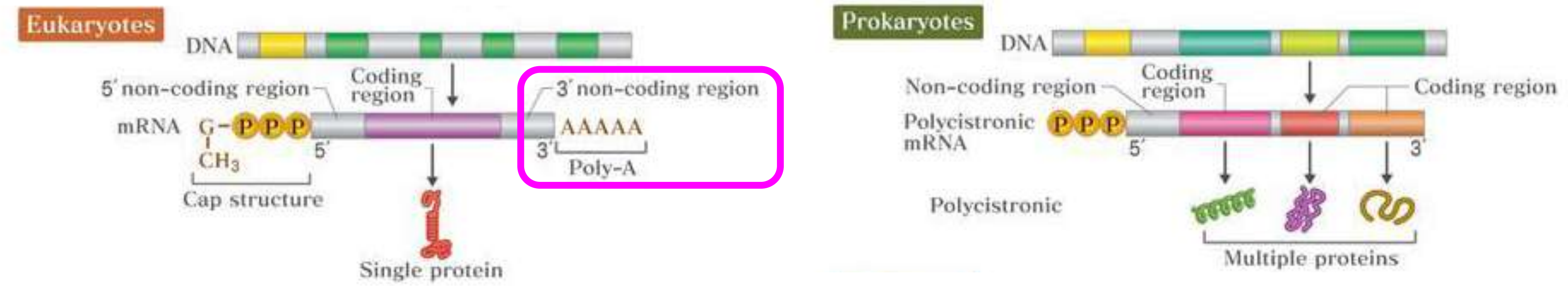
Library preparation

Sequencing

Eukaryotic rRNA

Prokaryotic rRNA

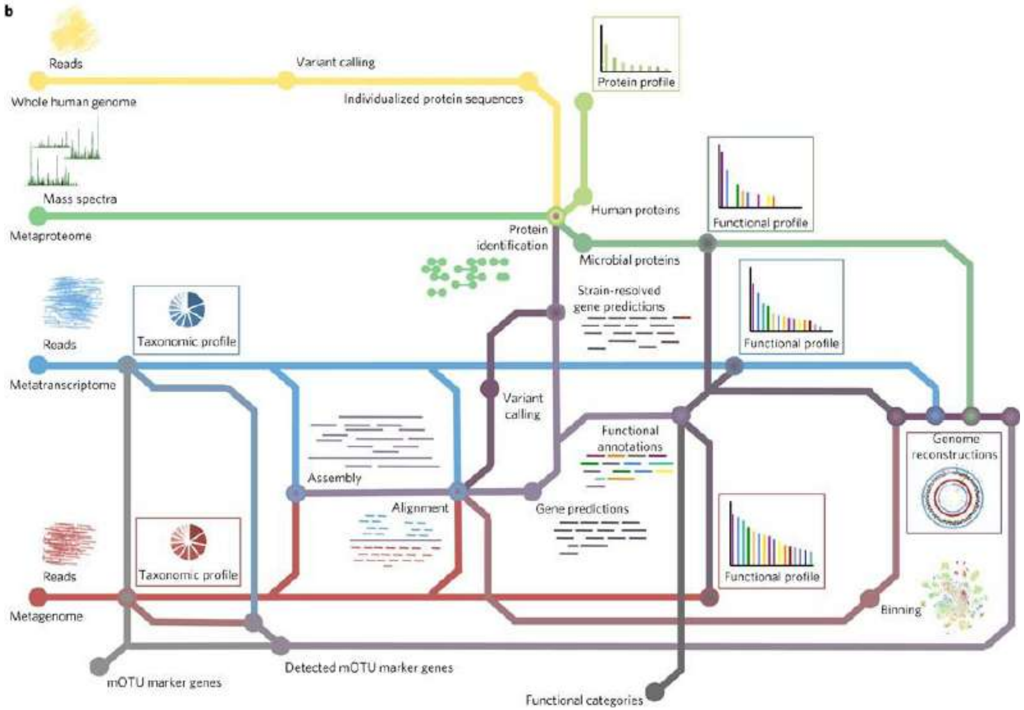
Gene expression



Mass spectrometry-based

Proteomics
Metabolomics

Data integration



Heintz-Buschart et al. 2016

Nature Microbiology

FishMed-PhD

Hologenomics

applied to host-microbiota interactions in marine organisms

Antton Alberdi

University of Copenhagen

antton.alberdi@sund.ku.dk

alberdilab.dk

CEH | CENTER FOR EVOLUTIONARY HOLOGENOMICS

UNIVERSITY OF COPENHAGEN

FishMed-PhD

Session 4

APPLICATIONS of knowledge on animal-microbiota interactions

FishMed-PhD

When and how should we implement hologenomics?

Hologenomics

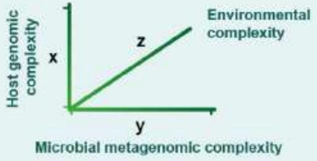
Complex

Expensive

Hologenomics

What is the complexity of the study system?

The complexity of a hologenomic system can be decomposed into three elements: the host genomic complexity, the microbial metagenomic complexity and the environmental complexity.



Which variables can be controlled by the researcher?

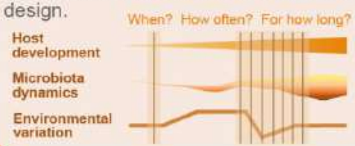
What information can be recovered from host-microbiota systems will depend on whether, and to what degree, host genomic, microbial metagenomic and environmental complexity can be controlled, minimised or removed by the researcher.

What is the genomic resolution at which hologenomic variation is studied?

The resolution at which the host genome and the microbial metagenome are characterised, and in consequence how genotypes, metagenotypes and combined hologenotypes are defined, also determines the genomic level of complexity to study a host-microbiota system.

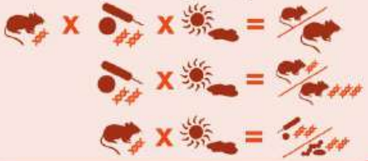
What are the spatio-temporal features of the studied hologenomic variation?

Which questions can be addressed also depends on the intrinsic spatial and temporal features of hosts and microbial communities considered, as well as the spatial and temporal features of the study design.

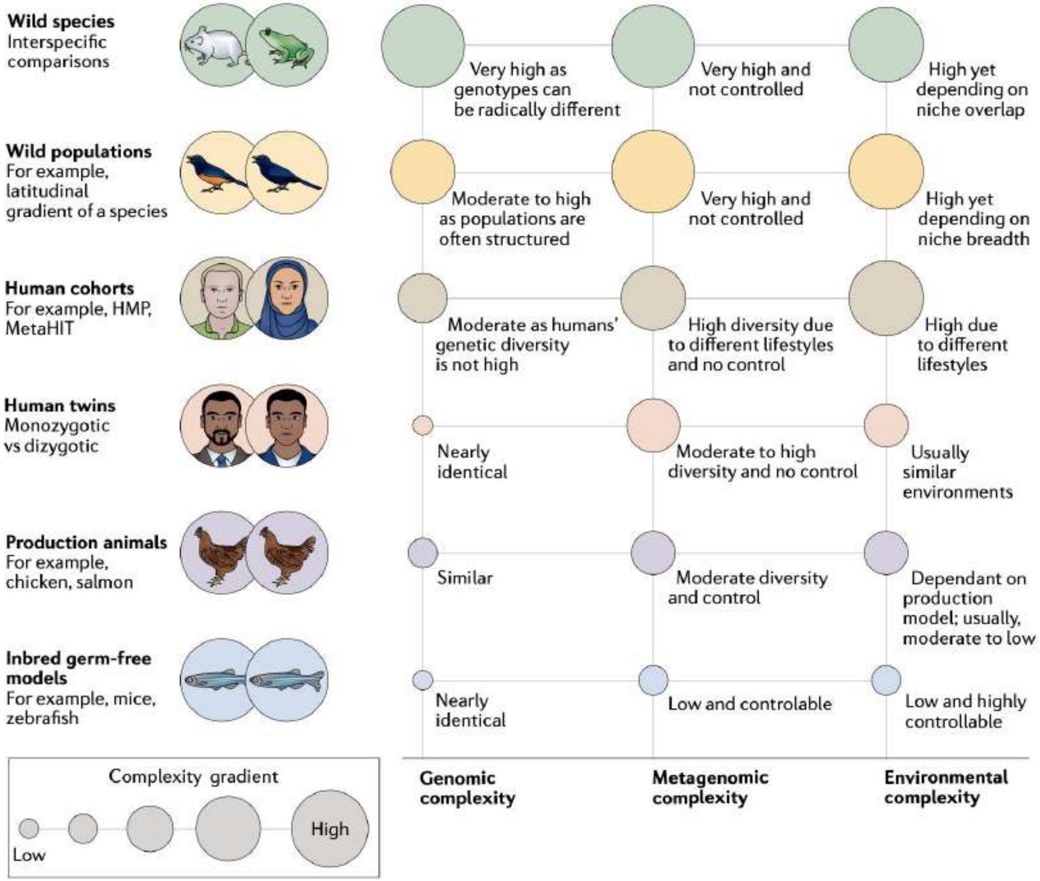


Which are the explanatory and response variables?

Due to the multi-layered nature and bi-directional interactions in host-microbiota systems, the definition of explanatory and response variables, and thus the question researchers want to answer, will often be determined by the study design.



Hologenomics

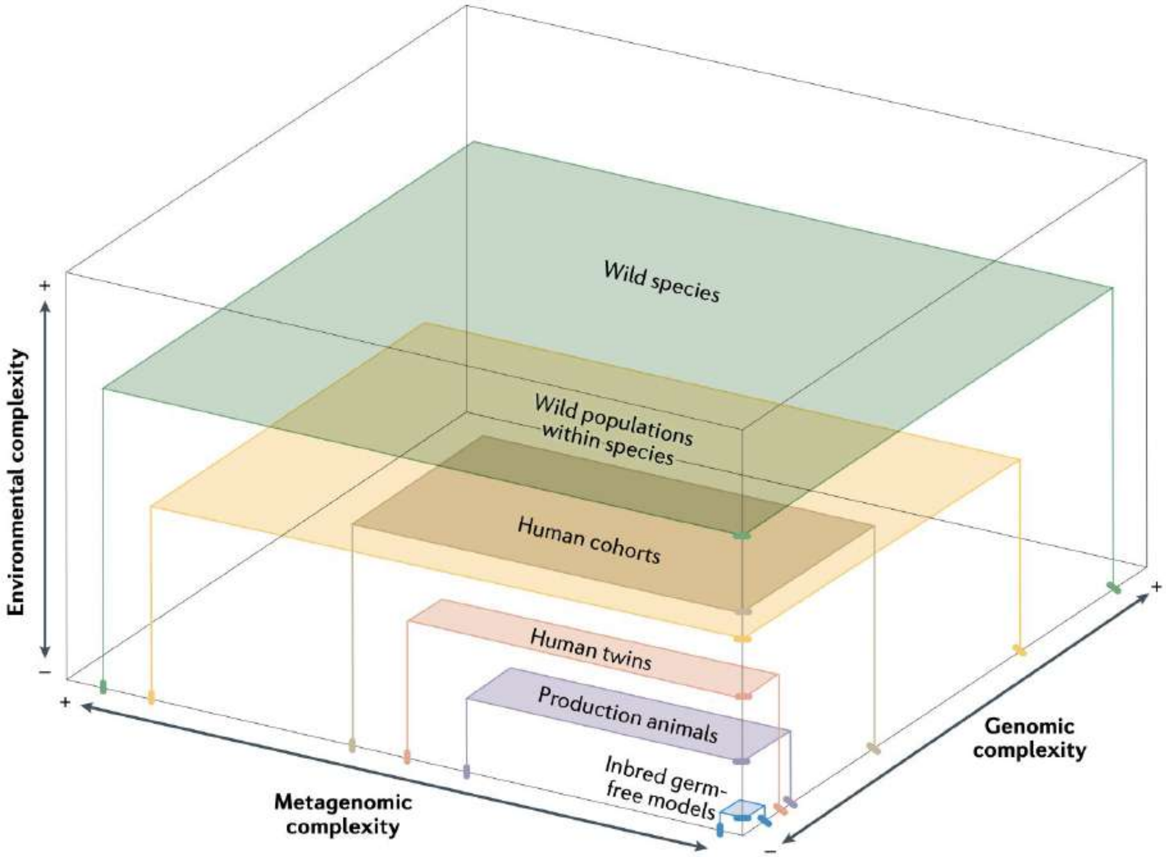


	Genomic complexity	Metagenomic complexity	Environmental complexity
Wild species Interspecific comparisons	Very high as genotypes can be radically different	Very high and not controlled	High yet depending on niche overlap
Wild populations For example, latitudinal gradient of a species	Moderate to high as populations are often structured	Very high and not controlled	High yet depending on niche breadth
Human cohorts For example, HMP, MetaHIT	Moderate as humans' genetic diversity is not high	High diversity due to different lifestyles and no control	High due to different lifestyles
Human twins Monozygotic vs dizygotic	Nearly identical	Moderate to high diversity and no control	Usually similar environments
Production animals For example, chicken, salmon	Similar	Moderate diversity and control	Dependant on production model; usually, moderate to low
Inbred germ-free models For example, mice, zebrafish	Nearly identical	Low and controlable	Low and highly controllable

Alberdi et al. 2021

Nature Reviews Genetics

Hologenomics



Alberdi et al. 2021

Nature Reviews Genetics

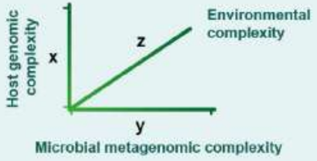
Hologenomics

FishMed-PhD

1

What is the complexity of the study system?

The complexity of a hologenomic system can be decomposed into three elements: the host genomic complexity, the microbial metagenomic complexity and the environmental complexity.



2

Which variables can be controlled by the researcher?

What information can be recovered from host-microbiota systems will depend on whether, and to what degree, host genomic, microbial metagenomic and environmental complexity can be controlled, minimised or removed by the researcher.

3

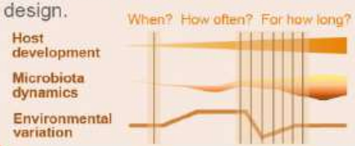
What is the genomic resolution at which hologenomic variation is studied?

The resolution at which the host genome and the microbial metagenome are characterised, and in consequence how genotypes, metagenotypes and combined hologenotypes are defined, also determines the genomic level of complexity to study a host-microbiota system.

4

What are the spatio-temporal features of the studied hologenomic variation?

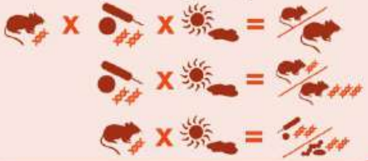
Which questions can be addressed also depends on the intrinsic spatial and temporal features of hosts and microbial communities considered, as well as the spatial and temporal features of the study design.



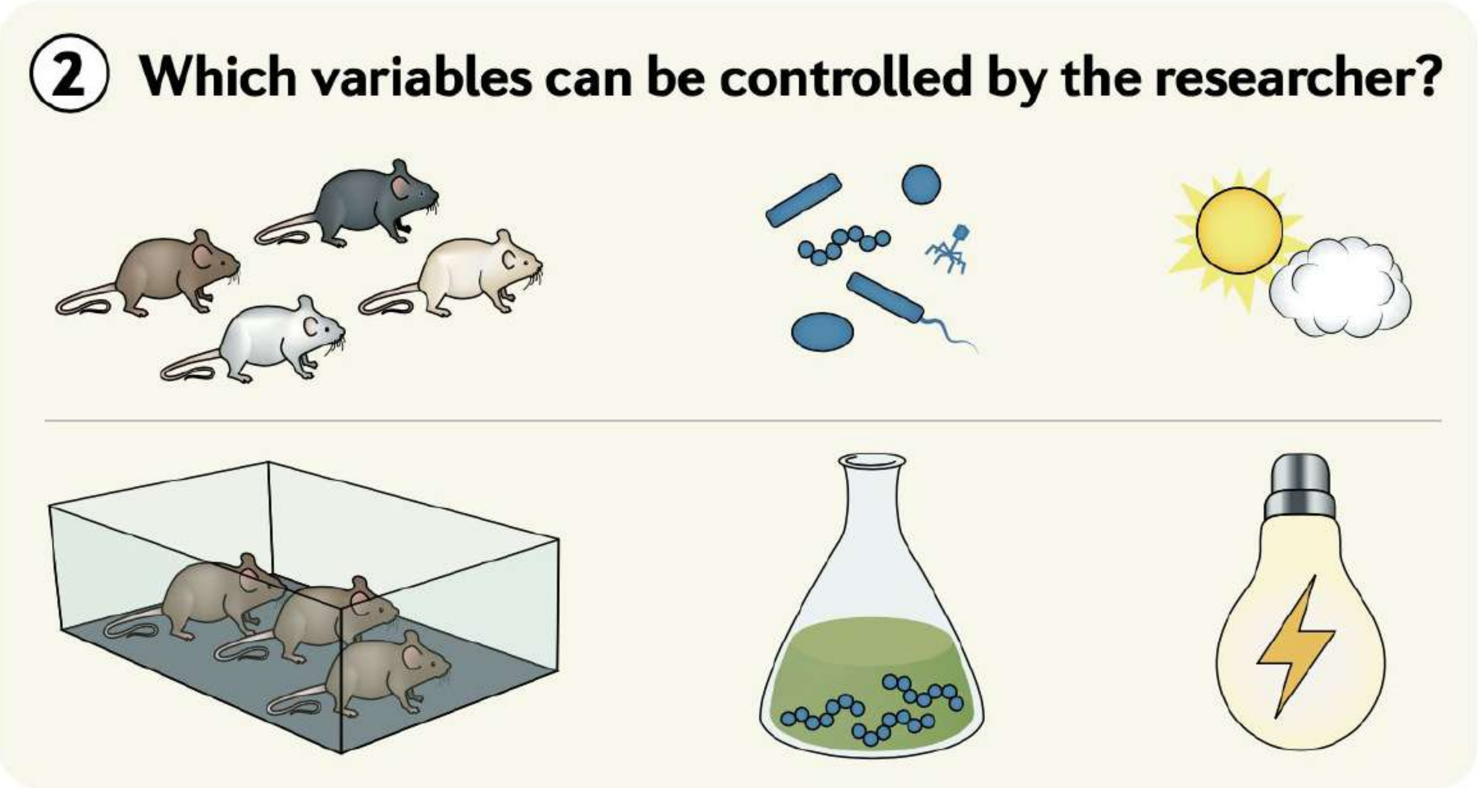
5

Which are the explanatory and response variables?

Due to the multi-layered nature and bi-directional interactions in host-microbiota systems, the definition of explanatory and response variables, and thus the question researchers want to answer, will often be determined by the study design.



Hologenomics

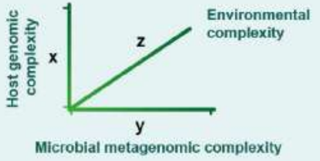


② Which variables can be controlled by the researcher?

Hologenomics

1 What is the complexity of the study system?

The complexity of a hologenomic system can be decomposed into three elements: the host genomic complexity, the microbial metagenomic complexity and the environmental complexity.



2 Which variables can be controlled by the researcher?

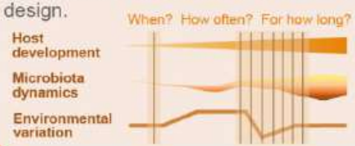
What information can be recovered from host-microbiota systems will depend on whether, and to what degree, host genomic, microbial metagenomic and environmental complexity can be controlled, minimised or removed by the researcher.

3 What is the genomic resolution at which hologenomic variation is studied?

The resolution at which the host genome and the microbial metagenome are characterised, and in consequence how genotypes, metagenotypes and combined hologenotypes are defined, also determines the genomic level of complexity to study a host-microbiota system.

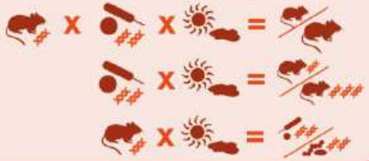
4 What are the spatio-temporal features of the studied hologenomic variation?

Which questions can be addressed also depends on the intrinsic spatial and temporal features of hosts and microbial communities considered, as well as the spatial and temporal features of the study design.

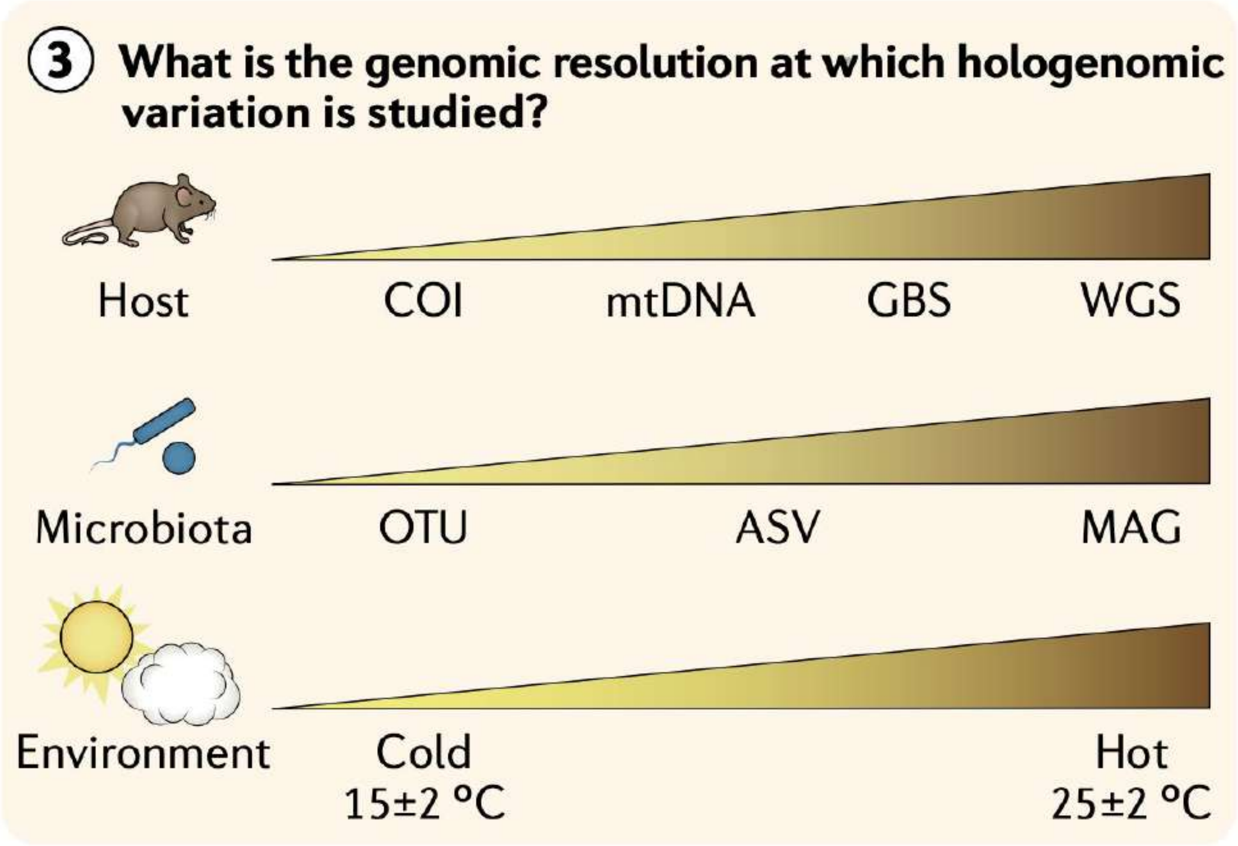


5 Which are the explanatory and response variables?

Due to the multi-layered nature and bi-directional interactions in host-microbiota systems, the definition of explanatory and response variables, and thus the question researchers want to answer, will often be determined by the study design.



Hologenomics

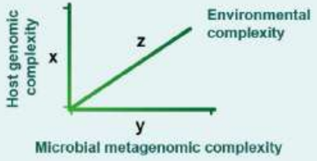


③ What is the genomic resolution at which hologenomic variation is studied?

Hologenomics

What is the complexity of the study system?

The complexity of a hologenomic system can be decomposed into three elements: the host genomic complexity, the microbial metagenomic complexity and the environmental complexity.



Which variables can be controlled by the researcher?

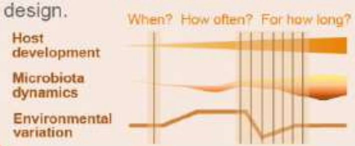
What information can be recovered from host-microbiota systems will depend on whether, and to what degree, host genomic, microbial metagenomic and environmental complexity can be controlled, minimised or removed by the researcher.

What is the genomic resolution at which hologenomic variation is studied?

The resolution at which the host genome and the microbial metagenome are characterised, and in consequence how genotypes, metagenotypes and combined hologenotypes are defined, also determines the genomic level of complexity to study a host-microbiota system.

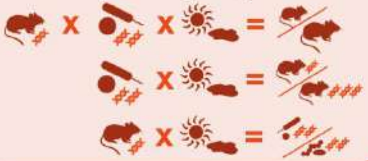
What are the spatio-temporal features of the studied hologenomic variation?

Which questions can be addressed also depends on the intrinsic spatial and temporal features of hosts and microbial communities considered, as well as the spatial and temporal features of the study design.

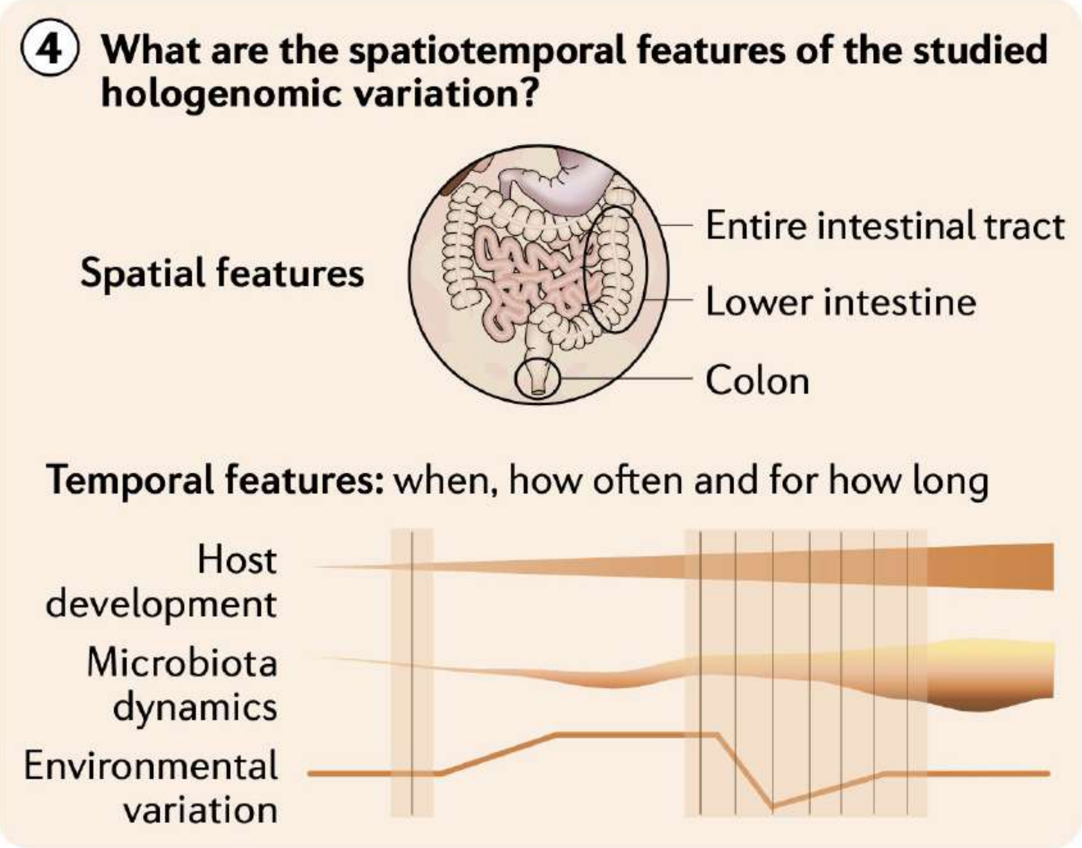


Which are the explanatory and response variables?

Due to the multi-layered nature and bi-directional interactions in host-microbiota systems, the definition of explanatory and response variables, and thus the question researchers want to answer, will often be determined by the study design.



Hologenomics

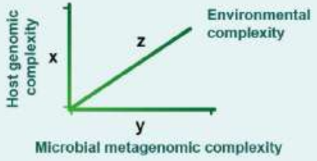


④ **What are the spatiotemporal features of the studied hologenomic variation?**

Hologenomics

1 What is the complexity of the study system?

The complexity of a hologenomic system can be decomposed into three elements: the host genomic complexity, the microbial metagenomic complexity and the environmental complexity.



2 Which variables can be controlled by the researcher?

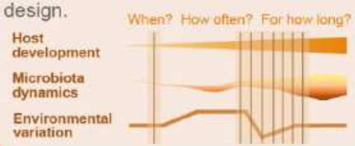
What information can be recovered from host-microbiota systems will depend on whether, and to what degree, host genomic, microbial metagenomic and environmental complexity can be controlled, minimised or removed by the researcher.

3 What is the genomic resolution at which hologenomic variation is studied?

The resolution at which the host genome and the microbial metagenome are characterised, and in consequence how genotypes, metagenotypes and combined hologenotypes are defined, also determines the genomic level of complexity to study a host-microbiota system.

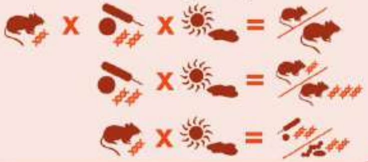
4 What are the spatio-temporal features of the studied hologenomic variation?

Which questions can be addressed also depends on the intrinsic spatial and temporal features of hosts and microbial communities considered, as well as the spatial and temporal features of the study design.



5 Which are the explanatory and response variables?

Due to the multi-layered nature and bi-directional interactions in host-microbiota systems, the definition of explanatory and response variables, and thus the question researchers want to answer, will often be determined by the study design.



Hologenomics

$$P = G \times MG \times E$$

Hologenomics

$$G \sim MG \times E$$

Hologenomics

$$MG \sim G \times E$$

Which research questions could be tackled with hologenomics?

Hologenomics

FishMed-PhD

- Effect of host-microbiota interactions on production
- Joint variations of domestication process to genomes and metagenomes
- Joint (genomic+metagenomic) means of adaptation to environmental change
- …