metagWGS: a nextflow workflow to analyze metagenomic data

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Journées du PEPI IBIS – 16/18 novembre 2021

The samples we want to analyze

https://oceans.taraexpeditions.org/

Marine sample

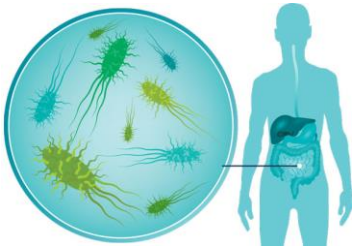


Image: newannyart/Thinkstock
https://www.health.harvard.edu

Gut sample

https://www6.inrae.fr/isite-agroecologie-bfc/

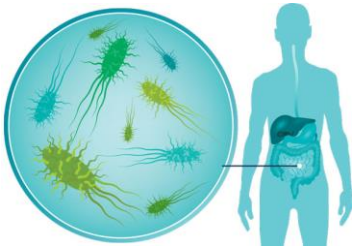
Soil sample

Environmental samples

The biological questions we want to answer

https://oceans.taraexpeditions.org/

Marine sample



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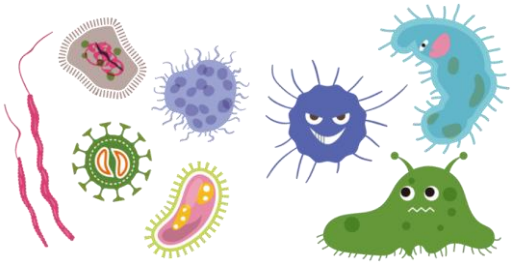
Gut sample

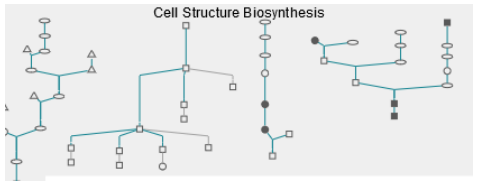
https://www6.inrae.fr/isite-agroecologie-bfc/

Soil sample

Who is there?



What genes are present in our samples?
What are their functions?



Metagenomics data: from sample to files

Samples

DNA extraction
+
Fragmentation
+
Library preparation

Whole genome DNA samples

Sequencing

Files of sequences = raw data

Whole genome shotgun sequencing data 2x150bp (Illumina)

? ? ? Data analysis ? ? ? ? ? ?

?

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Whole genome shotgun sequencing data 2x150bp (Illumina)

Bioinformatics

Data analysis

Statistics

Bioinformatics analysis:
- Output files
- Output graphs

Biostatistical analysis

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Bioinformatics

Data analysis

Statistics

Bioinformatics analysis:
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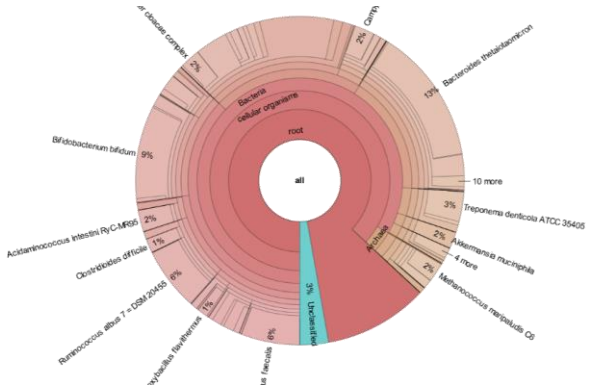
- Output graphs

Biostatistical analysis

Definition of bioinformatics output data

Who is there?

Taxonomic affiliation of reads



Taxonomic affiliation (gene, contig)

Sequence id	consensus taxid	consensus lineage
Sequence 1	210	cellular organisms; Bacteria; Proteobacteria; delta/epsilon subdivisions; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter; Helicobacter pylori
Sequence 2	1681	cellular organisms; Bacteria; Terrabacteria group; Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium; Bifidobacterium bifidum
Sequence 3	1358	cellular organisms; Bacteria; Terrabacteria group; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Lactococcus; Lactococcus lactis

What genes are present in our samples?
What are their functions?

Read quantification by gene

Gene	Sample 1	Sample 2
Gene 1	844	887
Gene 2	847	891
Gene 3	4092	4389
Gene 4	5279	3702
Gene 5	584	611

Read quantification by function

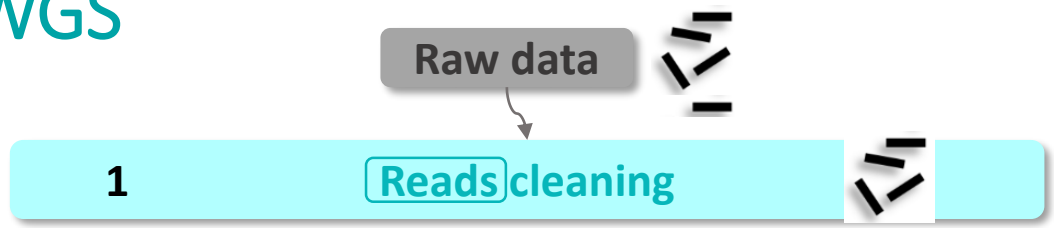
Function	Sample 1	Sample 2
Function 1	1840	5857
Function 2	4010	4506
Function 3	6005	7052
Function 4	9500	4506
Function 5	1234	5678

metagWGS

Raw data

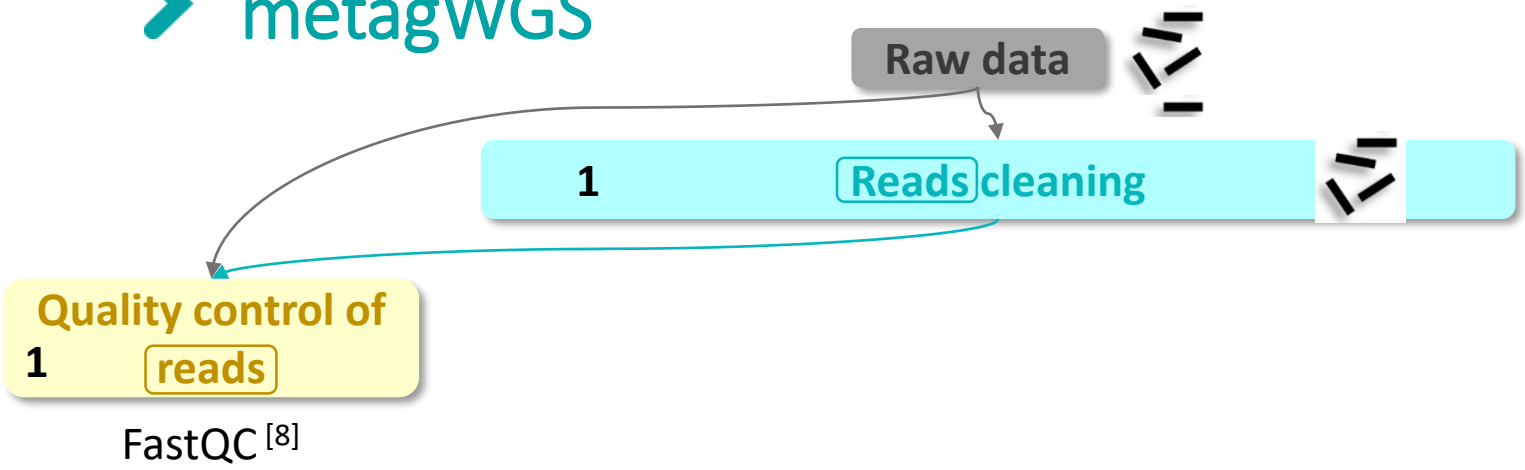
INRAE

metagWGS



cutadapt [5], sickle [6], bwa [7] (remove host reads)

metagWGS



FastQC [8]

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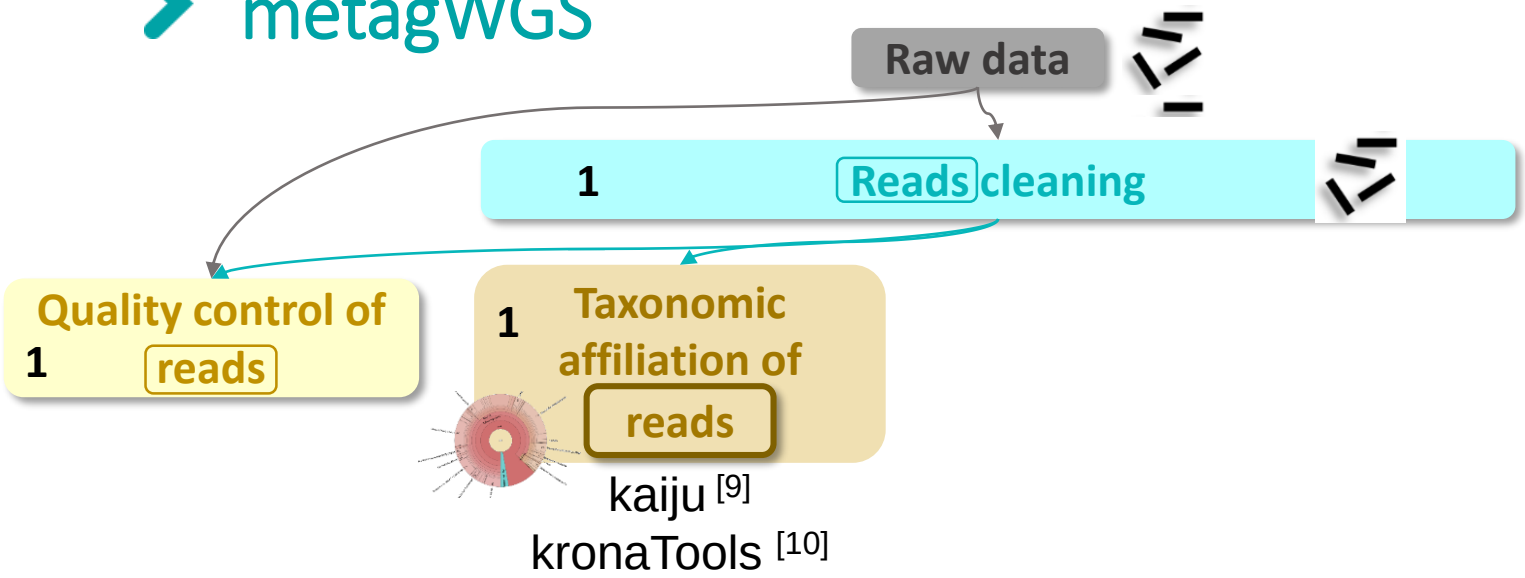
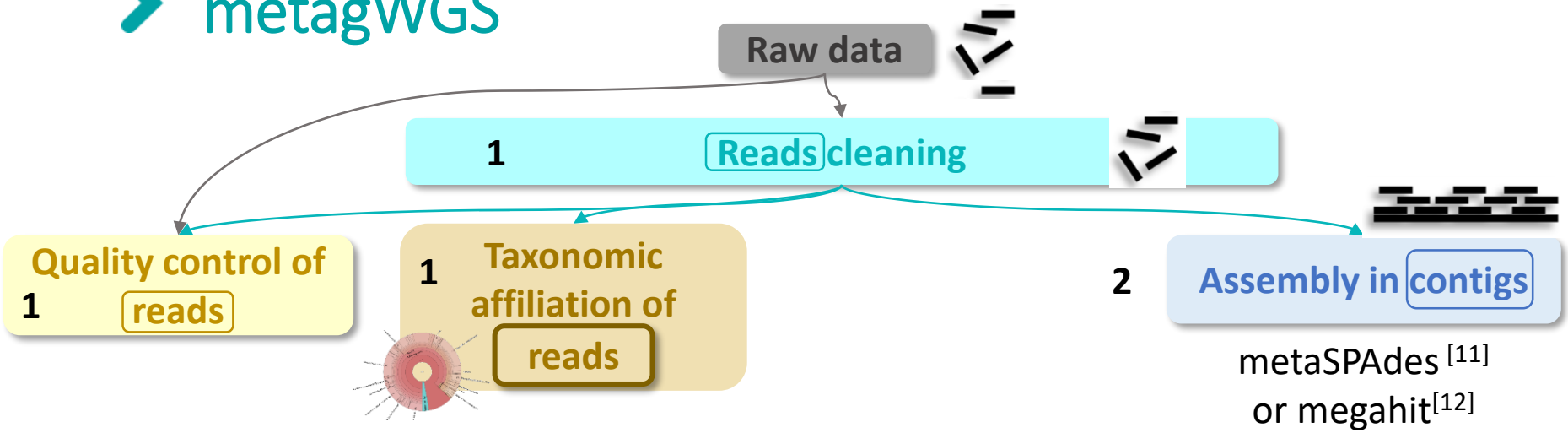
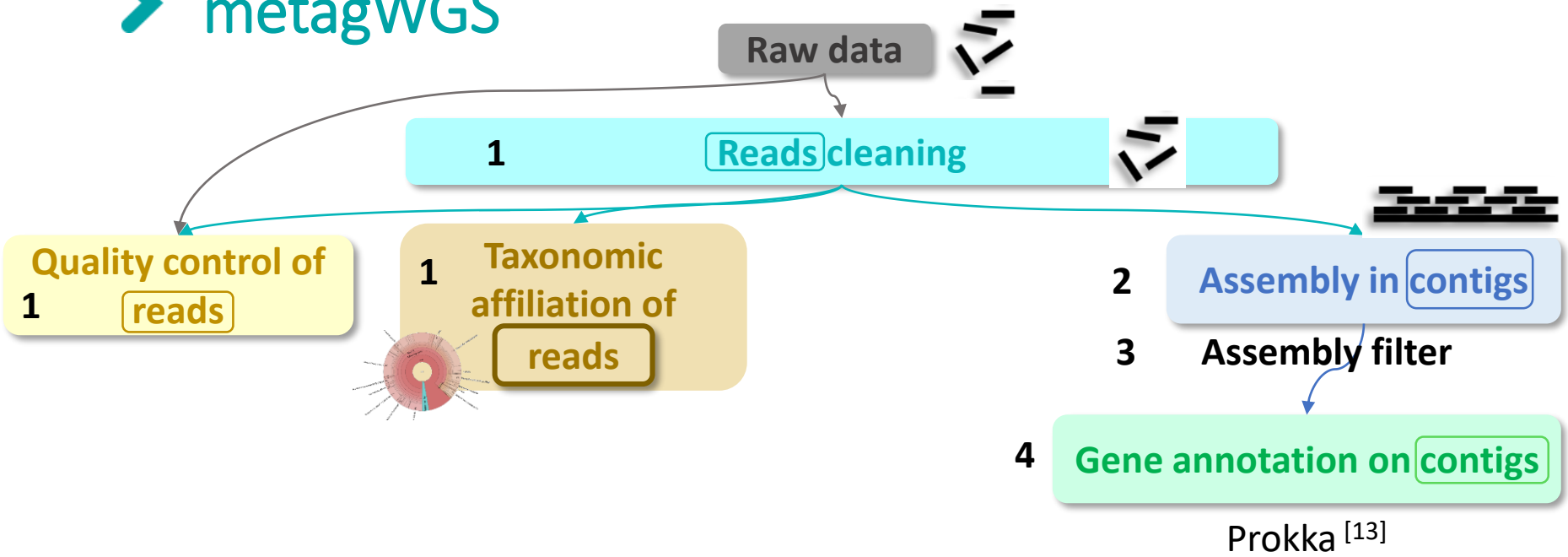

Raw data
1 Reads cleaning
Quality control of
1 reads
1 Taxonomic affiliation of
reads
kaiju [9]
kronaTools [10]

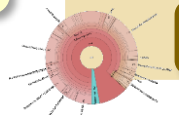
metagWGS


Raw data
1 Reads cleaning
Quality control of
1 reads
1 Taxonomic affiliation of reads
2 Assembly in contigs
metaSPAdes [11]
or megahit[12]

metagWGS


Raw data
1 Reads cleaning
Quality control of
1 reads
1 Taxonomic affiliation of reads
2 Assembly in contigs
3 Assembly filter
4 Gene annotation on contigs
Prokka [13]

metagWGS



Raw data

1 Reads cleaning

Quality control of 1 reads

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DIAMOND [14] 5 Alignment vs protein database

Python script 7 Taxonomic affiliation

genes contigs

H. pylori
Helicobacter

Gene A Gene B Gene C Gene D

→ Contig: H. pylori

Sequence id	consensus taxid	consensus lineage
Sequence 1	210	cellular organisms; Bacteria; Proteobacteria; delta/epsilon subdivisions; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter; Helicobacter pylori
Sequence 2	1681	cellular organisms; Bacteria; Terrabacteria group; Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium; Bifidobacterium bifidum

metagWGS

Raw data

1 Reads cleaning

Quality control of 1 reads

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2 Assembly in contigs

3 Assembly filter

4 Gene annotation on contigs

5 Alignment vs protein database

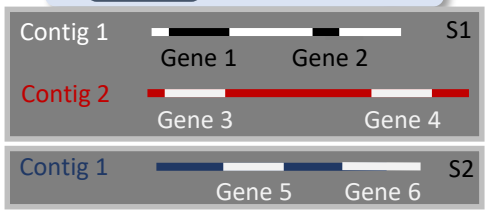
7 Taxonomic affiliation

genes | contigs

OTU	Contigs name	Nb reads	Mean of depth
Helicobacter pylori	...	4367697975	35
Bifidobacterium bifidum	...	3467585	3

metagWGS

Gene	Sample 1	Sample 2
Gene 1	844	887
Gene 2	847	891
...	...	...



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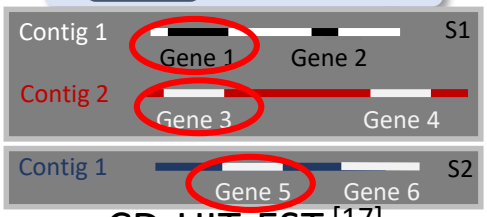
...	...	...



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Bifidobacterium bifidum	...	3467585	3

metagWGS

Gene	Sample 1	Sample 2
Gene 1	844	887
Gene 2	847	891
...	...	...

Function	Sample 1	Sample 2
Function 1	1840	5857
Function 2	4010	4506
...	...	...

OTU	Contigs name	Nb reads	Mean of depth
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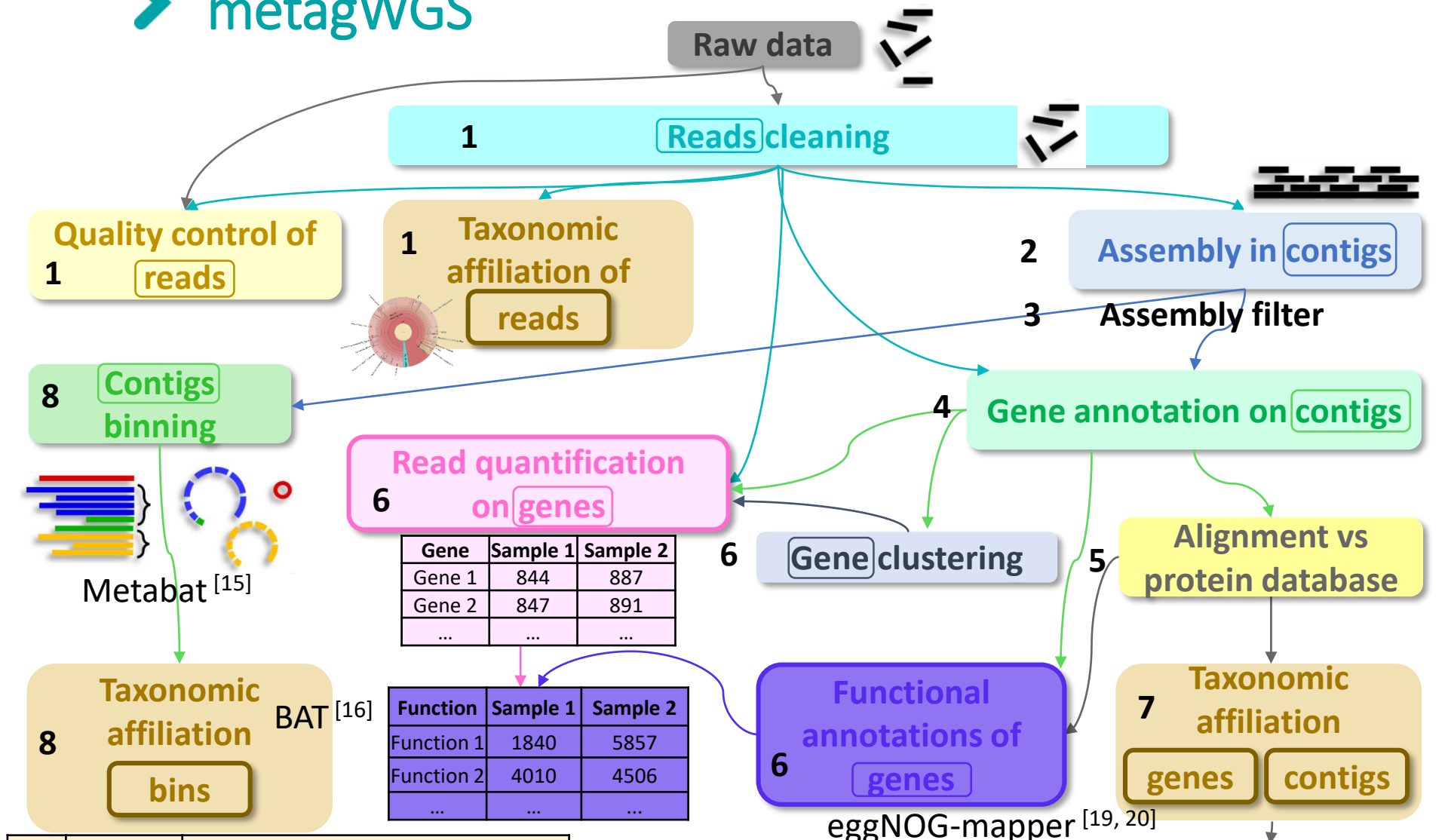
Bifidobacterium bifidum	...	3467585	3

metagWGS



Gene	Sample 1	Sample 2
Gene 1	844	887
Gene 2	847	891
...	...	...

Function	Sample 1	Sample 2
Function 1	1840	5857
Function 2	4010	4506
...	...	...

Bin id	consensus taxid	consensus lineage
Bin 1	210	cellular organisms; Bacteria; Proteobacteria; delta/epsilon subdivisions; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter; Helicobacter pylori

OTU	Contigs name	Nb reads	Mean of depth
Helicobacter pylori	...	4367697975	35
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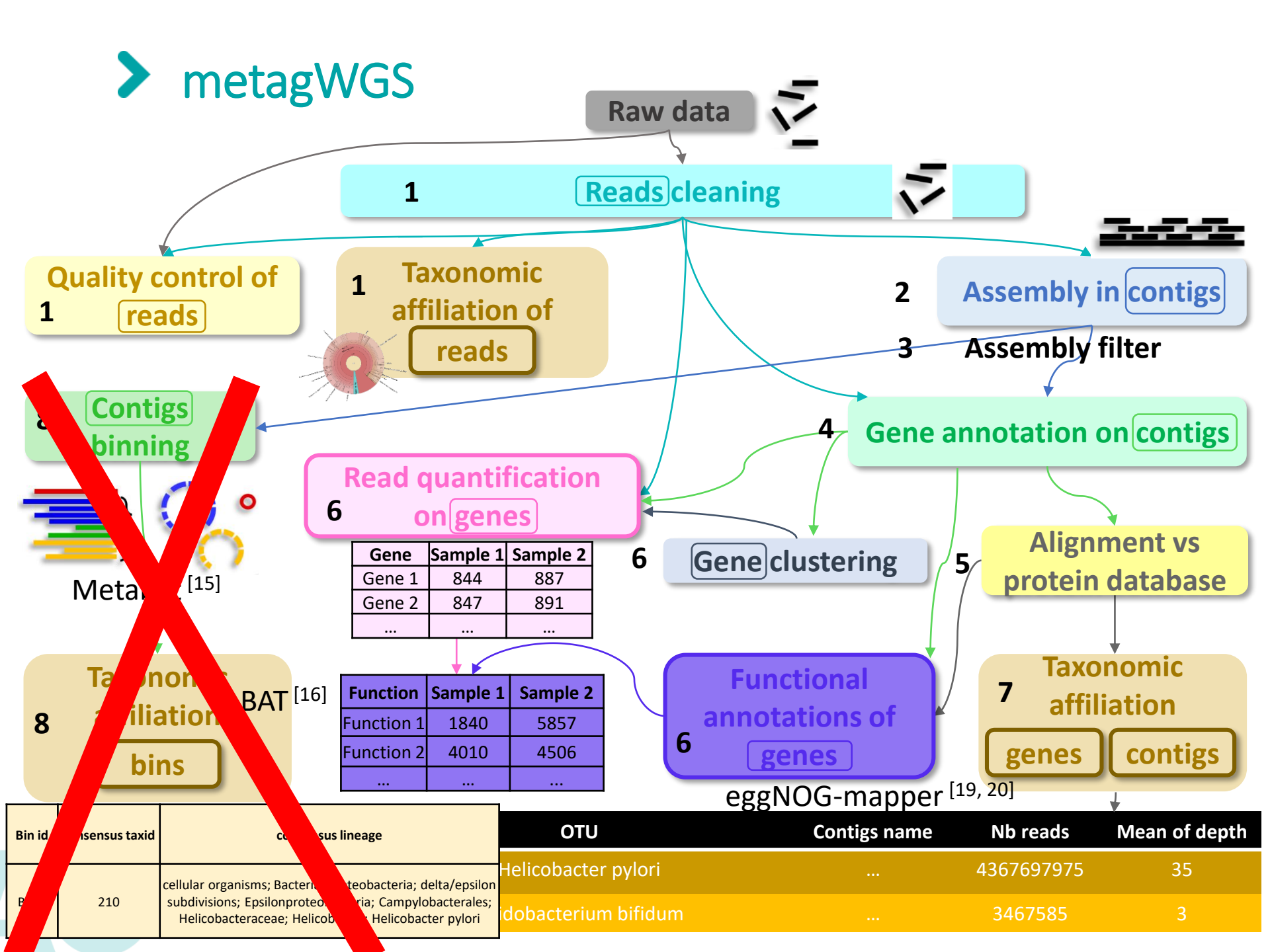
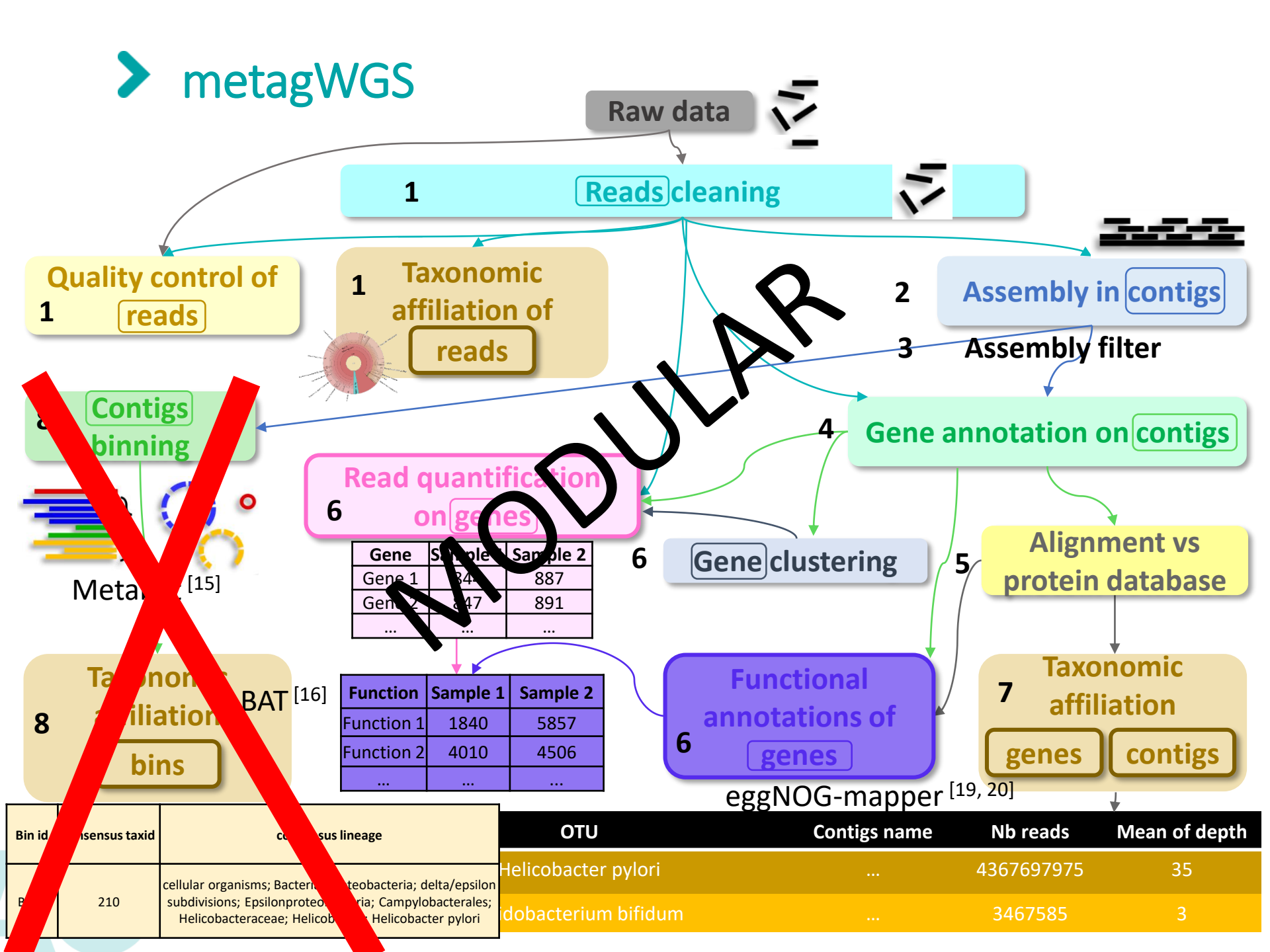
metagWGS


Raw data
1 Reads cleaning
Quality control of reads
1
1 Taxonomic affiliation of reads
2 Assembly in contigs
3 Assembly filter
Contigs binning
4 Gene annotation on contigs
Read quantification on genes
6
Gene clustering
6
5 Alignment vs protein database
Gene | Sample 1 | Sample 2
Gene 1 | 844 | 887
Gene 2 | 847 | 891
... | ... | ...
Function | Sample 1 | Sample 2
Function 1 | 1840 | 5857
Function 2 | 4010 | 4506
... | ... | ...
Functional annotations of genes
6
eggNOG-mapper [19, 20]
7 Taxonomic affiliation
genes
contigs
8 Taxonomic affiliation
bins
BAT [16]
Bin id | sensus taxid | cus lineage
210 | cellular organisms; Bacteri teobacteria; delta/epsilon subdivisions; Epsilonproteo ria; Campylobacterales; Helicobacteraceae; Helicob Helicobacter pylori
OTU | Contigs name | Nb reads | Mean of depth
Helicobacter pylori | ... | 4367697975 | 35
idobacterium bifidum | ... | 3467585 | 3

metagWGS



Gene	Sample 1	Sample 2
Gene 1	344	887
Gene 2	647	891
...	...	...

Function	Sample 1	Sample 2
Function 1	1840	5857
Function 2	4010	4506
...	...	...

Bin id	...sensus taxid	c... ...sus lineage
B...	210	cellular organisms; Bacteri... ...teobacteria; delta/epsilon subdivisions; Epsilonproteo... ...ria; Campylobacterales; Helicobacteraceae; Helicob... Helicobacter pylori

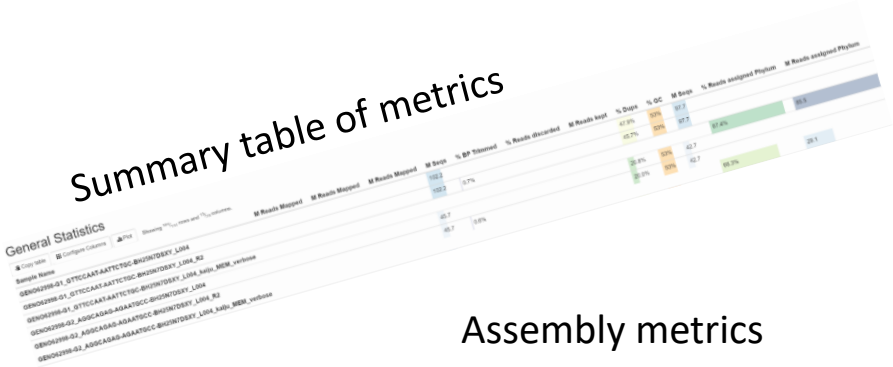
OTU	Contigs name	Nb reads	Mean of depth
Helicobacter pylori	...	4367697975	35
...idobacterium bifidum	...	3467585	3

metagWGS output files

Metrics reports with multiQC[23]

To complete the tabulated files of taxonomic and functional abundance matrices, other files are generated such as html reports of metrics on many steps of the workflow

Summary table of metrics

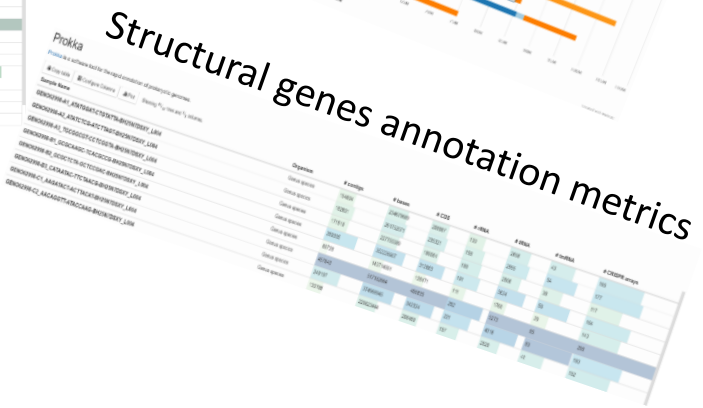


Taxonomic affiliation of reads metrics

Assembly metrics

Reads assignment to genes metrics

Structural genes annotation metrics



metagWGS is freely available

https://forgemia.inra.fr/genotoul-bioinfo/metagwgs

GPL Free Software
Free as in Freedom
GNU General Public License

- Master branch:
  - Version 2.1
  - Documentation:
    - Installation
    - Output
    - Usage
    - Use-case
  - Functional tests and associated documentation

Conclusion and future work

- Workflow easy to use, well documented, able to build taxonomic and functional profiles and many metrics.
- You can choose the step you want to run
- Next version 2.2 (main issues):
  - Binning strategies
  - Improve performances
  - …
  - Currently: upgrading to nextflow DSL2
- Add long reads (HiFi) component ➔ objective: an integrated and modular workflow able to deal with short or long reads metagenomic data

Tool reference

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[20] Huerta-Cepas et al. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res*, 2019

Questions ?

Merci de votre attention