Février_2017_Formation Métagénomique avec le logiciel FROGS

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Training on Galaxy: Metagenomics

February 2017

Find Rapidly OTU with Galaxy Solution

FRÉDÉRIC ESCUDIÉ* and LUCAS AUER*, MARIA BERNARD, LAURENT CAUQUIL, KATIA VIDAL, SARAH MAMAN,
MAHENDRA MARIADASSOU, SYLVIE COMBES, GUILLERMINA HERNANDEZ-RAQUET, GÉRALDINE PASCAL

*THESE AUTHORS HAVE CONTRIBUTED EQUALLY TO THE PRESENT WORK.

Feedback:

What are your needs in “metagenomics"?

454 / MiSeq ?

Your background ?

Monday
afternoon

- Frogs
- Coffee break
- Frogs

Tuesday

- Frogs
- Coffee break
- Frogs
- Lunch
- Frogs
- Coffee break
- Frogs

Wednesday

- Frogs
- Coffee break
- Frogs
- Lunch
- Statistics
- Coffee break
- Statistics

Thrusday

- Statistics
- Coffee break
- Statistics
- Lunch
- Statistics
- Coffee break
- Statistics

9 am to 5 pm

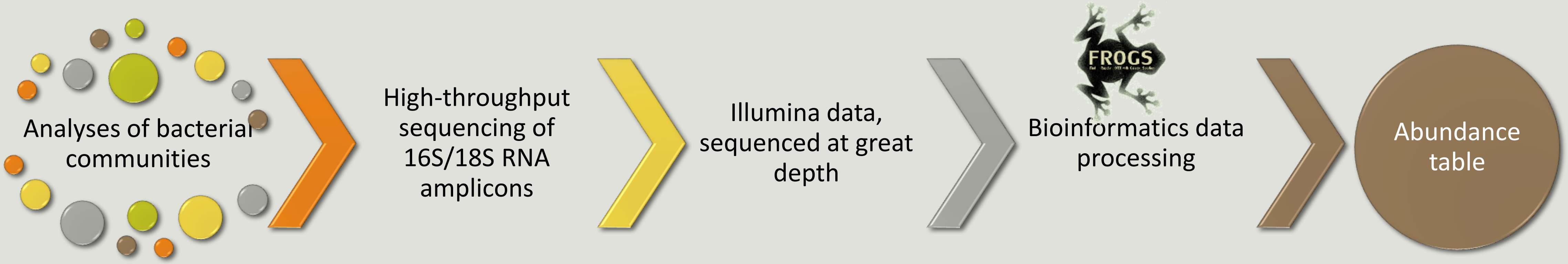
2 short coffee breaks
morning and afternoon

Lunch
12.30 to 2.00 pm

Overview

- Objectives
- Material: data + FROGS
- Data upload into galaxy environment
- Demultiplex tool
- Preprocessing
- Clustering + Cluster Statistics
- Chimera removal
- Filtering
- Affiliation + Affiliation Statistics
- Normalization
- Tool descriptions
- Format transformation
- Workflow creation
- Download data
- Some figures

Objectives



Analyses of bacterial communities

High-throughput sequencing of 16S/18S RNA amplicons

Illumina data, sequenced at great depth

Bioinformatics data processing

Abundance table

WITH
operational taxonomic units (OTUs) and
their taxonomic affiliation.

OTUs for ecology

Operational Taxonomy Unit:
a grouping of similar sequences that can be treated as a single « species »

Strengths:

- Conceptually simple
- Mask effect of poor quality data
  - Sequencing error
  - In vitro recombination (chimera)

Weaknesses:

- Limited resolution
- Logically inconsistent definition

Objectives

	Affiliation	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
OTU1	Species A	0	100	0	45	75	18645
OTU2	Species B	741	0	456	4421	1255	23
OTU3	Species C	12786	45	3	0	0	0
OTU4	Species D	127	4534	80	456	756	108
OTU5	Species E	8766	7578	56	0	0	200

Why we have developed FROGS

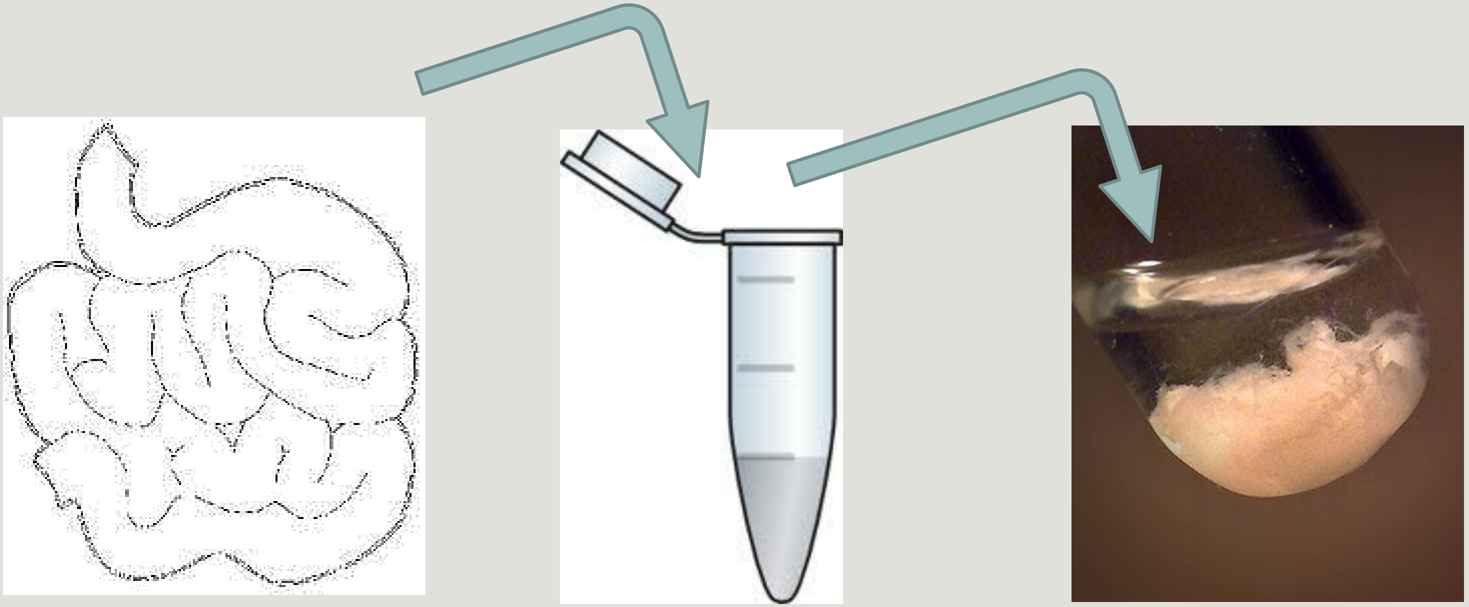
The current processing pipelines struggle to run in a reasonable time.

The most effective solutions are often designed for specialists making access difficult for the whole community.

In this context we developed the pipeline FROGS*: « Find Rapidly OTU with Galaxy Solution ».*

Material

Sample collection and DNA extraction



« Meta-omics » using next-generation sequencing (NGS)

DNA

RNA

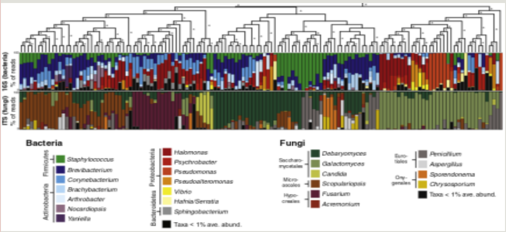
Metagenomics

Metatranscriptomics

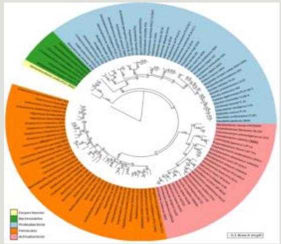
Amplicon sequencing

Shotgun sequencing

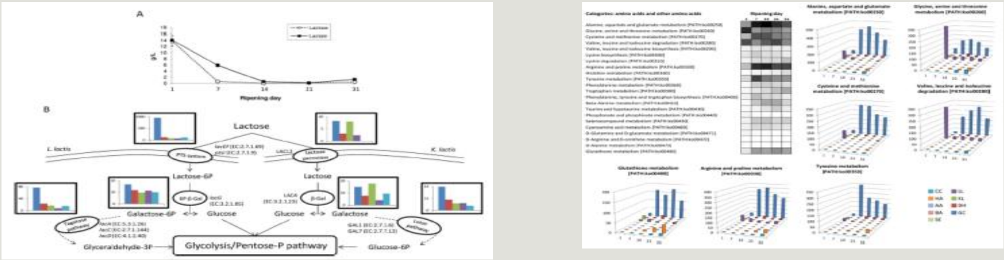
RNA sequencing



Wolfe *et al.*, 2014



Almeida *et al.*, 2014



Dugat-Bony *et al.*, 2015

Who is here?

What can they do?

What are they doing?

The gene encoding the small subunit of the ribosomal RNA

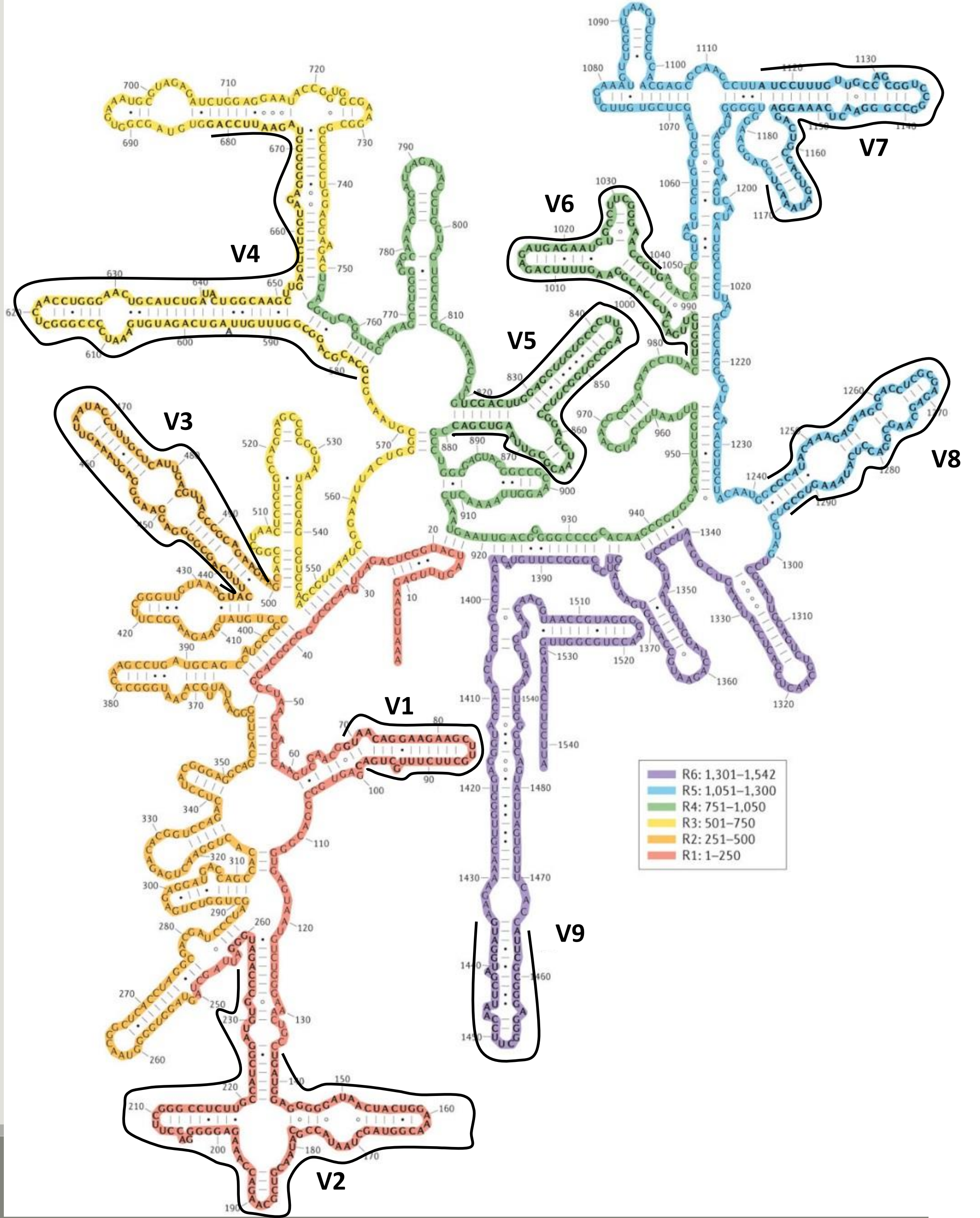
The most widely used gene in **molecular phylogenetic** studies

Ubiquist gene : **16S rDNA** in prokayotes ; **18S rDNA** in eukaryotes

Gene encoding a ribosomal RNA : non-coding RNA (not translated), part of the small subunit of the ribosome which is responsible for the translation of mRNA in proteins

Not submitted to lateral gene transfer

Availability of databases facilitating comparison
(Silva 2015: >22000 type strains)

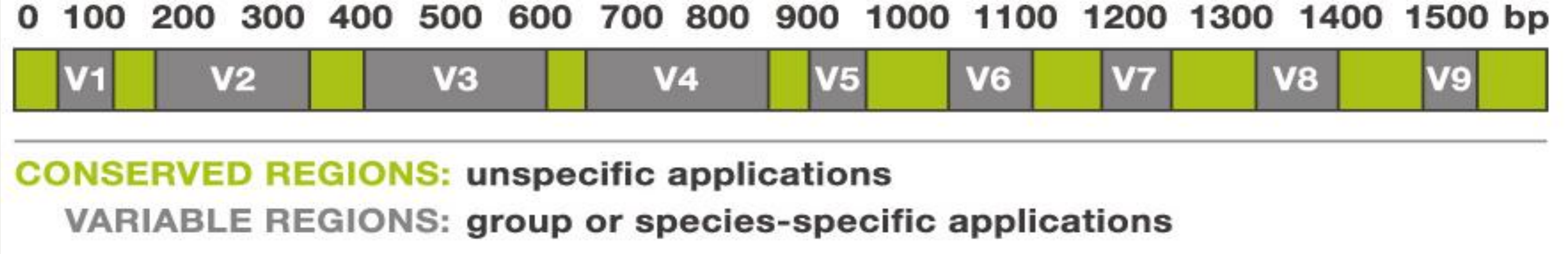

V1
V2
V3
V4
V5
V6
V7
V8
V9
R6: 1,301–1,542
R5: 1,051–1,300
R4: 751–1,050
R3: 501–750
R2: 251–500
R1: 1–250

Secondary structure of the 16S rRNA of *Escherichia coli*

In red, fragment R1 including regions V1 and V2;
in orange, fragment R2 including region V3;
in yellow, fragment R3 including region V4;
in green, fragment R4 including regions V5 and V6;
in blue, fragment R5 including regions V7 and V8;
and in purple, fragment R6 including region V9.

Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences
Pablo Yarza, et al.
Nature Reviews Microbiology 12, 635–645 (2014) doi:10.1038/nrmicro3330

The gene encoding the small subunit of the ribosomal RNA

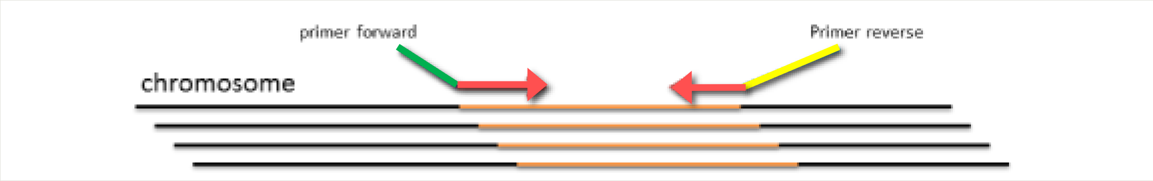

0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 bp
V1
V2
V3
V4
V5
V6
V7
V8
V9

CONSERVED REGIONS: unspecific applications

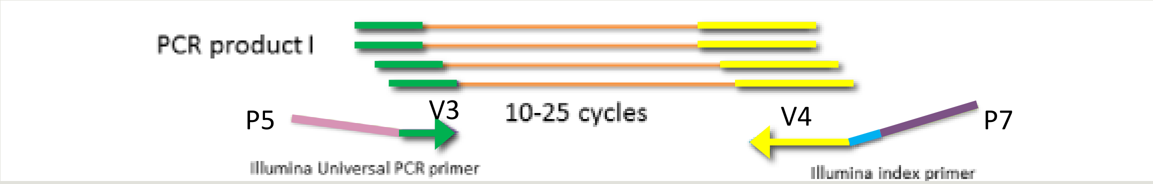
VARIABLE REGIONS: group or species-specific applications

Steps for Illumina sequencing

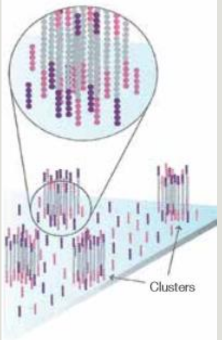
- 1st step : one PCR

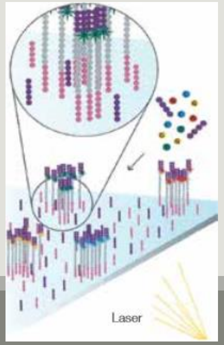

primer forward
Primer reverse
chromosome

- 2nd step: one PCR


PCR product I
P5
V3
10-25 cycles
V4
P7
Illumina Universal PCR primer
Illumina index primer

- 3rd step: on flow cell, the cluster generations
- 4th step: sequencing

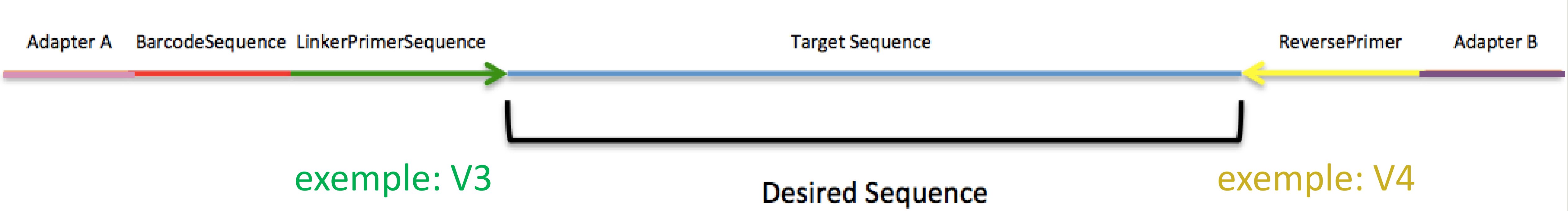




Amplification and sequencing

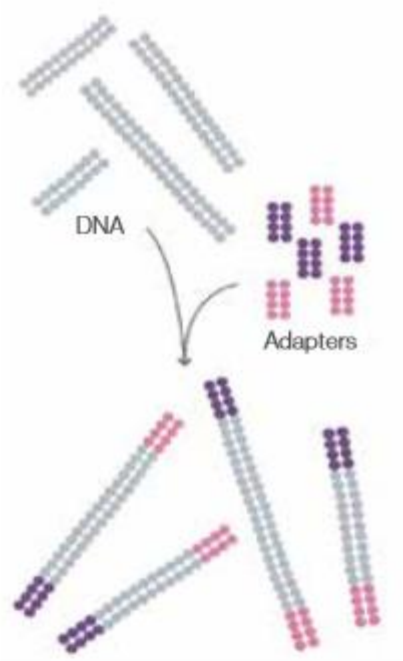
« Universal » primer sets are used for **PCR amplification** of the phylogenetic biomarker

The primers contain adapters used for the sequencing step and barcodes (= tags = MIDs) to distinguish the samples (multiplexing = sequencing several samples on the same run)


Adapter A
BarcodeSequence
LinkerPrimerSequence
Target Sequence
ReversePrimer
Adapter B
exemple: V3
Desired Sequence
exemple: V4

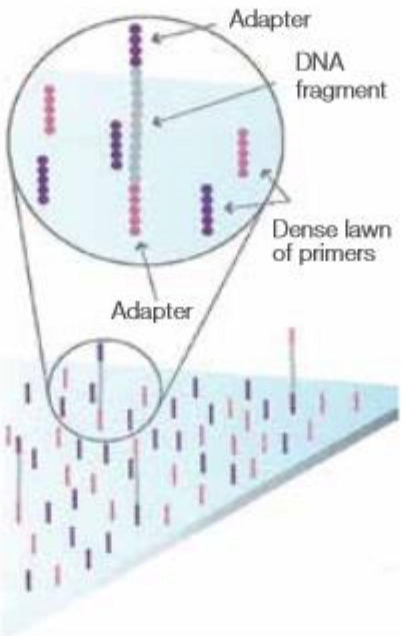
Cluster generation

Prepare Genomic DNA Sample


DNA
Adapters

Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

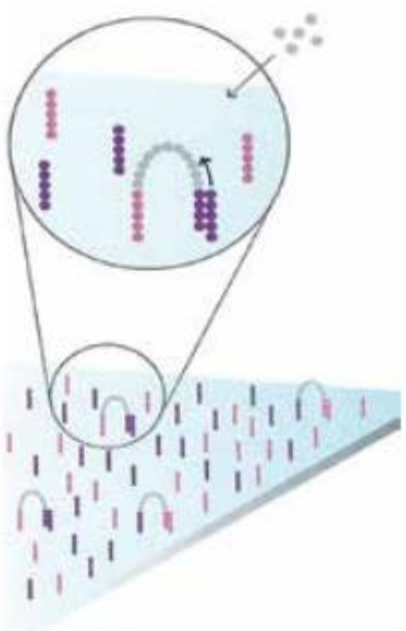
Attach DNA to Surface


Adapter
DNA fragment
Dense lawn of primers
Adapter

Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

Attach DNA to surface

Bridge Amplification

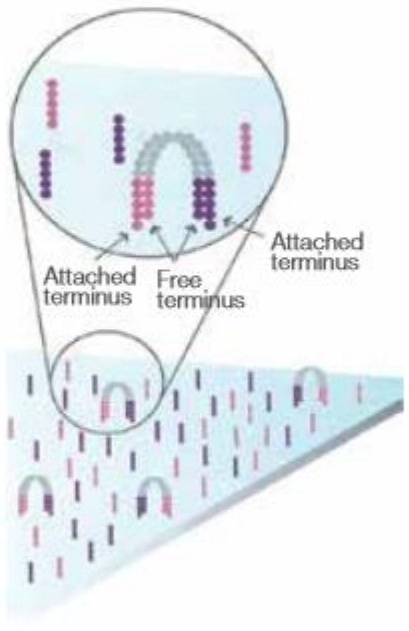


Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

Bridge amplification

Cluster generation

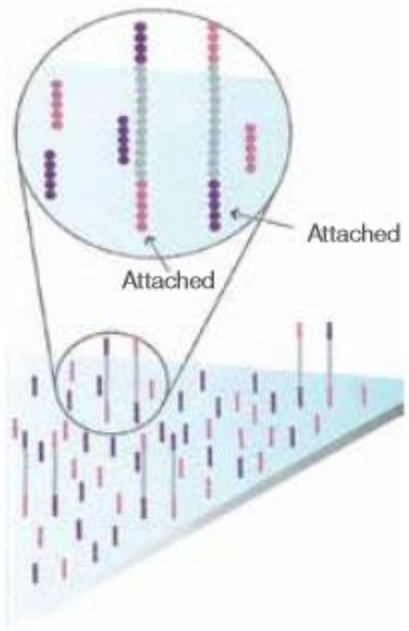
Fragments Become Double Stranded


Attached terminus
Attached terminus
Free terminus

The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

Fragments become double stranded

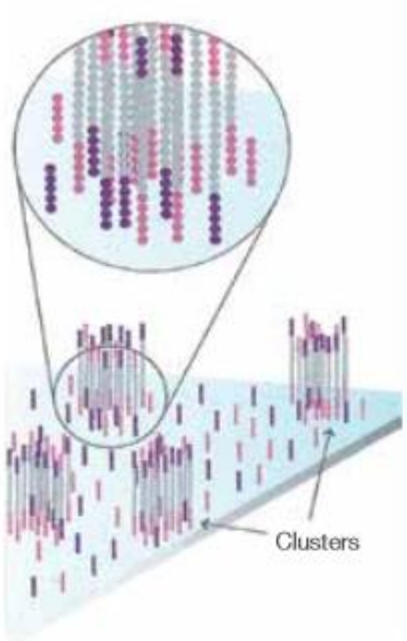
Denature the Double-Stranded Molecules


Attached
Attached

Denaturation leaves single-stranded templates anchored to the substrate.

Denature the double-stranded molecule

Complete Amplification

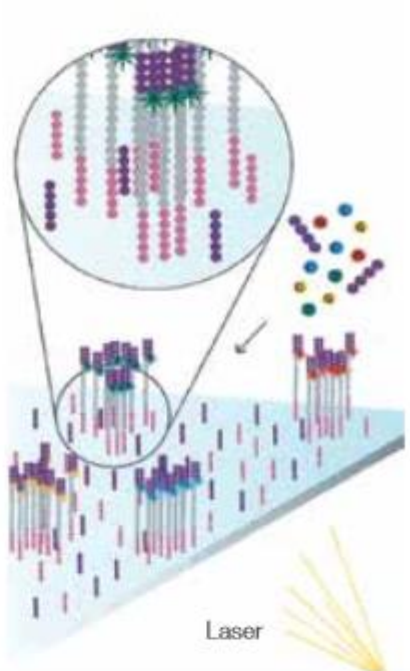


Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

Cycle of new strand synthesis and denaturation to make multiple copies of the same sequence (amplification)
Reverse strands are washed

Sequencing by synthesis

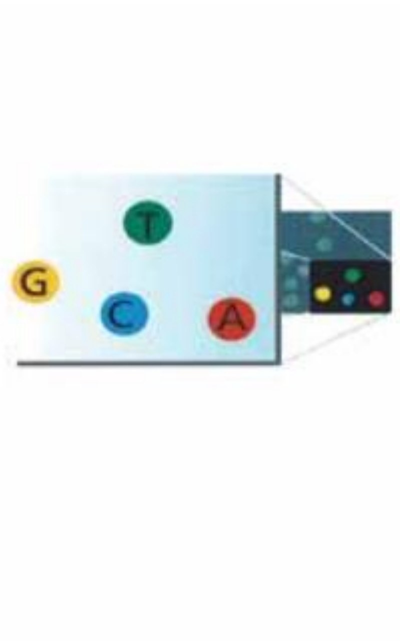
Determine First Base



The first sequencing cycle begins by adding four labeled reversible terminators, primers, and DNA polymerase.

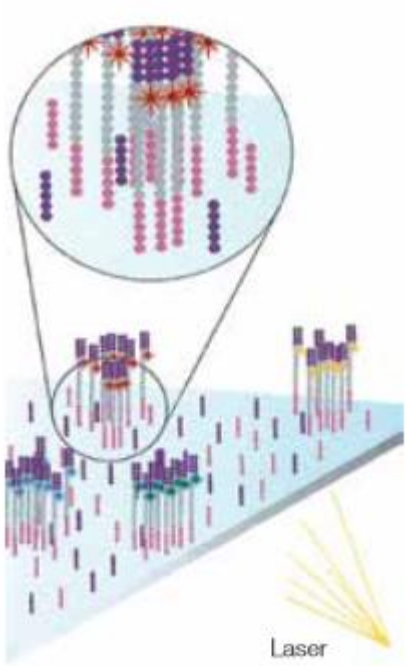
Light signal is more strong in cluster

Image First Base



After laser excitation, the emitted fluorescence from each cluster is captured and the first base is identified.

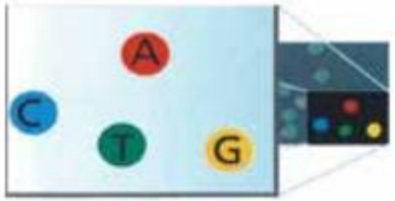
Determine Second Base



The next cycle repeats the incorporation of four labeled reversible terminators, primers, and DNA polymerase.

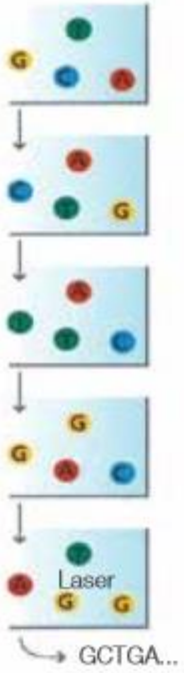
Sequencing by synthesis

Image Second Chemistry Cycle



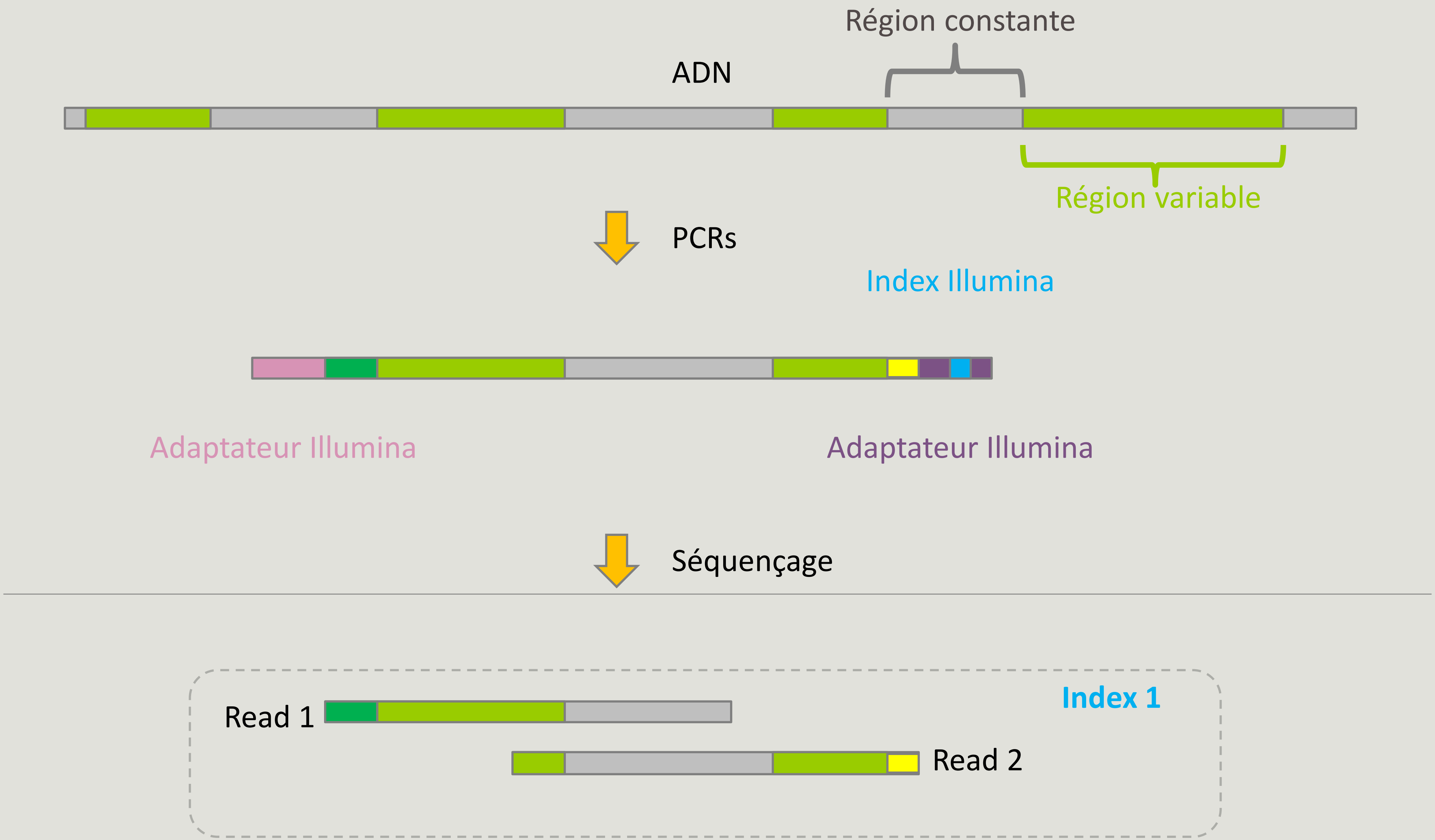
After laser excitation, the image is captured as before, and the identity of the second base is recorded.

Sequencing Over Multiple Chemistry Cycles

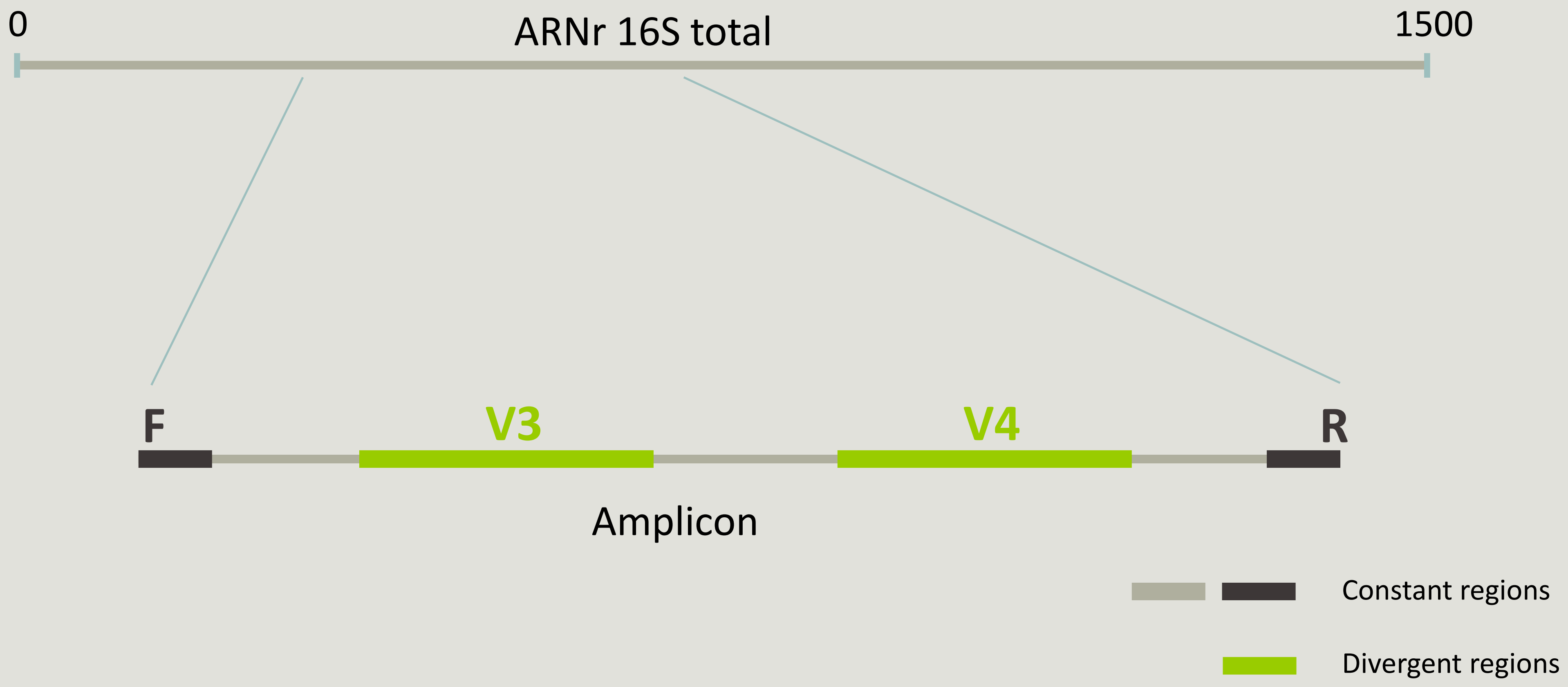


The sequencing cycles are repeated to determine the sequence of bases in a fragment, one base at a time.

Barcode is read, so cluster is identified.
After first sequencing (250 or 300 nt of Reverse strand), fragment form bridges again and Forward strand can be sequenced also.


Région constante
ADN
Région variable
PCRs
Index Illumina
Adaptateur Illumina
Adaptateur Illumina
Séquençage
Index 1
Read 1
Read 2

Identification of bacterial populations may be not discriminating



0

ARNr 16S total

1500

F

V3

V4

R

Amplicon

▬ ▬ Constant regions

🟩 Divergent regions

Amplification and sequencing

Sequencing is generally perform on **Roche-454** or **Illumina MiSeq** platforms.

Roche-454 generally produce ~ 10 000 reads per sample

MiSeq ~ 30 000 reads per sample

Sequence length is **>650 bp** for pyrosequencing technology (Roche-454) and **2 x 300 bp** for the MiSeq technology in paired-end mode.

GS Junior
Roche

Methods

Which bioinformatics solutions ?

	Disadvantages
QIIME	Installation problem Command lines
UPARSE	Global clustering command lines
MOTHUR	Not MiSeq data without normalization Global hierarchical clustering Command lines
MG-RAST	No modularity No transparence

FROGS
Find Rapdly OTU with Galaxy Solution

QIIME allows analysis of high-throughput community sequencing data
J Gregory Caporaso et al, Nature Methods, 2010; doi:10.1038/nmeth.f.303
Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities.
Schloss, P.D., et al., Appl Environ Microbiol, 2009, doi: 10.1128/AEM.01541-09

UPARSE: Highly accurate OTU sequences from microbial amplicon reads
Edgar, R.C. et al, *Nature Methods*, 2013, dx.doi.org/10.1038/nmeth.2604
The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes
F Meyer et al, BMC Bioinformatics, 2008, doi:10.1186/1471-2105-9-386

FROGS ?

Use platform Galaxy

Set of modules = Tools to analyze your “big” data

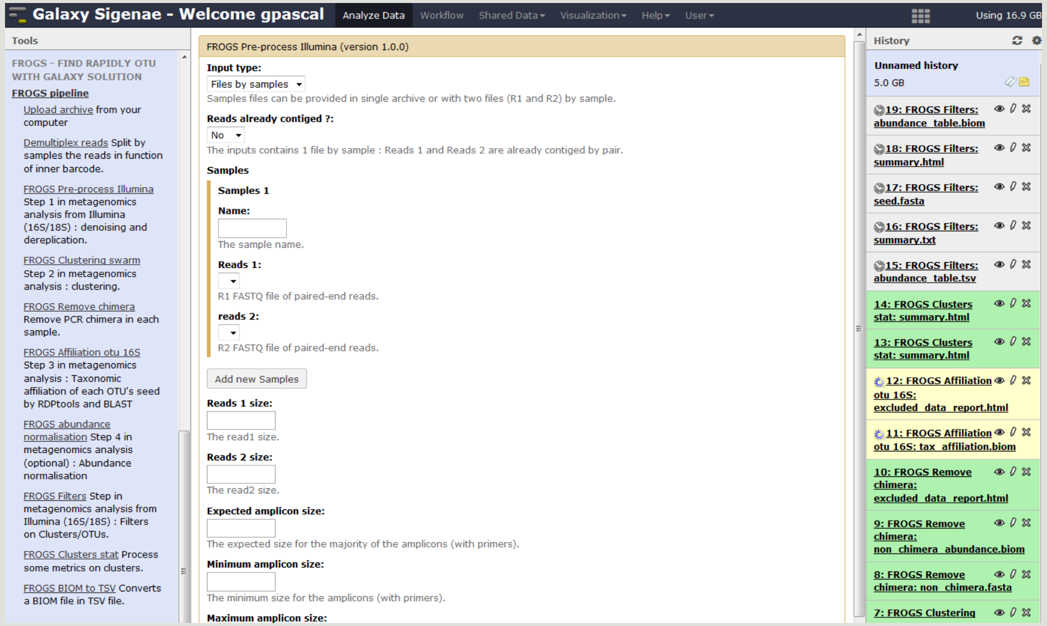
Independent modules

Run on Illumina/454 data 16S, 18S, and 23S

New clustering method

Many graphics for interpretation

User friendly, hiding bioinformatics infrastructure/complexity



FROGS - FIND RAPIDLY OTU WITH GALAXY SOLUTION

FROGS pipeline

Upload archive from your computer

Demultiplex reads Split by samples the reads in function of inner barcode.

FROGS Pre-process Illumina Step 1 in metagenomics analysis from Illumina (16S/18S) : denoising and dereplication.

FROGS Clustering swarm Step 2 in metagenomics analysis : clustering.

FROGS Remove chimera Remove PCR chimera in each sample.

FROGS Affiliation otu 16S Step 3 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST

FROGS abundance normalisation Step 4 in metagenomics analysis (optional) : Abundance normalisation

FROGS Filters Step in metagenomics analysis from Illumina (16S/18S) : Filters on Clusters/OTUs.

FROGS Clusters stat Process some metrics on clusters.

FROGS BIOM to TSV Converts a BIOM file in TSV file.

FROGS Pre-process Illumina (version 1.0.0)

Input type:
Files by samples
Samples files can be provided in single archive or with two files (R1 and R2) by sample.

Reads already contiged ?:
No
The inputs contains 1 file by sample : Reads 1 and Reads 2 are already contiged by pair.

Samples

Samples 1

Name:
The sample name.

Reads 1:
R1 FASTQ file of paired-end reads.

reads 2:
R2 FASTQ file of paired-end reads.

Reads 1 size:
The read1 size.

Reads 2 size:
The read2 size.

Expected amplicon size:
The expected size for the majority of the amplicons (with primers).

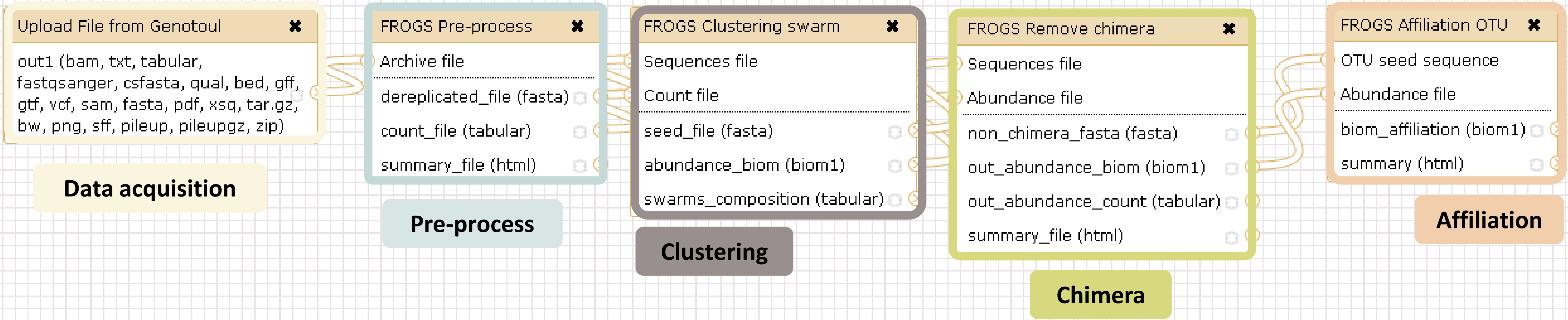
Minimum amplicon size:
The minimum size for the amplicons (with primers).

Maximum amplicon size:

Unnamed history
5.0 GB

19: FROGS Filters: abundance_table.biom
18: FROGS Filters: summary.html
17: FROGS Filters: seed.fasta
16: FROGS Filters: summary.txt
15: FROGS Filters: abundance_table.tsv
14: FROGS Clusters stat: summary.html
13: FROGS Clusters stat: summary.html
12: FROGS Affiliation otu 16S: excluded_data_report.html
11: FROGS Affiliation otu 16S: tax_affiliation.biom
10: FROGS Remove chimera: excluded_data_report.html
9: FROGS Remove chimera: non_chimera_abundance.biom
8: FROGS Remove chimera: non_chimera.fasta
7: FROGS Clustering

FROGS Pipeline



Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

Pre-process

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

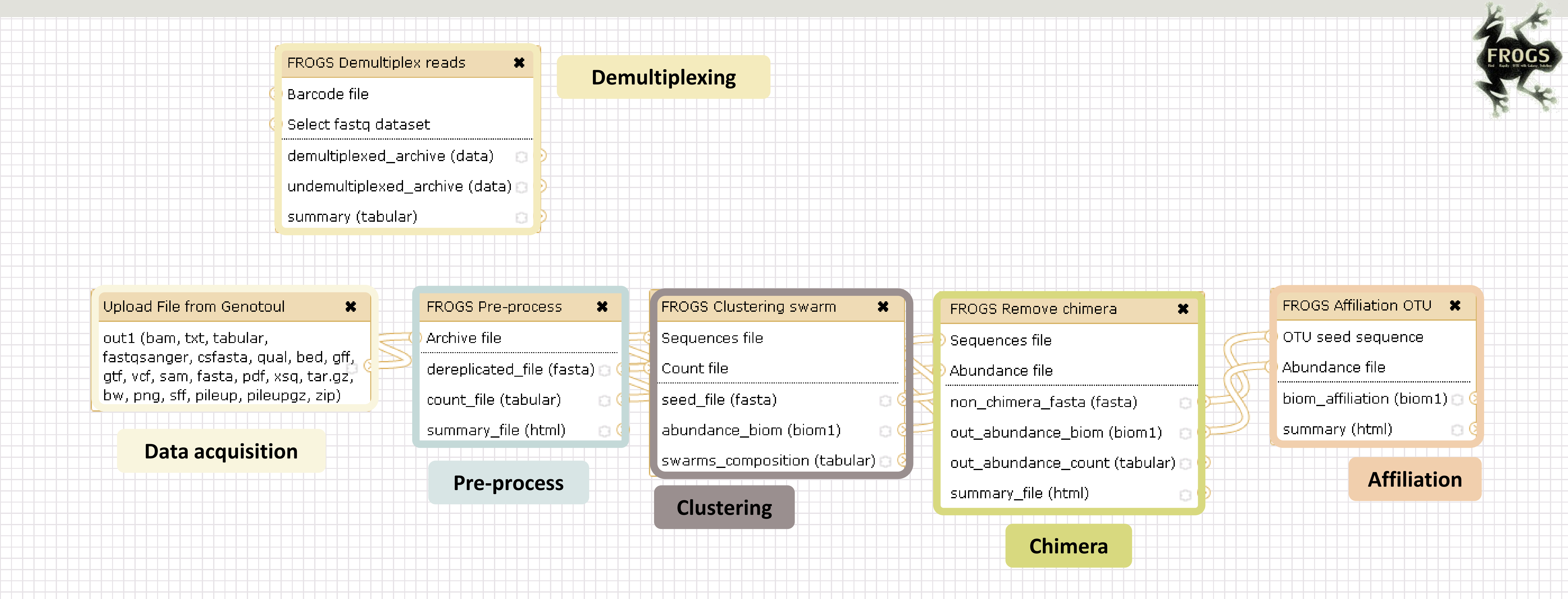
Clustering

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

Chimera

FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Demultiplexing

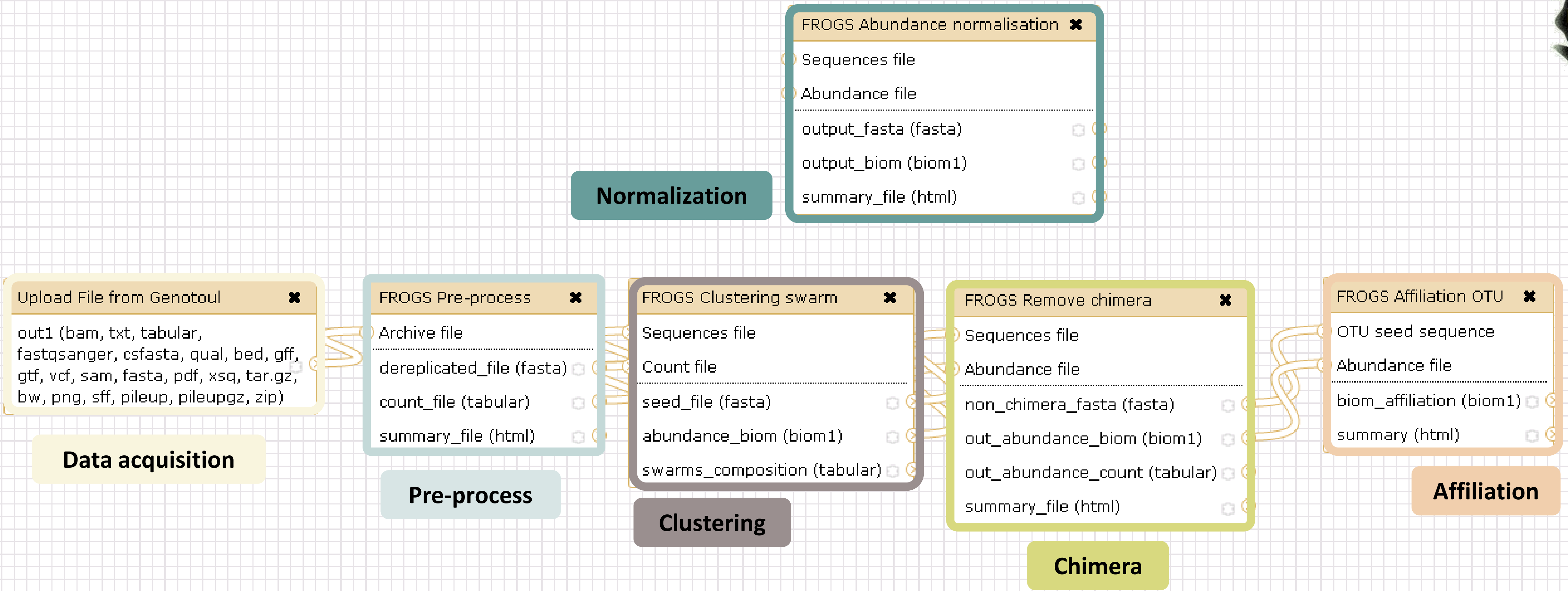
Data acquisition

Pre-process

Clustering

Chimera

Affiliation



FROGS Abundance normalisation ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
summary_file (html)

Normalization

Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

Pre-process

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

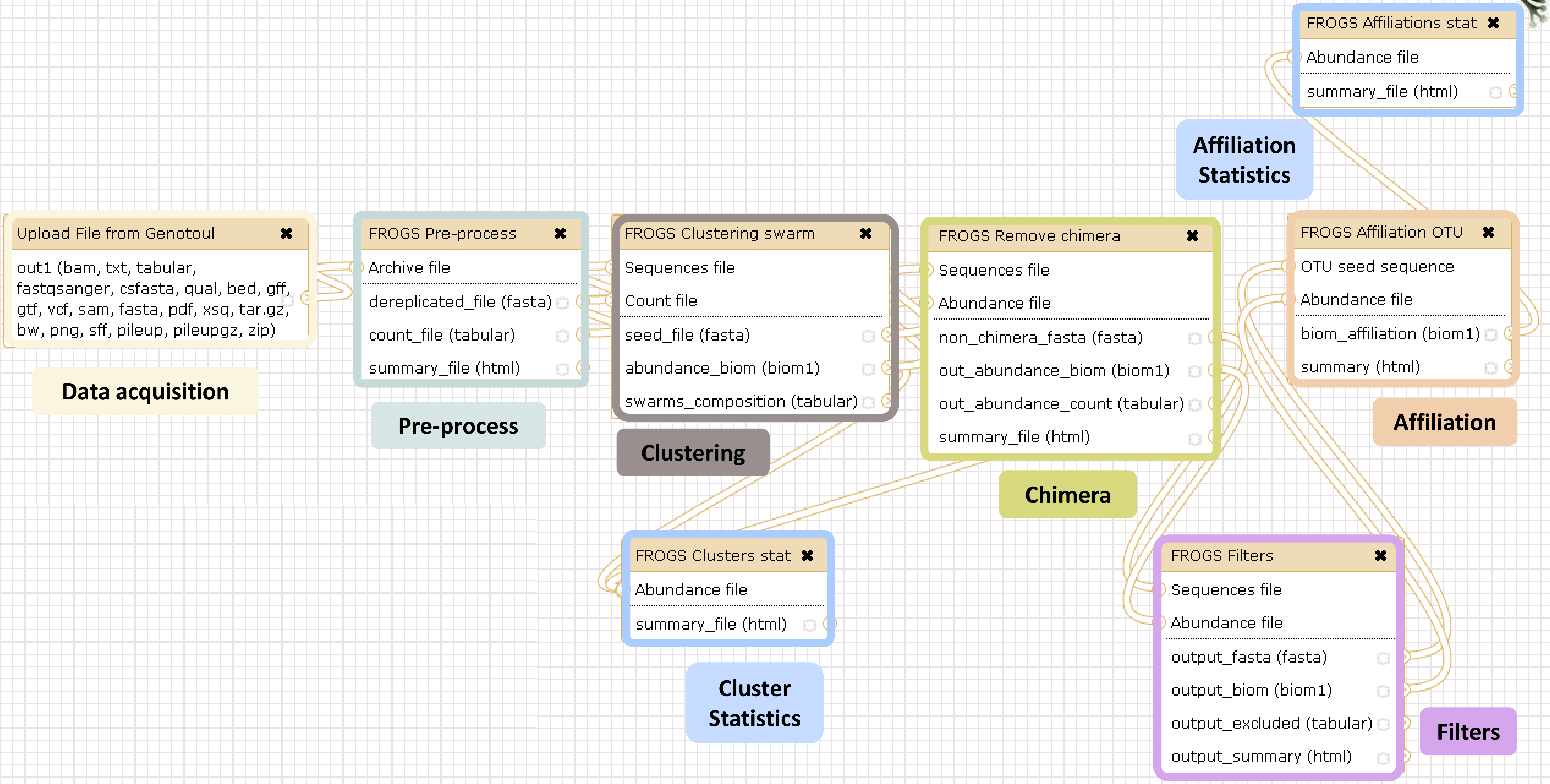
Clustering

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

Chimera

FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation



Upload File from Genotoul ✖

out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process ✖

Archive file

dereplicated_file (fasta)

count_file (tabular)

summary_file (html)

Pre-process

FROGS Clustering swarm ✖

Sequences file

Count file

seed_file (fasta)

abundance_biom (biom1)

swarms_composition (tabular)

Clustering

FROGS Clusters stat ✖

Abundance file

summary_file (html)

Cluster Statistics

FROGS Remove chimera ✖

Sequences file

Abundance file

non_chimera_fasta (fasta)

out_abundance_biom (biom1)

out_abundance_count (tabular)

summary_file (html)

Chimera

FROGS Filters ✖

Sequences file

Abundance file

output_fasta (fasta)

output_biom (biom1)

output_excluded (tabular)

output_summary (html)

Filters

FROGS Affiliation OTU ✖

OTU seed sequence

Abundance file

biom_affiliation (biom1)

summary (html)

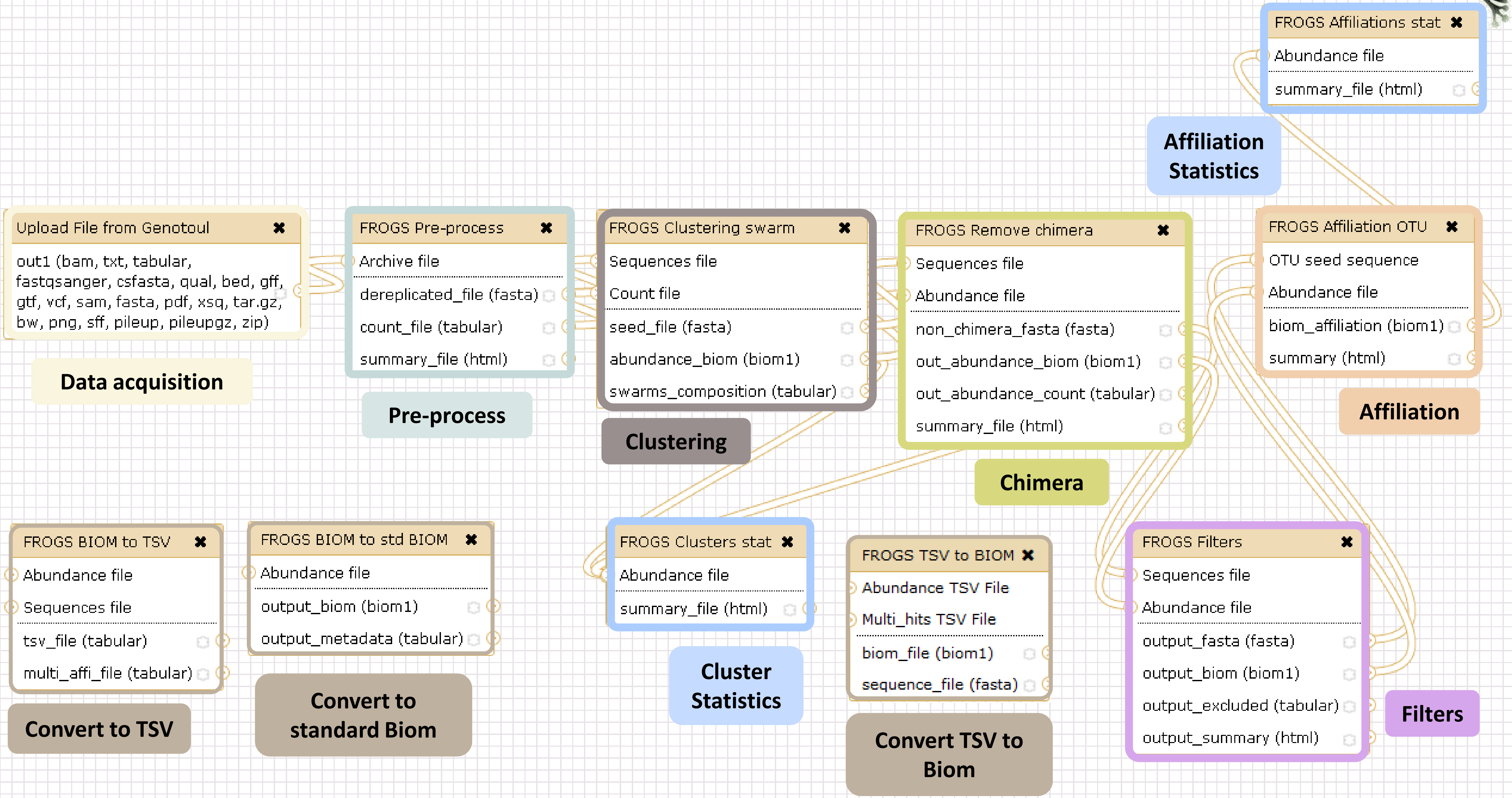
Affiliation

FROGS Affiliations stat ✖

Abundance file

summary_file (html)

Affiliation Statistics



Upload File from Genotoul

out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process

Archive file

dereplicated_file (fasta)

count_file (tabular)

summary_file (html)

Pre-process

FROGS Clustering swarm

Sequences file

Count file

seed_file (fasta)

abundance_biom (biom1)

swarms_composition (tabular)

Clustering

FROGS Remove chimera

Sequences file

Abundance file

non_chimera_fasta (fasta)

out_abundance_biom (biom1)

out_abundance_count (tabular)

summary_file (html)

Chimera

FROGS Affiliation OTU

OTU seed sequence

Abundance file

biom_affiliation (biom1)

summary (html)

Affiliation

FROGS Affiliations stat

Abundance file

summary_file (html)

Affiliation Statistics

FROGS BIOM to TSV

Abundance file

Sequences file

tsv_file (tabular)

multi_affi_file (tabular)

Convert to TSV

FROGS BIOM to std BIOM

Abundance file

output_biom (biom1)

output_metadata (tabular)

Convert to standard Biom

FROGS Clusters stat

Abundance file

summary_file (html)

Cluster Statistics

FROGS TSV to BIOM

Abundance TSV File

Multi_hits TSV File

biom_file (biom1)

sequence_file (fasta)

Convert TSV to Biom

FROGS Filters

Sequences file

Abundance file

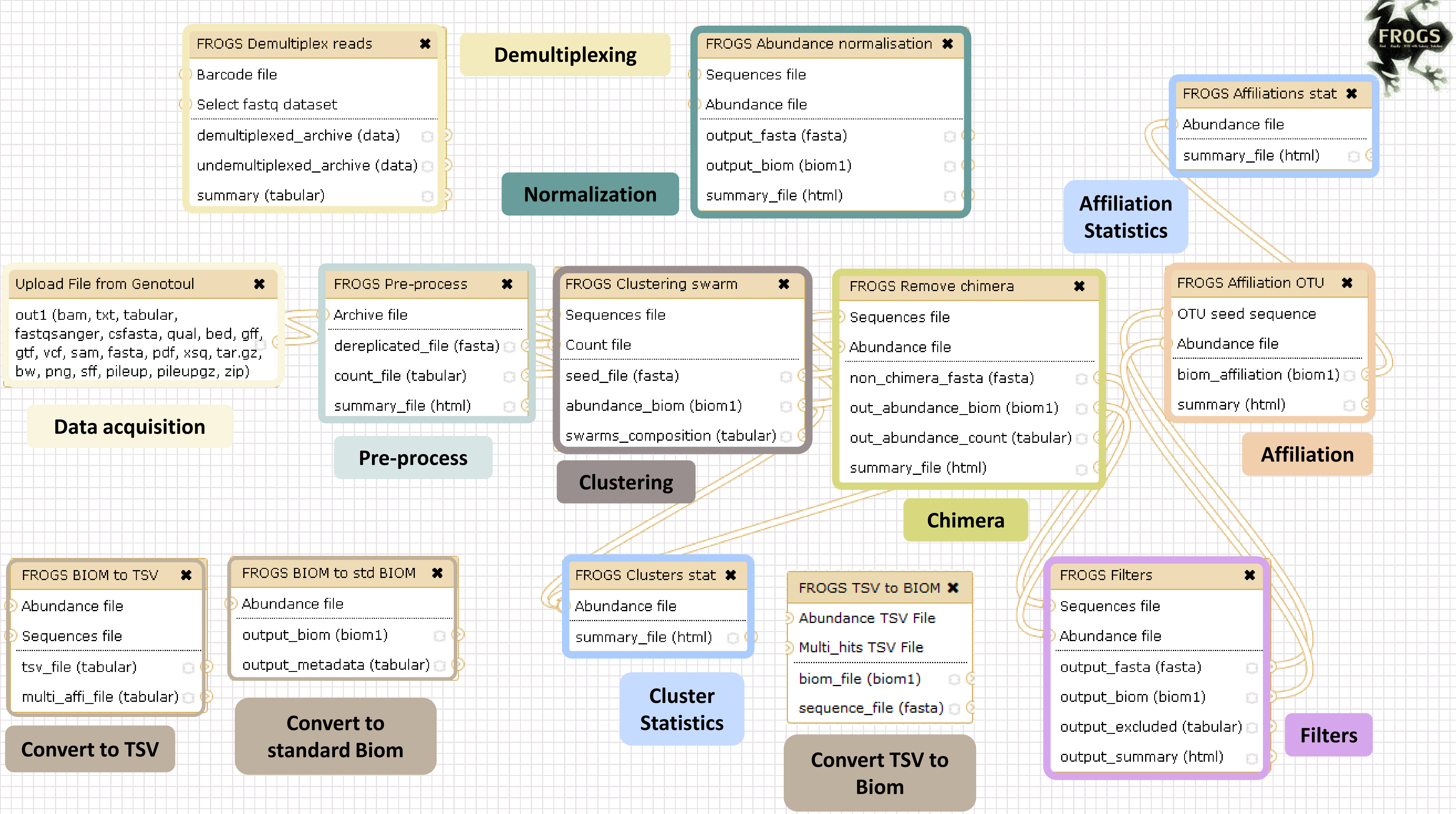
output_fasta (fasta)

output_biom (biom1)

output_excluded (tabular)

output_summary (html)

Filters



FROGS Demultiplex reads ✖
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)

Demultiplexing

Normalization

FROGS Abundance normalisation ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
summary_file (html)

FROGS Affiliations stat ✖
Abundance file
summary_file (html)

Affiliation Statistics

Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

Pre-process

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

Clustering

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

Chimera

FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation

FROGS BIOM to TSV ✖
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)

Convert to TSV

FROGS BIOM to std BIOM ✖
Abundance file
output_biom (biom1)
output_metadata (tabular)

Convert to standard Biom

FROGS Clusters stat ✖
Abundance file
summary_file (html)

Cluster Statistics

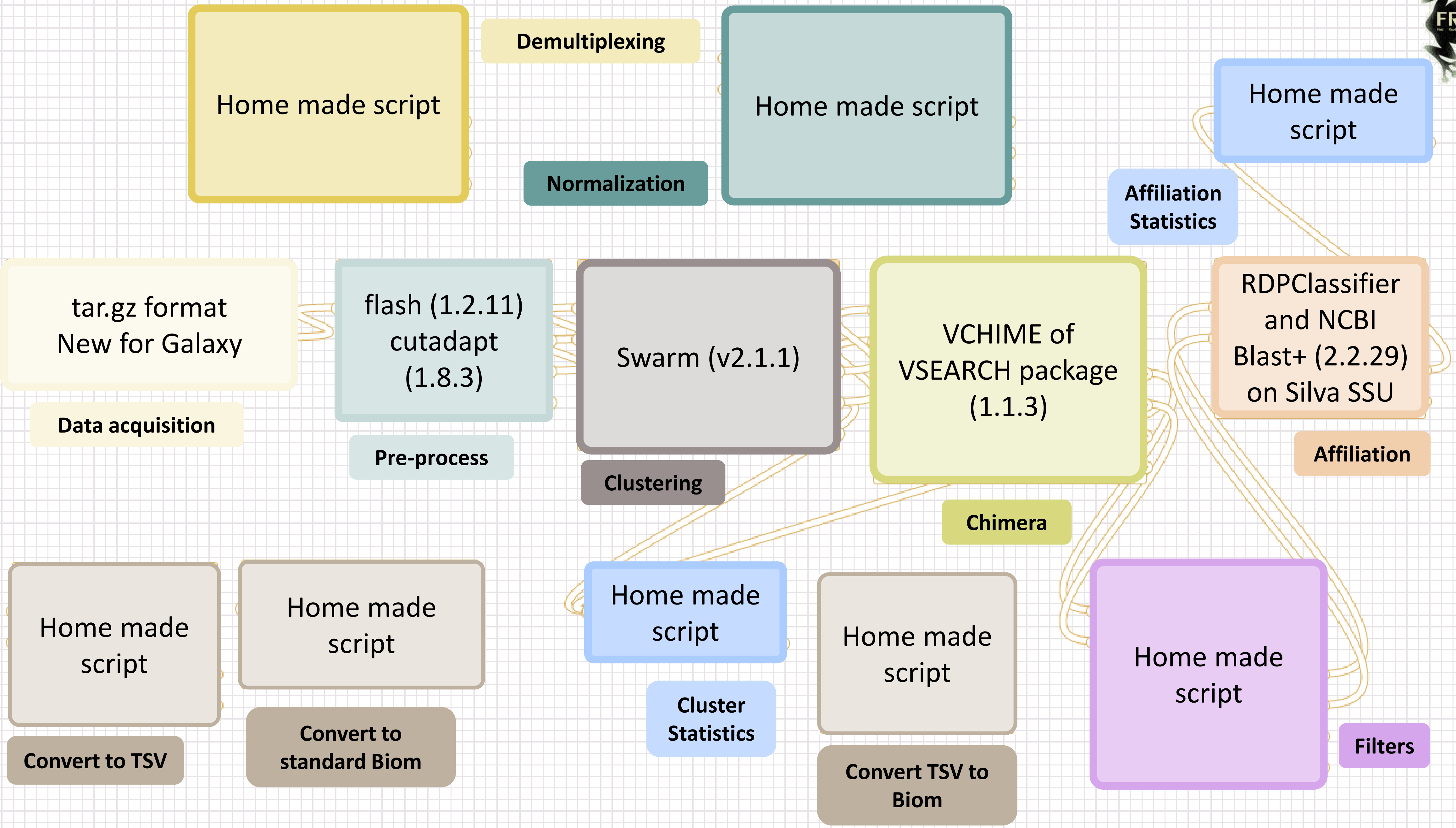
FROGS TSV to BIOM ✖
Abundance TSV File
Multi_hits TSV File
biom_file (biom1)
sequence_file (fasta)

Convert TSV to Biom

FROGS Filters ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

Filters

FROGS



Home made script

Demultiplexing

Normalization

Home made script

Home made script

Affiliation Statistics

tar.gz format
New for Galaxy

Data acquisition

flash (1.2.11)
cutadapt
(1.8.3)

Pre-process

Swarm (v2.1.1)

Clustering

VCHIME of
VSEARCH package
(1.1.3)

Chimera

RDPClassifier
and NCBI
Blast+ (2.2.29)
on Silva SSU

Affiliation

Home made script

Convert to TSV

Home made script

Convert to standard Biom

Home made script

Cluster Statistics

Home made script

Convert TSV to Biom

Home made script

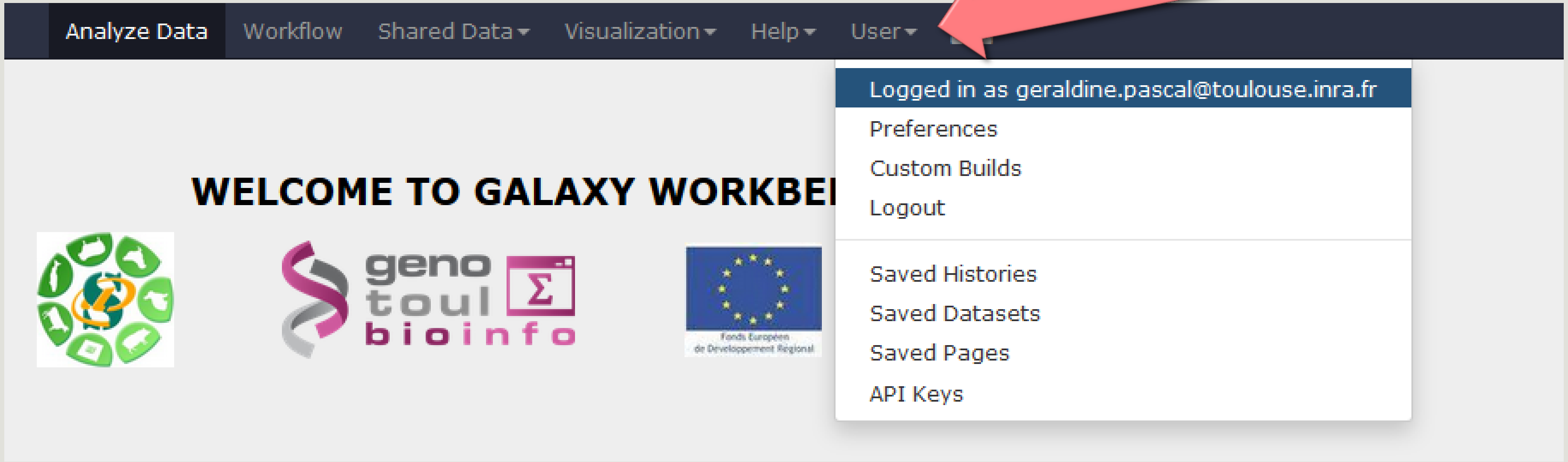
Filters

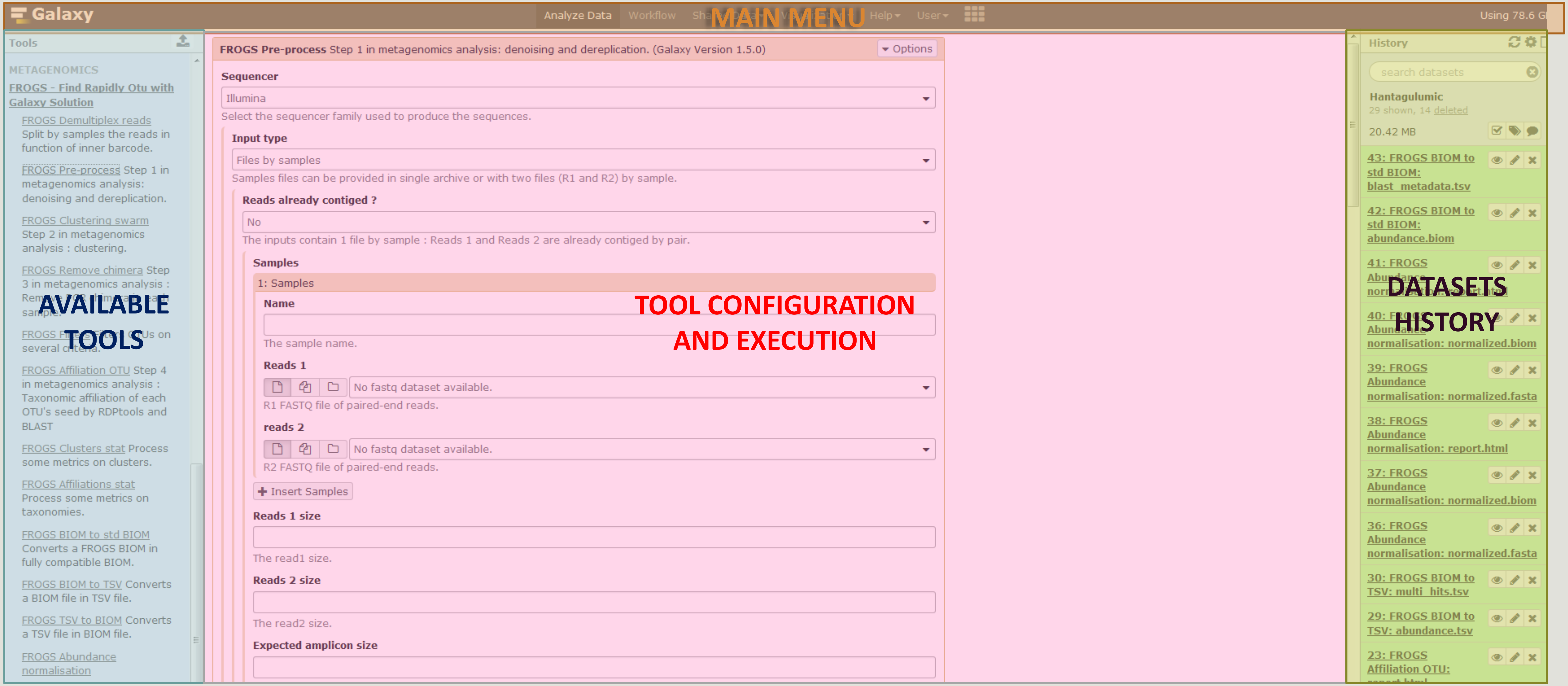
Together go to visit FROGS

In your internet browser (Firefox, chrome, Internet explorer) :

http://sigenae-workbench.toulouse.inra.fr/

Enter your email adress and password from GenoToul


Analyze Data
Workflow
Shared Data
Visualization
Help
User
Logged in as geraldine.pascal@toulouse.inra.fr
Preferences
Custom Builds
Logout
Saved Histories
Saved Datasets
Saved Pages
API Keys
WELCOME TO GALAXY WORKBE
geno toul bioinfo
Fonds Européen de Développement Régional



METAGENOMICS

FROGS - Find Rapidly Otu with Galaxy Solution

FROGS Demultiplex reads Split by samples the reads in function of inner barcode.

FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication.

FROGS Clustering swarm Step 2 in metagenomics analysis : clustering.

FROGS Remove chimera Step 3 in metagenomics analysis :

FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST

FROGS Clusters stat Process some metrics on clusters.

FROGS Affiliations stat Process some metrics on taxonomies.

FROGS BIOM to std BIOM Converts a FROGS BIOM in fully compatible BIOM.

FROGS BIOM to TSV Converts a BIOM file in TSV file.

FROGS TSV to BIOM Converts a TSV file in BIOM file.

FROGS Abundance normalisation

FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication. (Galaxy Version 1.5.0)

Sequencer
Illumina
Select the sequencer family used to produce the sequences.

Input type
Files by samples
Samples files can be provided in single archive or with two files (R1 and R2) by sample.

Reads already contiged ?
No
The inputs contain 1 file by sample : Reads 1 and Reads 2 are already contiged by pair.

Samples
1: Samples

Name
The sample name.

Reads 1
No fastq dataset available.
R1 FASTQ file of paired-end reads.

reads 2
No fastq dataset available.
R2 FASTQ file of paired-end reads.

+ Insert Samples

Reads 1 size
The read1 size.

Reads 2 size
The read2 size.

Expected amplicon size

Hantagulumic
29 shown, 14 deleted
20.42 MB

43: FROGS BIOM to std BIOM: blast_metadata.tsv
42: FROGS BIOM to std BIOM: abundance.biom
41: FROGS
40: FROGS normalisation: normalized.biom
39: FROGS Abundance normalisation: normalized.fasta
38: FROGS Abundance normalisation: report.html
37: FROGS Abundance normalisation: normalized.biom
36: FROGS Abundance normalisation: normalized.fasta
30: FROGS BIOM to TSV: multi_hits.tsv
29: FROGS BIOM to TSV: abundance.tsv
23: FROGS Affiliation OTU:

Demultiplexing

Pre-process

Clustering

Chimera

Filters

Affiliation

Cluster Stat

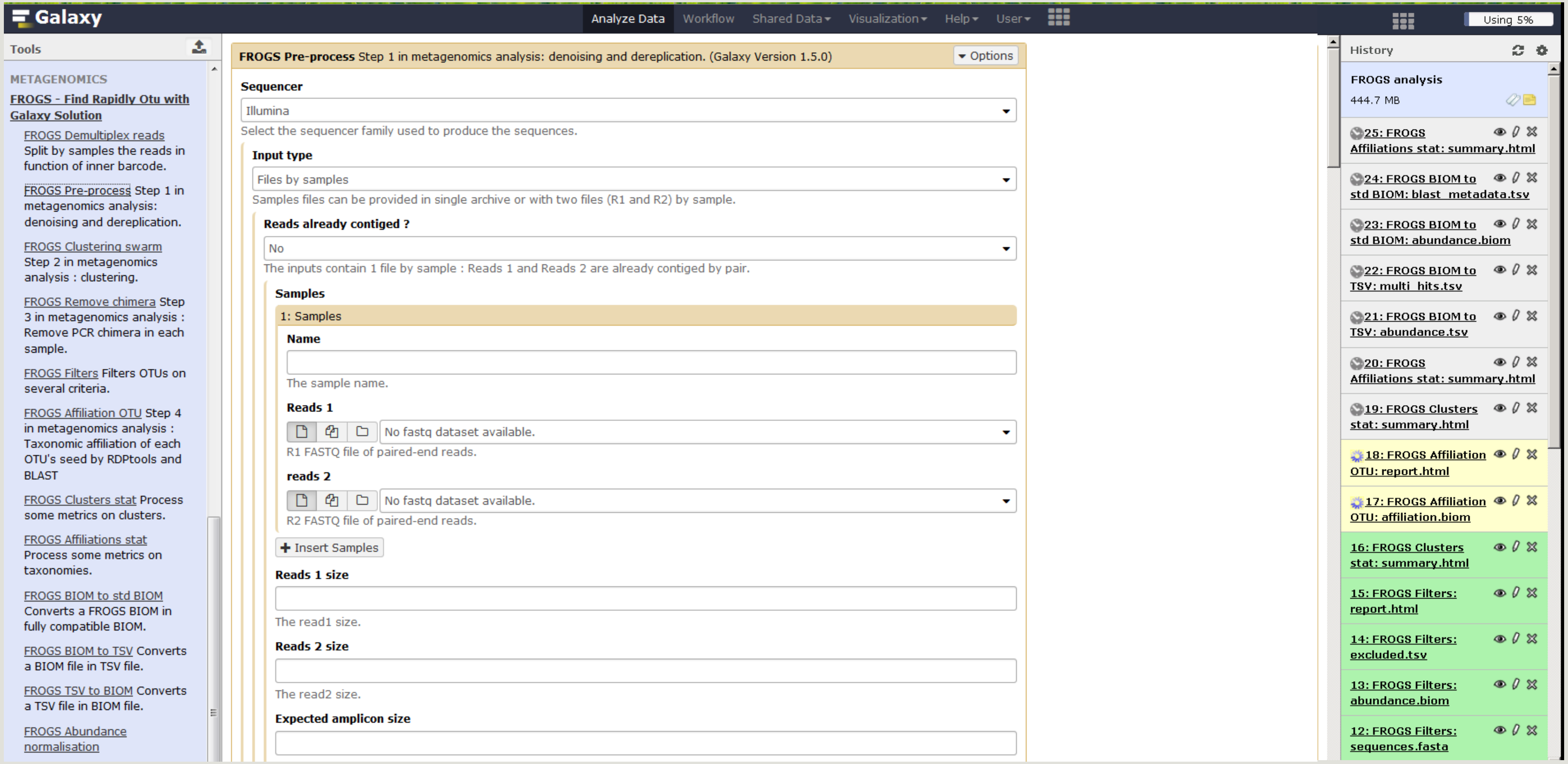
Affiliation Stat

Biom to std Biom

Biom to TSV

TSV to Biom

Normalization



METAGENOMICS

FROGS - Find Rapidly Otu with Galaxy Solution

- FROGS Demultiplex reads Split by samples the reads in function of inner barcode.
- FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication.
- FROGS Clustering swarm Step 2 in metagenomics analysis : clustering.
- FROGS Remove chimera Step 3 in metagenomics analysis : Remove PCR chimera in each sample.
- FROGS Filters Filters OTUs on several criteria.
- FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST
- FROGS Clusters stat Process some metrics on clusters.
- FROGS Affiliations stat Process some metrics on taxonomies.
- FROGS BIOM to std BIOM Converts a FROGS BIOM in fully compatible BIOM.
- FROGS BIOM to TSV Converts a BIOM file in TSV file.
- FROGS TSV to BIOM Converts a TSV file in BIOM file.
- FROGS Abundance normalisation

FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication. (Galaxy Version 1.5.0)

Sequencer
Illumina
Select the sequencer family used to produce the sequences.

Input type
Files by samples
Samples files can be provided in single archive or with two files (R1 and R2) by sample.

Reads already contiged ?
No
The inputs contain 1 file by sample : Reads 1 and Reads 2 are already contiged by pair.

Samples

1: Samples

Name
The sample name.

Reads 1
No fastq dataset available.
R1 FASTQ file of paired-end reads.

reads 2
No fastq dataset available.
R2 FASTQ file of paired-end reads.

Reads 1 size
The read1 size.

Reads 2 size
The read2 size.

Expected amplicon size

FROGS analysis
444.7 MB

- 25: FROGS Affiliations stat: summary.html
- 24: FROGS BIOM to std BIOM: blast_metadata.tsv
- 23: FROGS BIOM to std BIOM: abundance.biom
- 22: FROGS BIOM to TSV: multi_hits.tsv
- 21: FROGS BIOM to TSV: abundance.tsv
- 20: FROGS Affiliations stat: summary.html
- 19: FROGS Clusters stat: summary.html
- 18: FROGS Affiliation OTU: report.html
- 17: FROGS Affiliation OTU: affiliation.biom
- 16: FROGS Clusters stat: summary.html
- 15: FROGS Filters: report.html
- 14: FROGS Filters: excluded.tsv
- 13: FROGS Filters: abundance.biom
- 12: FROGS Filters: sequences.fasta

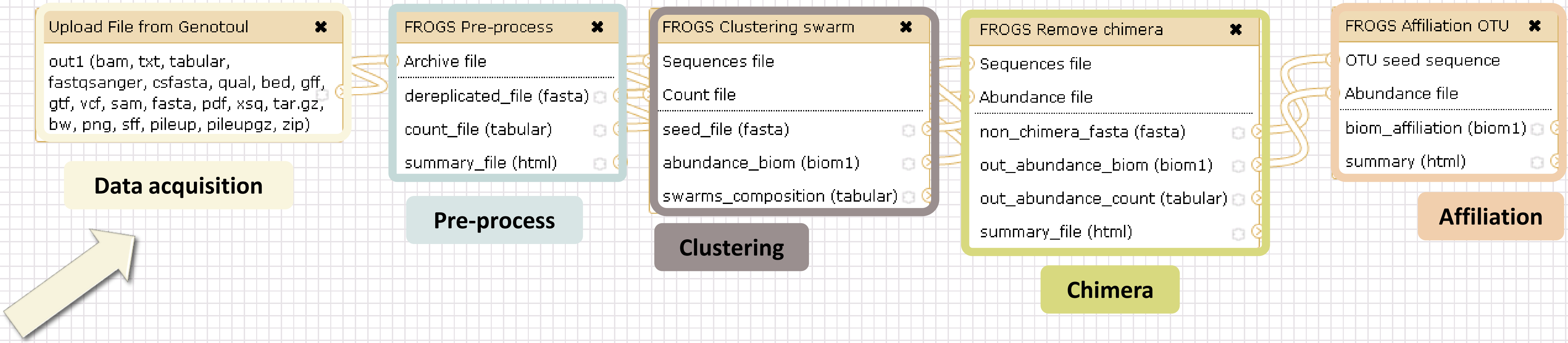
Waiting to run

Currently running

Result files

Upload data

Go to demultiplexing tool



Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Data acquisition

Pre-process

Clustering

Chimera

Affiliation

What kind of data ?

4 Upload → 4 Histories

Multiplexed data

Pathobiomes rodents and ticks

multiplex.fastq

barcode.tabular

454 data

Freshwater sediment metagenome

454.fastq.gz

SRA number
- SRR443364

MiSeq
R1 fastq + R2 fastq

Farm animal feces metagenome

sampleA_R1.fastq

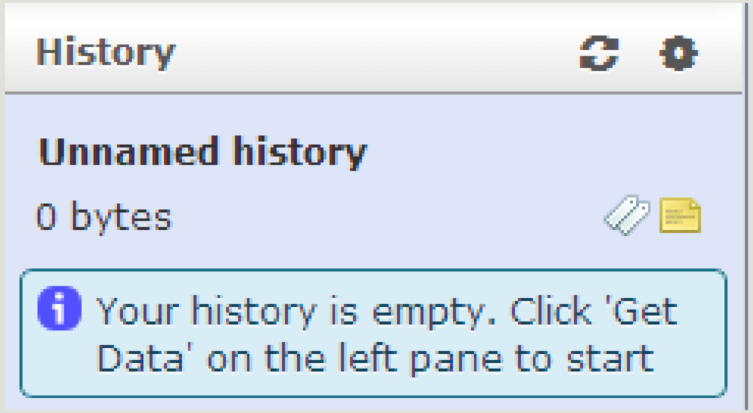
sampleA_R2.fastq

MiSeq contiged fastq in archive tar.gz

Farm animal feces metagenome

100spec_90000seq_9samples.tar.gz

1ST CONNEXION



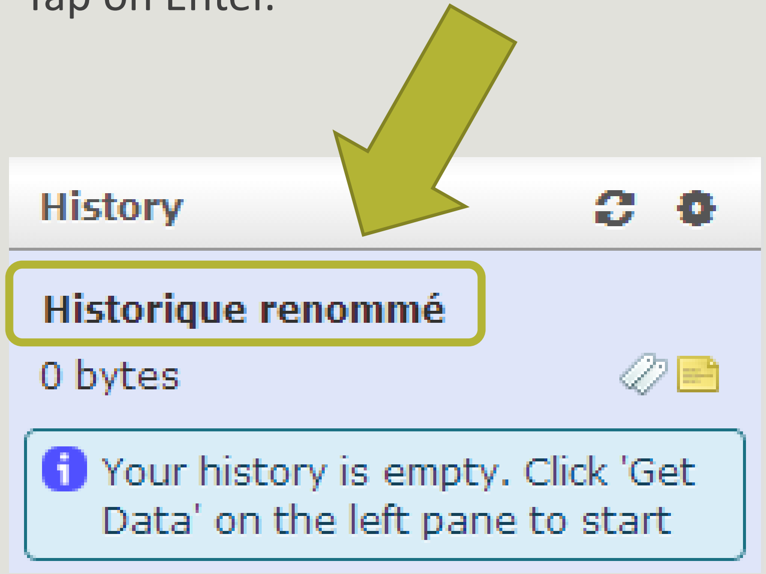
Unnamed history

0 bytes

Your history is empty. Click 'Get Data' on the left pane to start

RENAME HISTORY

- click on Unnamed history,
- Write your new name,
- Tap on Enter.



Historique renommé

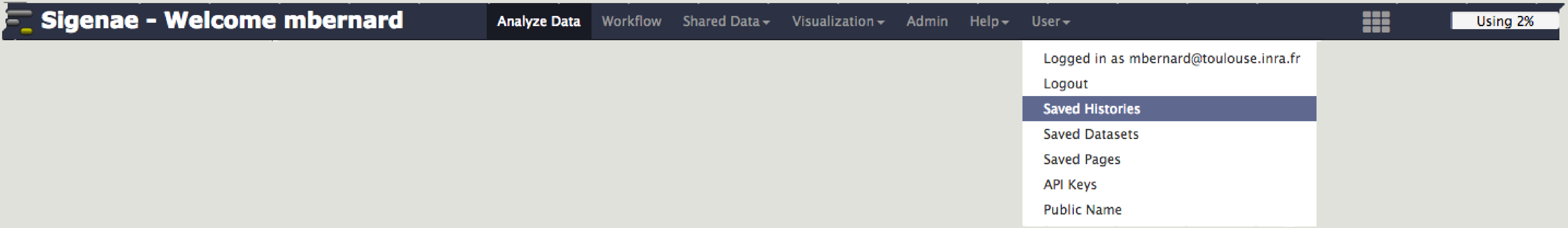
0 bytes

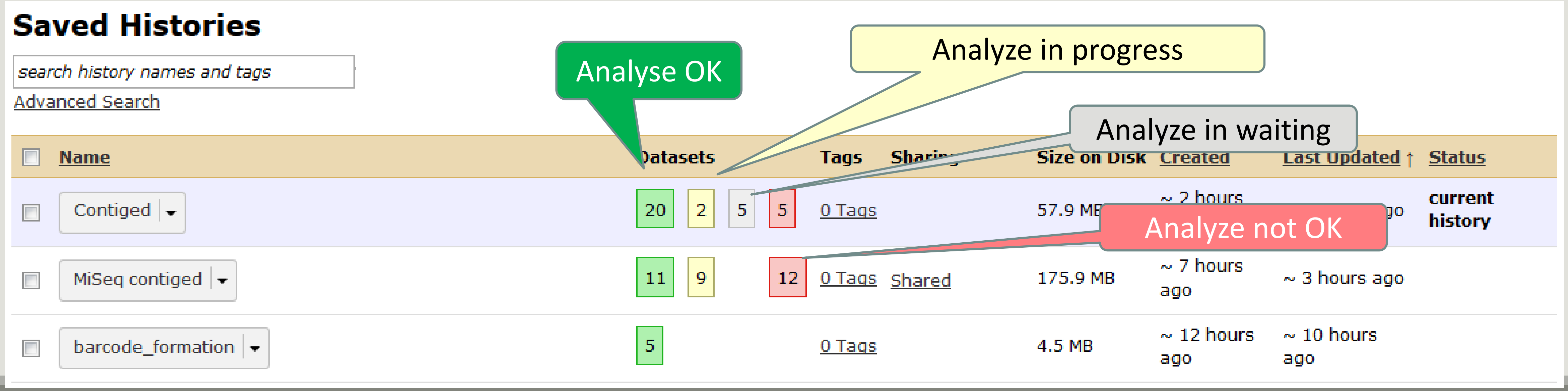
Your history is empty. Click 'Get Data' on the left pane to start

History gestion

- Keep all steps of your analysis.
- Share your analyzes.
- At each run of a tool, a new dataset is created. The data are not overwritten.
- Repeat, as many times as necessary, an analysis.
- All your logs are automatically saved.
- Your published histories are accessible to all users connected to Galaxy (Shared Data / Published Histories).
- Shared histories are accessible only to a specific user (History / Option / Histories Shared With Me).
- To share or publish a history: User / Saved histories / Click the history name / Share or Publish

Saved Histories


Sigenae - Welcome mbernard
Analyze Data
Workflow
Shared Data
Visualization
Admin
Help
User
Using 2%
Logged in as mbernard@toulouse.inra.fr
Logout
Saved Histories
Saved Datasets
Saved Pages
API Keys
Public Name



Saved Histories

search history names and tags

Advanced Search

☐	Name	Datasets	Tags	Sharing	Size on Disk	Created	Last Updated ↑	Status
☐	Contiged	20 2 5 5	0 Tags		57.9 MB	~ 2 hours	go	current history
☐	MiSeq contiged	11 9 12	0 Tags	Shared	175.9 MB	~ 7 hours ago	~ 3 hours ago	
☐	barcode_formation	5	0 Tags		4.5 MB	~ 12 hours ago	~ 10 hours ago	

Analyse OK

Analyze in progress

Analyze in waiting

Analyze not OK

Your turn! - 1

LAUNCH UPLOAD TOOLS

Accounts:

- anemone
- arome
- aster
- bleuet
- camelia
- capucine
- chardon
- clematite
- cobee
- coquelicot
- cosmos

- Password: f1o2r3!

Your turn: exo 1

Create the 1st history **multiplexed**

Import files « **multiplex.fastq** » and « **barcode.tabular** » present in the **Genotoul** folder /work/formation/FROGS/

Create the 2nd history **454**

Import file « **454.fastq.gz** » present in the **Genotoul** folder /work/formation/FROGS/
(datatype fastq or fastq.gz is the same !)

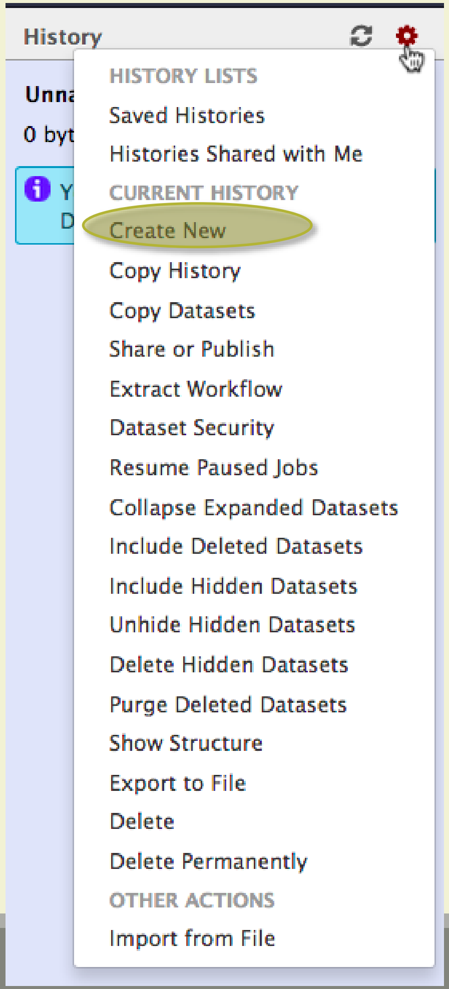
Create the 3rd history **MiSeq R1 R2**

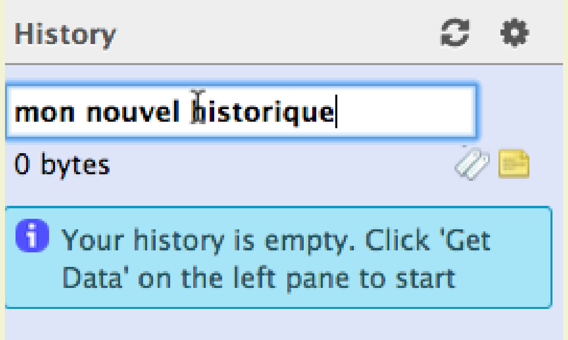
Import files « **sampleA_R1.fastq** » and « **sampleA_R2.fastq** » present in the **Genotoul** folder /work/formation/FROGS/

Create the 4th history **MiSeq contiged**

Import archive file « **100spec_90000seq_9samples.tar.gz** » present in the **Genotoul** folder /work/formation/FROGS/

History creation


History
Unna
0 byt
Y
D
HISTORY LISTS
Saved Histories
Histories Shared with Me
CURRENT HISTORY
Create New
Copy History
Copy Datasets
Share or Publish
Extract Workflow
Dataset Security
Resume Paused Jobs
Collapse Expanded Datasets
Include Deleted Datasets
Include Hidden Datasets
Unhide Hidden Datasets
Delete Hidden Datasets
Purge Deleted Datasets
Show Structure
Export to File
Delete
Delete Permanently
OTHER ACTIONS
Import from File

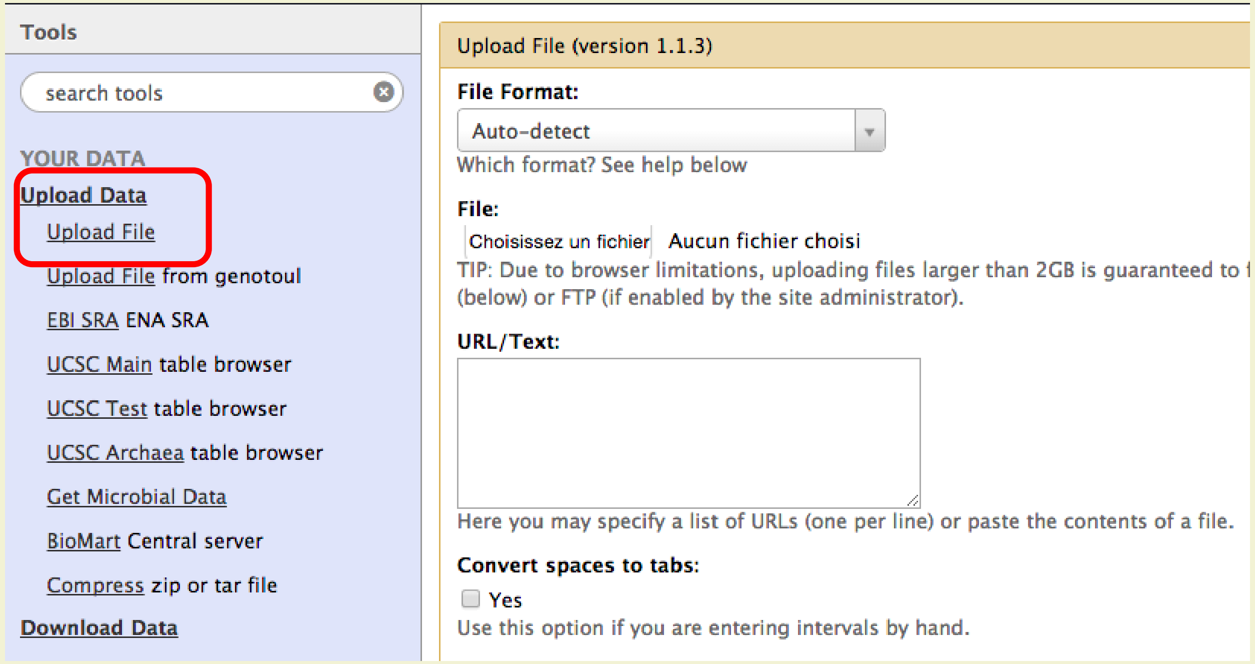


mon nouvel historique

0 bytes

Your history is empty. Click 'Get Data' on the left pane to start

Upload data: different methods


Tools
search tools
YOUR DATA
Upload Data
Upload File
Upload File from genotoul
EBI SRA ENA SRA
UCSC Main table browser
UCSC Test table browser
UCSC Archaea table browser
Get Microbial Data
BioMart Central server
Compress zip or tar file
Download Data

Upload File (version 1.1.3)

File Format:

Auto-detect

Which format? See help below

File:

Choisissez un fichier Aucun fichier choisi

TIP: Due to browser limitations, uploading files larger than 2GB is guaranteed to
(below) or FTP (if enabled by the site administrator).

URL/Text:

Here you may specify a list of URLs (one per line) or paste the contents of a file.

Convert spaces to tabs:

☐ Yes

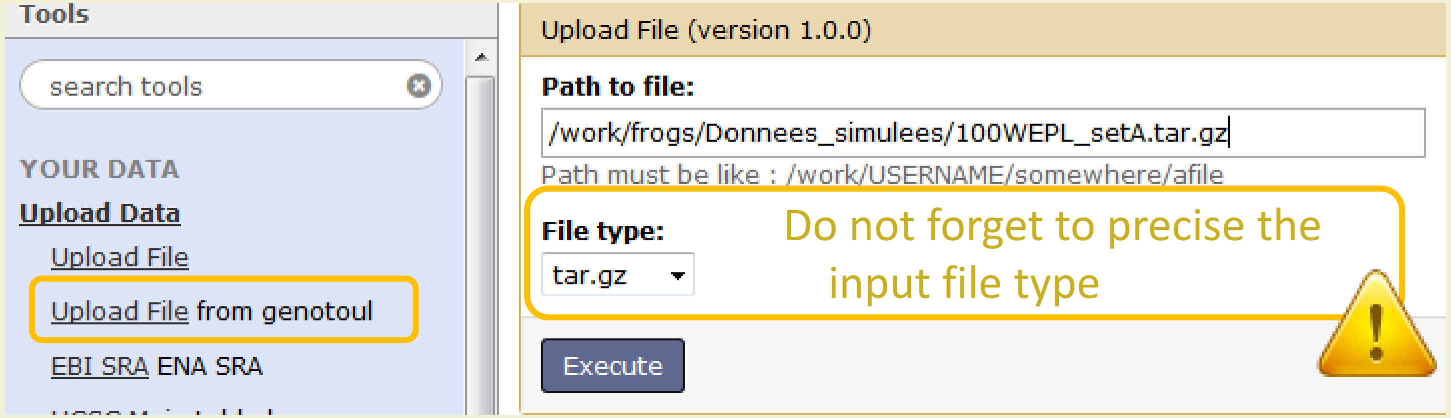
Use this option if you are entering intervals by hand.

Default method, your files are on **your** computer or accessible on the internet, they are copied on your Galaxy account

You can only upload one local file at a time
→ 10 samples ≥ 10 uploads
You can upload multiple files using URLs
but only smaller than 2Go

Each uploaded file will consume your Galaxy's quota!

Upload data: different methods


Tools
search tools
YOUR DATA
Upload Data
Upload File
Upload File from genotoul
EBI SRA ENA SRA
Upload File (version 1.0.0)
Execute

Path to file:

/work/frogs/Donnees_simulees/100WEPL_setA.tar.gz

Path must be like : /work/USERNAME/somewhere/afile

File type: tar.gz

Do not forget to precise the input file type

Specific SIGENAE GENOTOUL method. It allows you to access to your files in **your work account** on the Genotoul **without consuming your Galaxy quota**.

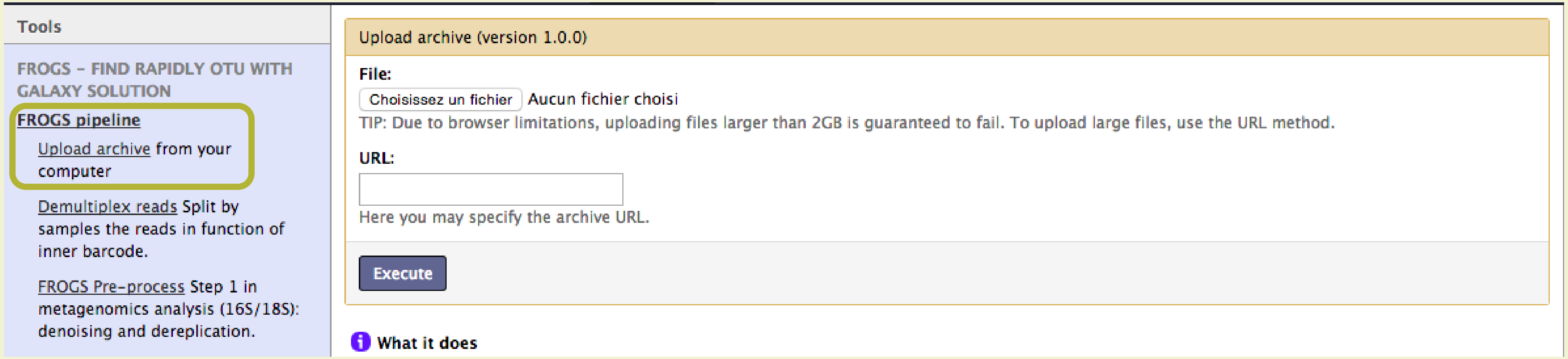
And if you have multiple samples ?

See How_to_create_an_archiveTAR.ppt

How to transfer files on /work of Genotoul?

See How_to_transfert_to_genotoul.ppt

Upload data: different methods



Tools

FROGS - FIND RAPIDLY OTU WITH GALAXY SOLUTION

FROGS pipeline

Upload archive from your computer

Demultiplex reads Split by samples the reads in function of inner barcode.

FROGS Pre-process Step 1 in metagenomics analysis (16S/18S): denoising and dereplication.

Upload archive (version 1.0.0)

File:

Choisissez un fichier Aucun fichier choisi

TIP: Due to browser limitations, uploading files larger than 2GB is guaranteed to fail. To upload large files, use the URL method.

URL:

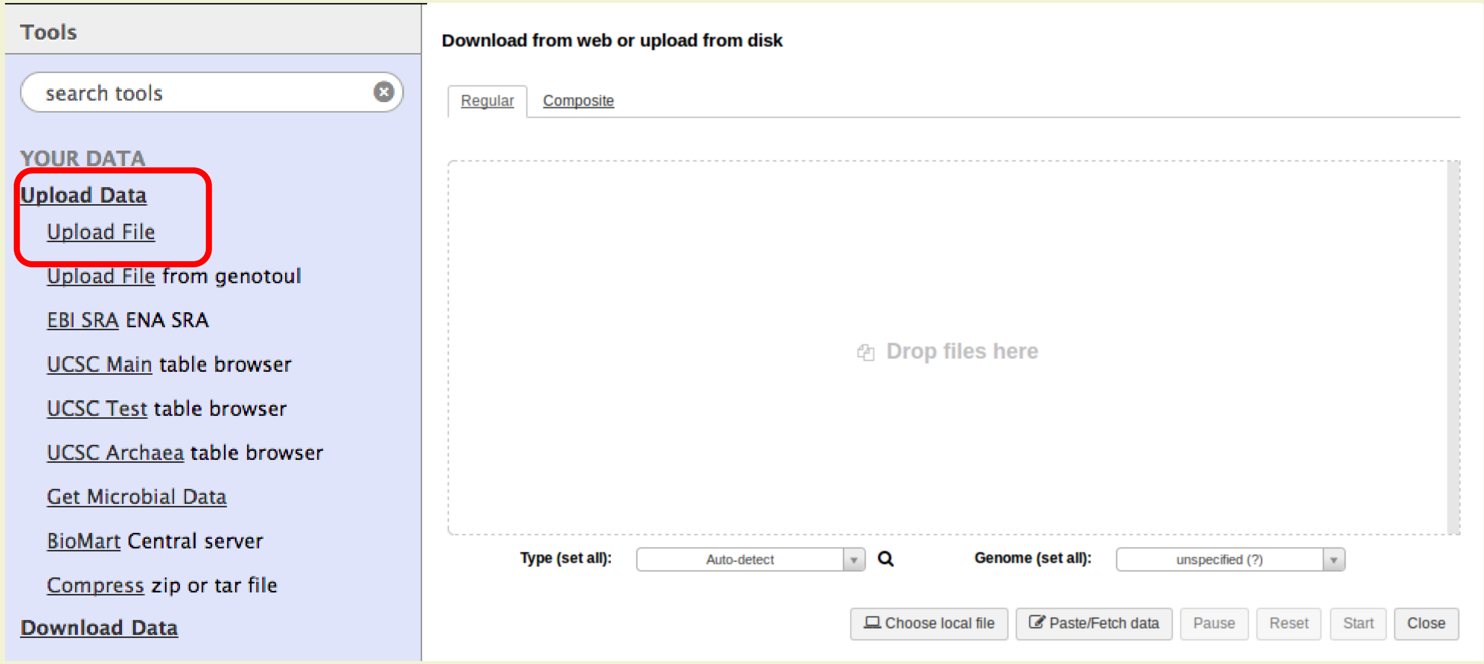
Here you may specify the archive URL.

Execute

What it does

If you have an archive on your own computer and smaller than 2Go, you may use this specific FROGS tool to upload your samples archive instead of the default « Upload File » of Galaxy.

Upload data: different methods



Tools

search tools

YOUR DATA

Upload Data

- Upload File
- Upload File from genotoul
- EBI SRA ENA SRA
- UCSC Main table browser
- UCSC Test table browser
- UCSC Archaea table browser
- Get Microbial Data
- BioMart Central server
- Compress zip or tar file

Download Data

Download from web or upload from disk

Type (set all): Auto-detect

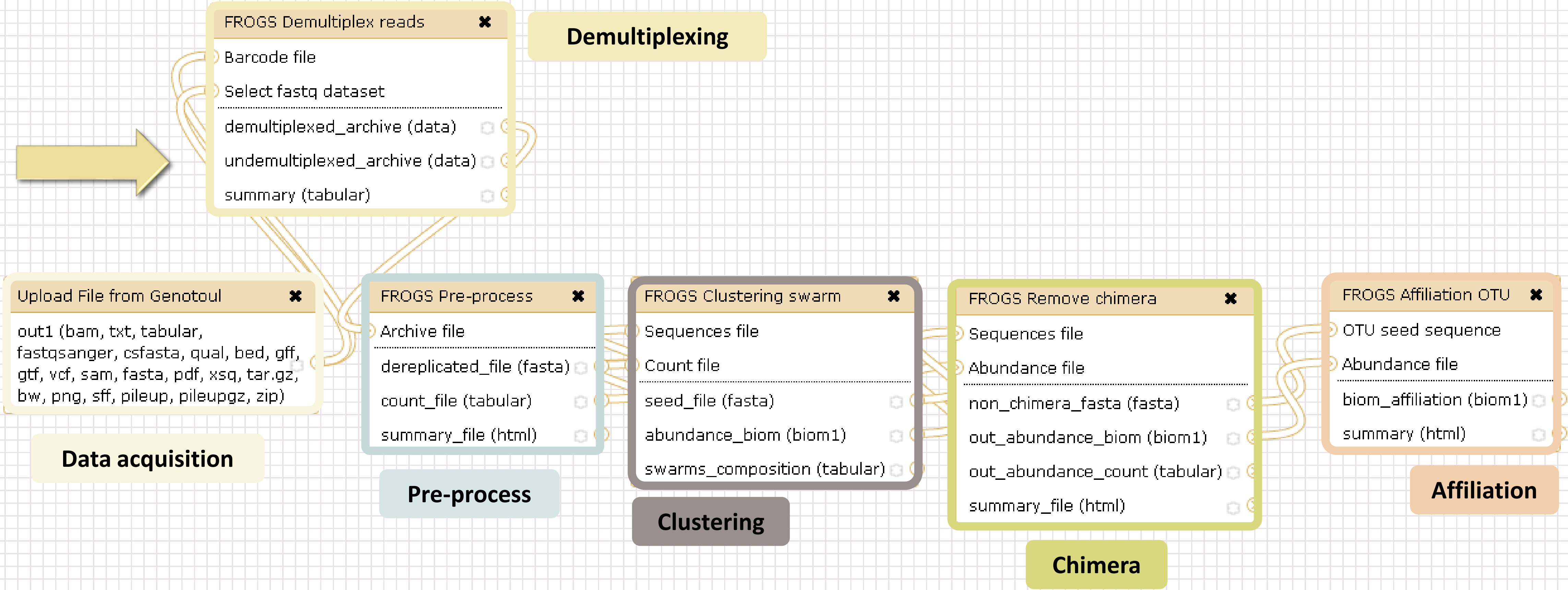
Genome (set all): unspecified (?)

Regular
Composite
Drop files here
Choose local file
Paste/Fetch data
Pause
Reset
Start
Close

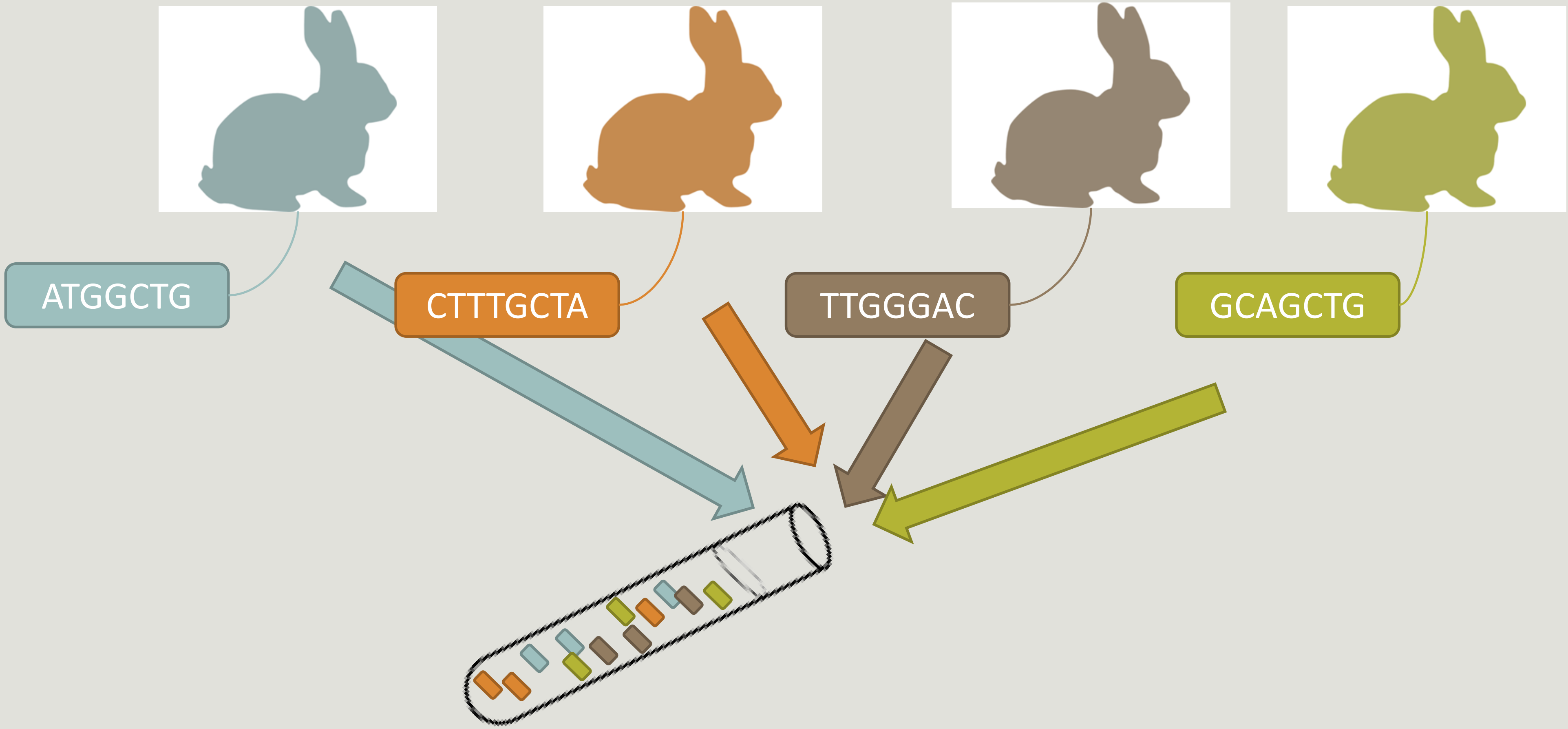
You can only upload multiple files at a time but only smaller than 2Go

New functionality in latest Galaxy version :
http://147.99.108.167/galaxy/

Demultiplexing tool


FROGS Demultiplex reads ✖
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Demultiplexing
Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
Data acquisition
FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
Pre-process
FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
Clustering
FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
Chimera
FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
Affiliation

Barcoding ?



ATGGCTG

CTTTGCTA

TTGGGAC

GCAGCTG

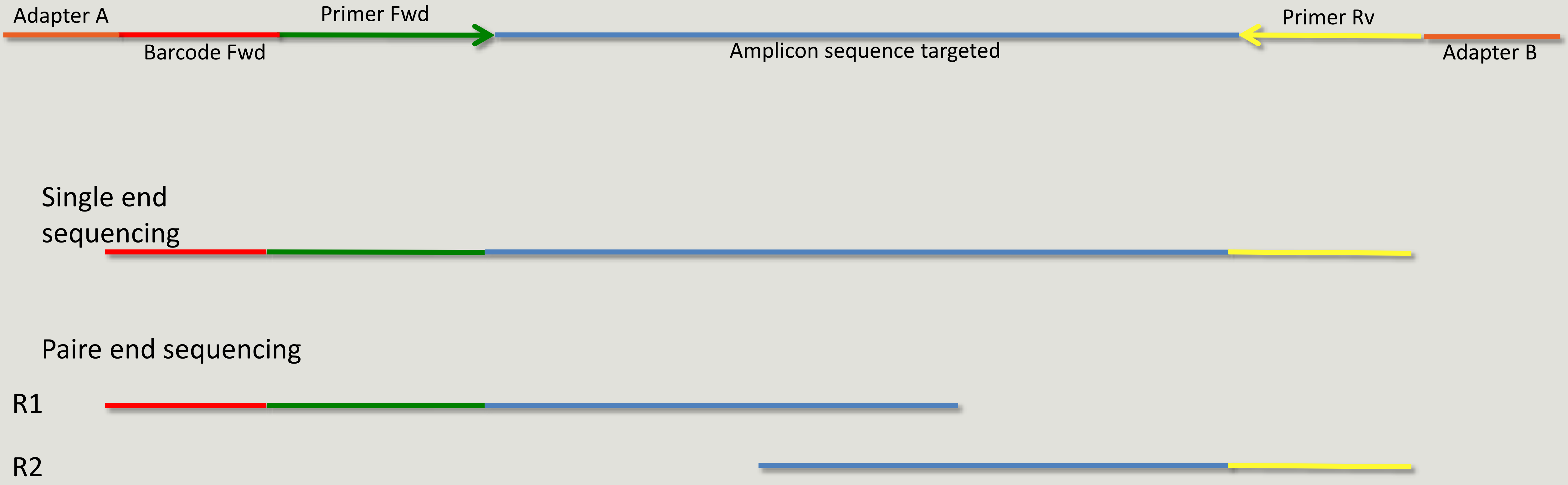
Demultiplexing

Sequence demultiplexing in function of barcode sequences :

- In forward
- In reverse
- In forward and reverse

Remove unbarcoded or ambiguous sequences

Demultiplexing forward



Adapter A

Primer Fwd

Primer Rv

Barcode Fwd

Amplicon sequence targeted

Adapter B

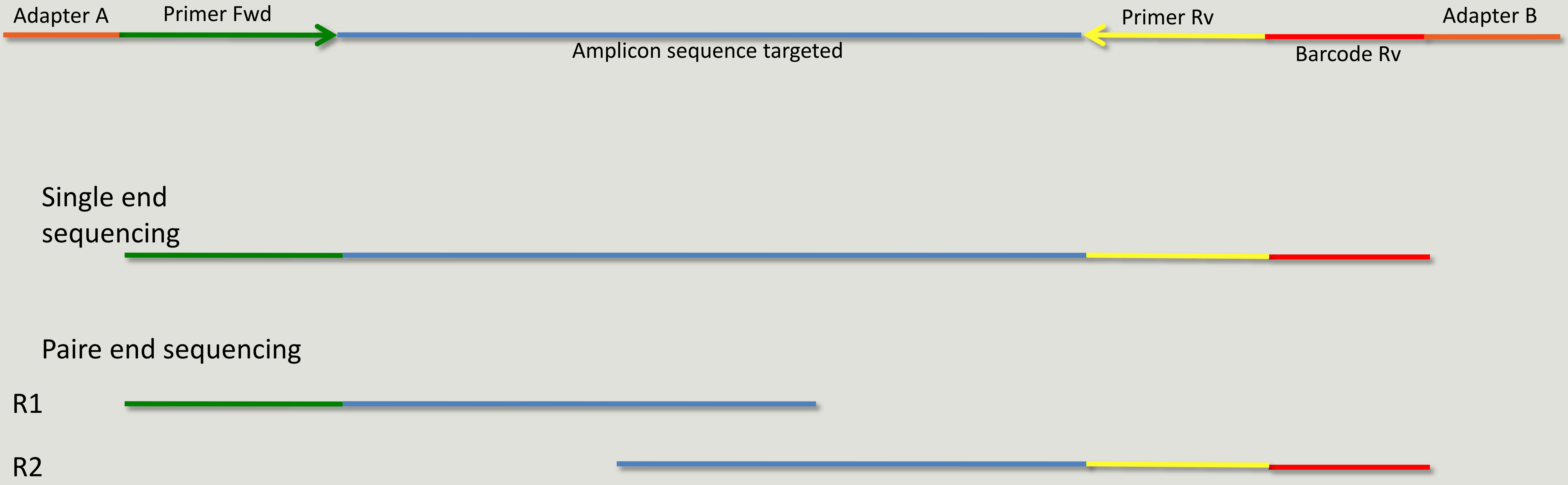
Single end sequencing

Paire end sequencing

R1

R2

Demultiplexing reverse



Adapter A

Primer Fwd

Amplicon sequence targeted

Primer Rv

Adapter B

Barcode Rv

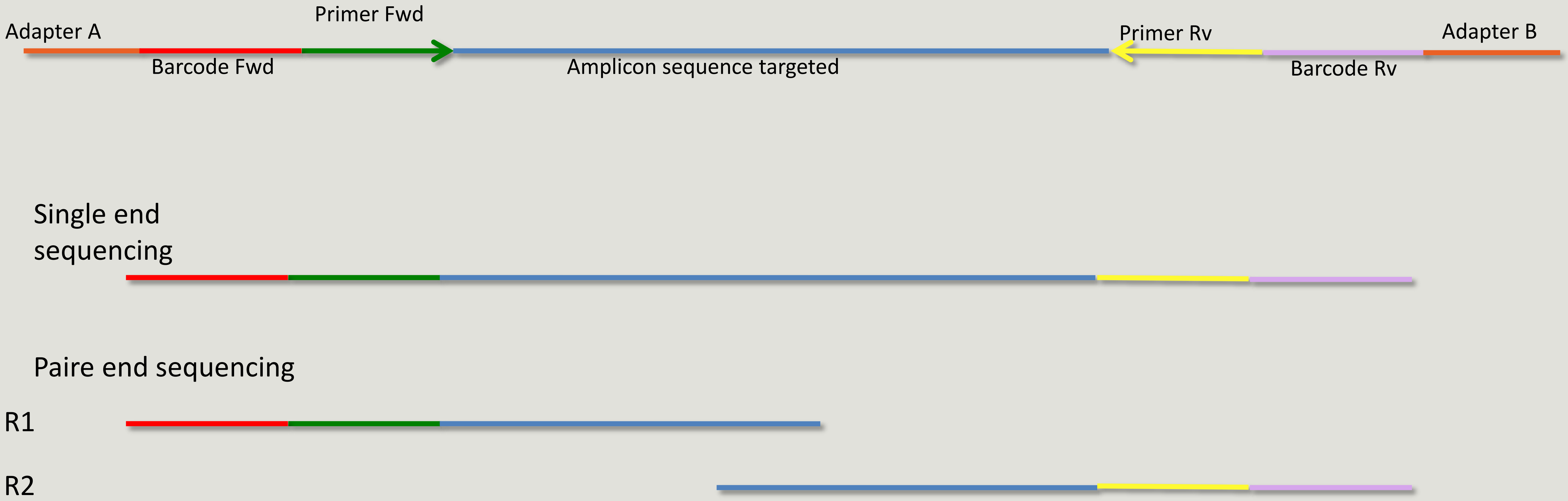
Single end sequencing

Paire end sequencing

R1

R2

Demultiplexing forward and reverse



Adapter A

Barcode Fwd

Primer Fwd

Amplicon sequence targeted

Primer Rv

Barcode Rv

Adapter B

Single end sequencing

Paire end sequencing

R1

R2

Your turn! - 2

LAUNCH DEMULTIPLEX READS TOOL

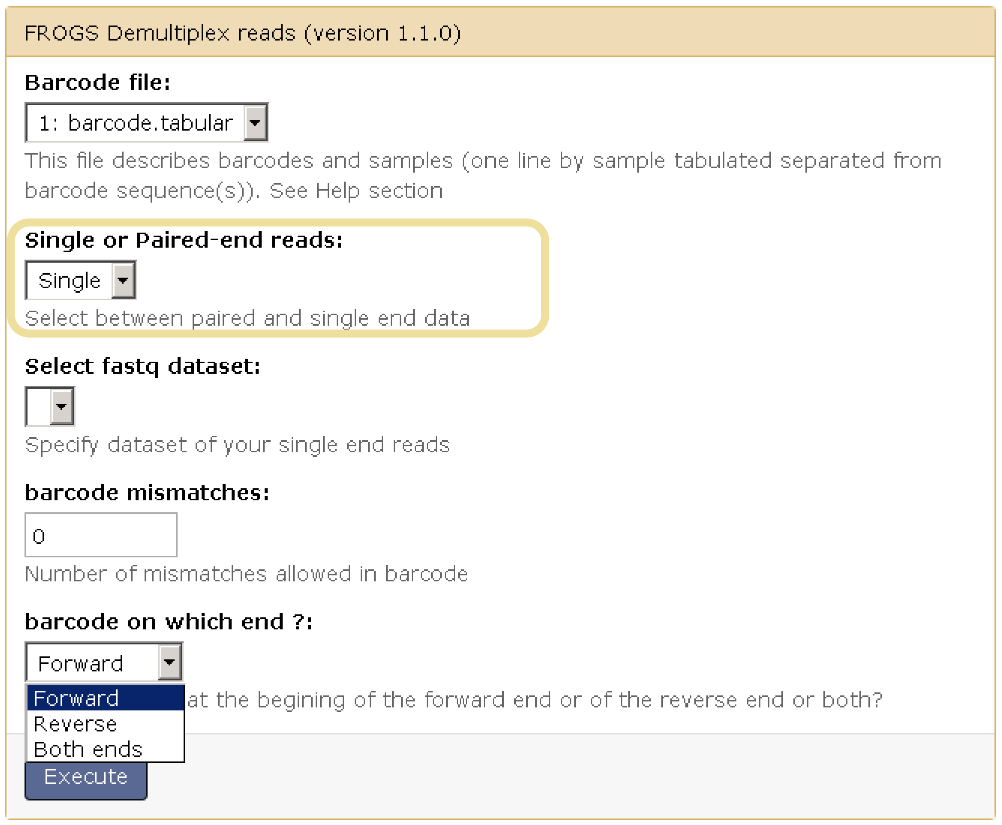
Accounts:

- anemone
- arome
- aster
- bleuet
- camelia
- capucine
- chardon
- clematite
- cobee
- coquelicot
- cosmos
- cyclamen

- Password: f1o2r3!

multiplexed

The tool parameters depend on the input data type



FROGS Demultiplex reads (version 1.1.0)

Barcode file:

1: barcode.tabular

This file describes barcodes and samples (one line by sample tabulated separated from barcode sequence(s)). See Help section

Single or Paired-end reads:

Single

Select between paired and single end data

Select fastq dataset:

Specify dataset of your single end reads

barcode mismatches:

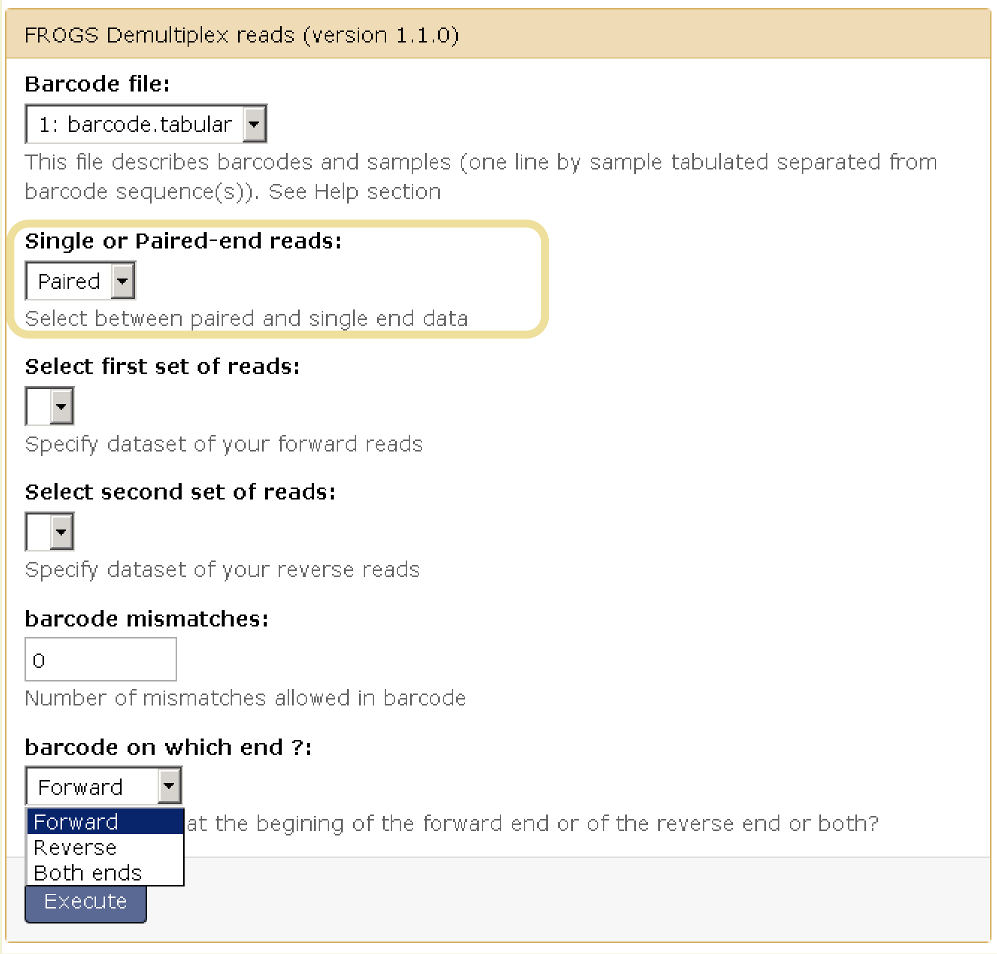
0

Number of mismatches allowed in barcode

barcode on which end ?:

Forward

at the begining of the forward end or of the reverse end or both?



FROGS Demultiplex reads (version 1.1.0)

Barcode file:

1: barcode.tabular

This file describes barcodes and samples (one line by sample tabulated separated from barcode sequence(s)). See Help section

Single or Paired-end reads:

Paired

Select between paired and single end data

Select first set of reads:

Specify dataset of your forward reads

Select second set of reads:

Specify dataset of your reverse reads

barcode mismatches:

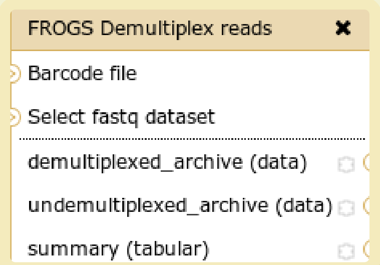
0

Number of mismatches allowed in barcode

barcode on which end ?:

Forward

at the begining of the forward end or of the reverse end or both?



FROGS Demultiplex reads

Barcode file

Select fastq dataset

demultiplexed_archive (data)

undemultiplexed_archive (data)

summary (tabular)

multiplexed

Exercise 2

In **multiplexed** history launch the demultiplex tool:

« The Patho-ID project, rodent and tick‘s pathobioms study, financed by the metaprogram INRA-MEM, studies zoonoses on rats and ticks from multiple places in the world, the co-infection systems and the interactions between pathogens. In this aim, thay have extracted hundreads of or rats and ticks samples from which they have extracted 16S DNA and sequenced them first time on Roche 454 plateform and in a second time on Illumina Miseq plateform. For this courses, they authorized us to publicly shared some parts of these samples. »

Parasites & Vectors (2015) 8:172 DOI 10.1186/s13071-015-0784-7. **Detection of Orientia sp. DNA in rodents from Asia, West Africa and Europe.** Jean François Cosson, Maxime Galan, Emilie Bard, Maria Razzauti, Maria Bernard, Serge Morand, Carine Brouat, Ambroise Dalecky, Khalilou Bâ, Nathalie Charbonnel and Muriel Vayssier-Taussat

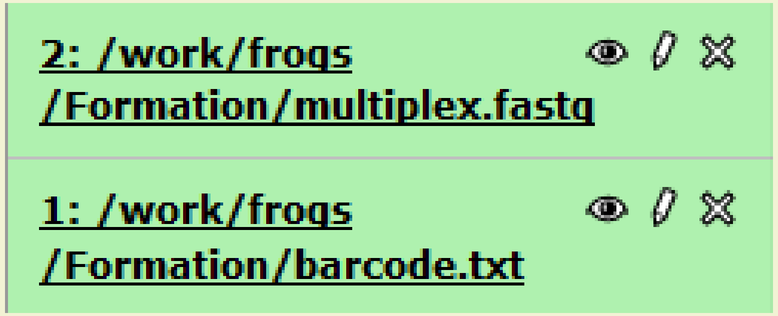
multiplexed

Exercise 2

In **multiplexed** history launch the demultiplex tool:

Data are single end reads
→ only 1 fastq file

Samples are characterized by an association of two barcodes in forward and reverse strands
→ multiplexing « both ends »



2: /work/frogs/Formation/multiplex.fastq

1: /work/frogs/Formation/barcode.txt

multiplexed

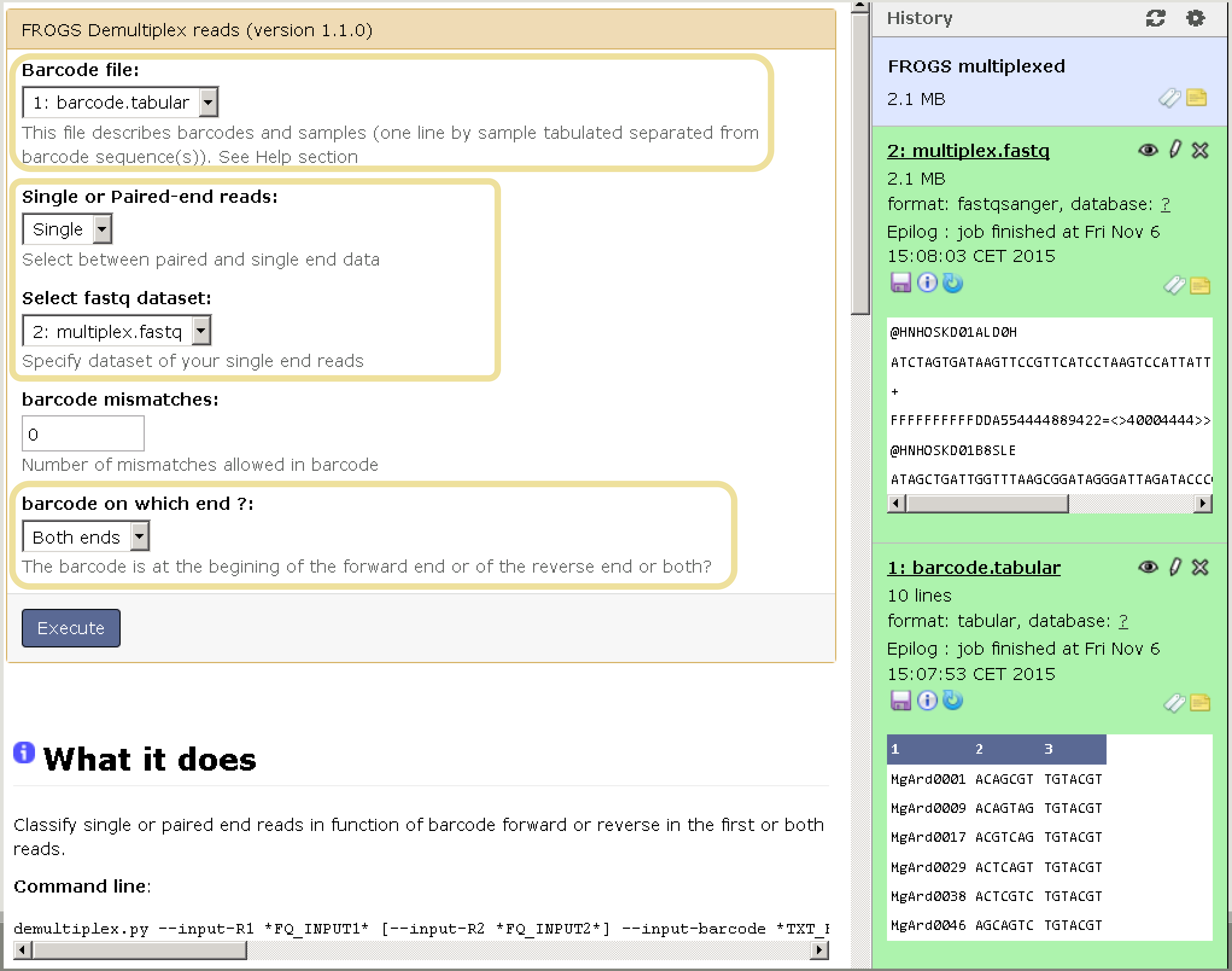
Exercise 2

Demultiplex tool asks for 2 files: one « fastq » and one « tabular »

1. Play with pictograms 👁 ✏

2. Observe how is built a fastq file. 👁
3. Look at the stdout, stderr when available (in the ⓘ pictogram)

multiplexed



FROGS Demultiplex reads (version 1.1.0)

Barcode file: 1: barcode.tabular

This file describes barcodes and samples (one line by sample tabulated separated from barcode sequence(s)). See Help section

Single or Paired-end reads: Single

Select between paired and single end data

Select fastq dataset: 2: multiplex.fastq

Specify dataset of your single end reads

barcode mismatches: 0

Number of mismatches allowed in barcode

barcode on which end ?: Both ends

The barcode is at the begining of the forward end or of the reverse end or both?

What it does

Classify single or paired end reads in function of barcode forward or reverse in the first or both reads.

Command line:

```
demultiplex.py --input-R1 *FQ_INPUT1* [--input-R2 *FQ_INPUT2*] --input-barcode *TXT_
```

FROGS multiplexed

2.1 MB

2: multiplex.fastq

2.1 MB

format: fastqsanger, database: ?

Epilog : job finished at Fri Nov 6 15:08:03 CET 2015

```
@HNHOSKD01ALD0H
ATCTAGTGATAAGTTCCGTTCATCCTAAGTCCATTATT
+
FFFFFFFFFFDDA554444889422=<>40004444>>
@HNHOSKD01B8SLE
ATAGCTGATTGGTTTAAGCGGATAGGGATTAGATACCC
```

1: barcode.tabular

10 lines

format: tabular, database: ?

Epilog : job finished at Fri Nov 6 15:07:53 CET 2015

1	2	3
MgArd0001	ACAGCGT	TGTACGT
MgArd0009	ACAGTAG	TGTACGT
MgArd0017	ACGTCAG	TGTACGT
MgArd0029	ACTCAGT	TGTACGT
MgArd0038	ACTCGTC	TGTACGT
MgArd0046	AGCAGTC	TGTACGT

multiplexed

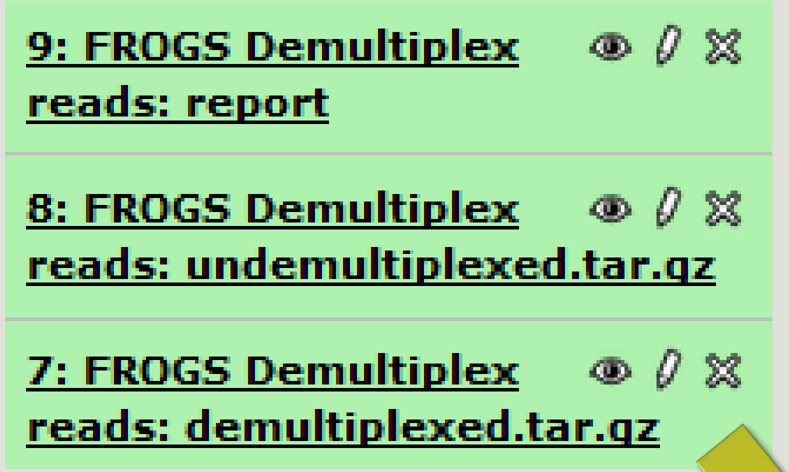
Advices

For your own data

- Do not forget to indicate barcode sequence as they are in the fastq sequence file, especially if you have data multiplexed via the reverse strand.
- For the mismatch threshold, we advised you to let the threshold to 0, and if you are not satisfied by the result, try with 1. The number of mismatch depends on the length of the barcode, but often those sequences are very short so 1 mismatch is already more than the sequencing error rate.
- If you have different barcode lengths, you must demultiplex your data in different times beginning by the longest barcode set and used the "unmatched" or "ambiguous" sequence with smaller barcode and so on.
- If you have Roche 454 sequences in sff format, you must convert them with some program like sff2fastq

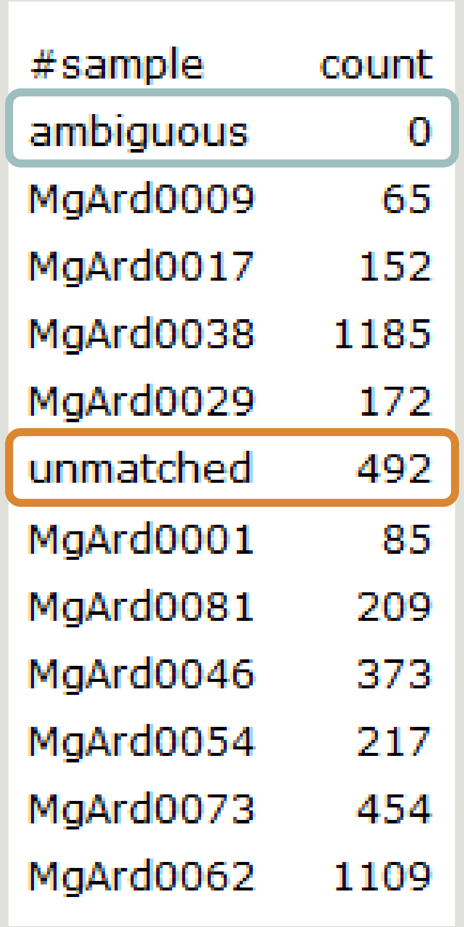
multiplexed

Results


9: FROGS Demultiplex reads: report

8: FROGS Demultiplex reads: undemultiplexed.tar.gz

7: FROGS Demultiplex reads: demultiplexed.tar.gz



#sample	count
ambiguous	0
MgArd0009	65
MgArd0017	152
MgArd0038	1185
MgArd0029	172
unmatched	492
MgArd0001	85
MgArd0081	209
MgArd0046	373
MgArd0054	217
MgArd0073	454
MgArd0062	1109

With barcode mismatches >1 sequence can corresponding to several samples.
So these sequences are non-affected to a sample.

Sequences without known barcode.
So these sequences are non-affected to a sample.

A tar archive is created by grouping one (or a pair of) fastq file per sample with the names indicated in the first column of the barcode tabular file

Format: Barcode

BARCODE FILE is expected to be tabulated:

- first column corresponds to the sample name (unique, without space)
- second to the forward sequence barcode used (None if only reverse barcode)
- optional third is the reverse sequence barcode (optional)

Take care to indicate sequence barcode in the strand of the read, so you may need to reverse complement the reverse barcode sequence. Barcode sequence must have the same length.

Example of barcode file.
The last column is optional, like this, it describes sample multiplexed by both fragment ends.

```
MgArd00001        ACAGCGT      ACGTACA
```

multiplexed

Format : FastQ

FASTQ : Text file describing biological sequence in 4 lines format:

- first line start by "@" correspond to the sequence identifier and optionally the sequence description. "@Sequence_1 description1"
- second line is the sequence itself. "ACAGC"
- third line is a "+" following by the sequence identifier or not depending on the version
- fourth line is the quality sequence, one code per base. The code depends on the version and the sequencer

```
@HNHOSKD01ALD0H
ACAGCGTCAGAGGGGTACCAGTCAGCCATGACGTAGCACGTACA
+
CCCFFFFFFHHHHHJJIJJJHHFF@DEDDDDDDD@CDDDDACDD
```

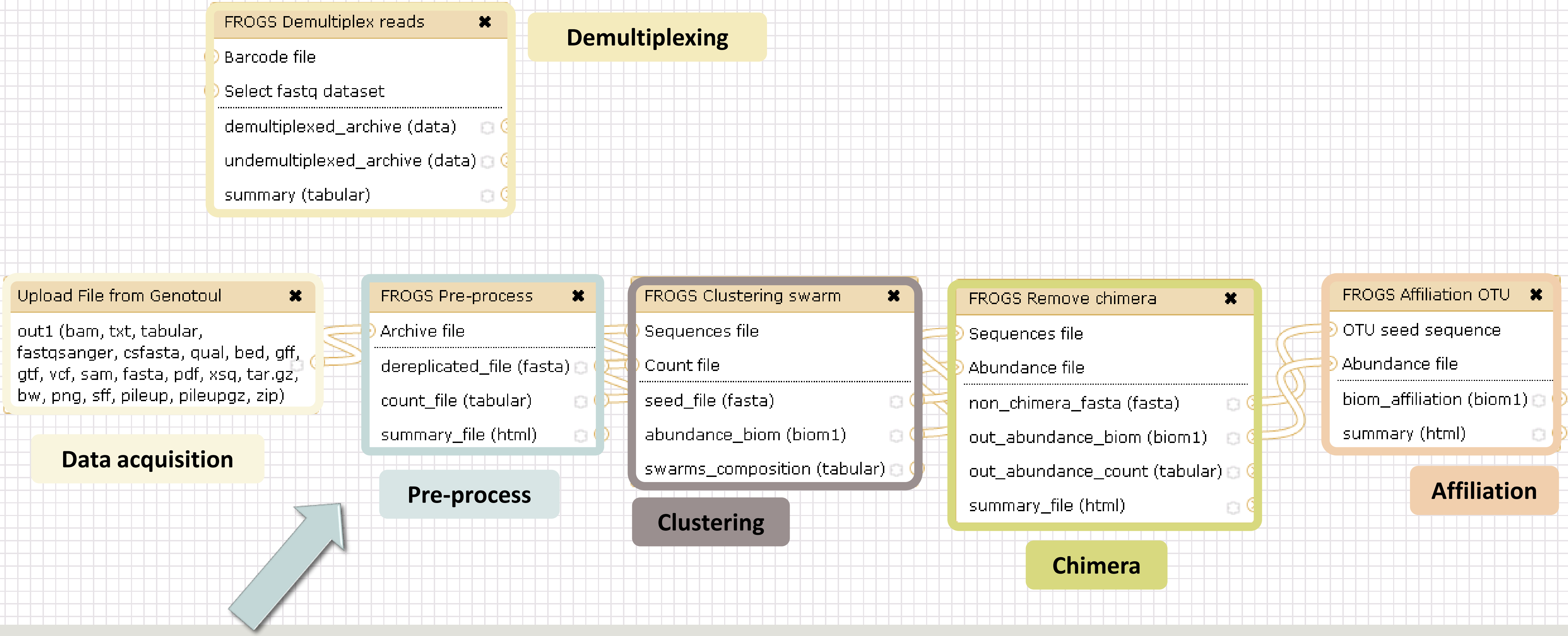
How it works ?

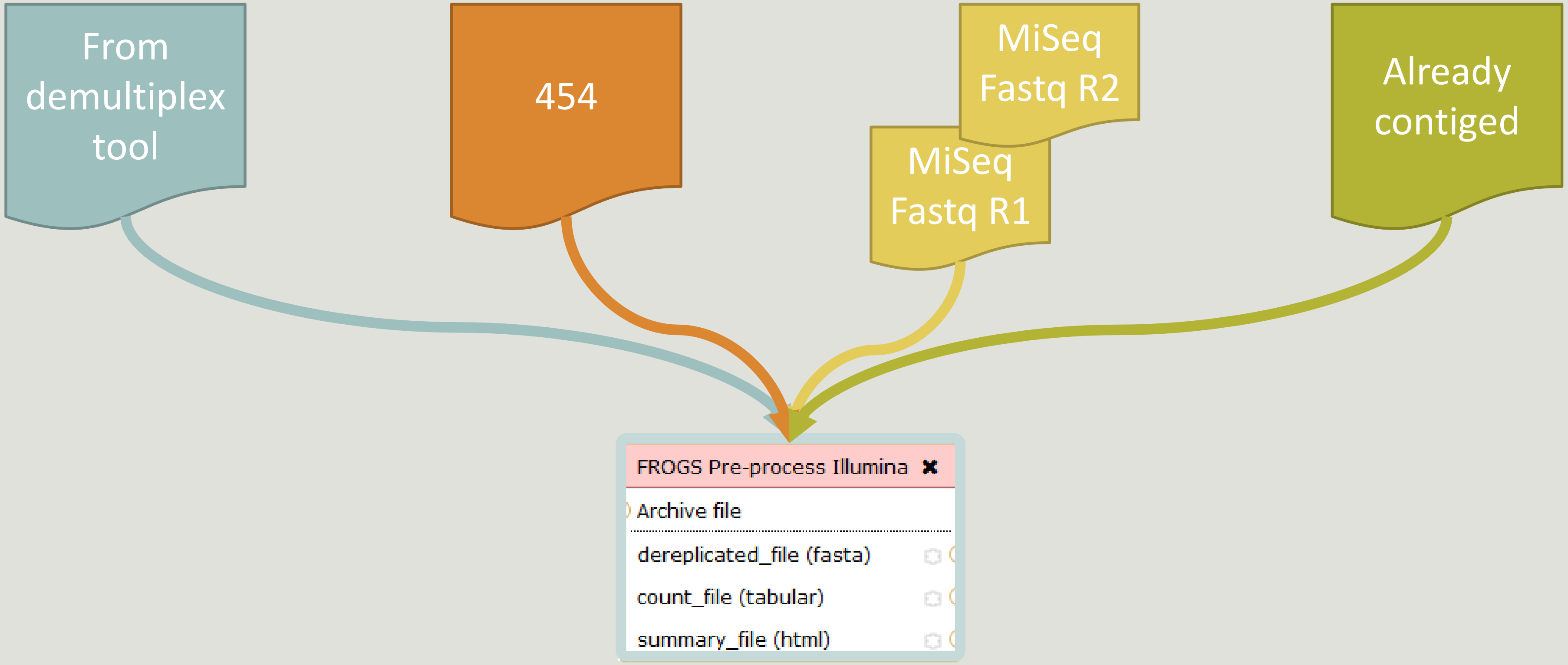
For each sequence or sequence pair the sequence fragment at the beginning (forward multiplexing) of the (first) read or at the end (reverse multiplexing) of the (second) read will be compare to all barcode sequence.

If this fragment is equal (with less or equal mismatch than the threshold) to one (and only one) barcode, the fragment is trimmed and the sequence will be attributed to the corresponding sample.

Finally fastq files (or pair of fastq files) for each sample are included in an archive, and a summary describes how many sequence are attributed for each sample.

Pre-process tool


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Demultiplexing
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
Data acquisition
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
Pre-process
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
Clustering
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
Chimera
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
Affiliation


From demultiplex tool

454

MiSeq Fastq R2

MiSeq Fastq R1

Already contiged

FROGS Pre-process Illumina ✖

Archive file

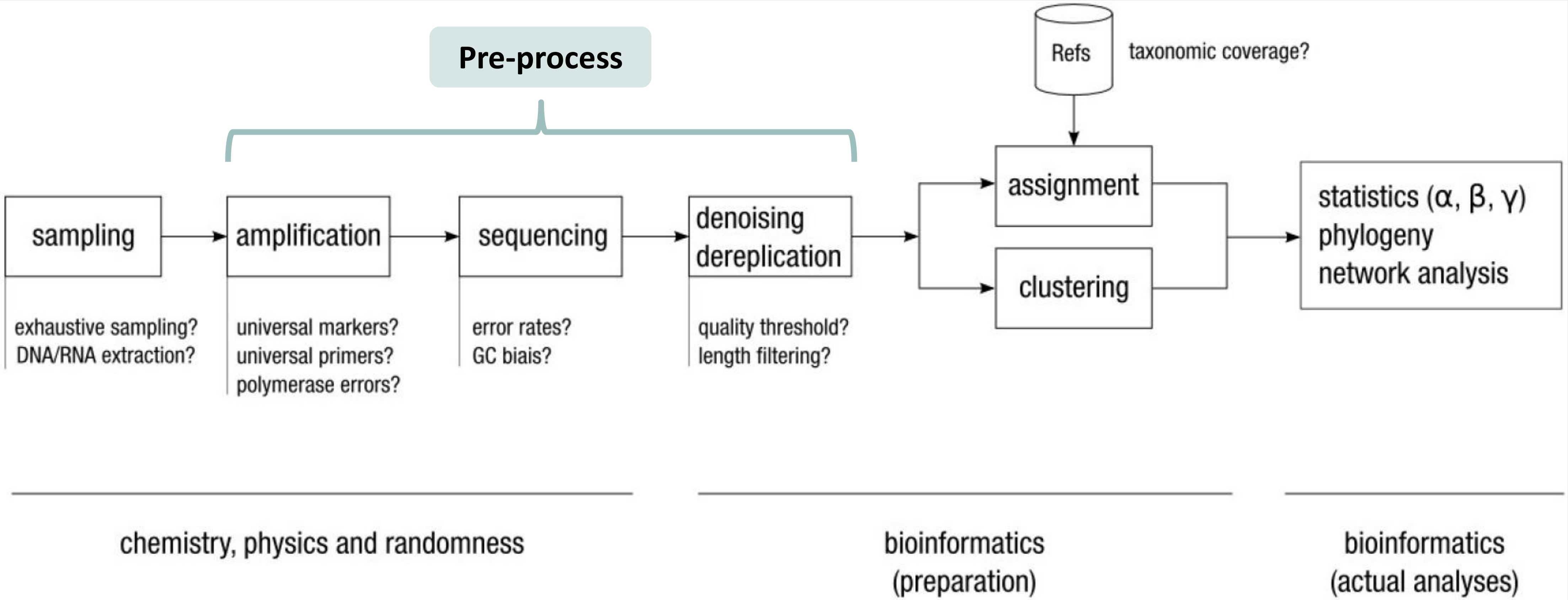
dereplicated_file (fasta)

count_file (tabular)

summary_file (html)

Pre-process

Amplicon-based studies general pipeline



Pre-process

Refs

taxonomic coverage?

sampling

amplification

sequencing

denoising
dereplication

assignment

clustering

statistics (α, β, γ)
phylogeny
network analysis

exhaustive sampling?
DNA/RNA extraction?

universal markers?
universal primers?
polymerase errors?

error rates?
GC biais?

quality threshold?
length filtering?

chemistry, physics and randomness

bioinformatics
(preparation)

bioinformatics
(actual analyses)

Pre-process

- Delete sequence with not expected lengths
- Delete sequences with ambiguous bases (N)
- Delete sequences do not contain good primers
- Dereplication

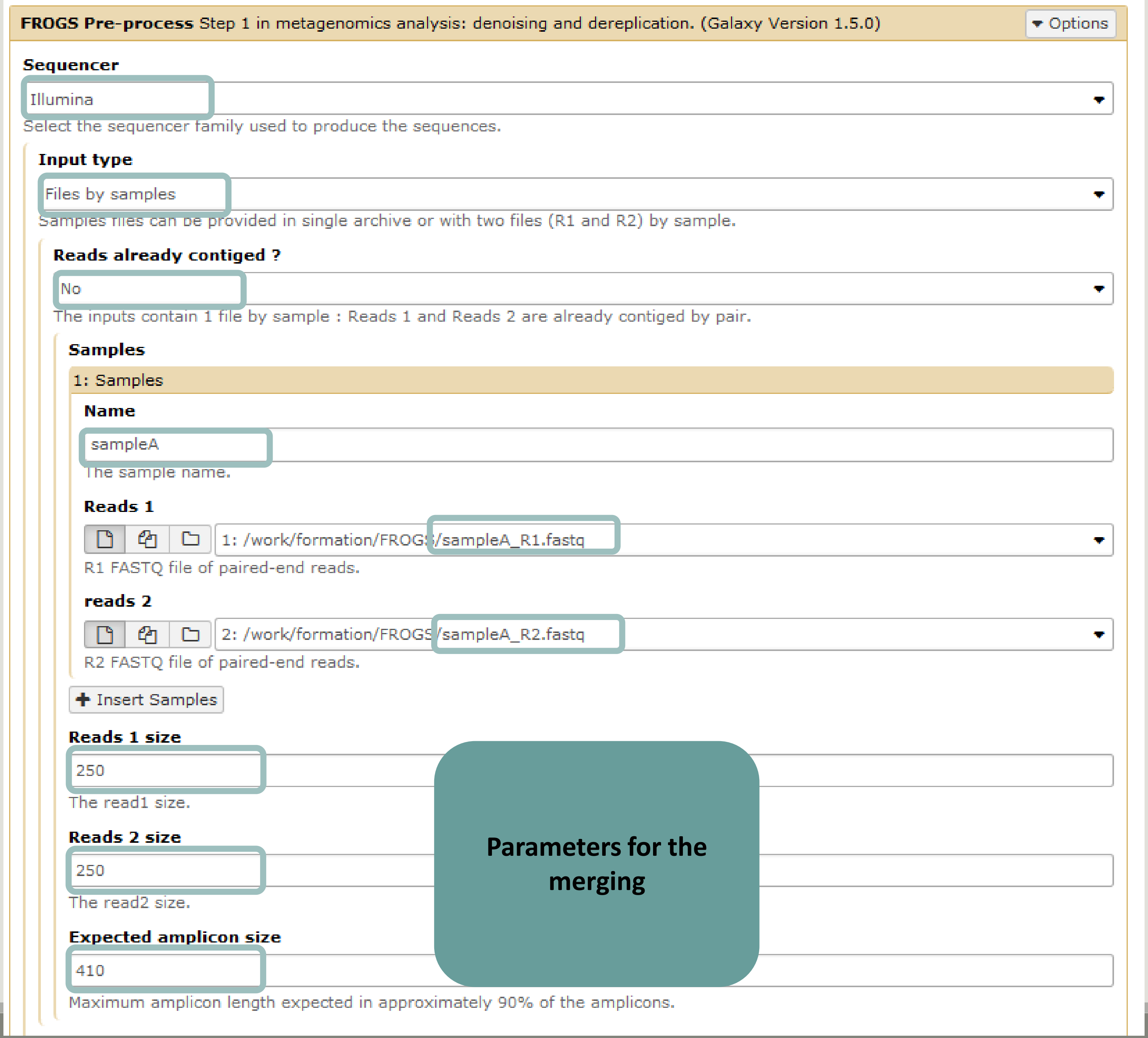
- + removing homopolymers (size = 8) for 454 data
- + quality filter for 454 data

EMBnet Journal, Vol17 no1. doi : 10.14806/ej.17.1.200
Cutadapt removes adapter sequences from high-throughput sequencing reads
Marcel Martin

Bioinformatics (2011) 27 (21):2957-2963. doi:10.1093/bioinformatics/btr507
FLASH: fast length adjustment of short reads to improve genome assemblies
TanjaMagoc, Steven L. Salzberg

Example for:

- Illumina MiSeq data
- 1 sample
- Non joined



FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication. (Galaxy Version 1.5.0)

Sequencer

Illumina

Select the sequencer family used to produce the sequences.

Input type

Files by samples

Samples files can be provided in single archive or with two files (R1 and R2) by sample.

Reads already contiged ?

No

The inputs contain 1 file by sample : Reads 1 and Reads 2 are already contiged by pair.

Samples

1: Samples

Name

sampleA

The sample name.

Reads 1

1: /work/formation/FROGS/sampleA_R1.fastq

R1 FASTQ file of paired-end reads.

reads 2

2: /work/formation/FROGS/sampleA_R2.fastq

R2 FASTQ file of paired-end reads.

Reads 1 size

250

The read1 size.

Reads 2 size

250

The read2 size.

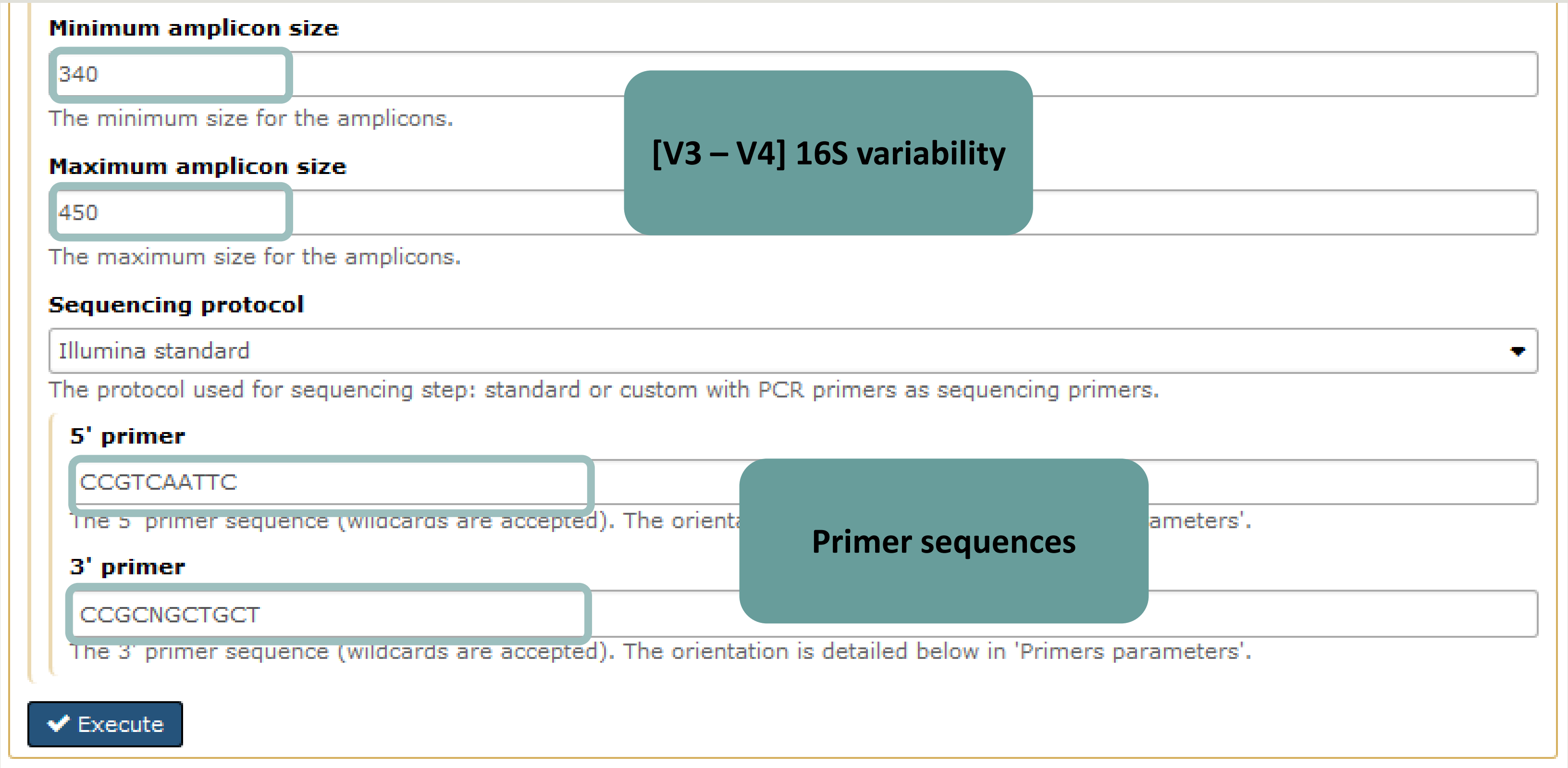
Expected amplicon size

410

Maximum amplicon length expected in approximately 90% of the amplicons.

Parameters for the merging

Pre-process example 1



Minimum amplicon size

340

The minimum size for the amplicons.

Maximum amplicon size

450

The maximum size for the amplicons.

Sequencing protocol

Illumina standard

The protocol used for sequencing step: standard or custom with PCR primers as sequencing primers.

5' primer

CCGTCAATTC

The 5' primer sequence (wildcards are accepted). The orienta ameters'.

3' primer

CCGCNGCTGCT

The 3' primer sequence (wildcards are accepted). The orientation is detailed below in 'Primers parameters'.

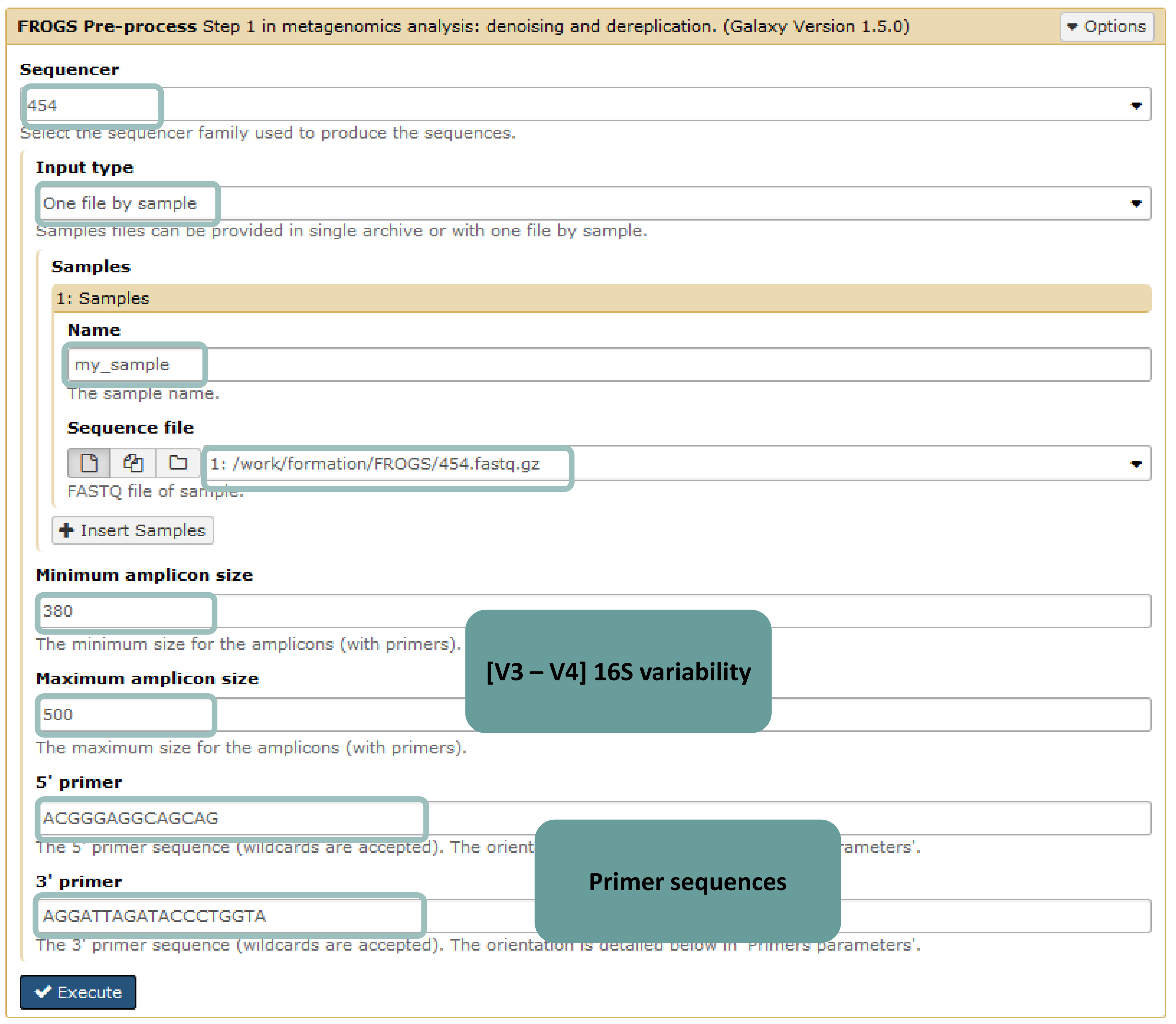
[V3 – V4] 16S variability

Primer sequences

Pre-process example 1

Example for:

- Sanger 454 data
- 1 sample
- Joined



FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication. (Galaxy Version 1.5.0)

Sequencer

454

Select the sequencer family used to produce the sequences.

Input type

One file by sample

Samples files can be provided in single archive or with one file by sample.

Samples

1: Samples

Name

my_sample

The sample name.

Sequence file

1: /work/formation/FROGS/454.fastq.gz

FASTQ file of sample.

Minimum amplicon size

380

The minimum size for the amplicons (with primers).

Maximum amplicon size

500

The maximum size for the amplicons (with primers).

5' primer

ACGGGAGGCAGCAG

The 5' primer sequence (wildcards are accepted). The orient rameters'.

3' primer

AGGATTAGATACCCTGGTA

The 3' primer sequence (wildcards are accepted). The orientation is detailed below in 'Primers parameters'.

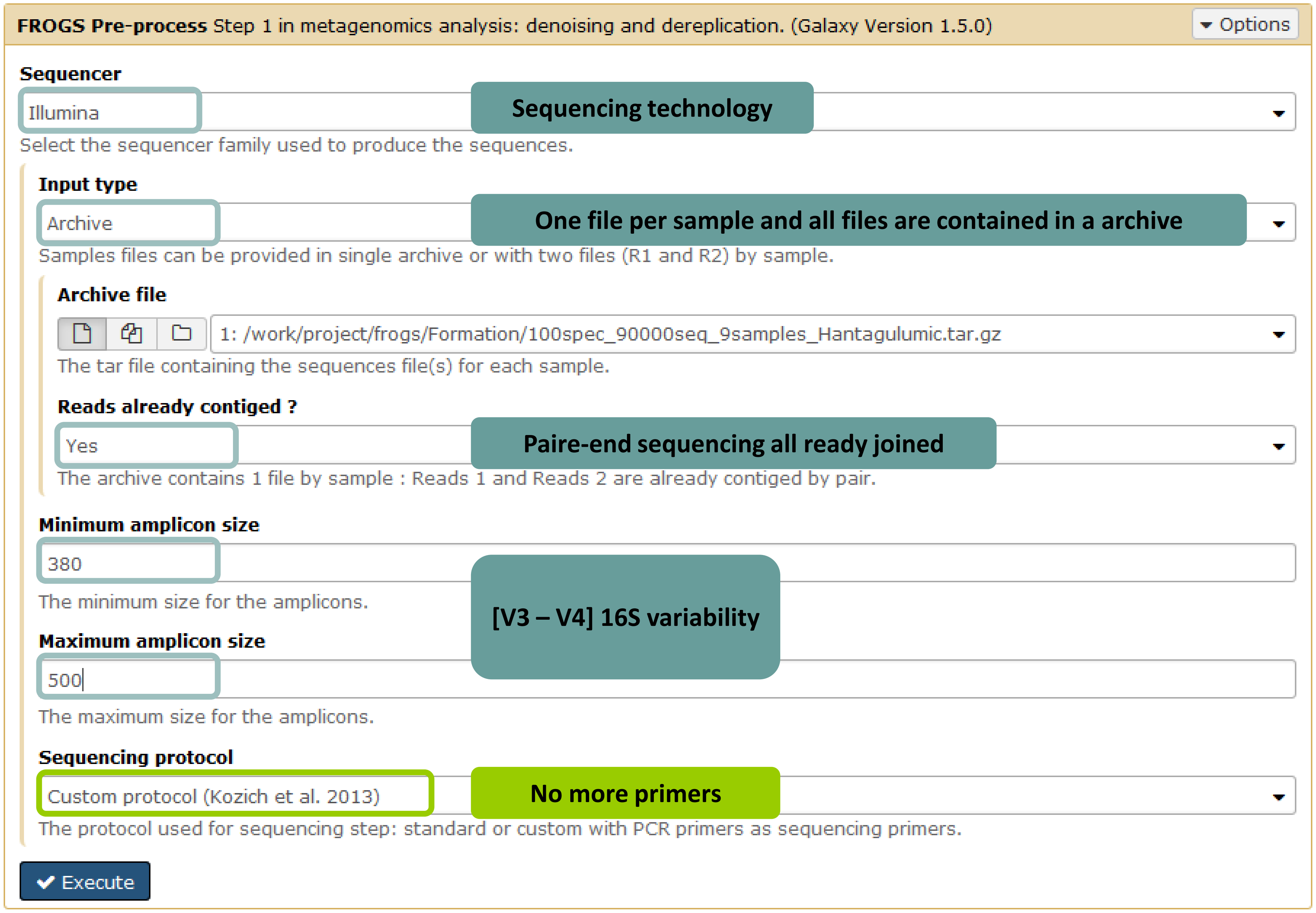
[V3 – V4] 16S variability

Primer sequences

Pre-process example 2

Example for:

- Illumina MiSeq data
- 9 samples in 1 archive
- Joined
- Without sequenced PCR primers (Kozich protocol)



FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication. (Galaxy Version 1.5.0)

Sequencer

Illumina

Select the sequencer family used to produce the sequences.

Input type

Archive

Samples files can be provided in single archive or with two files (R1 and R2) by sample.

Archive file

1: /work/project/frogs/Formation/100spec_90000seq_9samples_Hantagulumic.tar.gz

The tar file containing the sequences file(s) for each sample.

Reads already contiged ?

Yes

The archive contains 1 file by sample : Reads 1 and Reads 2 are already contiged by pair.

Minimum amplicon size

380

The minimum size for the amplicons.

Maximum amplicon size

500

The maximum size for the amplicons.

Sequencing protocol

Custom protocol (Kozich et al. 2013)

The protocol used for sequencing step: standard or custom with PCR primers as sequencing primers.

Sequencing technology

One file per sample and all files are contained in a archive

Paire-end sequencing all ready joined

[V3 – V4] 16S variability

No more primers

Pre-process example 3

Your turn! - 3

GO TO EXERCISES 3

454

Exercise 3.1

Go to « 454 » history

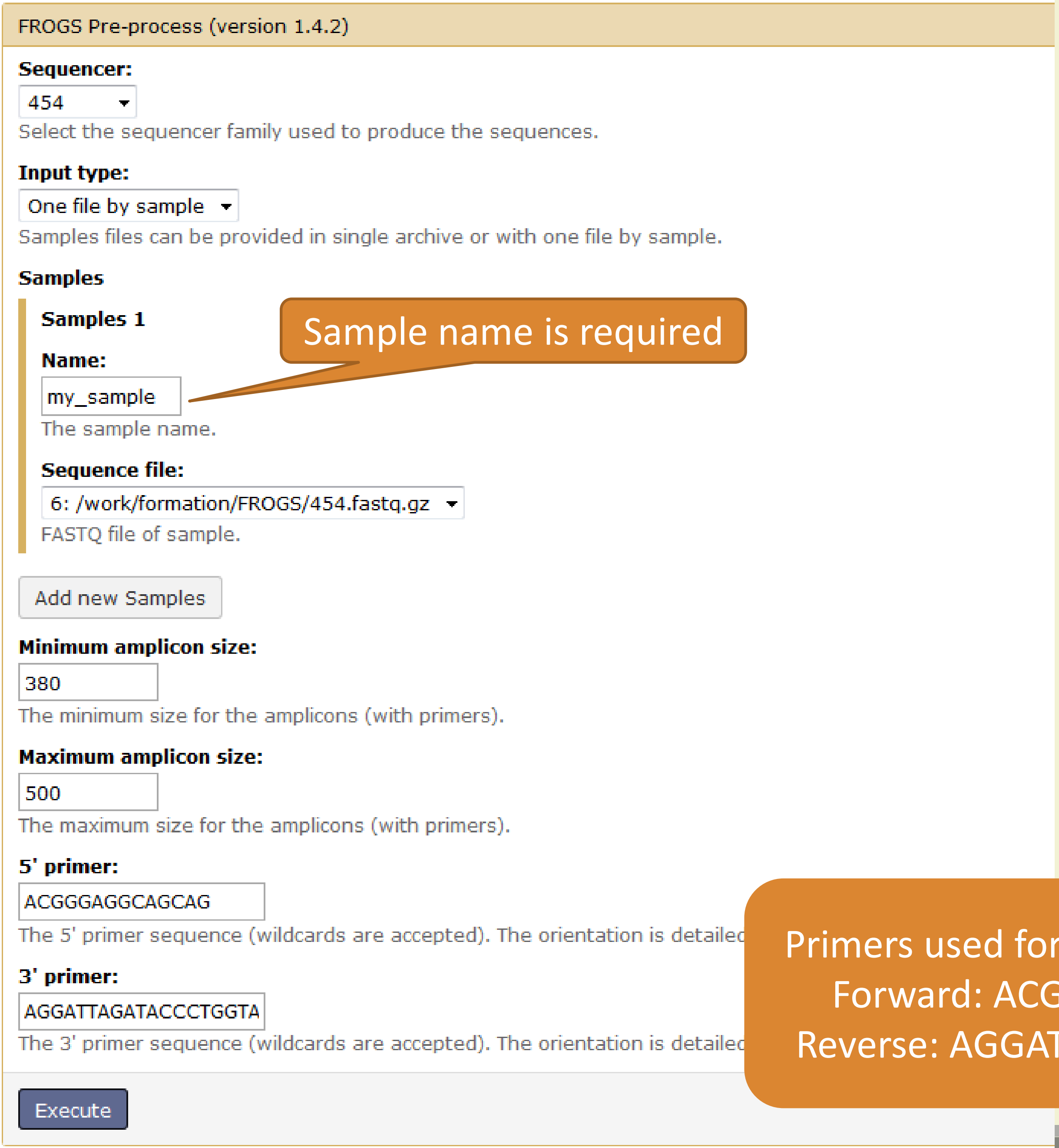
Launch the pre-process tool on that data set

→ objective : understand the parameters

1- Test different parameters for « minimum and maximum amplicon size »

2- Enter these primers: Forward: ACGGGAGGCAGCAG   Reverse: AGGATTAGATACCCTGGTA

454



FROGS Pre-process (version 1.4.2)

Sequencer:
454
Select the sequencer family used to produce the sequences.

Input type:
One file by sample
Samples files can be provided in single archive or with one file by sample.

Samples

Samples 1

Name:
my_sample
The sample name.

Sequence file:
6: /work/formation/FROGS/454.fastq.gz
FASTQ file of sample.

Minimum amplicon size:
380
The minimum size for the amplicons (with primers).

Maximum amplicon size:
500
The maximum size for the amplicons (with primers).

5' primer:
ACGGGAGGCAGCAG
The 5' primer sequence (wildcards are accepted). The orientation is detaile

3' primer:
AGGATTAGATACCCTGGTA
The 3' primer sequence (wildcards are accepted). The orientation is detaile

Sample name is required

Size range of 16S V3-V4:
[380 – 500]

Primers used for sequencing V3-V4:
Forward: ACGGGAGGCAGCAG
Reverse: AGGATTAGATACCCTGGTA

454

Exercise 3.1

What do you understand about amplicon size, which file can help you ?

What is the length of your reads before preprocessing ?

Do you understand how enter your primers ? 👁

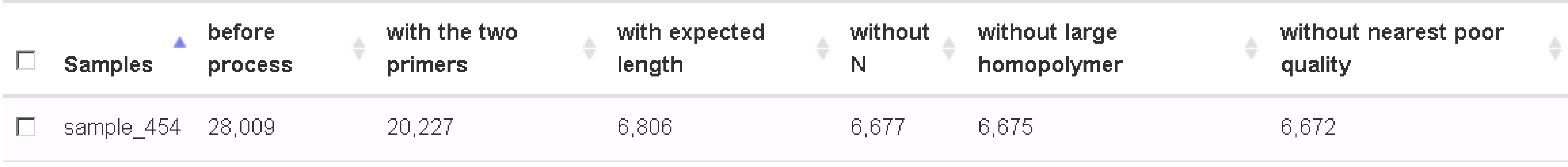
What is the « FROGS Pre-process: dereplicated.fasta » file ? 👁

What is the « FROGS Pre-process: count.tsv » file ? 👁

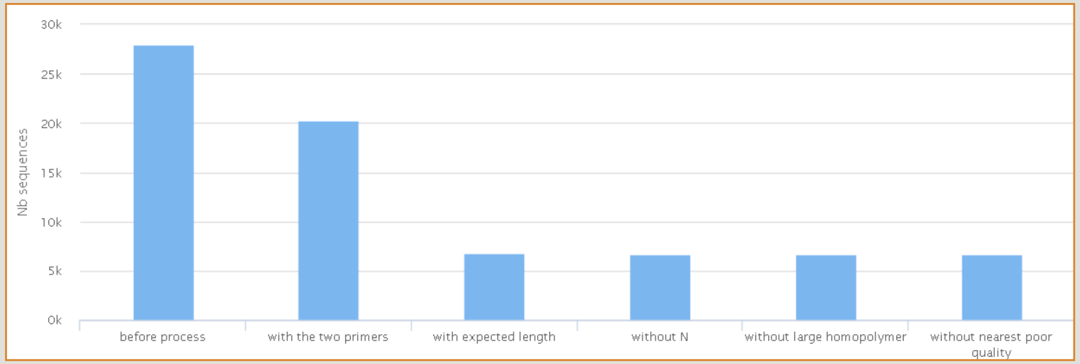
Explore the file « FROGS Pre-process: report.html »

Who loose a lot of sequences ?

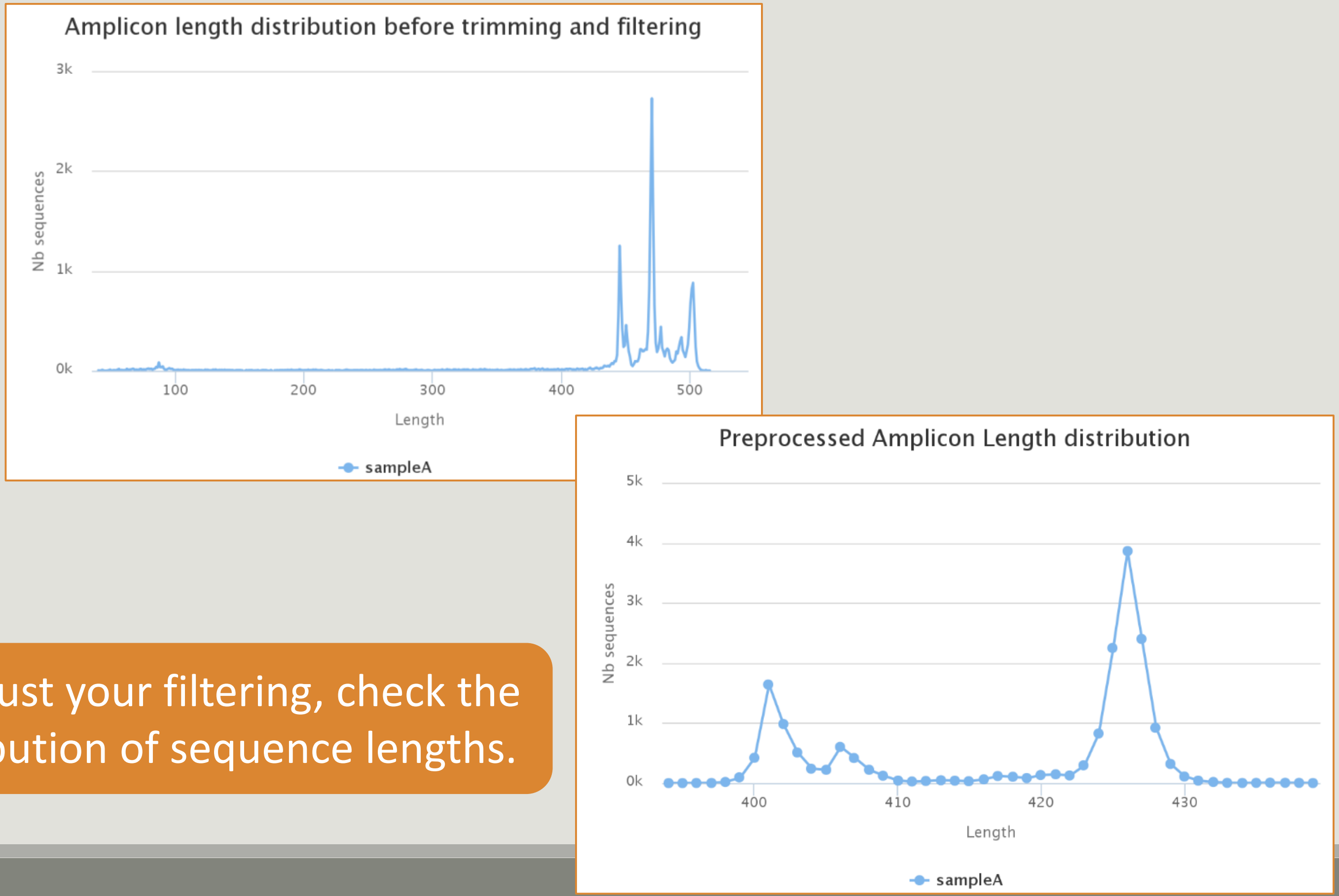
454



☐	Samples	before process	with the two primers	with expected length	without N	without large homopolymer	without nearest poor quality
☐	sample_454	28,009	20,227	6,806	6,677	6,675	6,672

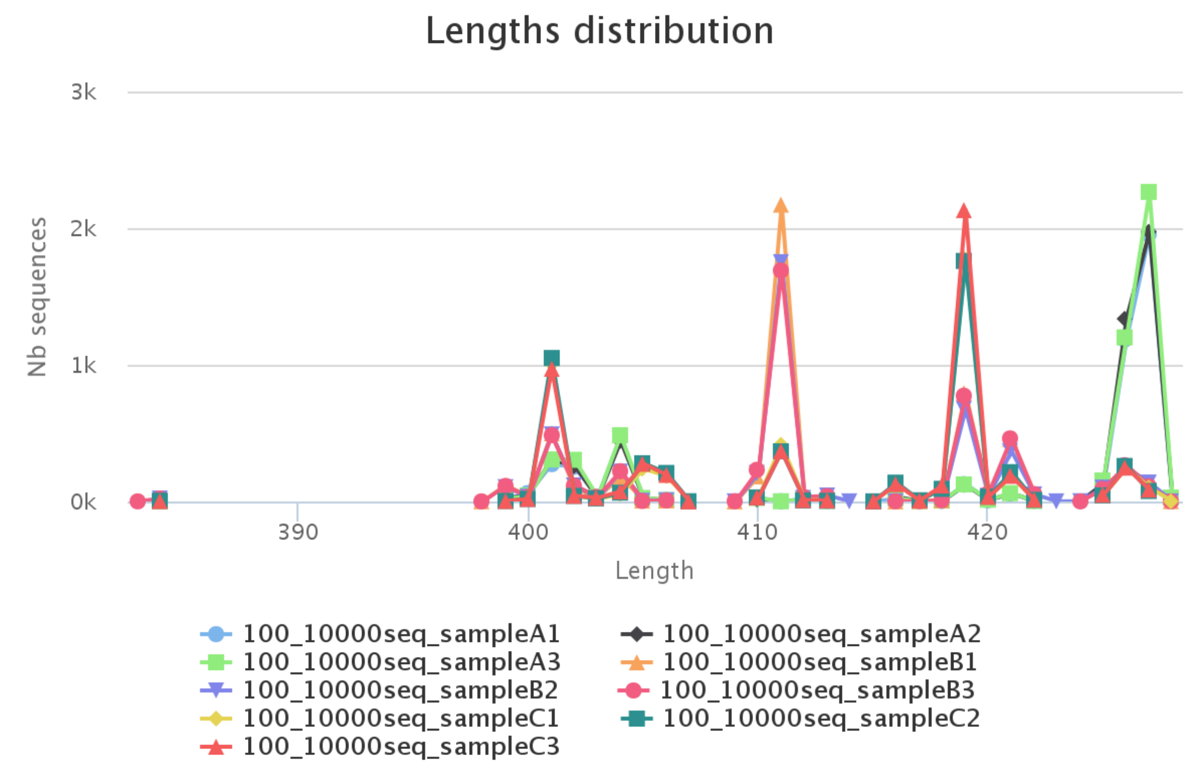


To be kept, sequences must have the 2 primers

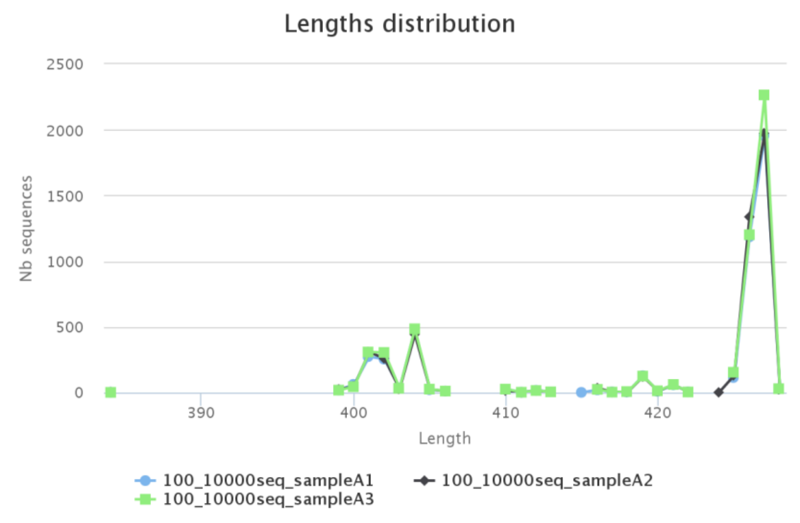


To adjust your filtering, check the distribution of sequence lengths.

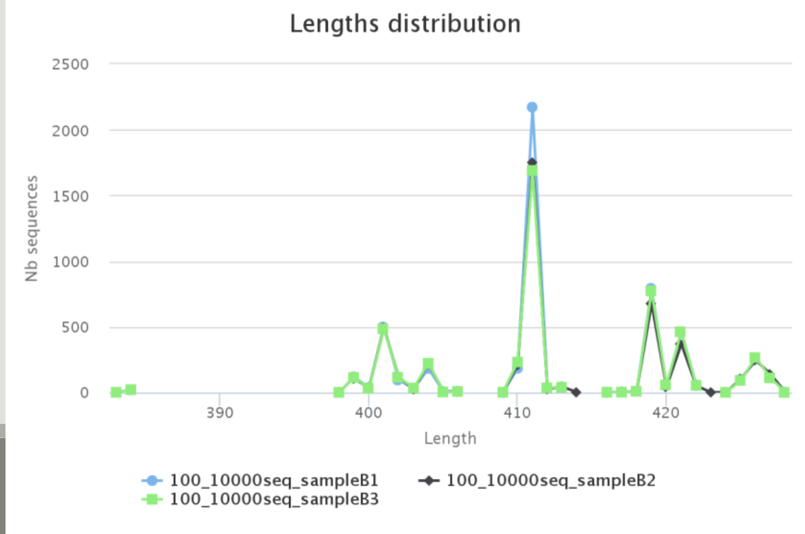
454



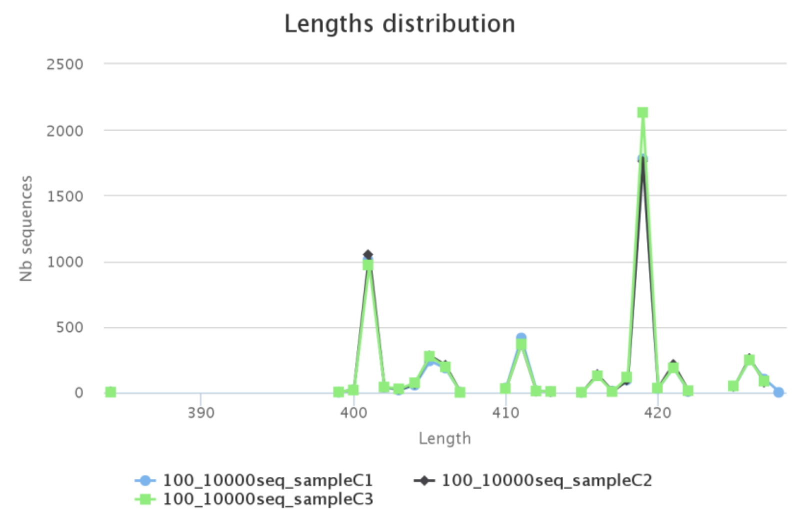
Samples A only



Samples B only



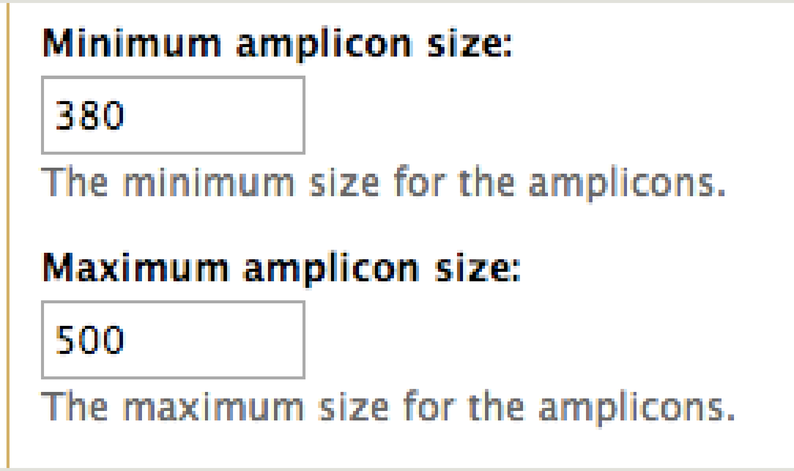
Samples C only



454

Cleaning, how it work ?

Filter contig sequence on its length which must be between min-amplicon-size and max-amplicon-size



Minimum amplicon size:

380

The minimum size for the amplicons.

Maximum amplicon size:

500

The maximum size for the amplicons.

use cutadapt to search and trim primers sequences with less than 10% differences

454

Cleaning, how it work ?

dereplicate sequences and return one uniq fasta file for all sample and a count table to indicate sequence abundances among sample.

In the HTML report file, you will find for each filter the number of sequences passing it, and a table that details these filters for each sample.

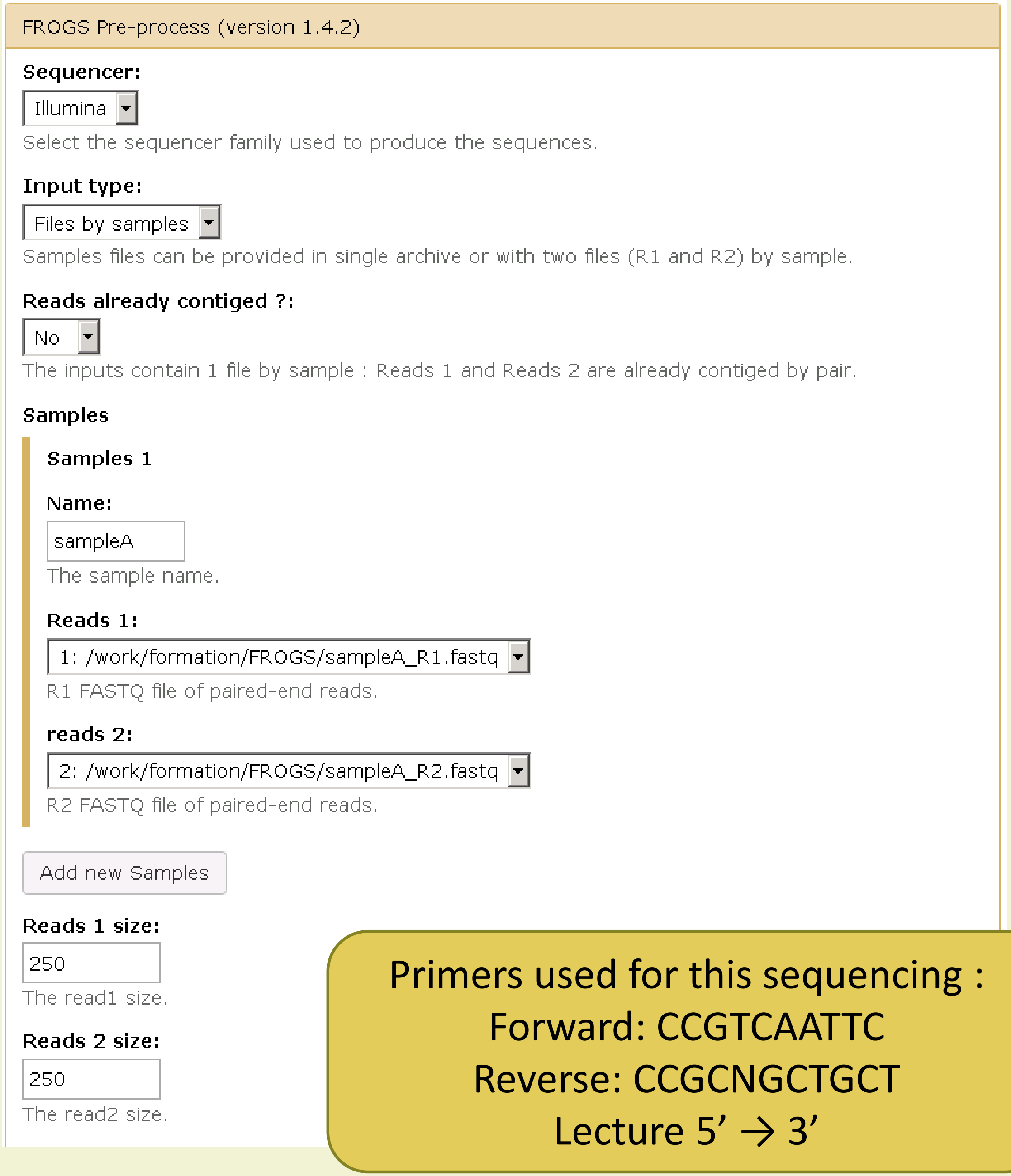
MiSeq
R1 R2

Exercise 3.2

Go to « MiSeq R1 R2 » history

Launch the pre-process tool on that data set

→ objective: understand flash software



FROGS Pre-process (version 1.4.2)

Sequencer:

Illumina

Select the sequencer family used to produce the sequences.

Input type:

Files by samples

Samples files can be provided in single archive or with two files (R1 and R2) by sample.

Reads already contiged ?:

No

The inputs contain 1 file by sample : Reads 1 and Reads 2 are already contiged by pair.

Samples

Samples 1

Name:

sampleA

The sample name.

Reads 1:

1: /work/formation/FROGS/sampleA_R1.fastq

R1 FASTQ file of paired-end reads.

reads 2:

2: /work/formation/FROGS/sampleA_R2.fastq

R2 FASTQ file of paired-end reads.

Reads 1 size:

250

The read1 size.

Reads 2 size:

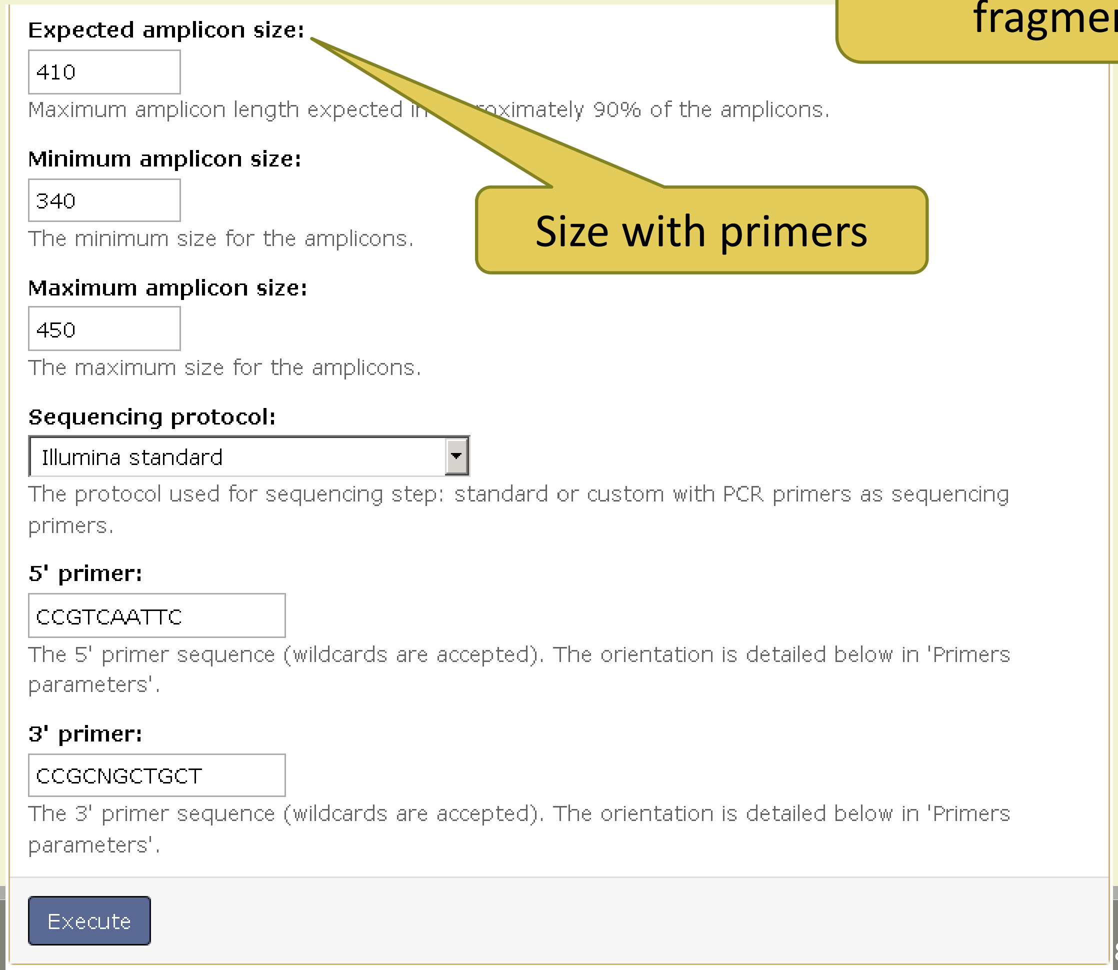
250

The read2 size.

```
>ERR619083.M00704
CCGTCAATTCATTGAGTTTCAACCTTGCGGCCGTACTTCCCAGGCGGTACGTT
TATCGCGTTAGCTTCGCCAAGCACAGCATCCTGCGCTTAGCCAACGTACATCG
TTTAGGGTGTGGACTACCCGGGTATCTAATCCTGTTCGCTACCCACGCTTTCG
AGCCTCAGCGTCAGTGACAGACCAGAGAGCCGCTTTCGCCACTGGTGTTCCTC
CATATATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCCCTTCTGC
ACTCAAGTCAGACAGTTTCCAGAGCACTCTATGGTTGAGCCATAGCCTTTTAC
TCCAGACTTTCCTGACCGACTGCACTCGCTTTACGCCCAATAAATCCGGACAA
CGCTTGCCACCTACGTATTACCGCNGCTGCT
```

MiSeq
R1 R2

Real 16S sequenced fragment



Expected amplicon size:

410

Maximum amplicon length expected in approximately 90% of the amplicons.

Minimum amplicon size:

340

The minimum size for the amplicons.

Maximum amplicon size:

450

The maximum size for the amplicons.

Sequencing protocol:

Illumina standard

The protocol used for sequencing step: standard or custom with PCR primers as sequencing primers.

5' primer:

CCGTCAATTC

The 5' primer sequence (wildcards are accepted). The orientation is detailed below in 'Primers parameters'.

3' primer:

CCGCNGCTGCT

The 3' primer sequence (wildcards are accepted). The orientation is detailed below in 'Primers parameters'.

Size with primers

Primers used for this sequencing :
Forward: CCGTCAATTC
Reverse: CCGCNGCTGCT
Lecture 5’ → 3’

MiSeq
R1 R2

Flash, how it works ?

To contig read1 and read2 with FLASh with :

a minimum overlap equals to

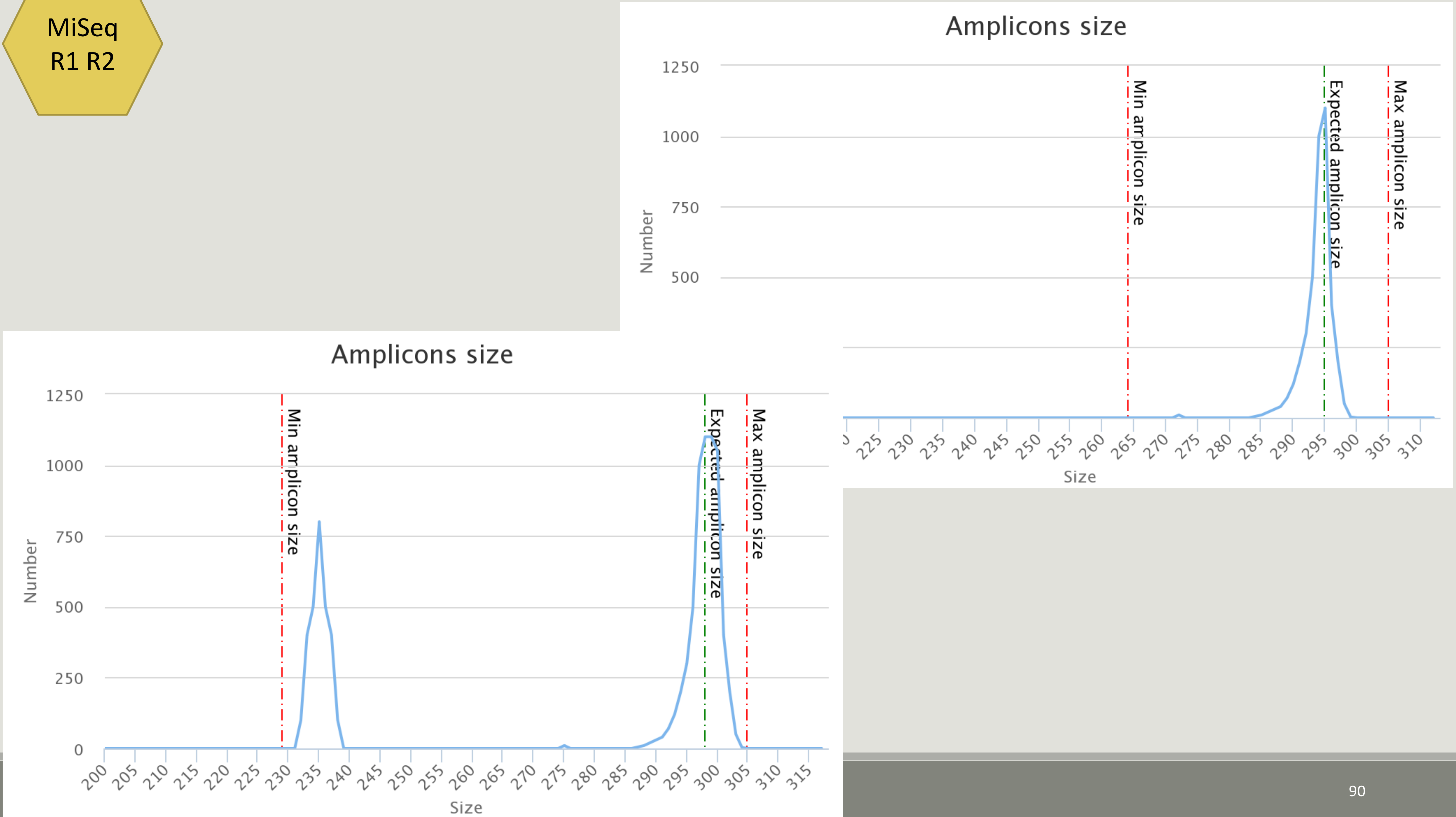
[(R1-size + R2-size) - expected-amplicon-size]

and a maximum overlap equal to [expected-amplicon-size] with a maximum of 10% mismatch among this overlap

ex: minimum overlap (250+250) - 410 = 50
maximum overlap 450

90% of the amplicon are smaller than [expected-amplicon-size]

MiSeq
R1 R2


Amplicons size
1250
1000
750
500
Number
Min amplicon size
Expected amplicon size
Max amplicon size
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
Size
Amplicons size
1250
1000
750
500
250
0
Number
Min amplicon size
Expected amplicon size
Max amplicon size
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
315
Size

MiSeq
R1 R2

Exercise 3.2

Interpret « FROGS Pre-process: report.html » file.

MiSeq
contiged

Exercise 3.3

Go to« MiSeq contiged » history

Launch the pre-process tool on that data set

→ objective: understand output files

MiSeq
contiged

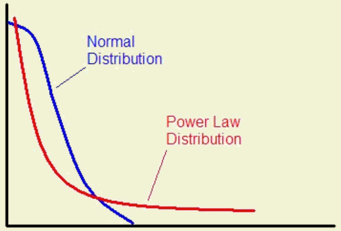
Exercise 3.3

3 samples are **technically replicated** 3 times : 9 samples of 10 000 sequences each.

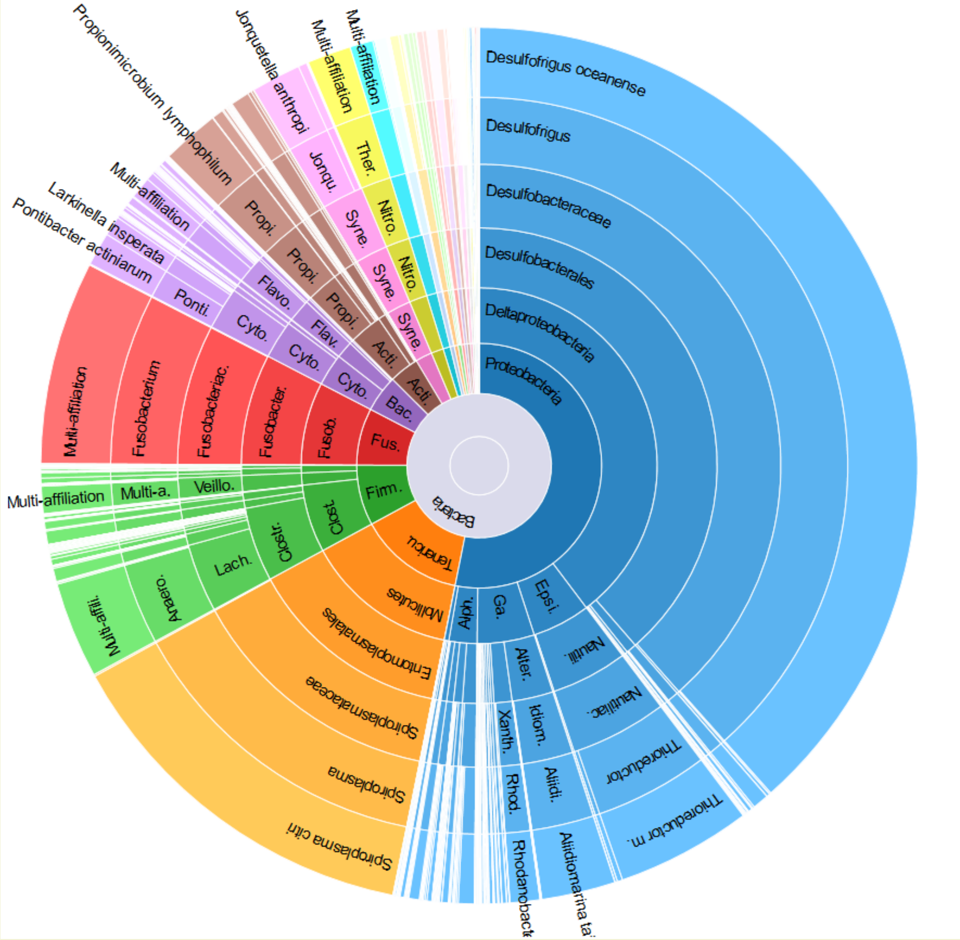
100_10000seq_sampleA1.fastq
100_10000seq_sampleA2.fastq
100_10000seq_sampleA3.fastq
100_10000seq_sampleB1.fastq
100_10000seq_sampleB2.fastq
100_10000seq_sampleB3.fastq
100_10000seq_sampleC1.fastq
100_10000seq_sampleC2.fastq
100_10000seq_sampleC3.fastq

MiSeq contiged

Exercise 3.3



- 100 species, covering all bacterial phyla
- Power Law distribution of the species abundances
- Error rate calibrated with real sequencing runs
- 10% chimeras
- 9 samples of 10 000 sequences each (90 000 sequences)



MiSeq contiged

Exercise 3.3

"Grinder (v 0.5.3) (Angly et al., 2012) was used to simulate the PCR amplification of full-length (V3-V4) sequences from reference databases. The reference database of size 100 were generated from the LTP SSU bank (version 115) (Yarza et al., 2008) by

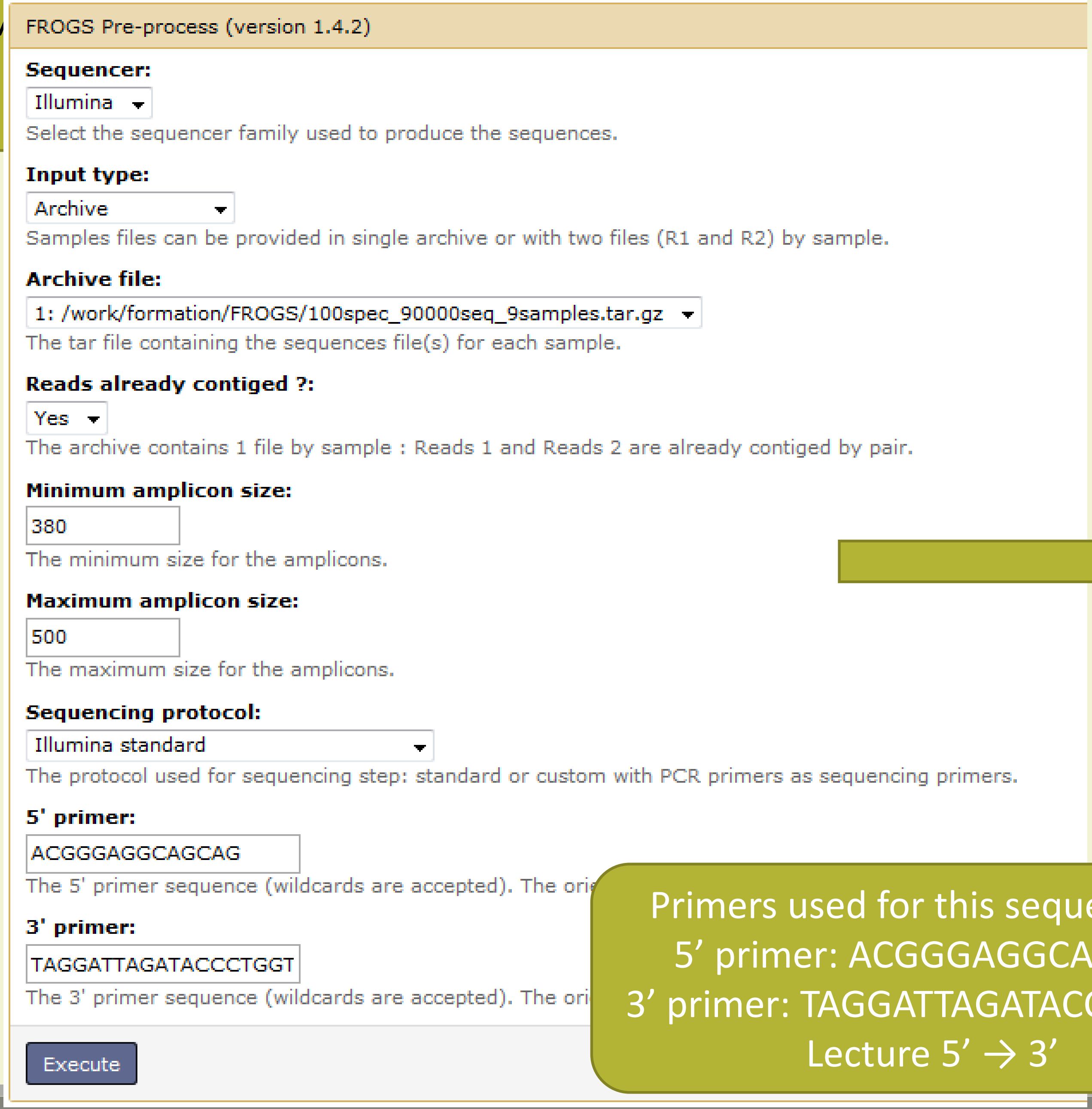
(1) filtering out sequences with a N,

(2) keeping only type species

(3) with a match for the forward (ACGGRAGGCAGCAG) and reverse (TACCAGGGTATCTAATCCTA) primers in the V3-V4 region and

(4) maximizing the phylogenetic diversity (PD) for a given database size. The PD was computed from the NJ tree distributed with the LTP."

M
co



FROGS Pre-process (version 1.4.2)

Sequencer:
Illumina
Select the sequencer family used to produce the sequences.

Input type:
Archive
Samples files can be provided in single archive or with two files (R1 and R2) by sample.

Archive file:
1: /work/formation/FROGS/100spec_90000seq_9samples.tar.gz
The tar file containing the sequences file(s) for each sample.

Reads already contiged ?:
Yes
The archive contains 1 file by sample : Reads 1 and Reads 2 are already contiged by pair.

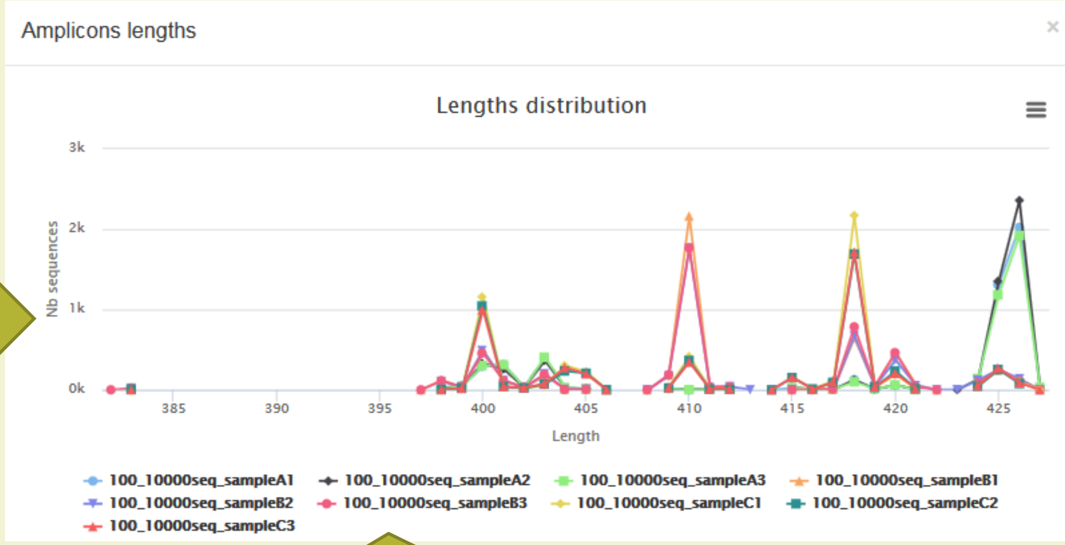
Minimum amplicon size:
380
The minimum size for the amplicons.

Maximum amplicon size:
500
The maximum size for the amplicons.

Sequencing protocol:
Illumina standard
The protocol used for sequencing step: standard or custom with PCR primers as sequencing primers.

5' primer:
ACGGGAGGCAGCAG
The 5' primer sequence (wildcards are accepted). The ori

3' primer:
TAGGATTAGATACCCTGGT
The 3' primer sequence (wildcards are accepted). The ori



Click on legend

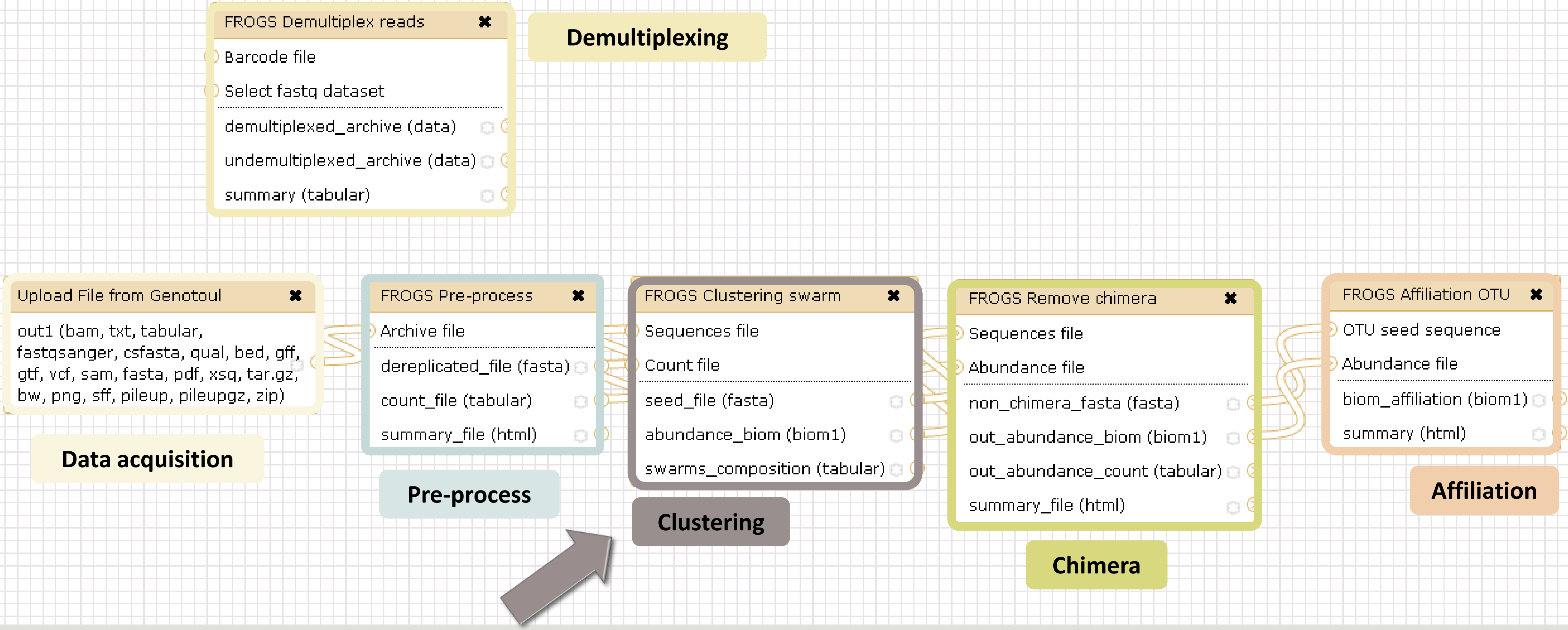
Primers used for this sequencing :
5’ primer: ACGGGAGGCAGCAG
3’ primer: TAGGATTAGATACCCTGGTA
Lecture 5’ → 3’

MiSeq
contiged

Exercise 3.3 - Questions

1. How many sequences are there in the input file ?
2. How many sequences did not have the 5’ primer?
3. How many sequences still are after pre-processing the data?
4. How much time did it take to pre-process the data ?
5. What can you tell about the sample based on sequence length distributions ?

Clustering tool


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Demultiplexing

Data acquisition

Pre-process

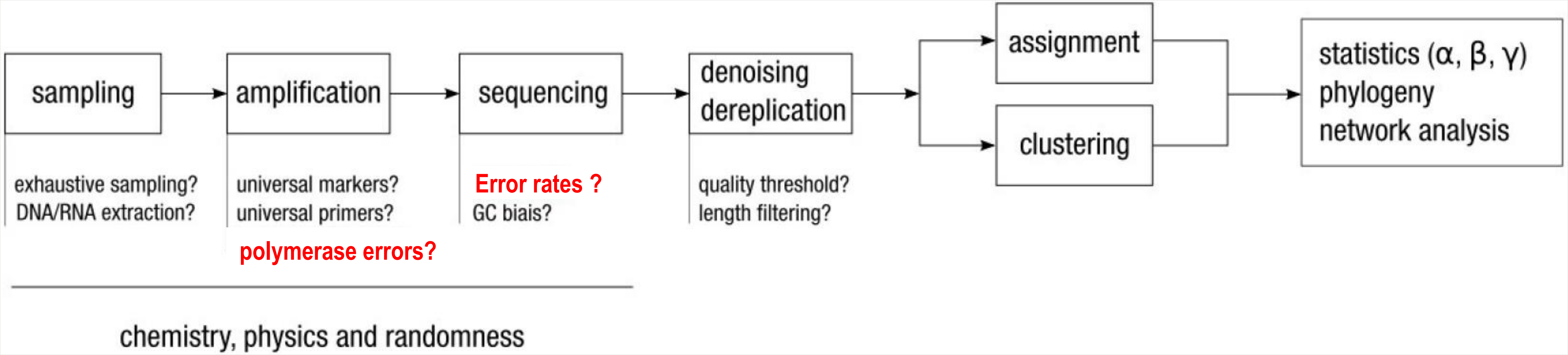
Clustering

Chimera

Affiliation

Why do we need clustering ?

Amplication and sequencing and are not perfect processes



sampling

amplification

sequencing

denoising
dereplication

assignment

clustering

statistics (α, β, γ)
phylogeny
network analysis

exhaustive sampling?
DNA/RNA extraction?

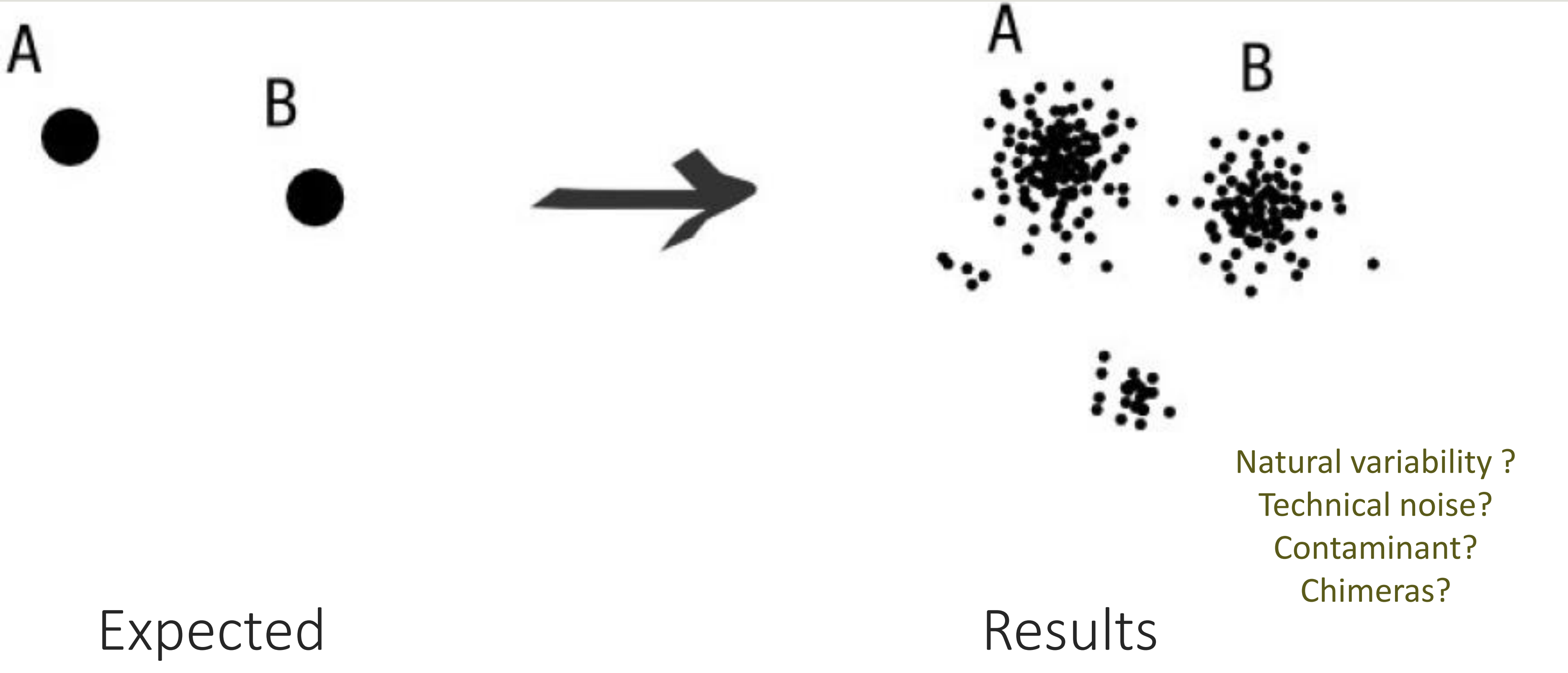
universal markers?
universal primers?

polymerase errors?

Error rates ?
GC biais?

quality threshold?
length filtering?

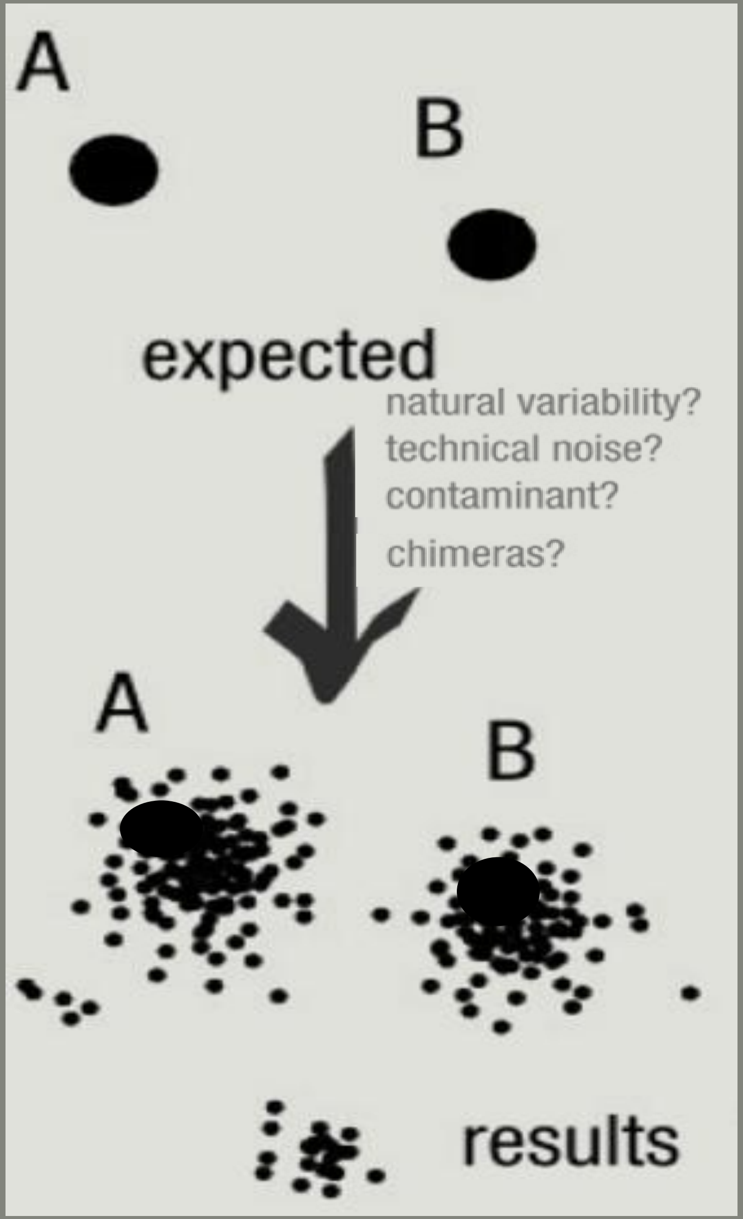
chemistry, physics and randomness



Natural variability ?
Technical noise?
Contaminant?
Chimeras?

Expected

Results


A
B
expected
natural variability?
technical noise?
contaminant?
chimeras?
A
B
results

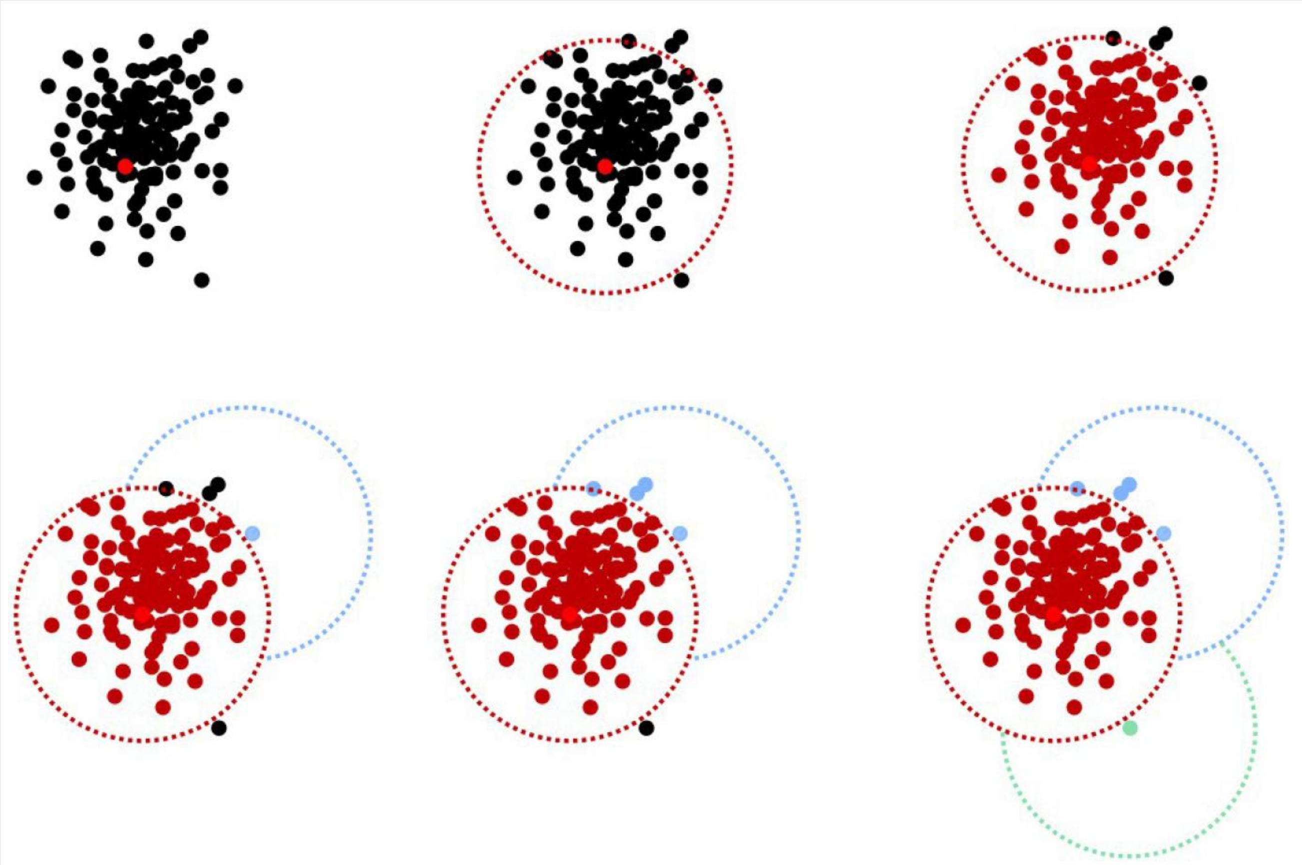
To have the best accuracy:

Method: All against all

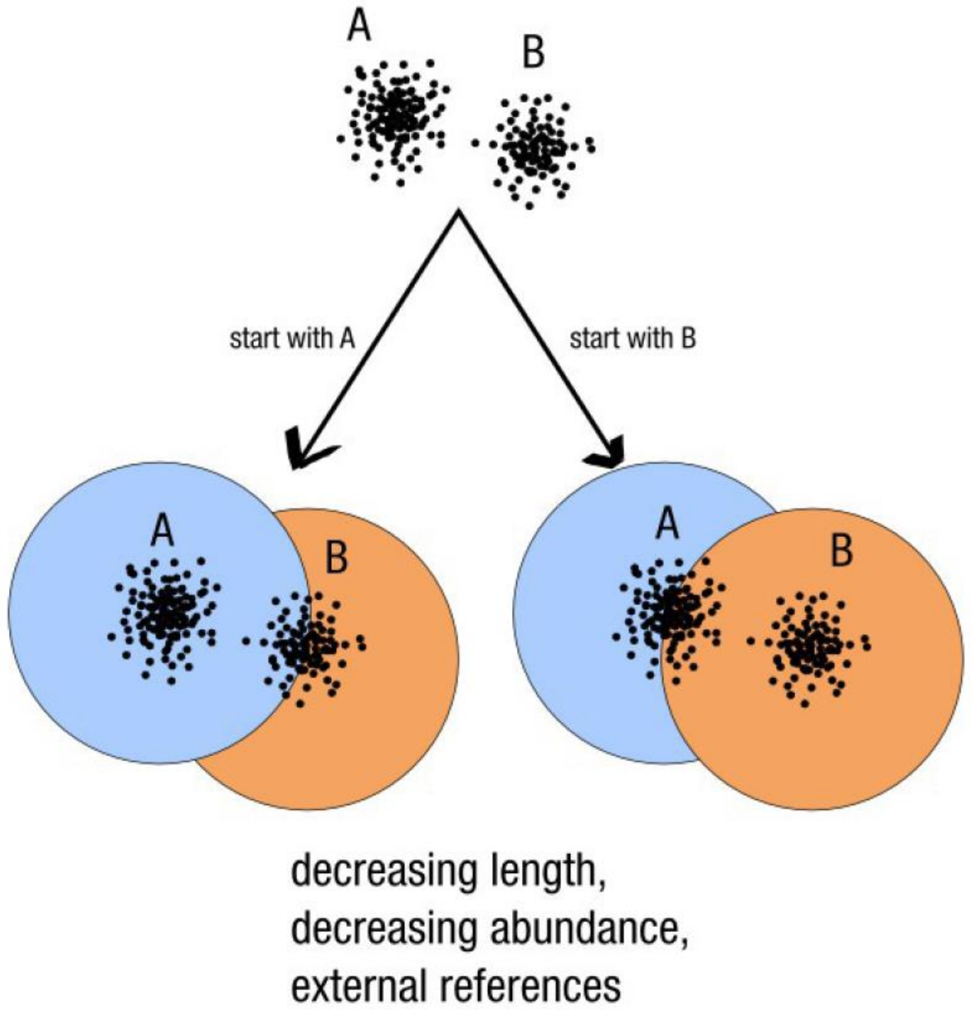
- Very accurate
- Requires a lot of memory and/or time

=> Impossible on very large datasets without strong filtering or sampling

How traditional clustering works ?

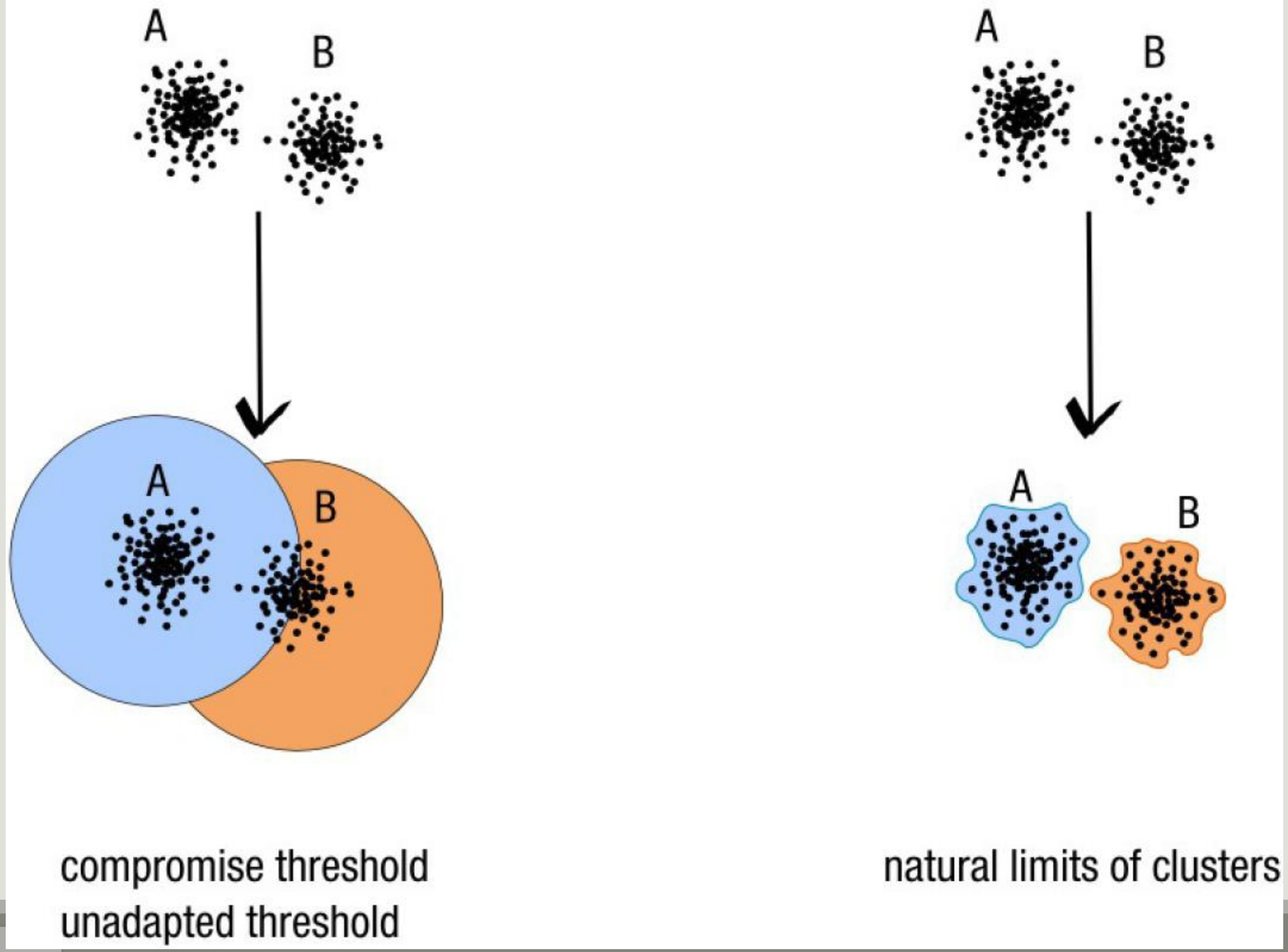


Input order dependent results


A
B
start with A
start with B
A
B
A
B

decreasing length,
decreasing abundance,
external references

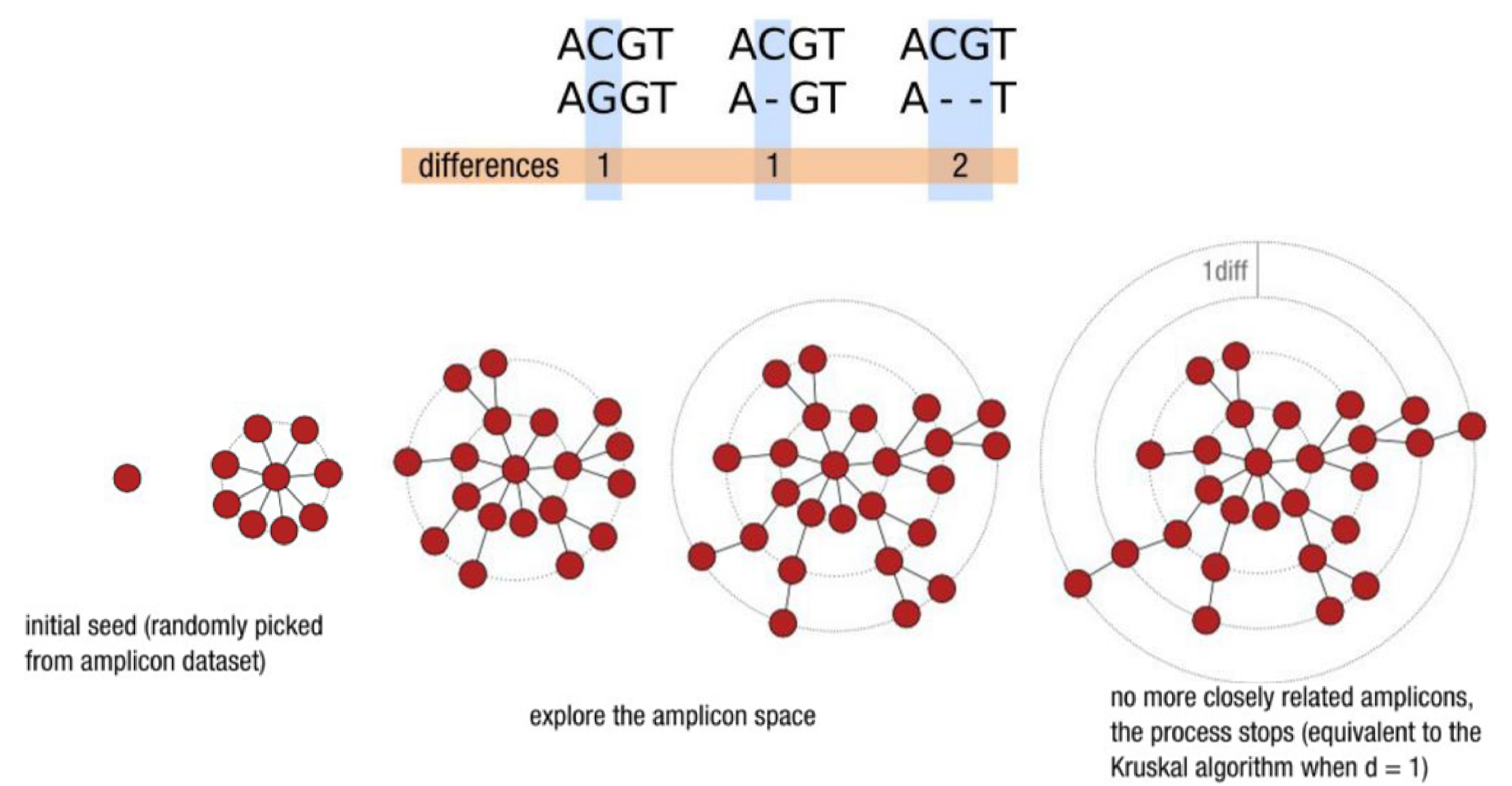
Single a priori clustering threshold



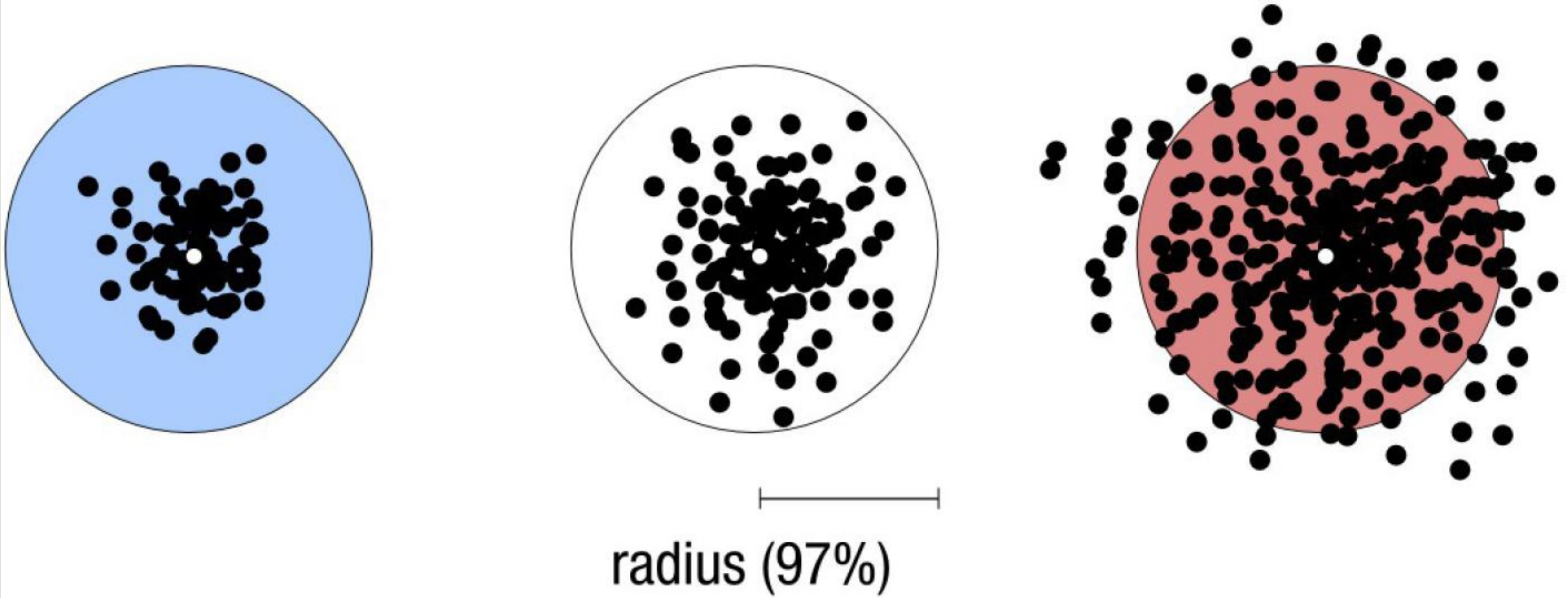
compromise threshold
unadapted threshold

natural limits of clusters

Swarm clustering method


ACGT
AGGT
ACGT
A - GT
ACGT
A - - T
differences 1 1 2
1diff
initial seed (randomly picked from amplicon dataset)
explore the amplicon space
no more closely related amplicons, the process stops (equivalent to the Kruskal algorithm when d = 1)

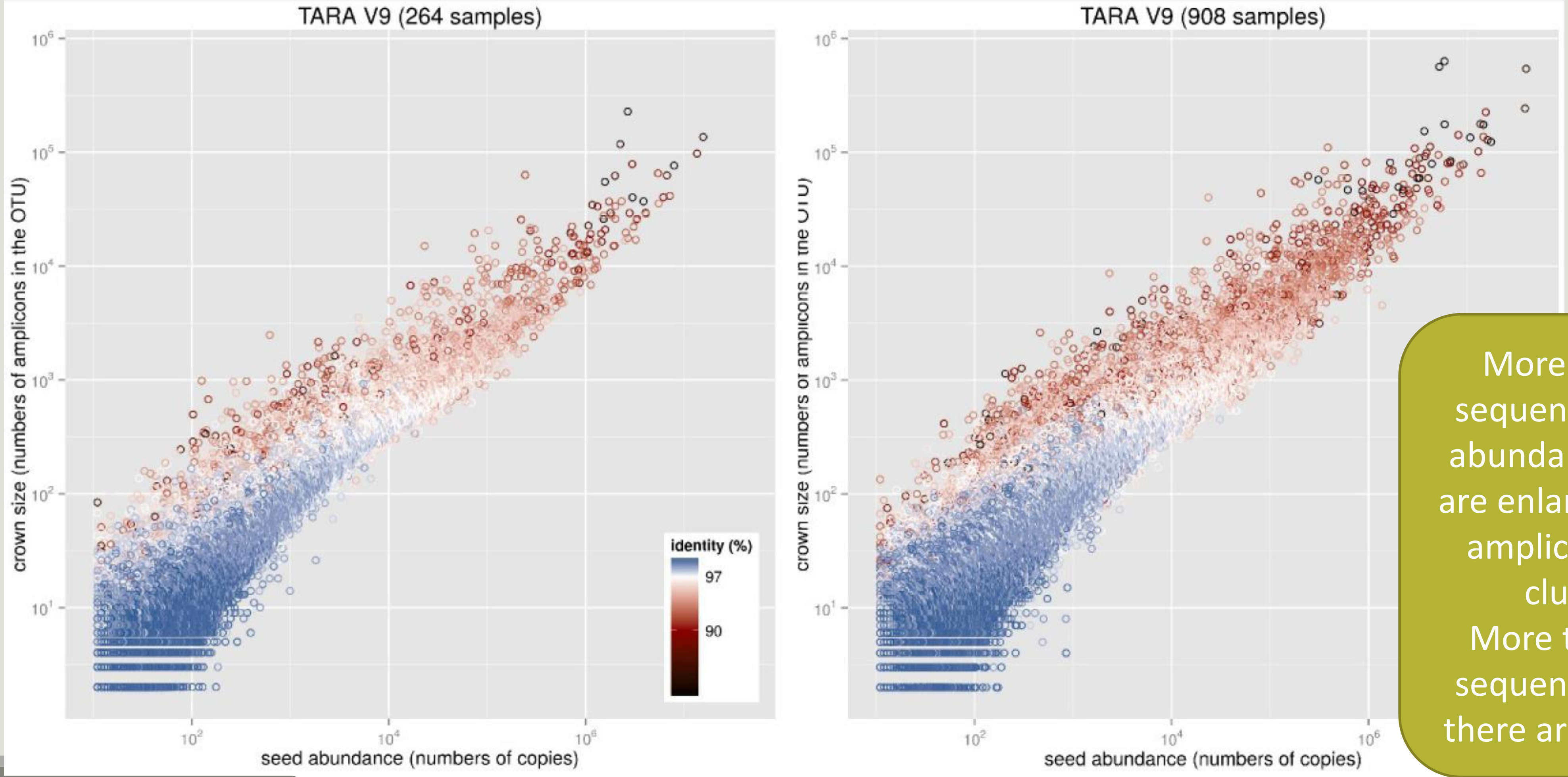
Comparison Swarm and 3% clusterings



Radius expressed as a percentage of identity with the central amplicon (97% is by far the most widely used clustering threshold)

Fréderic Mahé communication

Comparison Swarm and 3% clusterings



clusters produced with swarm using d = 1

More there is sequences, more abundant clusters are enlarged (more amplicon in the cluster).
More there are sequences, more there are artefacts

Fréderic Mahé communication

SWARM

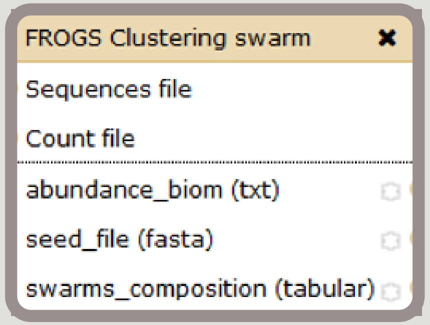
A robust and fast clustering method for amplicon-based studies.

The purpose of **swarm** is to provide a novel clustering algorithm to handle large sets of amplicons.

swarm results are resilient to input-order changes and rely on a small **local** linking threshold *d*, the maximum number of differences between two amplicons.

swarm forms stable high-resolution clusters, with a high yield of biological information.

Swarm: robust and fast clustering method for amplicon-based studies.
Mahé F, Rognes T, Quince C, de Vargas C, Dunthorn M.
PeerJ. 2014 Sep 25;2:e593. doi: 10.7717/peerj.593. eCollection 2014.
PMID:25276506


FROGS Clustering swarm

Sequences file

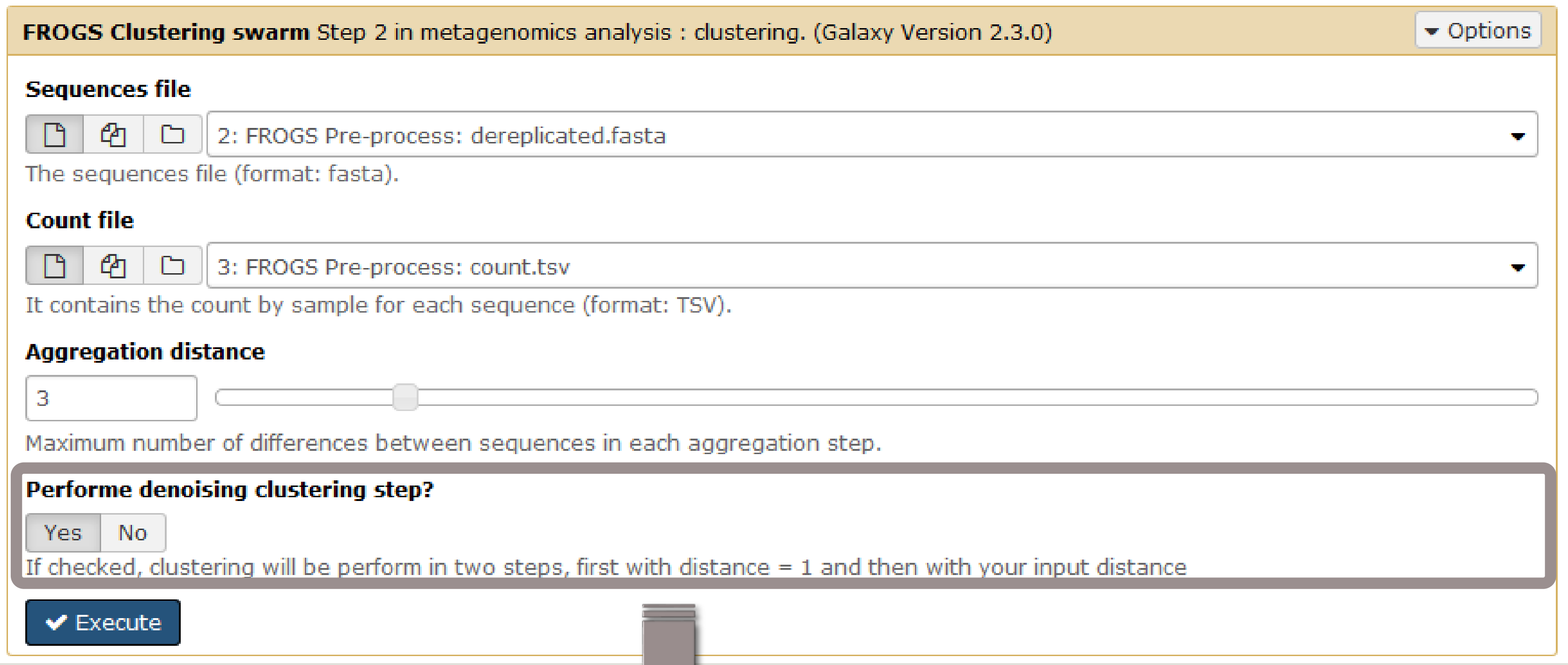
Count file

abundance_biom (txt)

seed_file (fasta)

swarms_composition (tabular)

Clustering


FROGS Clustering swarm Step 2 in metagenomics analysis : clustering. (Galaxy Version 2.3.0)

Sequences file

2: FROGS Pre-process: dereplicated.fasta

The sequences file (format: fasta).

Count file

3: FROGS Pre-process: count.tsv

It contains the count by sample for each sequence (format: TSV).

Aggregation distance

3

Maximum number of differences between sequences in each aggregation step.

Performe denoising clustering step?

Yes No

If checked, clustering will be perform in two steps, first with distance = 1 and then with your input distance

1st run for denoising:
Swarm with d = 1 -> high clusters definition
linear complexity

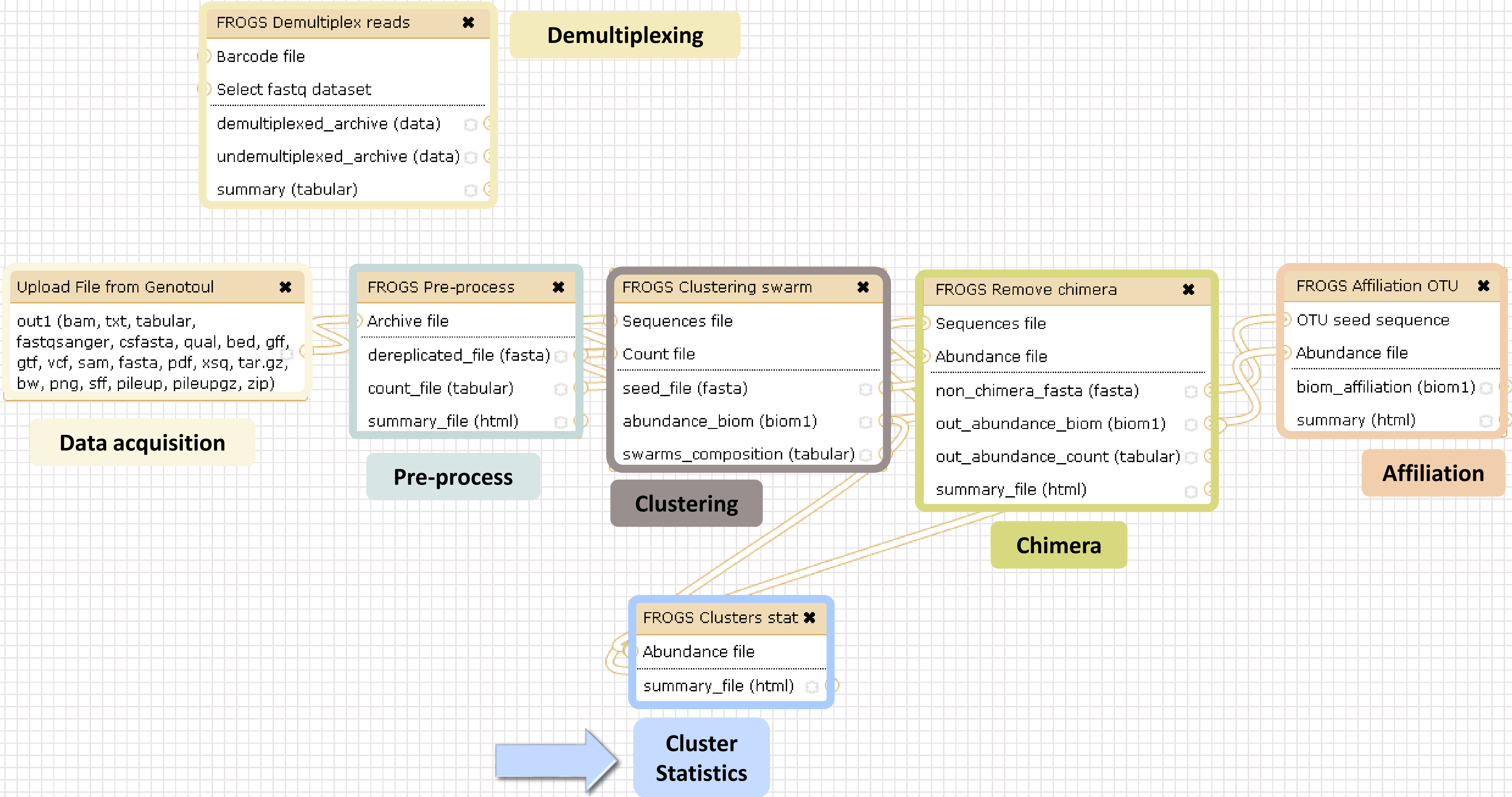
2nd run for clustering:
Swarm with d = 3 on the seeds of first Swarm
quadratic complexity

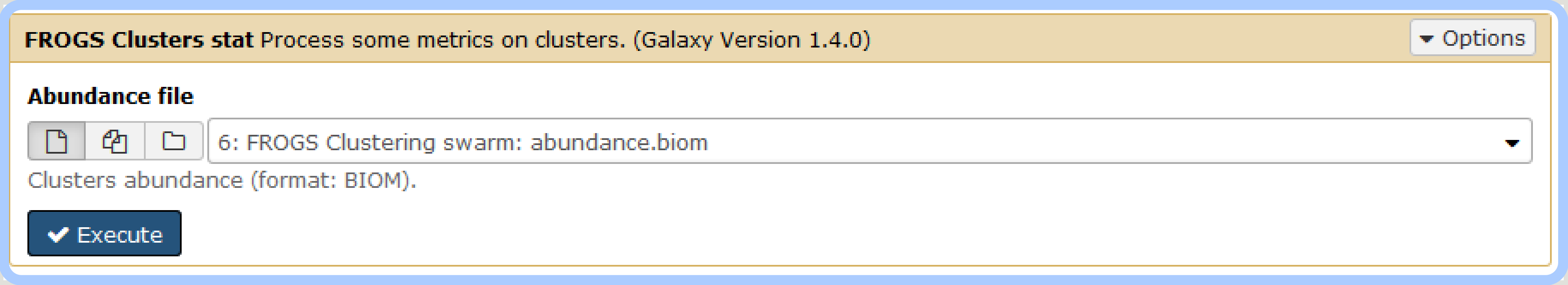
Gain time !

Remove false positives !

PeerJ PrePrints 2:e386v1. 2014. doi: 10.7287/peerj.preprints.386v1
Swarm: robust and fast clustering method for amplicon-based studies.
Mahé F, Rognes T, Quince C, de Vargas C, Dunthorn M.

Cluster stat tool


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Demultiplexing
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
Data acquisition
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
Pre-process
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
Clustering
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
Chimera
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
Affiliation
FROGS Clusters stat
Abundance file
summary_file (html)
Cluster Statistics


FROGS Clusters stat Process some metrics on clusters. (Galaxy Version 1.4.0)

Abundance file

6: FROGS Clustering swarm: abundance.biom

Clusters abundance (format: BIOM).

Your Turn! - 4

LAUNCH CLUSTERING AND CLUSTERSTAT TOOLS

MiSeq contiged

Exercise 4

Go to « MiSeq contiged » history

Launch the Clustering SWARM tool on that data set with aggregation distance = 3 and the denoising

→ objectives :

- understand the denoising efficiency
- understand the ClusterStat utility

MiSeq contiged

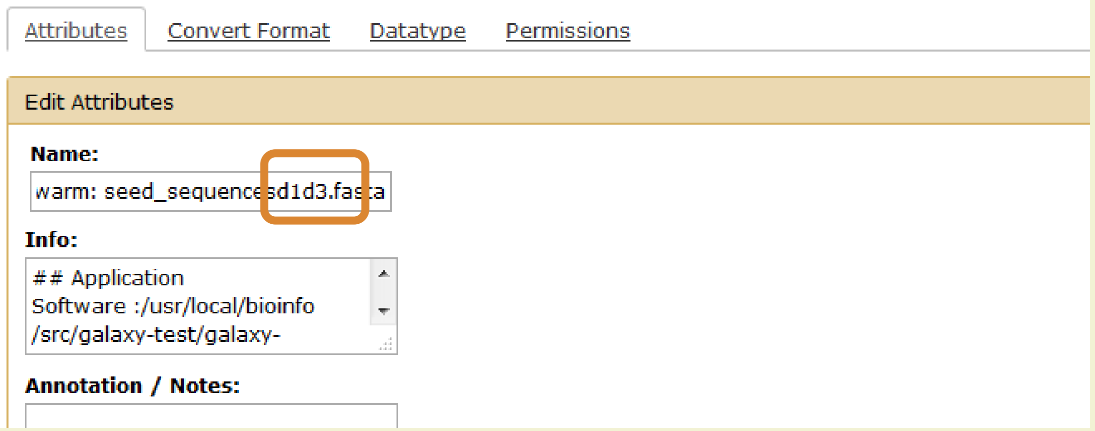
Exercise 4

1. How much time does it take to finish?
2. How many clusters do you get ?

MiSeq
contiged

Exercise 4

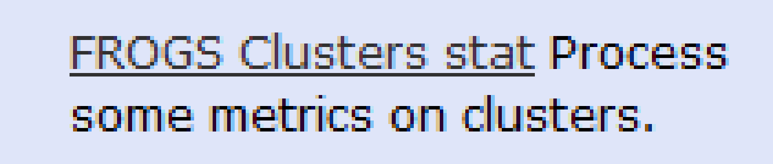
3. Edit the biom and fasta output dataset by adding d1d3


Attributes
Convert Format
Datatype
Permissions
Edit Attributes

Name:
warm: seed_sequencesd1d3.fasta

Info:
Application
Software :/usr/local/bioinfo
/src/galaxy-test/galaxy-

Annotation / Notes:



FROGS Clusters stat Process some metrics on clusters.

4. Launch FROGS Cluster Stat tools on the previous abundance biom file

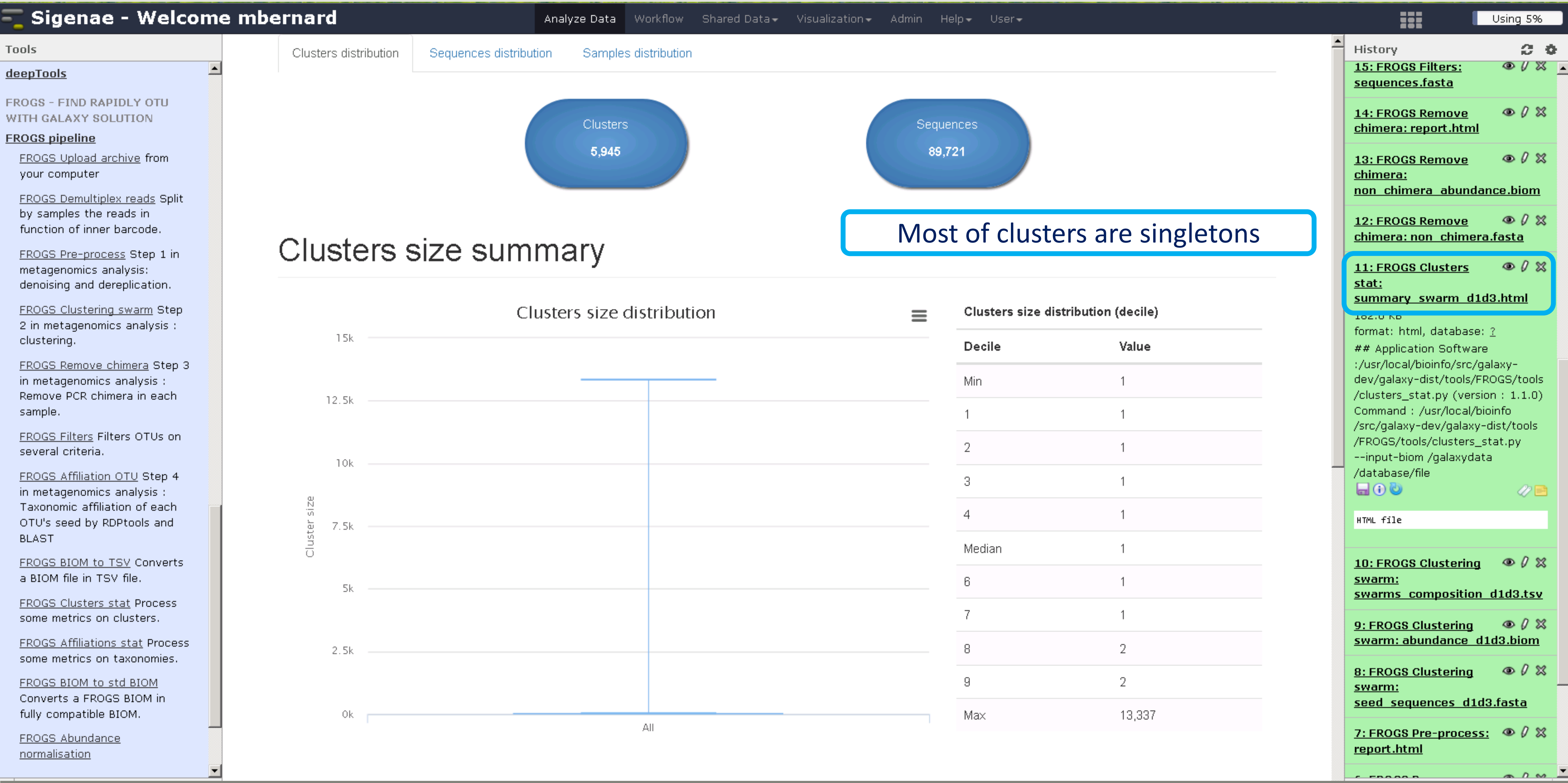
MiSeq contiged

Exercise 4

5. Interpret the boxplot: **Clusters size summary**
6. Interpret the table: **Clusters size details**
7. What can we say by observing the **sequence distribution**?
8. How many clusters share “sampleB3” with at least one other sample?
9. How many clusters could we expect to be shared ?
10. How many sequences represent the 550 specific clusters of “sampleC2”?
11. This represents what proportion of “sampleC2”?
12. What do you think about it?
13. How do you interpret the « Hierarchical clustering » ?

The « Hierachical clustering » is established with a Bray Curtis distance particularly well adapted to abundance table of very heterogenous values (very big and very small figures).

Most of clusters are singletons


Sigenae - Welcome mbernard
Analyze Data
Workflow
Shared Data
Visualization
Admin
Help
User
Using 5%
Tools
History
Clusters distribution
Sequences distribution
Samples distribution
Clusters
5,945
Sequences
89,721
Clusters size distribution
15k
12.5k
10k
7.5k
5k
2.5k
0k
Cluster size
All

deepTools

FROGS - FIND RAPIDLY OTU WITH GALAXY SOLUTION

FROGS pipeline

- FROGS Upload archive from your computer
- FROGS Demultiplex reads Split by samples the reads in function of inner barcode.
- FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication.
- FROGS Clustering swarm Step 2 in metagenomics analysis : clustering.
- FROGS Remove chimera Step 3 in metagenomics analysis : Remove PCR chimera in each sample.
- FROGS Filters Filters OTUs on several criteria.
- FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST
- FROGS BIOM to TSV Converts a BIOM file in TSV file.
- FROGS Clusters stat Process some metrics on clusters.
- FROGS Affiliations stat Process some metrics on taxonomies.
- FROGS BIOM to std BIOM Converts a FROGS BIOM in fully compatible BIOM.
- FROGS Abundance normalisation

Clusters size summary

Clusters size distribution (decile)

Decile	Value
Min	1
1	1
2	1
3	1
4	1
Median	1
6	1
7	1
8	2
9	2
Max	13,337

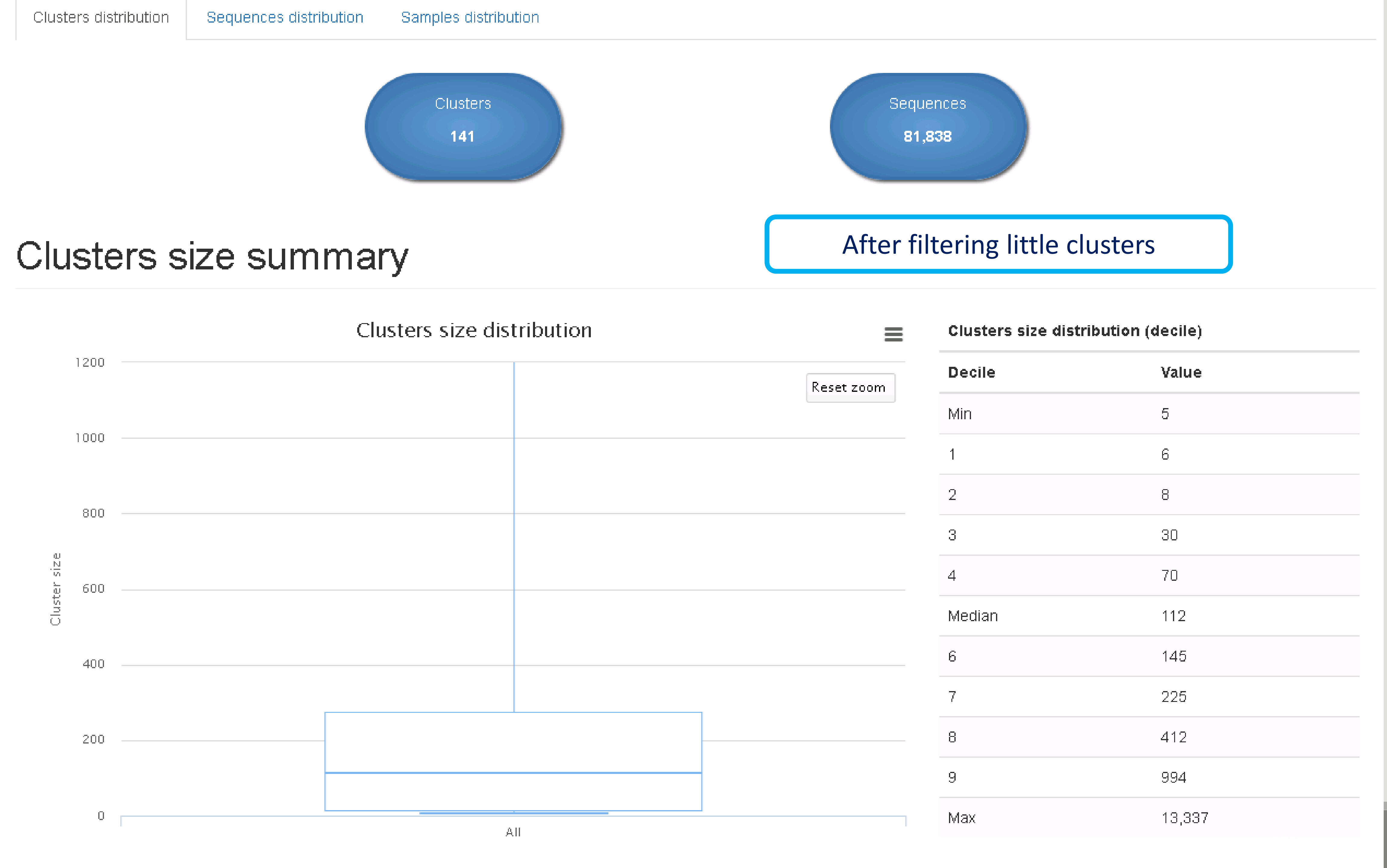
- 15: FROGS Filters: sequences.fasta
- 14: FROGS Remove chimera: report.html
- 13: FROGS Remove chimera: non_chimera_abundance.biom
- 12: FROGS Remove chimera: non_chimera.fasta
- 11: FROGS Clusters stat: summary_swarm_d1d3.html

format: html, database: ?

Application Software :/usr/local/bioinfo/src/galaxy-dev/galaxy-dist/tools/FROGS/tools/clusters_stat.py (version : 1.1.0) Command : /usr/local/bioinfo/src/galaxy-dev/galaxy-dist/tools/FROGS/tools/clusters_stat.py --input-biom /galaxydata/database/file

HTML file

- 10: FROGS Clustering swarm: swarms_composition_d1d3.tsv
- 9: FROGS Clustering swarm: abundance_d1d3.biom
- 8: FROGS Clustering swarm: seed_sequences_d1d3.fasta
- 7: FROGS Pre-process: report.html


Clusters distribution
Sequences distribution
Samples distribution
Reset zoom
1200
1000
800
600
400
200
0
Cluster size
All

Clusters
141

Sequences
81,838

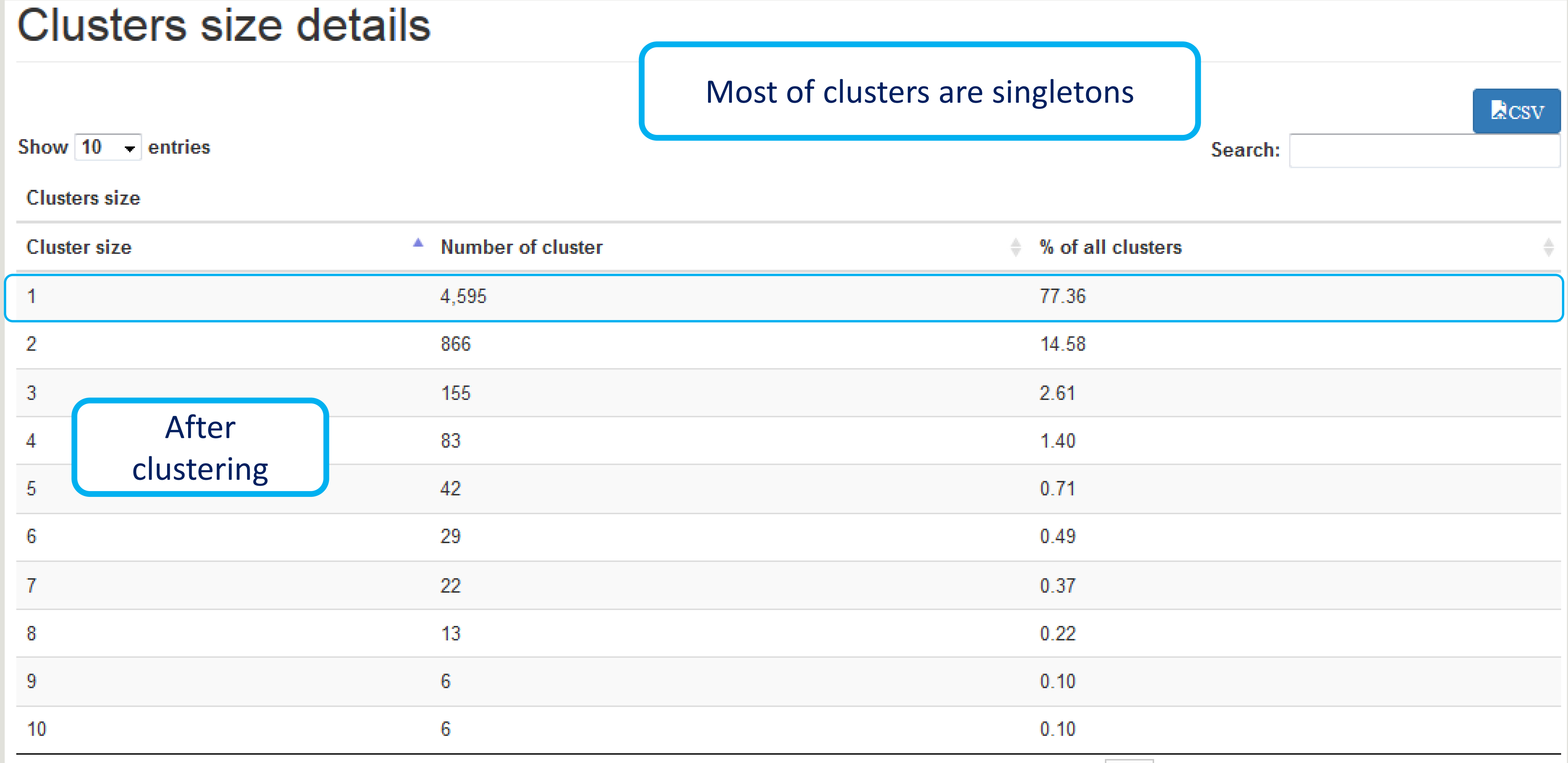
After filtering little clusters

Clusters size summary

Clusters size distribution

Clusters size distribution (decile)

Decile	Value
Min	5
1	6
2	8
3	30
4	70
Median	112
6	145
7	225
8	412
9	994
Max	13,337


CSV
Show 10 entries
Search:

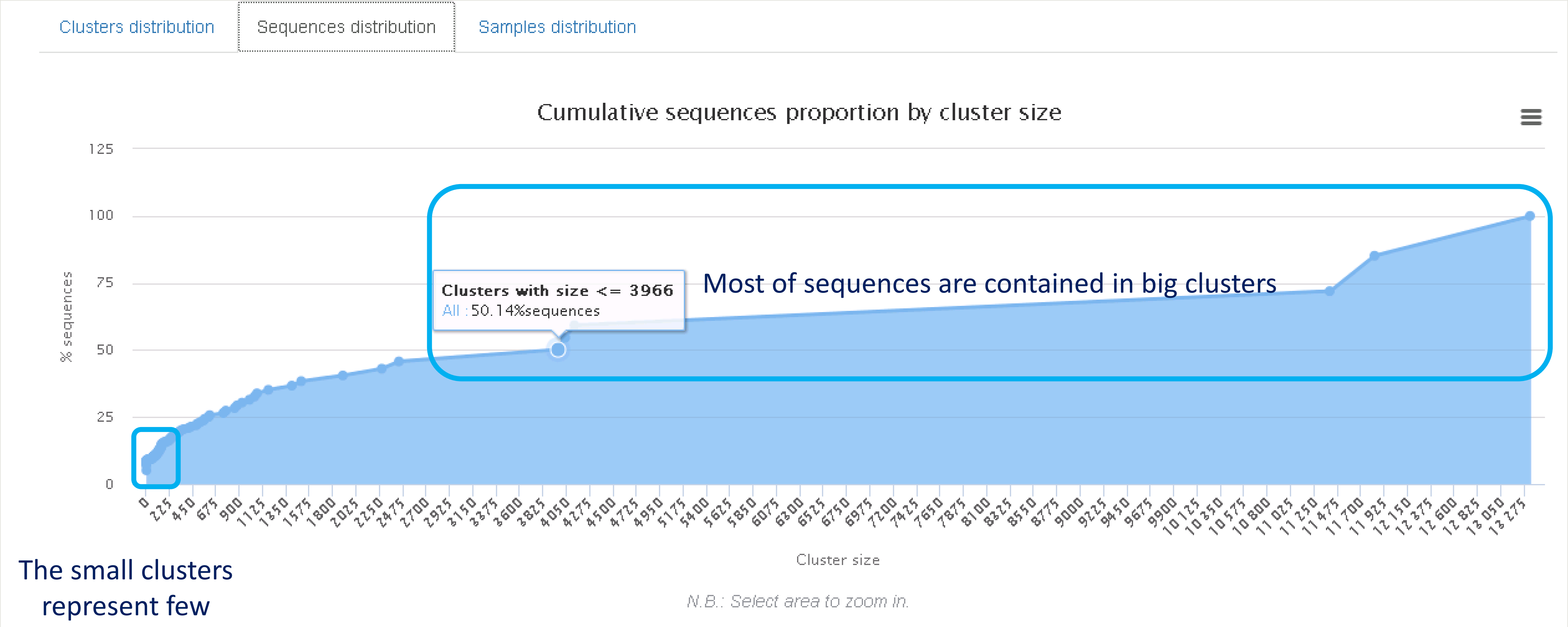
Clusters size details

Most of clusters are singletons

After clustering

Clusters size

Cluster size	Number of cluster	% of all clusters
1	4,595	77.36
2	866	14.58
3	155	2.61
4	83	1.40
5	42	0.71
6	29	0.49
7	22	0.37
8	13	0.22
9	6	0.10
10	6	0.10

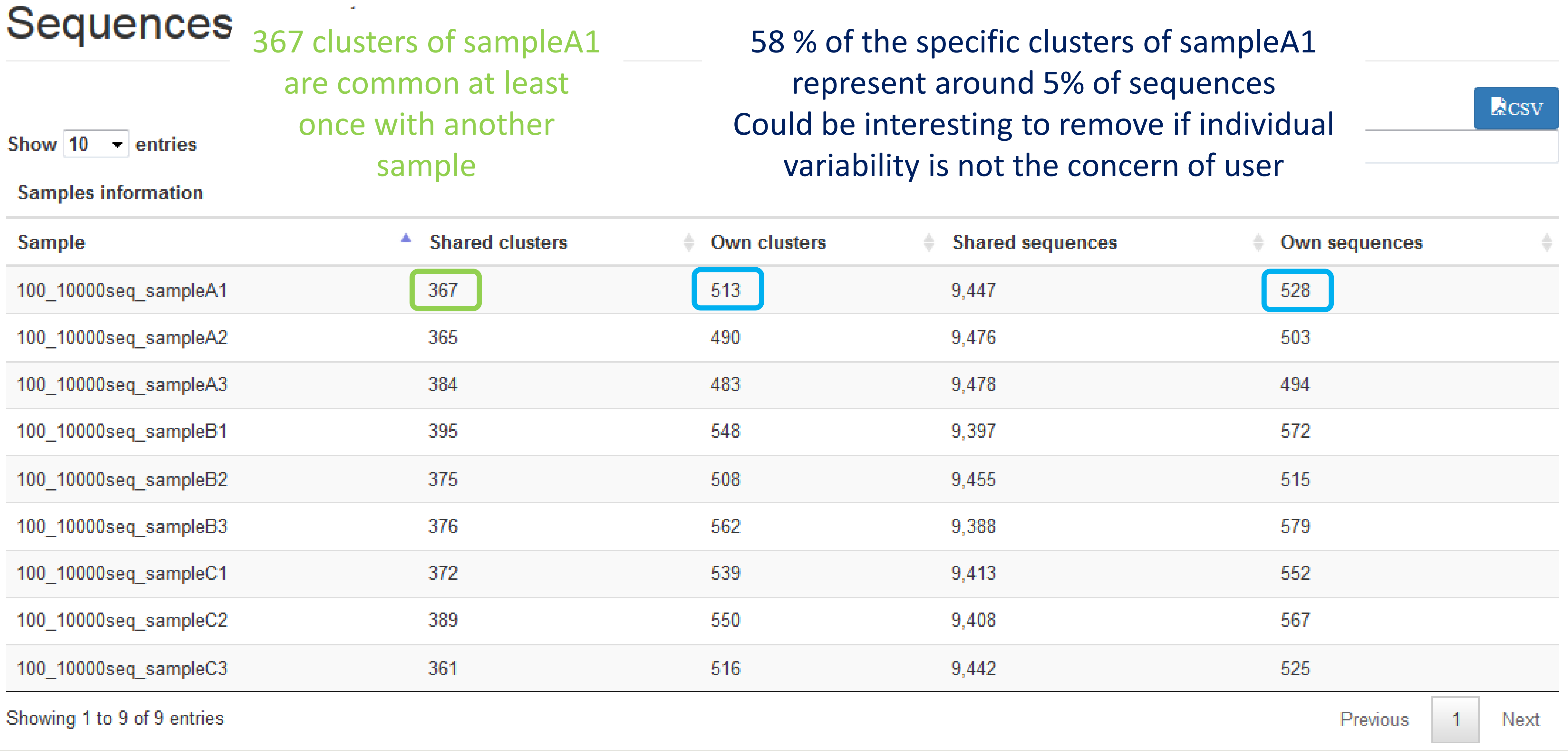

Clusters distribution
Sequences distribution
Samples distribution
Cumulative sequences proportion by cluster size
125
100
75
50
25
0
% sequences
0
225
450
675
900
1125
1350
1575
1800
2025
2250
2475
2700
2925
3150
3375
3600
3825
4050
4275
4500
4725
4950
5175
5400
5625
5850
6075
6300
6525
6750
6975
7200
7425
7650
7875
8100
8325
8550
8775
9000
9225
9450
9675
9900
10 125
10 350
10 575
10 800
11 025
11 250
11 475
11 700
11 925
12 150
12 375
12 600
12 825
13 050
13 275
Cluster size

Clusters with size <= 3966
All : 50.14%sequences

N.B.: Select area to zoom in.

Most of sequences are contained in big clusters

The small clusters represent few sequences



Sequences

367 clusters of sampleA1 are common at least once with another sample

58 % of the specific clusters of sampleA1 represent around 5% of sequences
Could be interesting to remove if individual variability is not the concern of user

Show 10 entries

Samples information

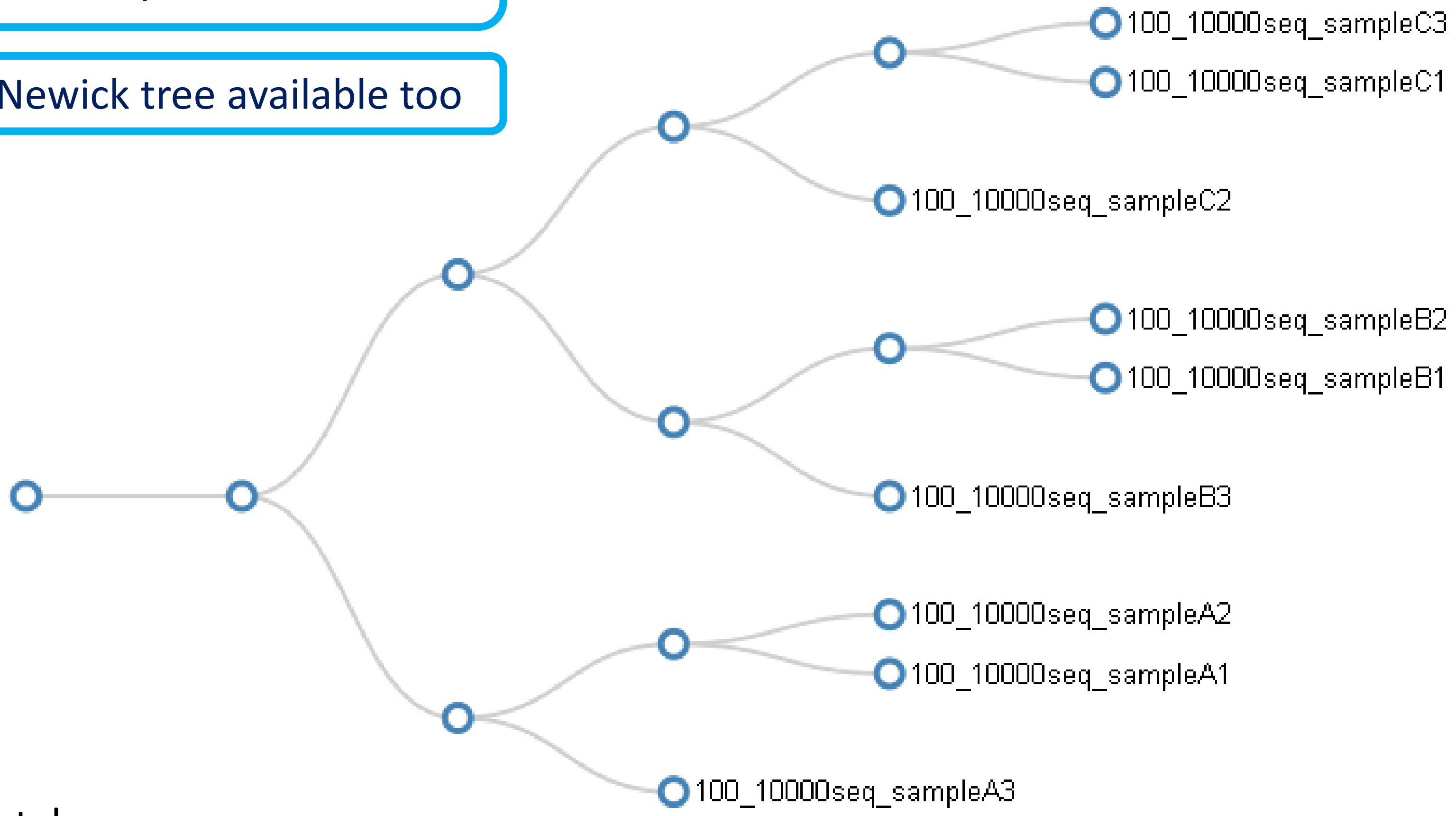
Sample	Shared clusters	Own clusters	Shared sequences	Own sequences
100_10000seq_sampleA1	367	513	9,447	528
100_10000seq_sampleA2	365	490	9,476	503
100_10000seq_sampleA3	384	483	9,478	494
100_10000seq_sampleB1	395	548	9,397	572
100_10000seq_sampleB2	375	508	9,455	515
100_10000seq_sampleB3	376	562	9,388	579
100_10000seq_sampleC1	372	539	9,413	552
100_10000seq_sampleC2	389	550	9,408	567
100_10000seq_sampleC3	361	516	9,442	525

Showing 1 to 9 of 9 entries

Hierachical clustering

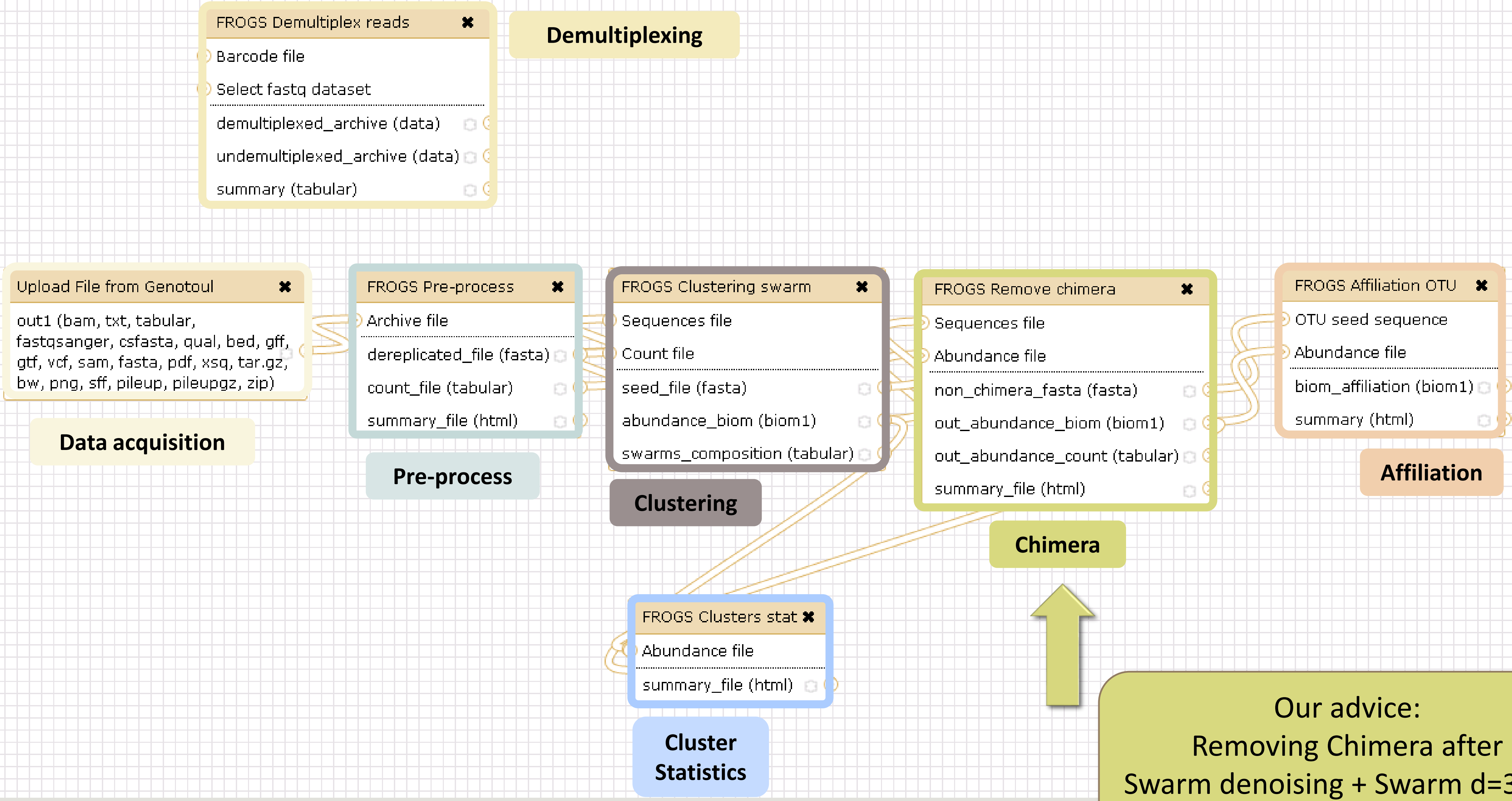
Hierarchical classification on Bray Curtis distance

Newick tree available too


100_10000seq_sampleC3
100_10000seq_sampleC1
100_10000seq_sampleC2
100_10000seq_sampleB2
100_10000seq_sampleB1
100_10000seq_sampleB3
100_10000seq_sampleA2
100_10000seq_sampleA1
100_10000seq_sampleA3

Samples distribution tab

Chimera removal tool


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
FROGS Clusters stat
Abundance file
summary_file (html)

Demultiplexing

Data acquisition

Pre-process

Clustering

Chimera

Affiliation

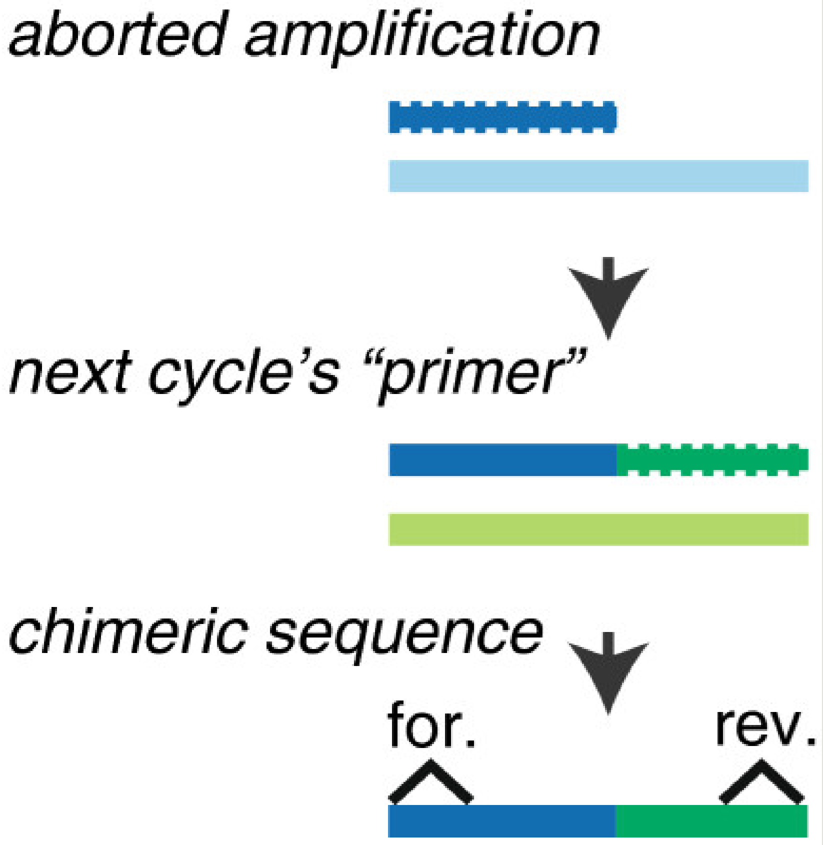
Cluster Statistics

Our advice:
Removing Chimera after
Swarm denoising + Swarm d=3, for saving time without sensitivity loss

What is chimera ?

PCR-generated chimeras are typically created when an aborted amplicon acts as a primer for a heterologous template. Subsequent chimeras are about the same length as the non-chimeric amplicon and contain the forward (for.) and reverse (rev.) primer sequence at each end of the amplicon.

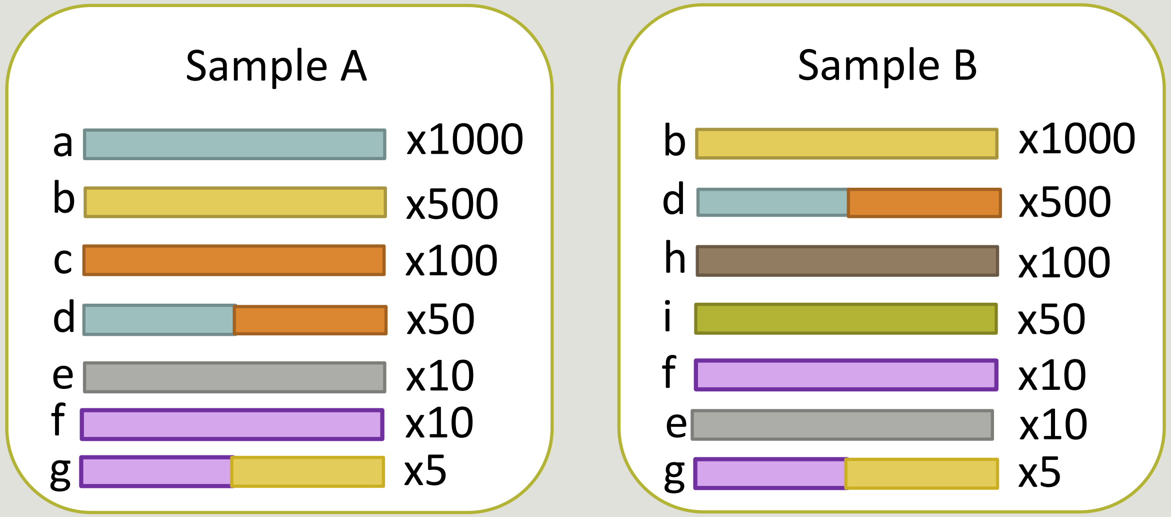
Chimera: from 5 to 45% of reads (Schloss 2011)


aborted amplification
next cycle's "primer"
chimeric sequence
for.
rev.

Fichot and Norman *Microbiome* 2013 **1**:10
doi:10.1186/2049-2618-1-10

A smart removal chimera to be accurate

We use a sample cross-validation



Sample A

a	x1000
b	x500
c	x100
d	x50
e	x10
f	x10
g	x5

Sample B

b	x1000
d	x500
h	x100
i	x50
f	x10
e	x10
g	x5

" **d** " is view as chimera by Vsearch
Its " parents " are presents

" **d** " is view as normal sequence by Vsearch
Its " parents " are absents

⇒ For FROGS "d" is not a chimera
⇒ For FROGS "g" is a chimera, "g" is removed
⇒ FROGS increases the detection specificity

Your Turn! - 5

LAUNCH THE REMOVE CHIMERA TOOL

MiSeq
contiged

Exercise 5

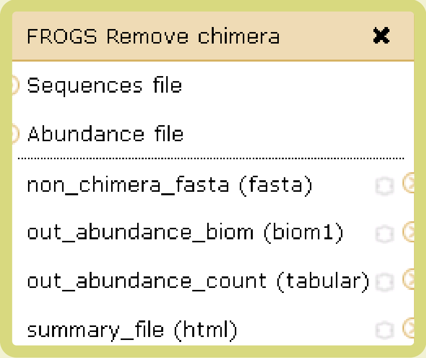
Go to « MiSeq contiged » history

Launch the « FROGS Remove Chimera » tool

Follow by the « FROGS ClusterStat » tool on the swarm d1d3 non chimera abundance biom

→ objectives :

- understand the efficiency of the chimera removal
- make links between small abundant OTUs and chimeras


FROGS Remove chimera

Sequences file

Abundance file

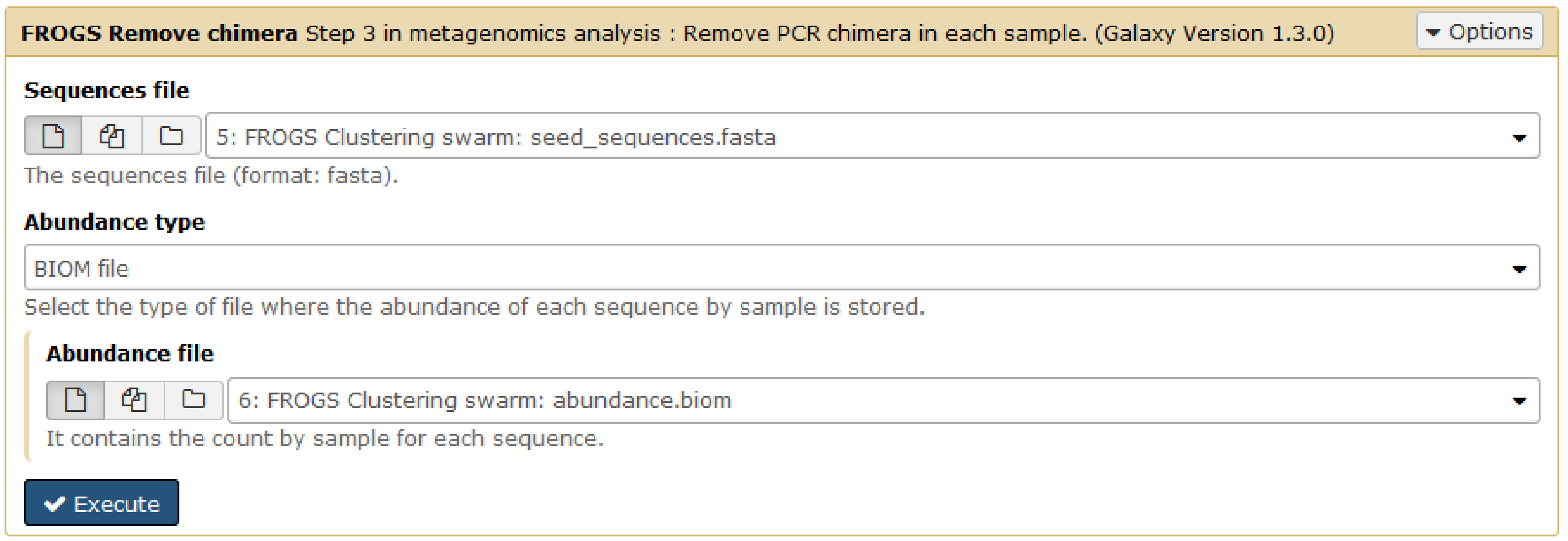
non_chimera_fasta (fasta)

out_abundance_biom (biom1)

out_abundance_count (tabular)

summary_file (html)

Chimera


FROGS Remove chimera Step 3 in metagenomics analysis : Remove PCR chimera in each sample. (Galaxy Version 1.3.0)

Sequences file

5: FROGS Clustering swarm: seed_sequences.fasta

The sequences file (format: fasta).

Abundance type

BIOM file

Select the type of file where the abundance of each sequence by sample is stored.

Abundance file

6: FROGS Clustering swarm: abundance.biom

It contains the count by sample for each sequence.

MiSeq contiged

Exercise 5

1. Understand the « FROGS remove chimera : report.html»
    a. How many clusters are kept after chimera removal?
    b. How many sequences that represent ? So what abundance?
    c. What do you conclude ?

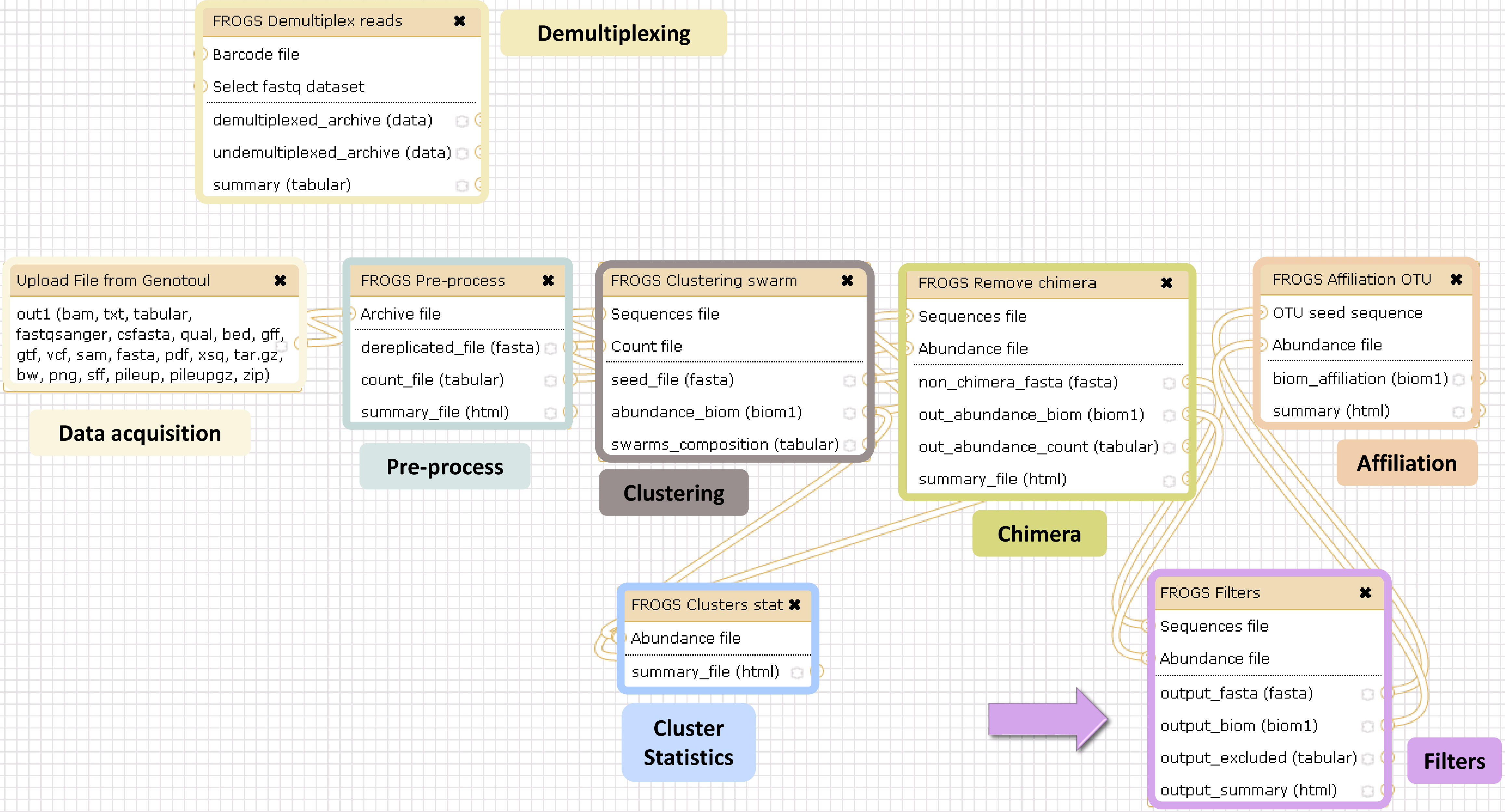
MiSeq contiged

Exercise 5

2. Launch « FROGS ClusterStat » tool on non_chimera_abundanced1d3.biom
3. Rename output in summary_nonchimera_d1d3.html
4. Compare the HTML files
    a. Of what are mainly composed singleton ? (compare with precedent summary.html)
    b. What are their abundance?
    c. What do you conclude ?

The weakly abundant OTUs are mainly false positives, our data would be much more exact if we remove them

Filters tool



FROGS Demultiplex reads ✖
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)

Demultiplexing

Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

Pre-process

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

Clustering

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

Chimera

FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation

FROGS Clusters stat ✖
Abundance file
summary_file (html)

Cluster Statistics

FROGS Filters ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

Filters

Affiliation runs long time

Advise:

Apply filters between “Chimera Removal ” and “Affiliation”.
Remove OTUs with weak abundance and non redundant before affiliation.

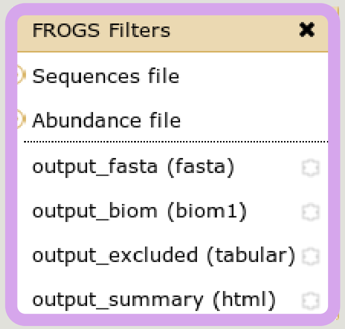
You will gain time !

Filters

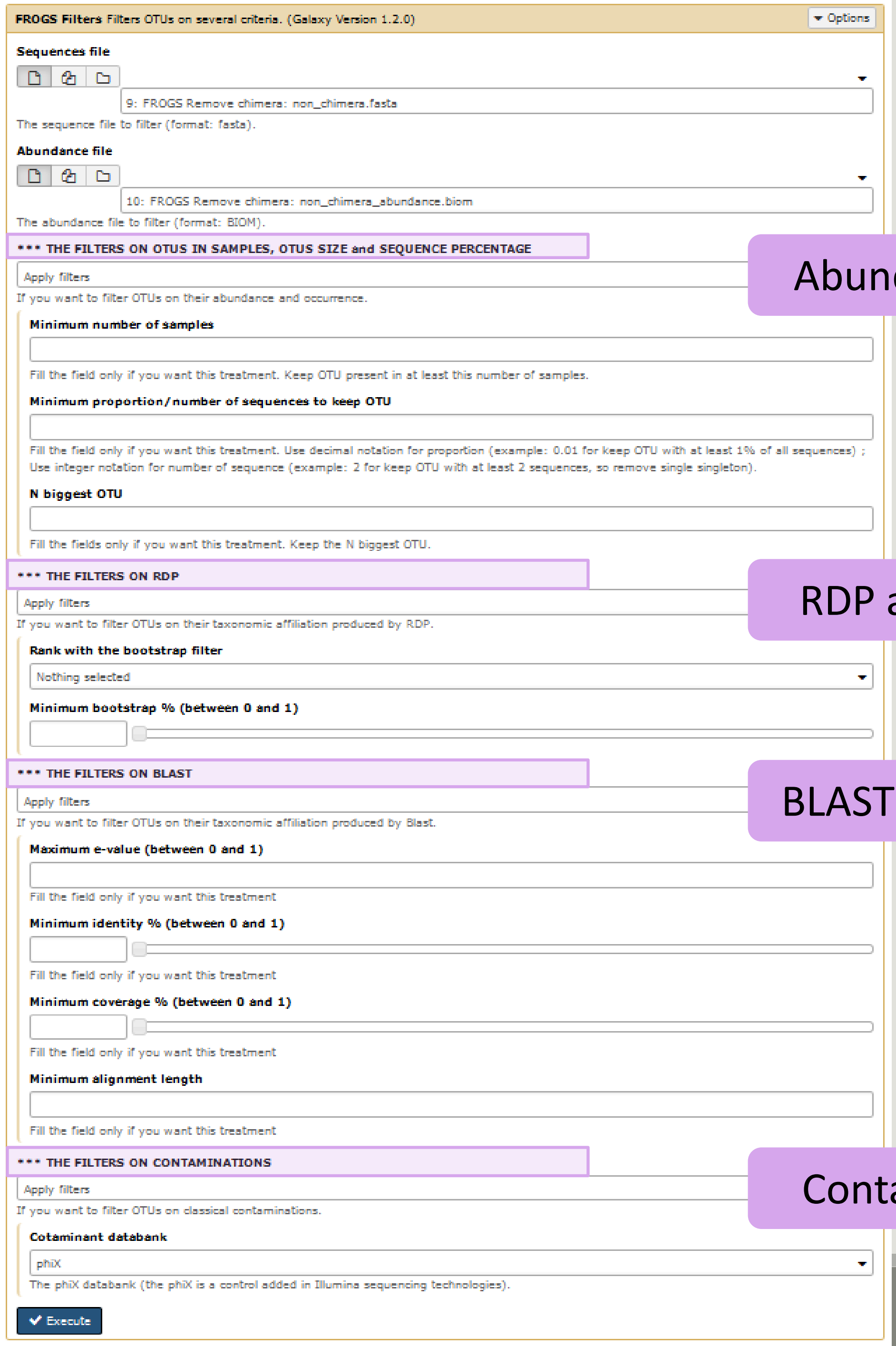
Filters allows to filter the result thanks to different criteria et may be used after different steps of pipeline :

- On the abundance
- On RDP affiliation
- On Blast affiliation
- On phix contaminant

After Affiliation tool


FROGS Filters
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

Filters



FROGS Filters Filters OTUs on several criteria. (Galaxy Version 1.2.0)

Sequences file
9: FROGS Remove chimera: non_chimera.fasta
The sequence file to filter (format: fasta).

Abundance file
10: FROGS Remove chimera: non_chimera_abundance.biom
The abundance file to filter (format: BIOM).

***** THE FILTERS ON OTUS IN SAMPLES, OTUS SIZE and SEQUENCE PERCENTAGE**
Apply filters
If you want to filter OTUs on their abundance and occurrence.

Minimum number of samples
Fill the field only if you want this treatment. Keep OTU present in at least this number of samples.

Minimum proportion/number of sequences to keep OTU
Fill the field only if you want this treatment. Use decimal notation for proportion (example: 0.01 for keep OTU with at least 1% of all sequences) ; Use integer notation for number of sequence (example: 2 for keep OTU with at least 2 sequences, so remove single singleton).

N biggest OTU
Fill the fields only if you want this treatment. Keep the N biggest OTU.

***** THE FILTERS ON RDP**
Apply filters
If you want to filter OTUs on their taxonomic affiliation produced by RDP.

Rank with the bootstrap filter
Nothing selected

Minimum bootstrap % (between 0 and 1)

***** THE FILTERS ON BLAST**
Apply filters
If you want to filter OTUs on their taxonomic affiliation produced by Blast.

Maximum e-value (between 0 and 1)
Fill the field only if you want this treatment

Minimum identity % (between 0 and 1)
Fill the field only if you want this treatment

Minimum coverage % (between 0 and 1)
Fill the field only if you want this treatment

Minimum alignment length
Fill the field only if you want this treatment

***** THE FILTERS ON CONTAMINATIONS**
Apply filters
If you want to filter OTUs on classical contaminations.

Cotaminant databank
phiX
The phiX databank (the phiX is a control added in Illumina sequencing technologies).

Abundance filters

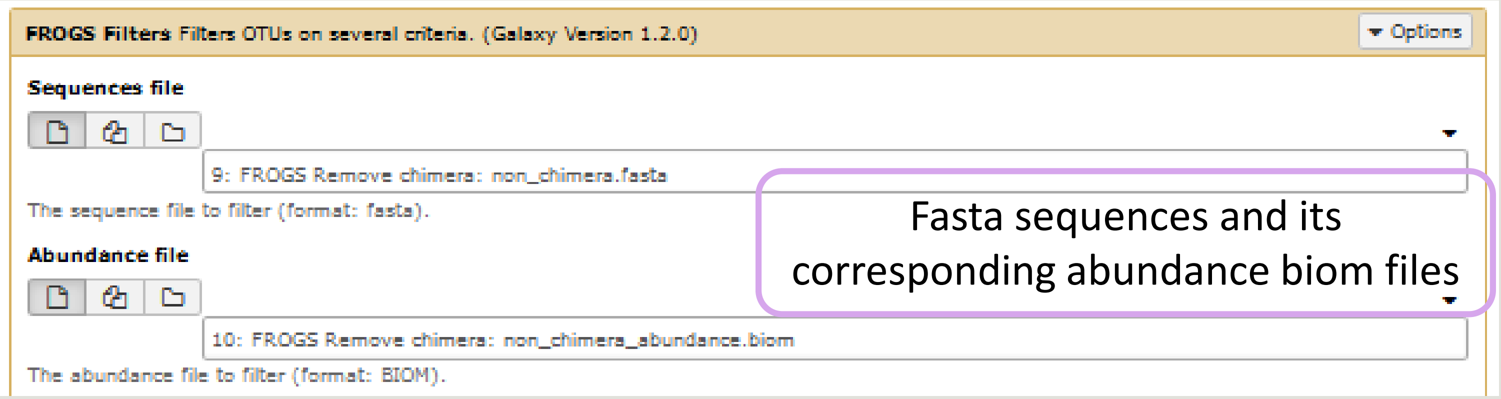
RDP affiliation filters

BLAST affiliation filters

Contamination filter

4 filter sections

Input



FROGS Filters Filters OTUs on several criteria. (Galaxy Version 1.2.0)

Sequences file

9: FROGS Remove chimera: non_chimera.fasta

The sequence file to filter (format: fasta).

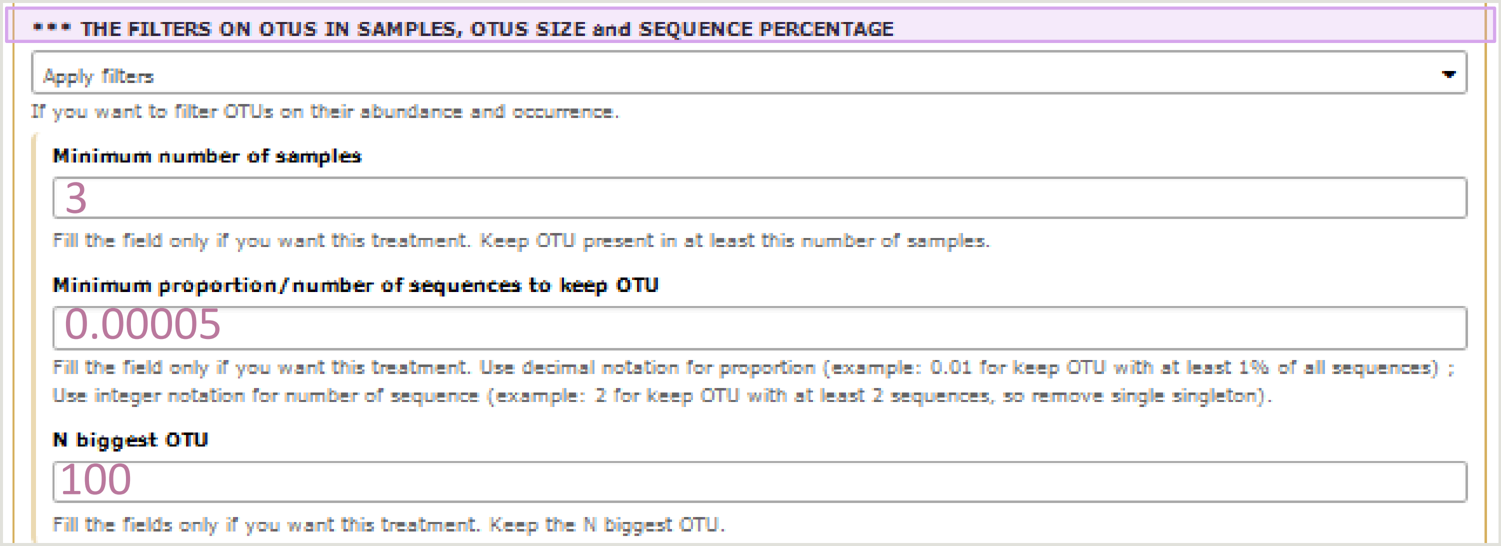
Abundance file

10: FROGS Remove chimera: non_chimera_abundance.biom

The abundance file to filter (format: BIOM).

Fasta sequences and its corresponding abundance biom files

Filter 1 : abundance



***** THE FILTERS ON OTUS IN SAMPLES, OTUS SIZE and SEQUENCE PERCENTAGE**

Apply filters

If you want to filter OTUs on their abundance and occurrence.

Minimum number of samples

3

Fill the field only if you want this treatment. Keep OTU present in at least this number of samples.

Minimum proportion/number of sequences to keep OTU

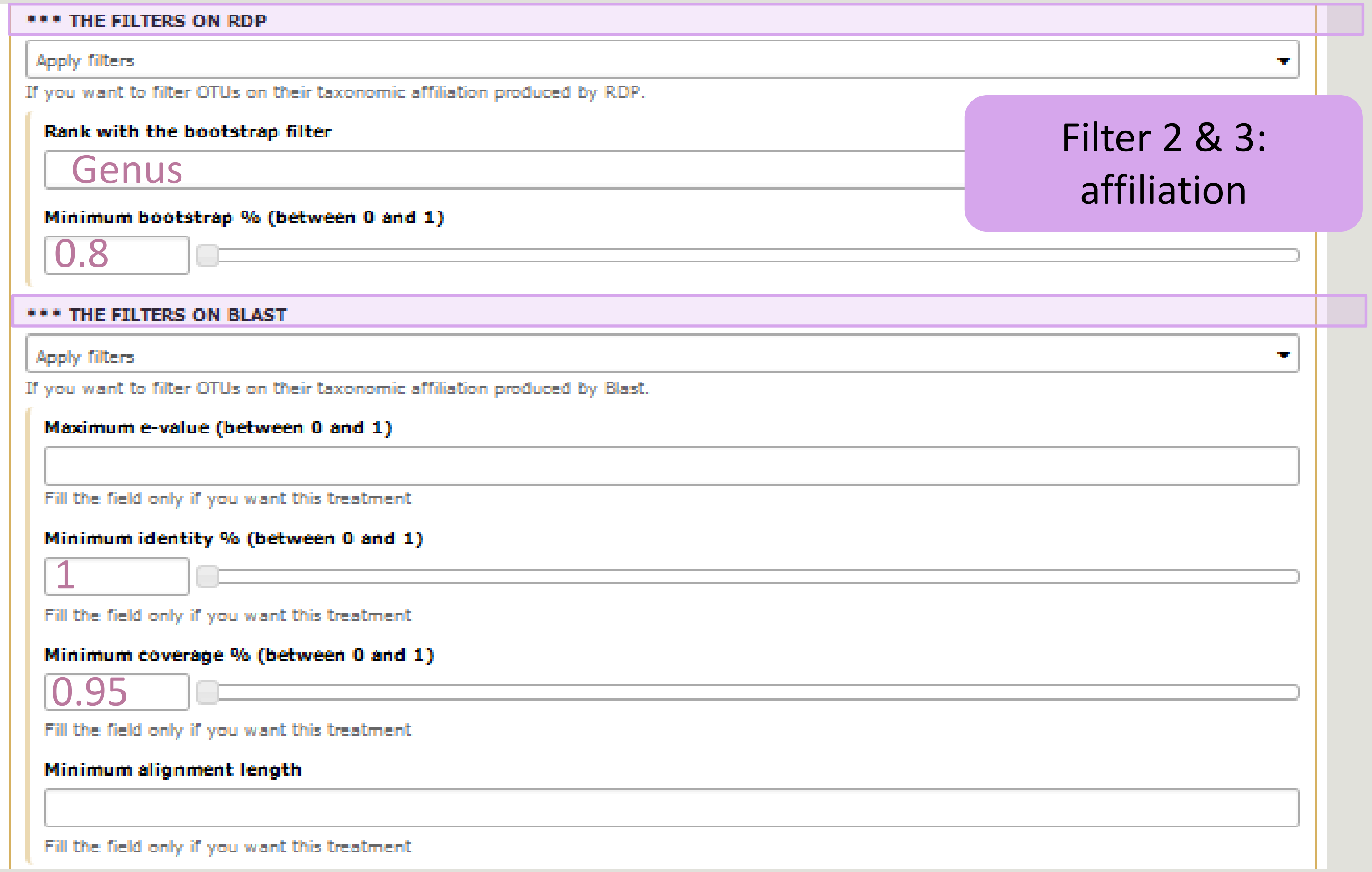
0.00005

Fill the field only if you want this treatment. Use decimal notation for proportion (example: 0.01 for keep OTU with at least 1% of all sequences) ; Use integer notation for number of sequence (example: 2 for keep OTU with at least 2 sequences, so remove single singleton).

N biggest OTU

100

Fill the fields only if you want this treatment. Keep the N biggest OTU.



Filter 2 & 3: affiliation

*** THE FILTERS ON RDP

Apply filters

If you want to filter OTUs on their taxonomic affiliation produced by RDP.

Rank with the bootstrap filter: Genus

Minimum bootstrap % (between 0 and 1): 0.8

*** THE FILTERS ON BLAST

Apply filters

If you want to filter OTUs on their taxonomic affiliation produced by Blast.

Maximum e-value (between 0 and 1)

Fill the field only if you want this treatment

Minimum identity % (between 0 and 1): 1

Fill the field only if you want this treatment

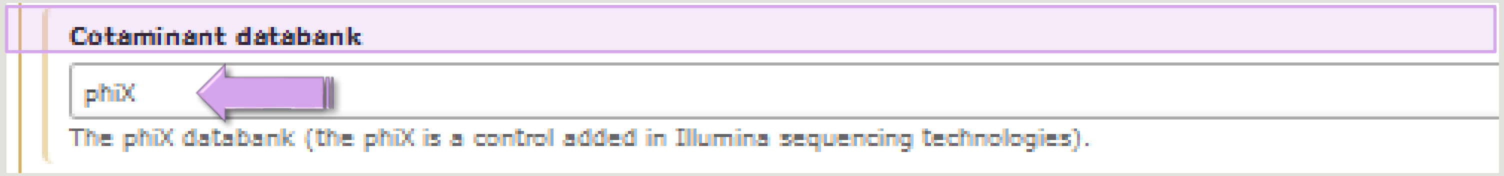
Minimum coverage % (between 0 and 1): 0.95

Fill the field only if you want this treatment

Minimum alignment length

Fill the field only if you want this treatment

Filter 4 : contamination



Cotaminant databank

phiX

The phiX databank (the phiX is a control added in Illumina sequencing technologies).

Soon, several contaminant banks

Your Turn! - 6

LAUNCH DE LA TOOL FILTERS

MiSeq
contiged

Exercise 6

Go to history « MiSeq contiged »

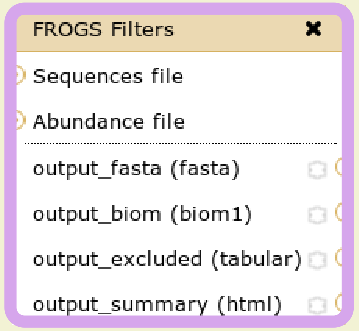
Launch « Filters » tool with non_chimera_abundanced1d3.biom, non_chimerad1d3.fasta

Apply 2 filters :

- Minimum proportion/number of sequences to keep OTU: 0.00005*
- Minimum number of samples: 3

→ objective : play with filters, understand their impacts on falses-positives OTUs

**Nat Methods. 2013 Jan;10(1):57-9. doi: 10.1038/nmeth.2276. Epub 2012 Dec 2.*
Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing.
Bokulich NA1, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG.



FROGS Filters

Sequences file

Abundance file

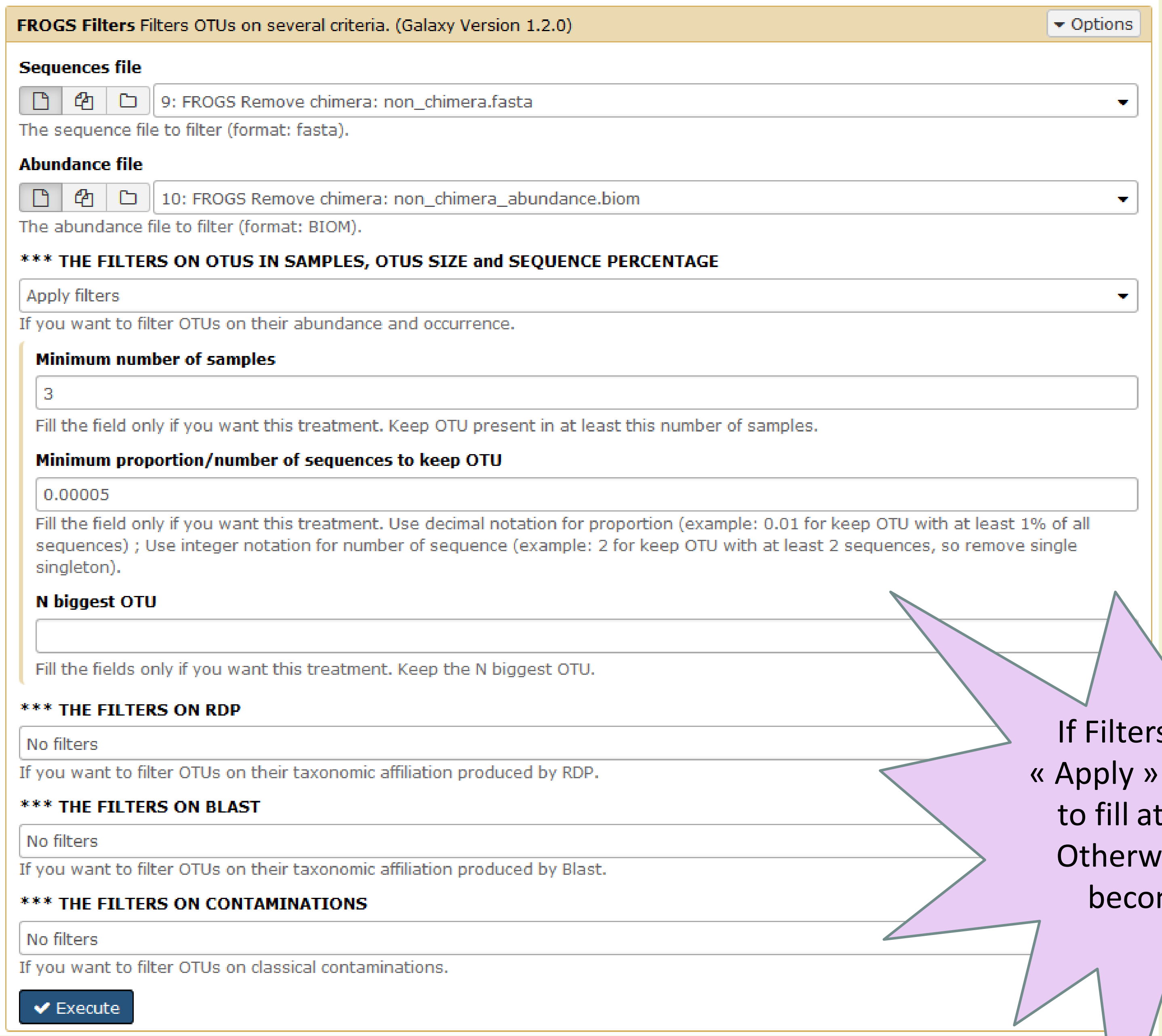
output_fasta (fasta)

output_biom (biom1)

output_excluded (tabular)

output_summary (html)

Filters



FROGS Filters Filters OTUs on several criteria. (Galaxy Version 1.2.0)

Sequences file

9: FROGS Remove chimera: non_chimera.fasta

The sequence file to filter (format: fasta).

Abundance file

10: FROGS Remove chimera: non_chimera_abundance.biom

The abundance file to filter (format: BIOM).

***** THE FILTERS ON OTUS IN SAMPLES, OTUS SIZE and SEQUENCE PERCENTAGE**

Apply filters

If you want to filter OTUs on their abundance and occurrence.

Minimum number of samples

3

Fill the field only if you want this treatment. Keep OTU present in at least this number of samples.

Minimum proportion/number of sequences to keep OTU

0.00005

Fill the field only if you want this treatment. Use decimal notation for proportion (example: 0.01 for keep OTU with at least 1% of all sequences) ; Use integer notation for number of sequence (example: 2 for keep OTU with at least 2 sequences, so remove single singleton).

N biggest OTU

Fill the fields only if you want this treatment. Keep the N biggest OTU.

***** THE FILTERS ON RDP**

No filters

If you want to filter OTUs on their taxonomic affiliation produced by RDP.

***** THE FILTERS ON BLAST**

No filters

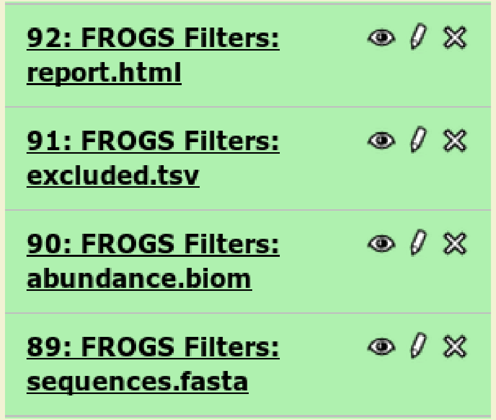
If you want to filter OTUs on their taxonomic affiliation produced by Blast.

***** THE FILTERS ON CONTAMINATIONS**

No filters

If you want to filter OTUs on classical contaminations.

Output



92: FROGS Filters: report.html

91: FROGS Filters: excluded.tsv

90: FROGS Filters: abundance.biom

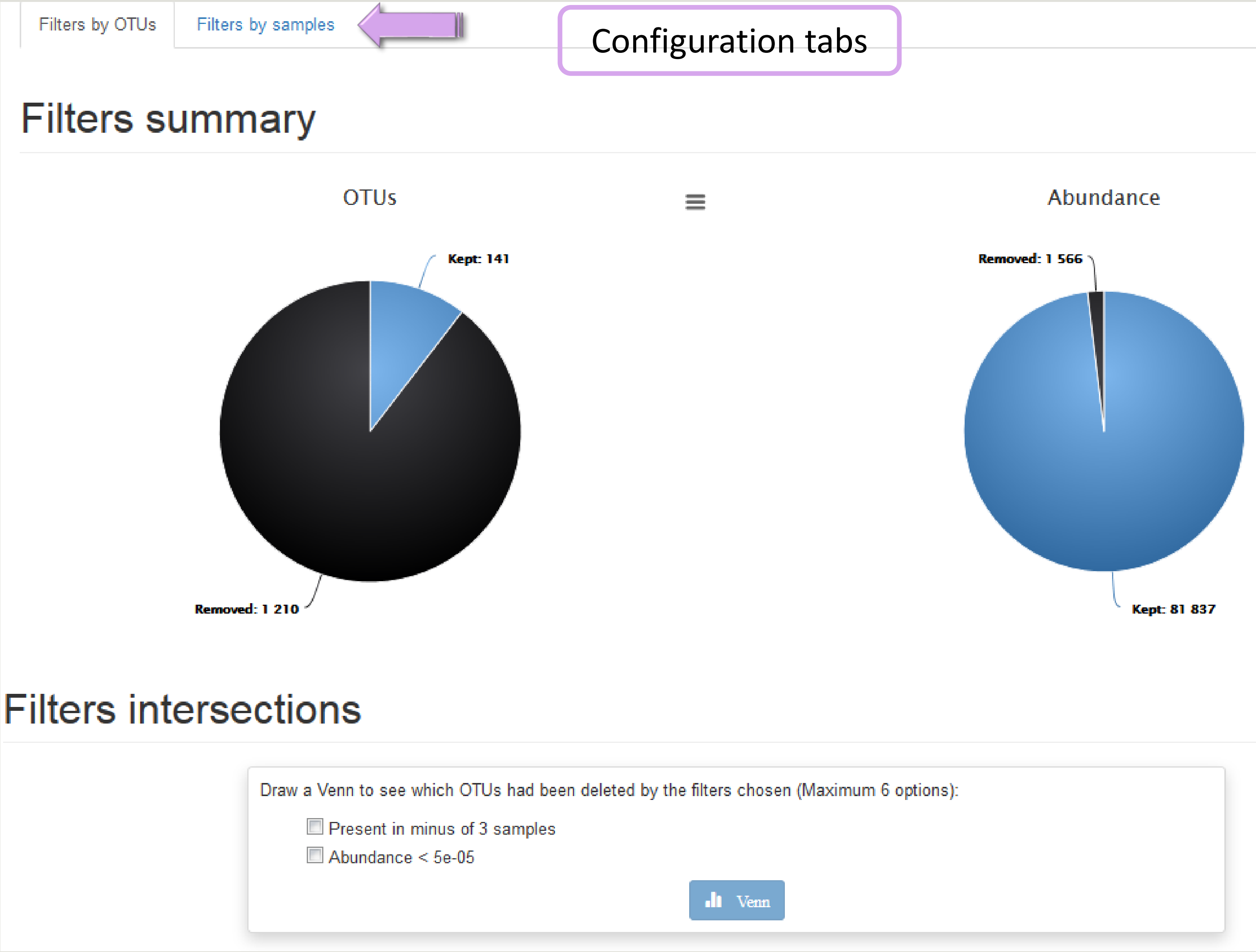
89: FROGS Filters: sequences.fasta

If Filters fields are « Apply » so you have to fill at one field. Otherwise, galaxy become red !

MiSeq
contiged

Exercise 6

1. What are the output files of “Filters” ?
2. Explore “FROGS Filter : report.html” file.
3. How many OTUs have you removed ?
4. Build the Venn diagram on the two filters.
5. How many OTUs have you removed with each filter “abundance > 0.005% ”, “Remove OTUs that are not present at least in 3 samples”?
6. How many OTUs do they remain ?
7. Is there a sample more impacted than the others ?
8. To characterize these new OTUs, do not forget to launch “FROGS Cluster Stat” tool, and rename the output HTML file.


Filters by OTUs
Filters by samples
OTUs
Kept: 141
Removed: 1 210
Abundance
Removed: 1 566
Kept: 81 837

Configuration tabs

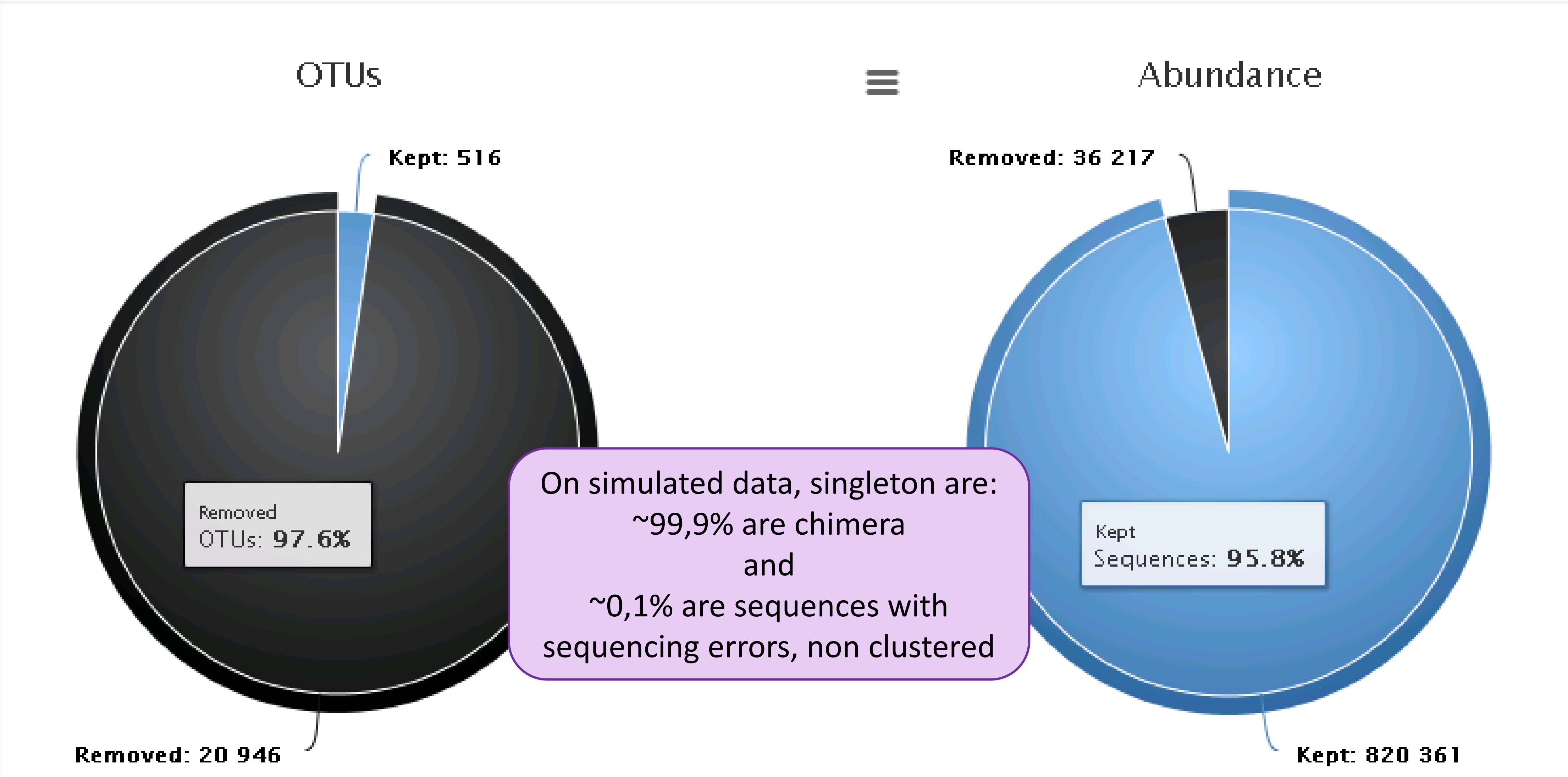
Filters summary

Filters intersections

Draw a Venn to see which OTUs had been deleted by the filters chosen (Maximum 6 options):

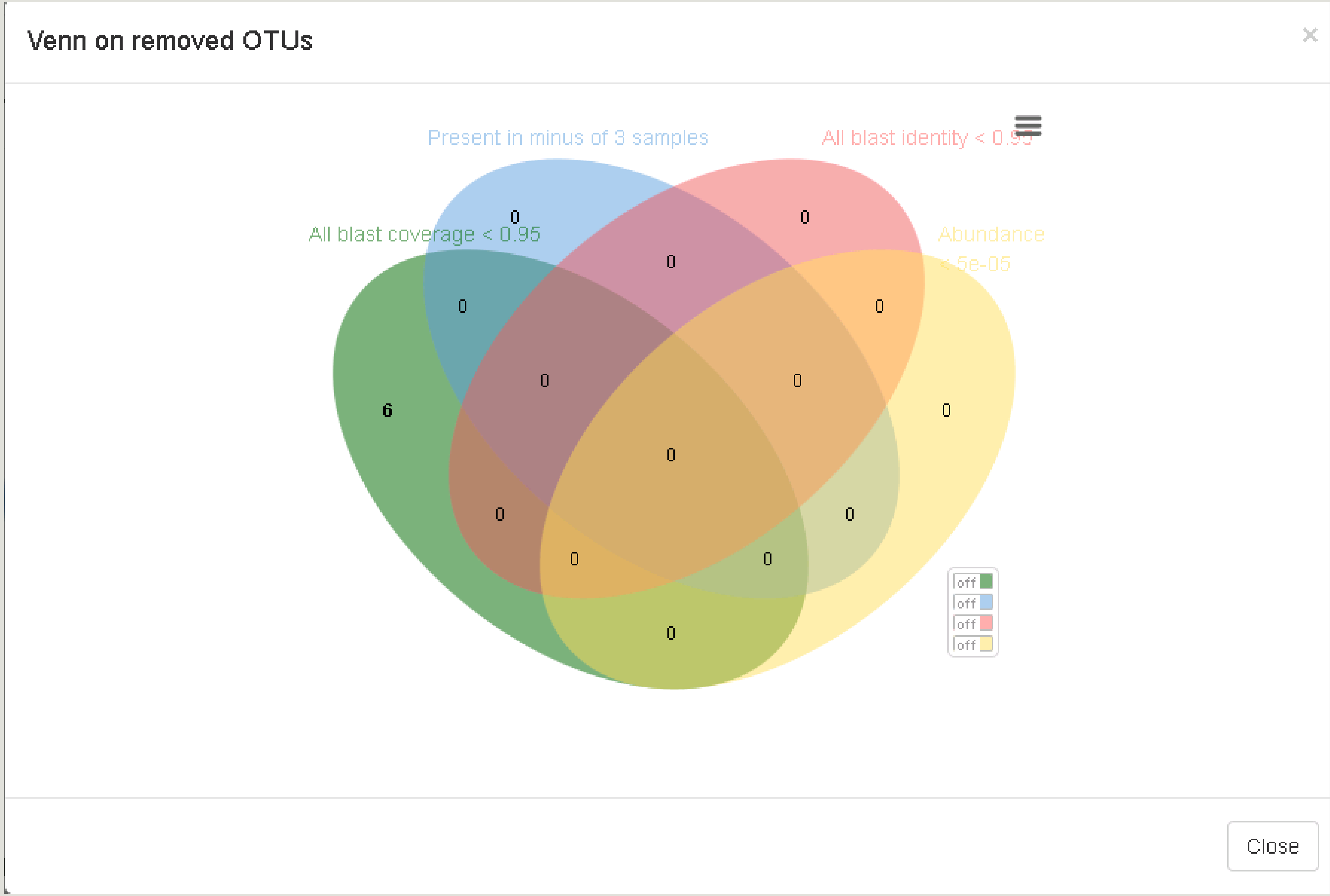
☐ Present in minus of 3 samples
☐ Abundance < 5e-05

Venn



On simulated data, singleton are:
~99,9% are chimera
and
~0,1% are sequences with
sequencing errors, non clustered

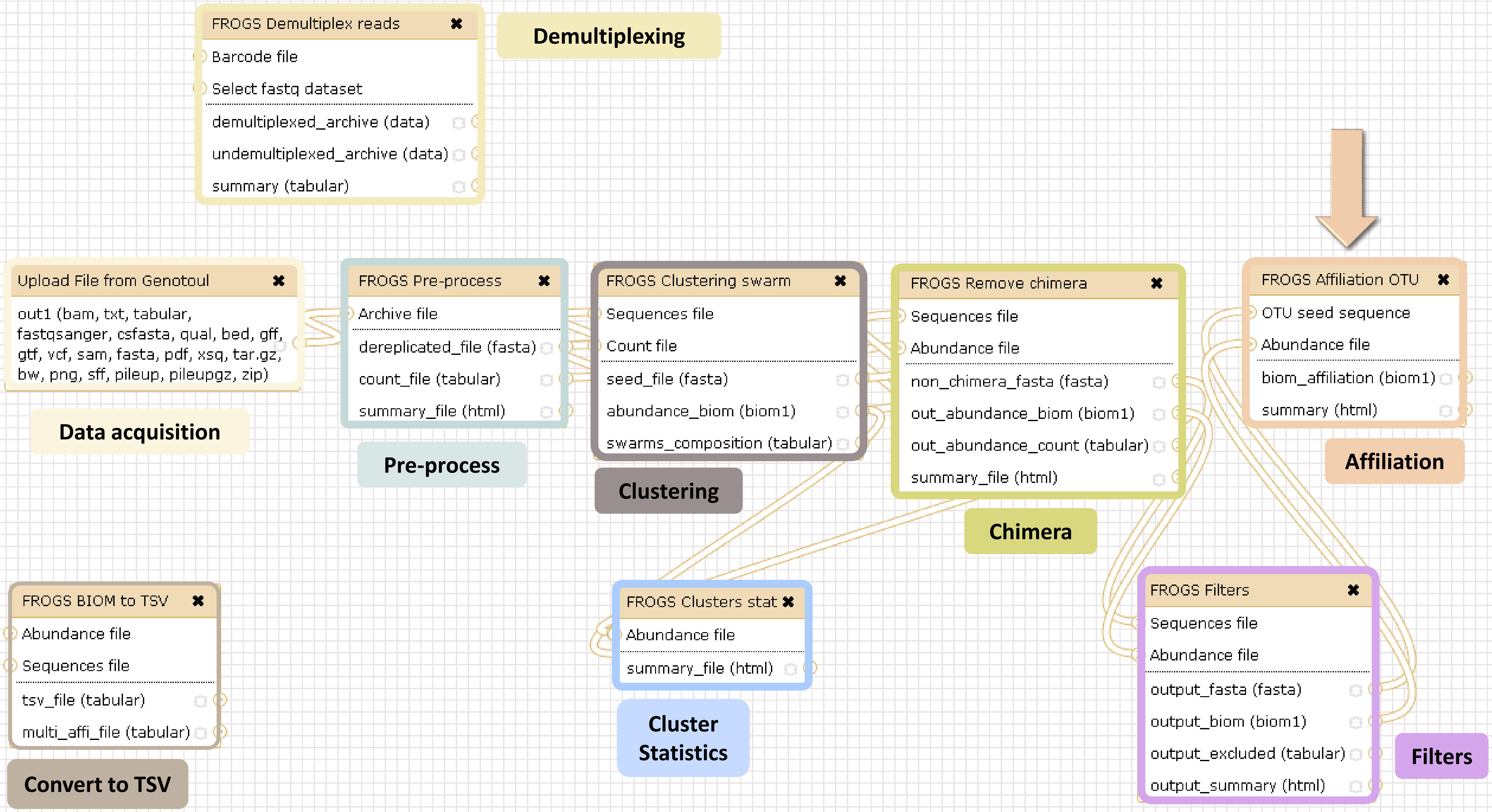
Removing little OTUs (conservation rate =0.005%)
and non shared OTU (in less than 2 samples)



Venn on removed OTUs

×
Present in minus of 3 samples
All blast identity < 0.95
All blast coverage < 0.95
Abundance
< 5e-05
0
0
0
0
0
0
6
0
0
0
0
0
0
0
0
off
off
off
off
Close

Affiliation tool



FROGS Demultiplex reads ✖
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)

Demultiplexing

Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

Pre-process

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

Clustering

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

Chimera

FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation

FROGS BIOM to TSV ✖
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)

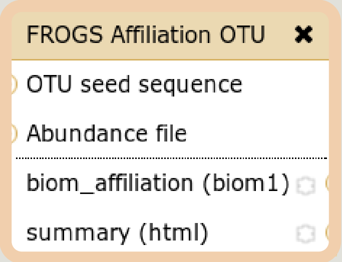
Convert to TSV

FROGS Clusters stat ✖
Abundance file
summary_file (html)

Cluster Statistics

FROGS Filters ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

Filters



FROGS Affiliation OTU ✖

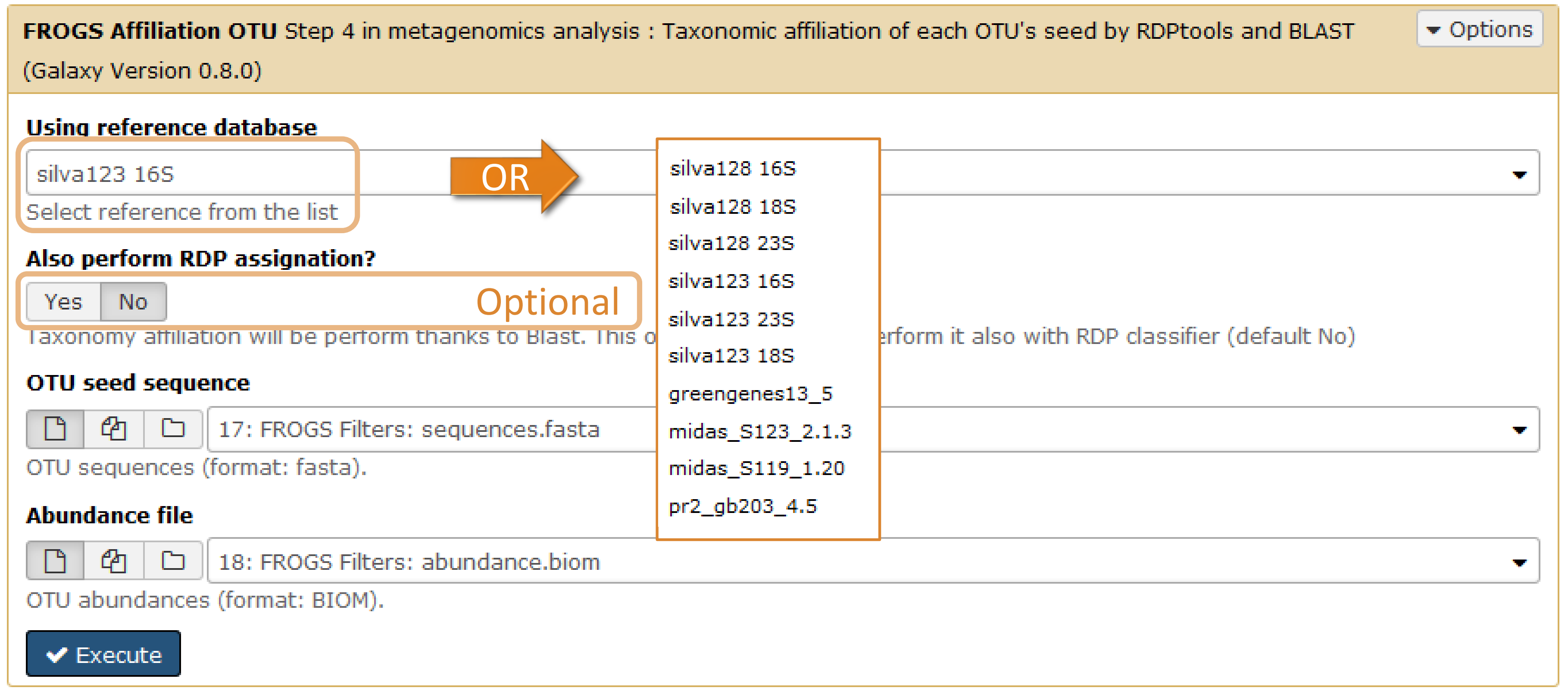
OTU seed sequence

Abundance file

biom_affiliation (biom1)

summary (html)

Affiliation



FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST (Galaxy Version 0.8.0)

Using reference database

silva123 16S

Select reference from the list

- silva128 16S
- silva128 18S
- silva128 23S
- silva123 16S
- silva123 23S
- silva123 18S
- greengenes13_5
- midas_S123_2.1.3
- midas_S119_1.20
- pr2_gb203_4.5

Also perform RDP assignation?

Yes [✓ No]

Taxonomy affiliation will be perform thanks to Blast. This o erform it also with RDP classifier (default No)

OTU seed sequence

17: FROGS Filters: sequences.fasta

OTU sequences (format: fasta).

Abundance file

18: FROGS Filters: abundance.biom

OTU abundances (format: BIOM).

1 Cluster = 2 affiliations

Double Affiliation vs SILVA 123 (for 16S, 18S or 23S), SILVA 119 (for 18S) or Greengenes **with :**

1. RDPClassifier* (Ribosomal Database Project): one affiliation with bootstrap, on each taxonomic subdivision.

Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);Lachnospiraceae(100);Pseudobutyrivibrio(80); Pseudobutyrivibrio xylanivorans (80)

2. NCBI Blastn+** : all identical Best Hits with identity %, coverage %, e-value, alignment length and a special tag “**Multi-affiliation”.**

Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Pseudobutyrivibrio;Pseudobutyrivibrio ruminis; Pseudobutyrivibrio xylanivorans

Identity: 100% and Coverage: 100%

* Appl. Environ. Microbiol. August 2007 vol. 73 no. 16 5261-5267. doi : 10.1128/AEM.00062-07
Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy.
Qiong Wang, George M.Garrity, James M. Tiedje and James R. Cole

** BMC Bioinformatics 2009, 10:421. doi:10.1186/1471-2105-10-421
BLAST+: architecture and applications
Christiam Camacho, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos,Kevin Bealer and Thomas L Madden

Affiliation Strategy of FROGS

Blastn+ with “**Multi-affiliation**” management

V3 – V4	Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S unknown species
V3 – V4	Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S Butyrivibrio fibrisolvens
V3 – V4	Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S rumen bacterium 8|9293-9
V3 – V4	Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S Pseudobutyrivibrio xylanivorans
V3 – V4	Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S Pseudobutyrivibrio ruminis

5 identical blast best hits on SILVA 123 databank

Affiliation Strategy of FROGS

Blastn+ with “**Multi-affiliation**” management

V3 – V4 Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S unknown species

V3 – V4 Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S Butyrivibrio fibrisolvens

V3 – V4 Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S rumen bacterium 8|9293-9

V3 – V4 Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S Pseudobutyrivibrio xylanivorans

V3 – V4 Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|16S Pseudobutyrivibrio ruminis

⇩

FROGS Affiliation: Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Pseudobutyrivibrio|**Multi-affiliation**

Your Turn! – 7

LAUNCH THE « FROGS AFFILIATION » TOOL

MiSeq contiged

Exercise 7.1

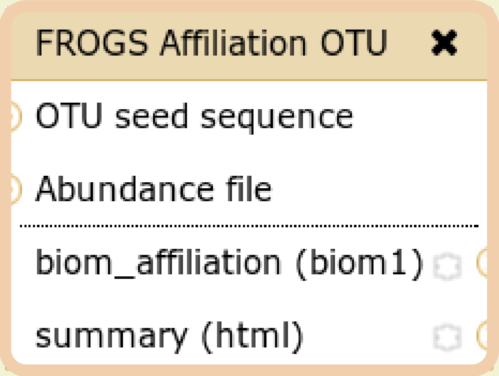
Go to « MiSeq contiged » history

Launch the « FROGS Affiliation » tool with

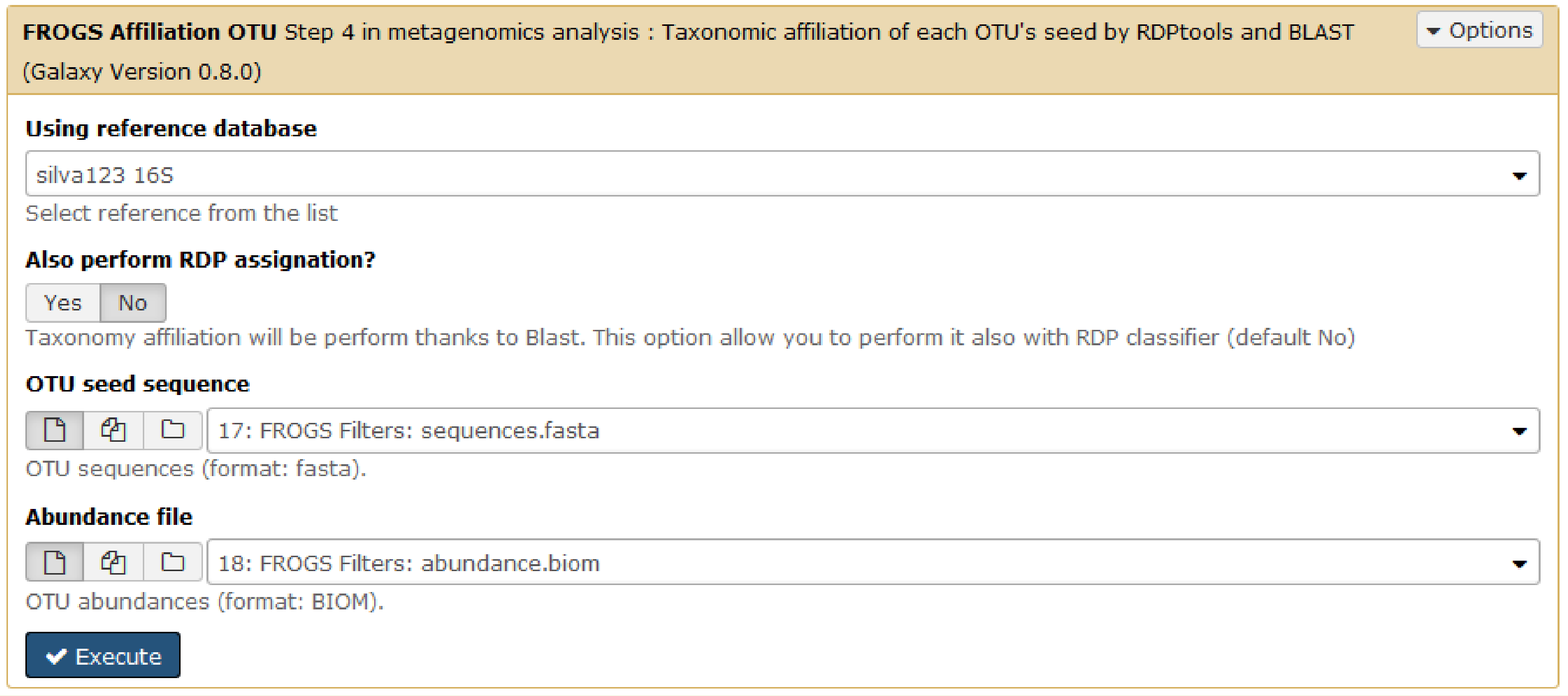
- SILVA 123 or 128 16S database
- FROGS Filters abundance biom and fasta files (after swarm d1d3, remove chimera and filter low abundances)

→ objectives :

  - understand abundance tables columns
  - understand the BLAST affiliation


FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation



FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST (Galaxy Version 0.8.0)

Using reference database

silva123 16S

Select reference from the list

Also perform RDP assignation?

Yes [✓ No]

Taxonomy affiliation will be perform thanks to Blast. This option allow you to perform it also with RDP classifier (default No)

OTU seed sequence

17: FROGS Filters: sequences.fasta

OTU sequences (format: fasta).

Abundance file

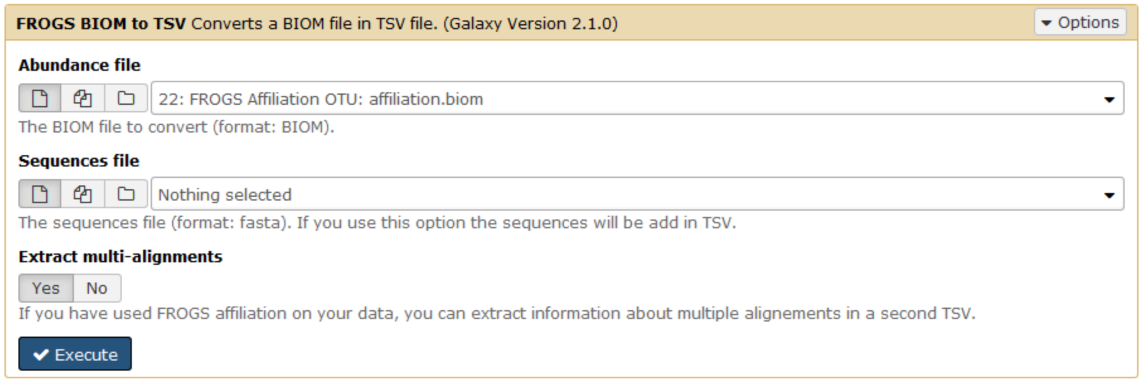
18: FROGS Filters: abundance.biom

OTU abundances (format: BIOM).

MiSeq contiged

Exercise 7.1

1. What are the « FROGS Affiliation » output files ?
2. How many sequences are affiliated by BLAST ?
3. Click on the « eye » button on the BIOM output file, what do you understand ? 👁
4. Use the Biom_to_TSV tool on this last file and click again on the ”eye” on the new output generated. 👁
   What do the columns ?
   What is the difference if we click on case or not ? What consequence about weight of your file ?



FROGS BIOM to TSV Converts a BIOM file in TSV file. (Galaxy Version 2.1.0)

Abundance file

22: FROGS Affiliation OTU: affiliation.biom

The BIOM file to convert (format: BIOM).

Sequences file

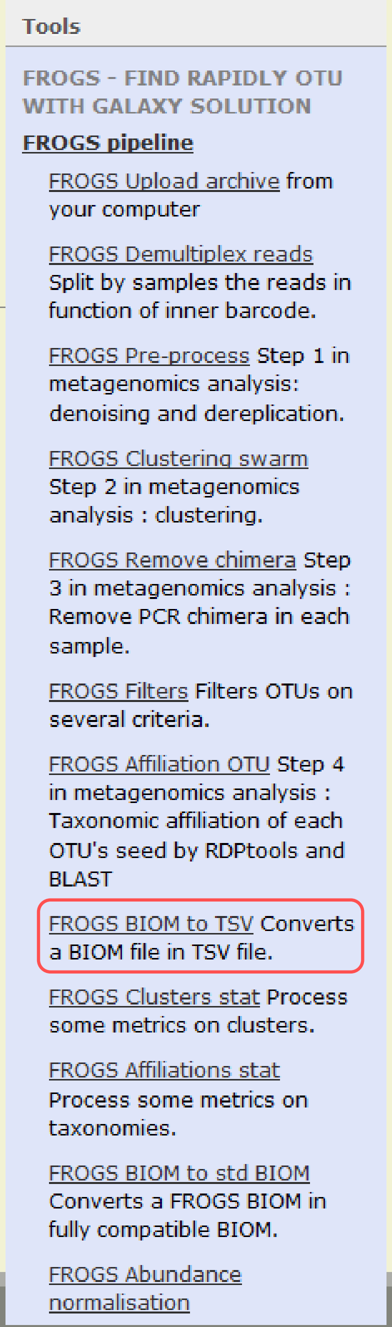
Nothing selected

The sequences file (format: fasta). If you use this option the sequences will be add in TSV.

Extract multi-alignments

Yes No

If you have used FROGS affiliation on your data, you can extract information about multiple alignements in a second TSV.



Tools

FROGS - FIND RAPIDLY OTU WITH GALAXY SOLUTION

FROGS pipeline

- FROGS Upload archive from your computer
- FROGS Demultiplex reads Split by samples the reads in function of inner barcode.
- FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication.
- FROGS Clustering swarm Step 2 in metagenomics analysis : clustering.
- FROGS Remove chimera Step 3 in metagenomics analysis : Remove PCR chimera in each sample.
- FROGS Filters Filters OTUs on several criteria.
- FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST
- FROGS BIOM to TSV Converts a BIOM file in TSV file.
- FROGS Clusters stat Process some metrics on clusters.
- FROGS Affiliations stat Process some metrics on taxonomies.
- FROGS BIOM to std BIOM Converts a FROGS BIOM in fully compatible BIOM.
- FROGS Abundance normalisation

MiSeq contiged

Exercise 7.1

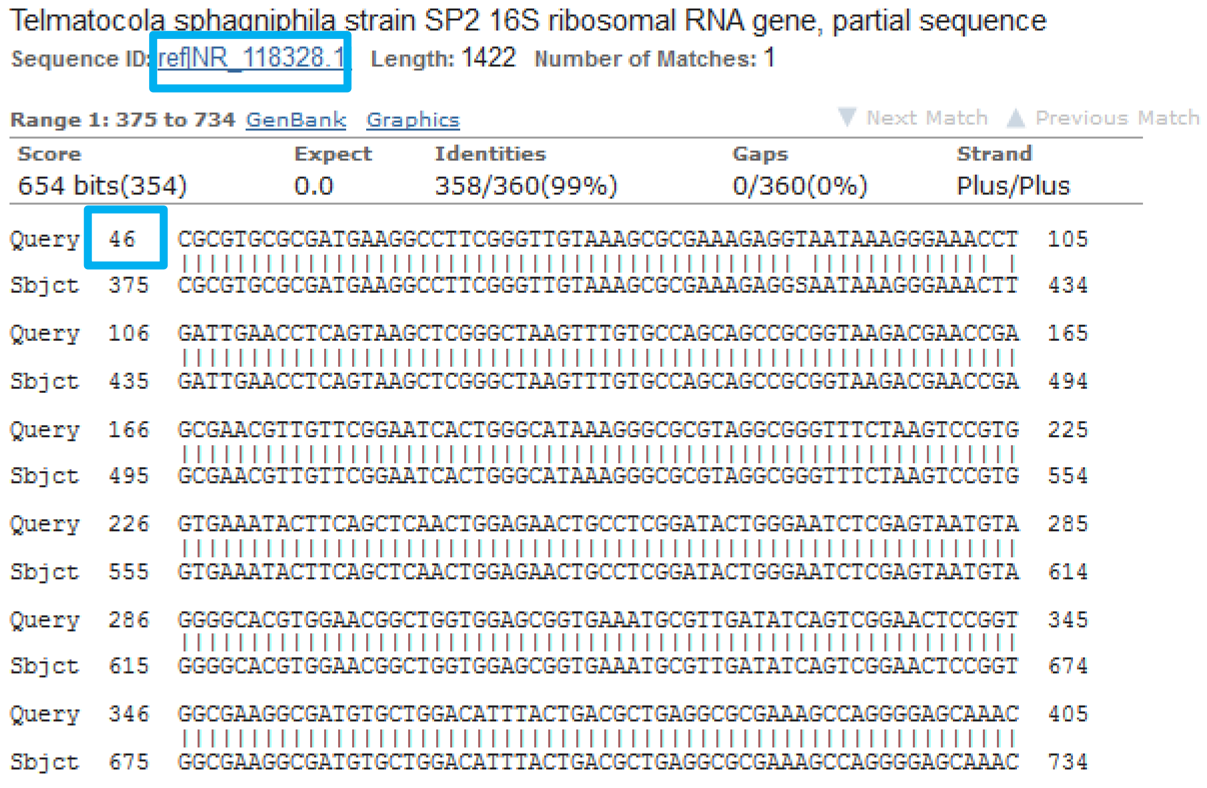
5. Understand Blast affiliations - Cluster_2388 (affiliation from silva 123)

blast_subject	blast_evalue	blast_len	blast_perc_query_coverage	blast_perc_identity	blast_taxonomy
JN880417.1.1422	0.0	360	88.88	99.44	Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Telmatocola;Telmatocola sphagniphila

Blast JN880417.1.1422 vs our OTU

OTU length : 405

Excellent blast but no matches at the beginning of OTU.



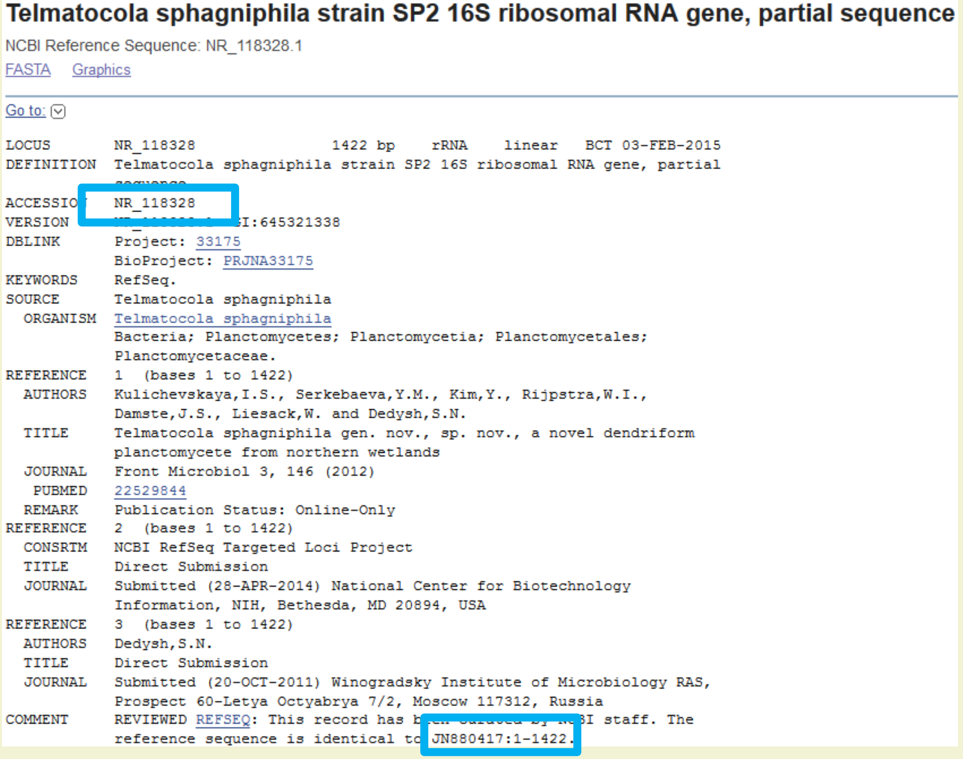
Telmatocola sphagniphila strain SP2 16S ribosomal RNA gene, partial sequence

Sequence ID: ref|NR_118328.1 **Length:** 1422 **Number of Matches:** 1

Range 1: 375 to 734 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
654 bits(354)	0.0	358/360(99%)	0/360(0%)	Plus/Plus

```
Query  46   CGCGTGCGCGATGAAGGCCTTCGGGTTGTAAAGCGCGAAAGAGGTAATAAAGGGAAACCT  105
            |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||| |
Sbjct  375  CGCGTGCGCGATGAAGGCCTTCGGGTTGTAAAGCGCGAAAGAGGSAATAAAGGGAAACTT  434

Query  106  GATTGAACCTCAGTAAGCTCGGGCTAAGTTTGTGCCAGCAGCCGCGGTAAGACGAACCGA  165
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435  GATTGAACCTCAGTAAGCTCGGGCTAAGTTTGTGCCAGCAGCCGCGGTAAGACGAACCGA  494

Query  166  GCGAACGTTGTTCGGAATCACTGGGCATAAAGGGCGCGTAGGCGGGTTTCTAAGTCCGTG  225
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  495  GCGAACGTTGTTCGGAATCACTGGGCATAAAGGGCGCGTAGGCGGGTTTCTAAGTCCGTG  554

Query  226  GTGAAATACTTCAGCTCAACTGGAGAACTGCCTCGGATACTGGGAATCTCGAGTAATGTA  285
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  555  GTGAAATACTTCAGCTCAACTGGAGAACTGCCTCGGATACTGGGAATCTCGAGTAATGTA  614

Query  286  GGGGCACGTGGAACGGCTGGTGGAGCGGTGAAATGCGTTGATATCAGTCGGAACTCCGGT  345
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  615  GGGGCACGTGGAACGGCTGGTGGAGCGGTGAAATGCGTTGATATCAGTCGGAACTCCGGT  674

Query  346  GGCGAAGGCGATGTGCTGGACATTTACTGACGCTGAGGCGCGAAAGCCAGGGGAGCAAAC  405
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  675  GGCGAAGGCGATGTGCTGGACATTTACTGACGCTGAGGCGCGAAAGCCAGGGGAGCAAAC  734
```



Telmatocola sphagniphila strain SP2 16S ribosomal RNA gene, partial sequence

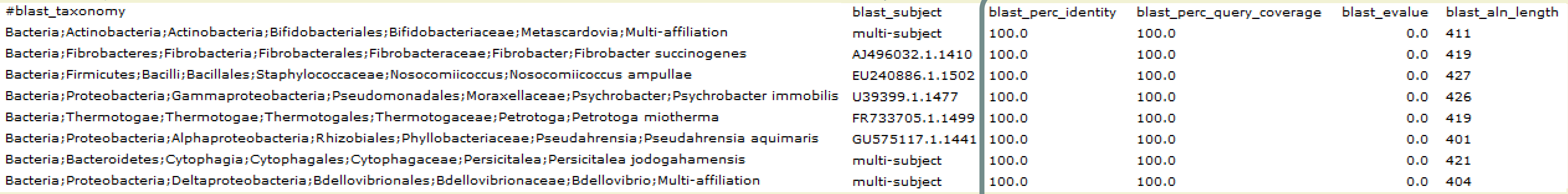
NCBI Reference Sequence: NR_118328.1

```
LOCUS       NR_118328               1422 bp    rRNA    linear   BCT 03-FEB-2015
DEFINITION  Telmatocola sphagniphila strain SP2 16S ribosomal RNA gene, partial
ACCESSIO    NR_118328
VERSION                 I:645321338
DBLINK      Project: 33175
            BioProject: PRJNA33175
KEYWORDS    RefSeq.
SOURCE      Telmatocola sphagniphila
  ORGANISM  Telmatocola sphagniphila
            Bacteria; Planctomycetes; Planctomycetia; Planctomycetales;
            Planctomycetaceae.
REFERENCE   1  (bases 1 to 1422)
  AUTHORS   Kulichevskaya,I.S., Serkebaeva,Y.M., Kim,Y., Rijpstra,W.I.,
            Damste,J.S., Liesack,W. and Dedysh,S.N.
  TITLE     Telmatocola sphagniphila gen. nov., sp. nov., a novel dendriform
            planctomycete from northern wetlands
  JOURNAL   Front Microbiol 3, 146 (2012)
   PUBMED   22529844
  REMARK    Publication Status: Online-Only
REFERENCE   2  (bases 1 to 1422)
  CONSRTM   NCBI RefSeq Targeted Loci Project
  TITLE     Direct Submission
  JOURNAL   Submitted (28-APR-2014) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   3  (bases 1 to 1422)
  AUTHORS   Dedysh,S.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-OCT-2011) Winogradsky Institute of Microbiology RAS,
            Prospect 60-Letya Octyabrya 7/2, Moscow 117312, Russia
COMMENT     REVIEWED REFSEQ: This record has b               I staff. The
            reference sequence is identical to JN880417:1-1422.
```

Blast columns

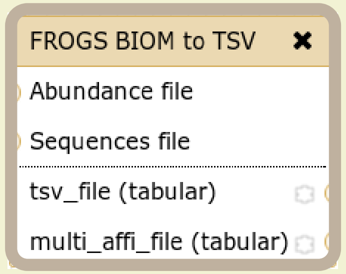
OTU_2 seed has a best BLAST hit with the reference sequence AJ496032.1.1410

The reference sequence taxonomic affiliation is this one.



#blast_taxonomy	blast_subject	blast_perc_identity	blast_perc_query_coverage	blast_evalue	blast_aln_length
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Metascardovia;Multi-affiliation	multi-subject	100.0	100.0	0.0	411
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter;Fibrobacter succinogenes	AJ496032.1.1410	100.0	100.0	0.0	419
Bacteria;Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Nosocomiicoccus;Nosocomiicoccus ampullae	EU240886.1.1502	100.0	100.0	0.0	427
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;Psychrobacter immobilis	U39399.1.1477	100.0	100.0	0.0	426
Bacteria;Thermotogae;Thermotogae;Thermotogales;Thermotogaceae;Petrotoga;Petrotoga miotherma	FR733705.1.1499	100.0	100.0	0.0	419
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia;Pseudahrensia aquimaris	GU575117.1.1441	100.0	100.0	0.0	401
Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;Persicitalea;Persicitalea jodogahamensis	multi-subject	100.0	100.0	0.0	421
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio;Multi-affiliation	multi-subject	100.0	100.0	0.0	404

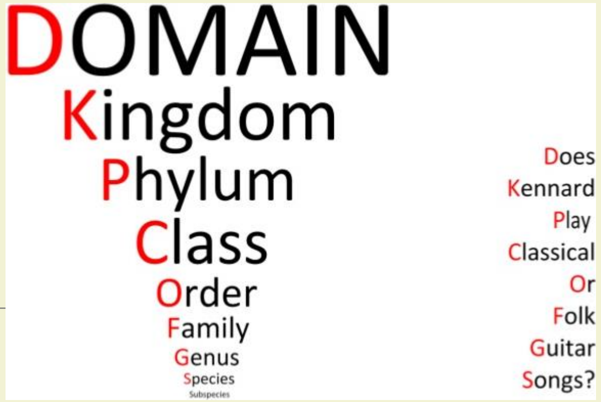
Convert to TSV



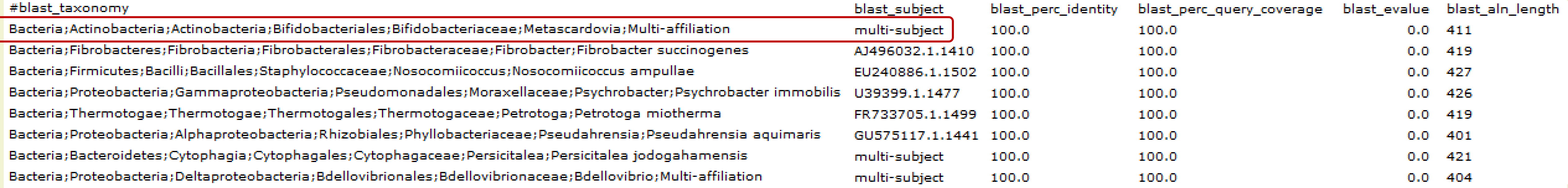
Evaluation variables of BLAST

Focus on “Multi-”

(affiliation from silva 123)



Observe line of Cluster 1 inside abundance.tsv and multi_hit.tsv files, what do you conclude ?



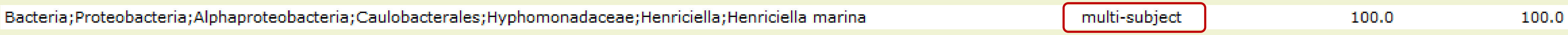
#blast_taxonomy	blast_subject	blast_perc_identity	blast_perc_query_coverage	blast_evalue	blast_aln_length
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Metascardovia;Multi-affiliation	multi-subject	100.0	100.0	0.0	411
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter;Fibrobacter succinogenes	AJ496032.1.1410	100.0	100.0	0.0	419
Bacteria;Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Nosocomiicoccus;Nosocomiicoccus ampullae	EU240886.1.1502	100.0	100.0	0.0	427
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;Psychrobacter immobilis	U39399.1.1477	100.0	100.0	0.0	426
Bacteria;Thermotogae;Thermotogae;Thermotogales;Thermotogaceae;Petrotoga;Petrotoga miotherma	FR733705.1.1499	100.0	100.0	0.0	419
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia;Pseudahrensia aquimaris	GU575117.1.1441	100.0	100.0	0.0	401
Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;Persicitalea;Persicitalea jodogahamensis	multi-subject	100.0	100.0	0.0	421
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio;Multi-affiliation	multi-subject	100.0	100.0	0.0	404

Cluster_1 has 5 identical blast hits, with different taxonomies as the species level

Focus on "Multi-"

(affiliation from silva 123)

Observe line of Cluster 11 inside abundance.tsv and multi_hit.tsv files, what do you conclude ?



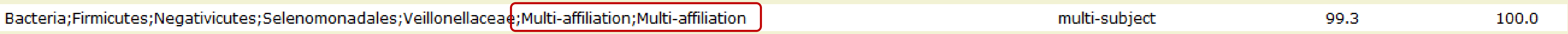
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Henriciella;Henriciella marina	multi-subject	100.0	100.0

Cluster_11 has 2 identical blast hits, with identical species but with different strains (strains are not written in our data)

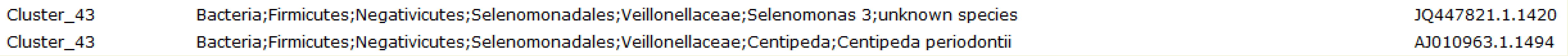
Focus on “Multi-”

(affiliation from silva 123)

Observe line of Cluster 43 inside abundance.tsv and multi_hit.tsv files, what do you conclude ?



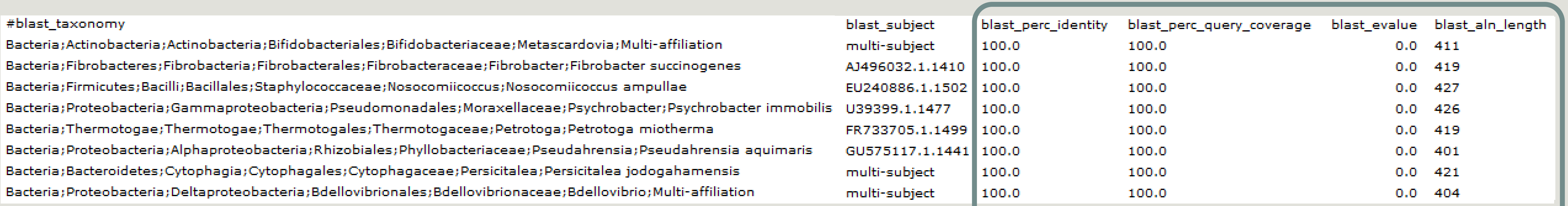
```
Bacteria;Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Multi-affiliation;Multi-affiliation                    multi-subject          99.3          100.0
```



```
Cluster_43        Bacteria;Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Selenomonas 3;unknown species          JQ447821.1.1420
Cluster_43        Bacteria;Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Centipeda;Centipeda periodontii         AJ010963.1.1494
```

Cluster_43 has 2 identical blast hits, with different taxonomies at the genus level

Back on Blast parameters



#blast_taxonomy	blast_subject	blast_perc_identity	blast_perc_query_coverage	blast_evalue	blast_aln_length
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Metascardovia;Multi-affiliation	multi-subject	100.0	100.0	0.0	411
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter;Fibrobacter succinogenes	AJ496032.1.1410	100.0	100.0	0.0	419
Bacteria;Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Nosocomiicoccus;Nosocomiicoccus ampullae	EU240886.1.1502	100.0	100.0	0.0	427
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;Psychrobacter immobilis	U39399.1.1477	100.0	100.0	0.0	426
Bacteria;Thermotogae;Thermotogae;Thermotogales;Thermotogaceae;Petrotoga;Petrotoga miotherma	FR733705.1.1499	100.0	100.0	0.0	419
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia;Pseudahrensia aquimaris	GU575117.1.1441	100.0	100.0	0.0	401
Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;Persicitalea;Persicitalea jodogahamensis	multi-subject	100.0	100.0	0.0	421
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio;Multi-affiliation	multi-subject	100.0	100.0	0.0	404

Evaluation variables of BLAST

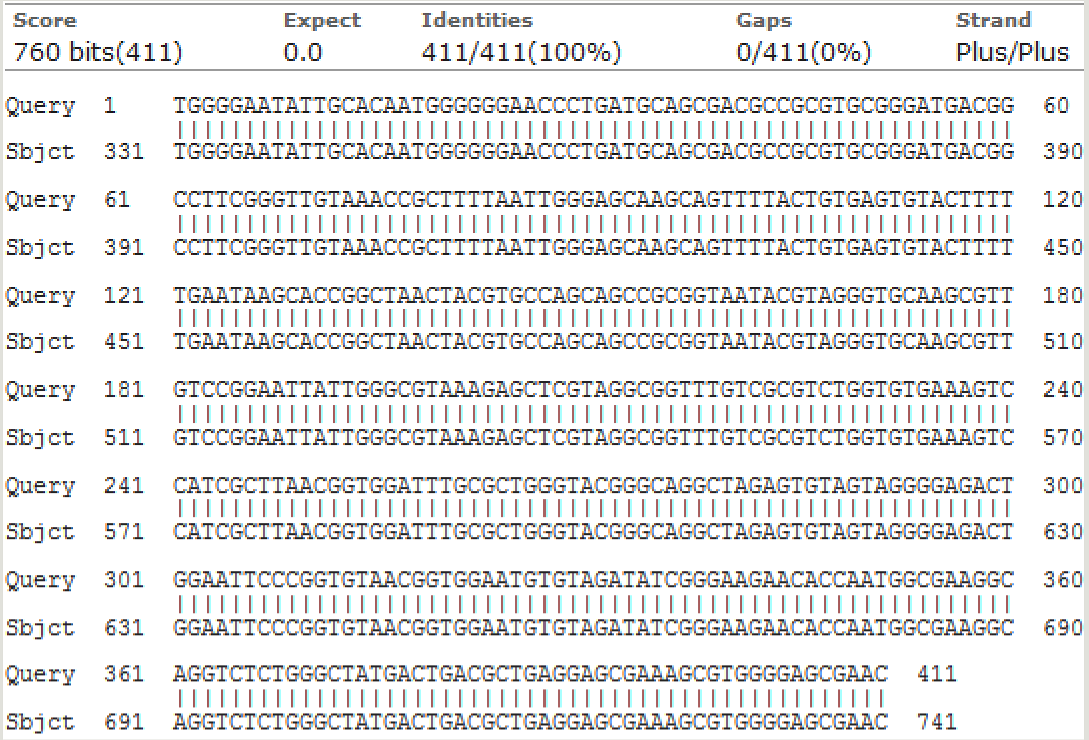
Blast variables : e-value

The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size.

The lower the E-value, or the closer it is to zero, the more "significant" the match is.

Blast variables : blast_perc_identity

Identity percentage between the Query (OTU) and the subject in the alignment (length subject = 1455 bases)

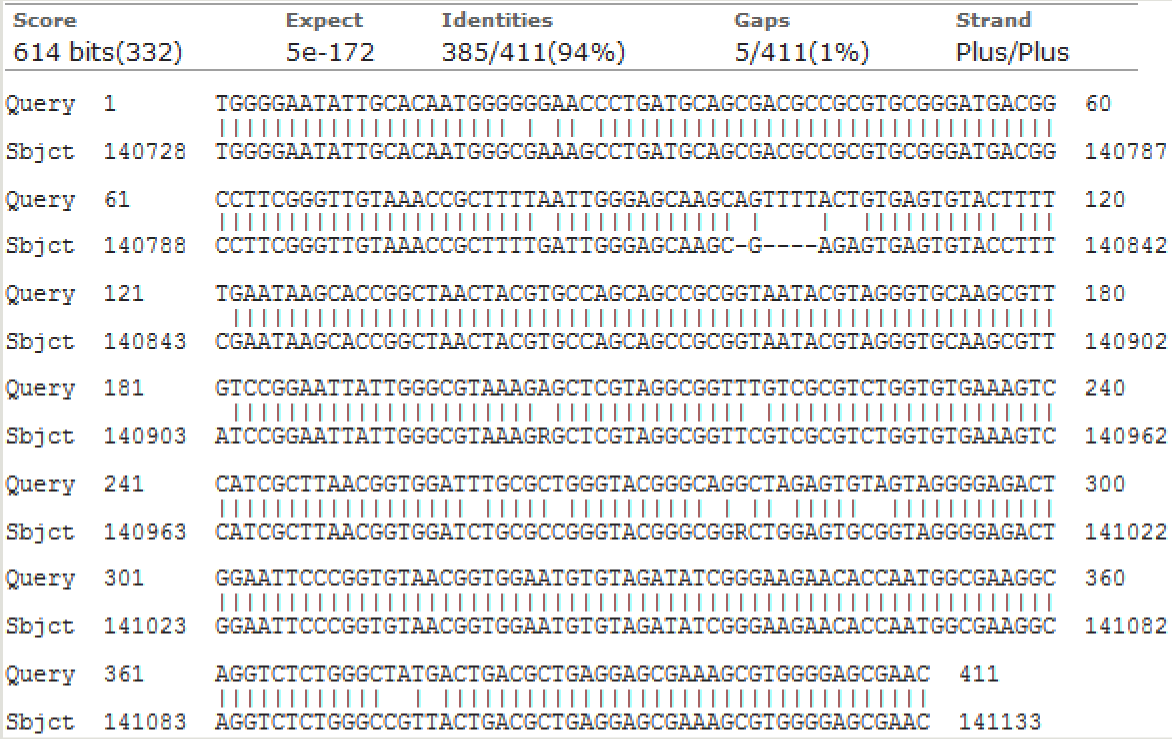


```
Score            Expect   Identities        Gaps          Strand
760 bits(411)    0.0      411/411(100%)     0/411(0%)     Plus/Plus

Query  1    TGGGGAATATTGCACAATGGGGGAACCCTGATGCAGCGACGCCGCGTGCGGGATGACGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  331  TGGGGAATATTGCACAATGGGGGAACCCTGATGCAGCGACGCCGCGTGCGGGATGACGG  390

Query  61   CCTTCGGGTTGTAAACCGCTTTTAATTGGGAGCAAGCAGTTTTACTGTGAGTGTACTTTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  391  CCTTCGGGTTGTAAACCGCTTTTAATTGGGAGCAAGCAGTTTTACTGTGAGTGTACTTTT  450

Query  121  TGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  451  TGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTT  510

Query  181  GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGGTGTGAAAGTC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  511  GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGGTGTGAAAGTC  570

Query  241  CATCGCTTAACGGTGGATTTGCGCTGGGTACGGGCAGGCTAGAGTGTAGTAGGGGAGACT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  571  CATCGCTTAACGGTGGATTTGCGCTGGGTACGGGCAGGCTAGAGTGTAGTAGGGGAGACT  630

Query  301  GGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  631  GGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGC  690

Query  361  AGGTCTCTGGGCTATGACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  411
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  691  AGGTCTCTGGGCTATGACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  741
```

Query length = 411
Alignment length = 411
0 mismatch
-> 100% identity

Blast variables : blast_perc_identity

Identity percentage between the Query (OTU) and the subject in the alignment
(length subject = 1455 bases)



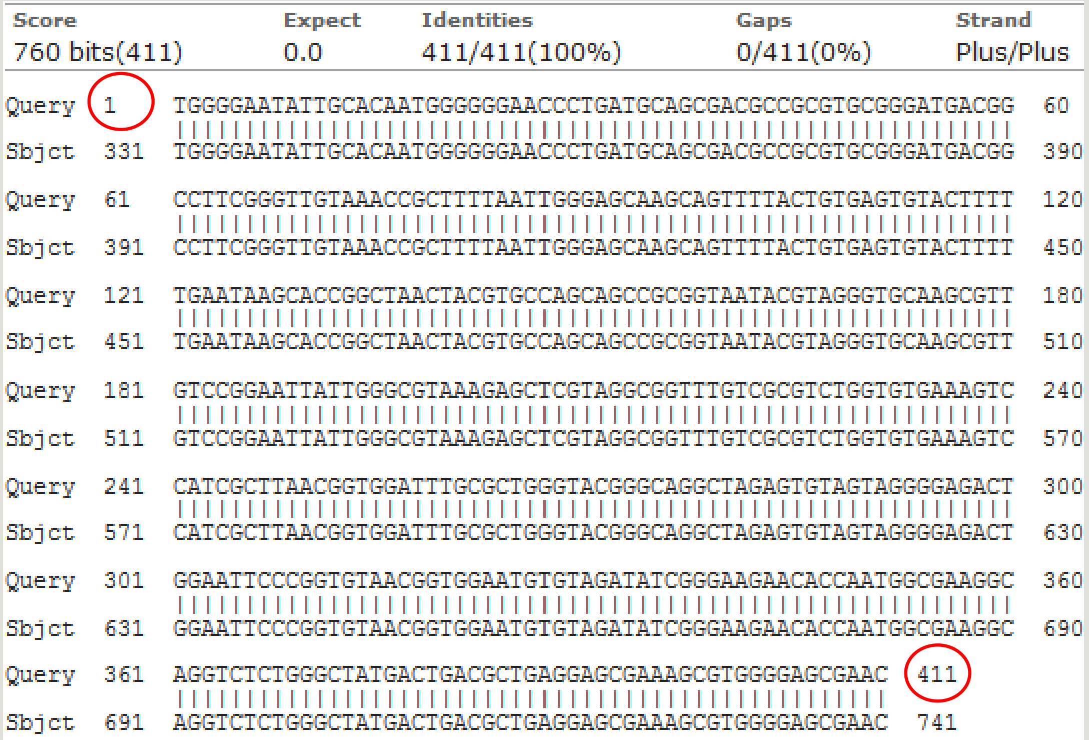
Score	Expect	Identities	Gaps	Strand
614 bits(332)	5e-172	385/411(94%)	5/411(1%)	Plus/Plus

```
Query  1       TGGGGAATATTGCACAATGGGGGGAACCCTGATGCAGCGACGCCGCGTGCGGGATGACGG  60
               ||||||||||||||||||||| | || |||||||||||||||||||||||||||||||||
Sbjct  140728  TGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGCGGGATGACGG  140787

Query  61      CCTTCGGGTTGTAAACCGCTTTTAATTGGGAGCAAGCAGTTTTACTGTGAGTGTACTTTT  120
               ||||||||||||||||||||||| ||||||||||||| |    |  |||||||||| |||
Sbjct  140788  CCTTCGGGTTGTAAACCGCTTTTGATTGGGAGCAAGC-G----AGAGTGAGTGTACCTTT  140842

Query  121     TGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTT  180
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  140843  CGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTT  140902

Query  181     GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGGTGTGAAAGTC  240
                |||||||||||||||||||||| |||||||||||||| |||||||||||||||||||||
Sbjct  140903  ATCCGGAATTATTGGGCGTAAAGRGCTCGTAGGCGGTTCGTCGCGTCTGGTGTGAAAGTC  140962

Query  241     CATCGCTTAACGGTGGATTTGCGCTGGGTACGGGCAGGCTAGAGTGTAGTAGGGGAGACT  300
               |||||||||||||||||| ||||| |||||||||| | || |||||  ||||||||||||
Sbjct  140963  CATCGCTTAACGGTGGATCTGCGCCGGGTACGGGCGGRCTGGAGTGCGGTAGGGGAGACT  141022

Query  301     GGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGC  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  141023  GGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGC  141082

Query  361     AGGTCTCTGGCTATGACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  411
               |||||||||||  | ||||||||||||||||||||||||||||||||||||
Sbjct  141083  AGGTCTCTGGCCGTTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  141133
```

Query length = 411
Alignment length = 411
26 mismatches (gaps included)
-> 94% identity

Blast variables : blast_perc_query_coverage

Coverage percentage of alignment on query (OTU)



```
Score            Expect   Identities      Gaps       Strand
760 bits(411)    0.0      411/411(100%)   0/411(0%)  Plus/Plus

Query  1    TGGGGAATATTGCACAATGGGGGGAACCCTGATGCAGCGACGCCGCGTGCGGGATGACGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  331  TGGGGAATATTGCACAATGGGGGGAACCCTGATGCAGCGACGCCGCGTGCGGGATGACGG  390

Query  61   CCTTCGGGTTGTAAACCGCTTTTAATTGGGAGCAAGCAGTTTTACTGTGAGTGTACTTTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  391  CCTTCGGGTTGTAAACCGCTTTTAATTGGGAGCAAGCAGTTTTACTGTGAGTGTACTTTT  450

Query  121  TGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  451  TGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTT  510

Query  181  GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGGTGTGAAAGTC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  511  GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGGTGTGAAAGTC  570

Query  241  CATCGCTTAACGGTGGATTTGCGCTGGGTACGGGCAGGCTAGAGTGTAGTAGGGGAGACT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  571  CATCGCTTAACGGTGGATTTGCGCTGGGTACGGGCAGGCTAGAGTGTAGTAGGGGAGACT  630

Query  301  GGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  631  GGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGC  690

Query  361  AGGTCTCTGGGCTATGACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  411
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  691  AGGTCTCTGGGCTATGACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  741
```

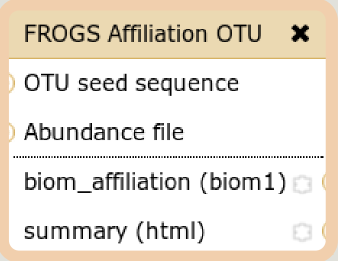
Query length = 411
100% coverage

Blast variables : blast-length

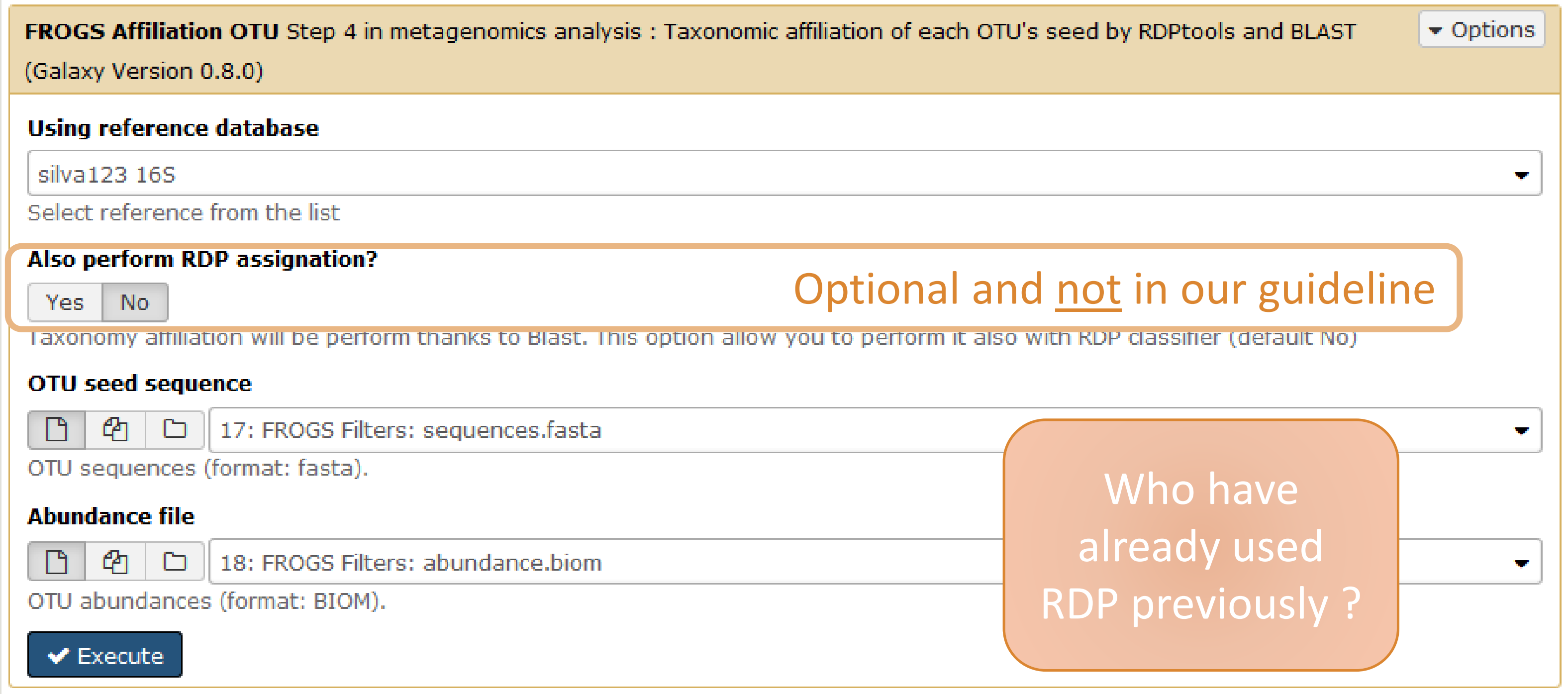
Length of alignment between the OTUs = “Query” and “subject” sequence of database

	Coverage %	Identity %	Length alignment
OTU1	100	98	400
OTU2	100	98	500

More mismatches/gaps


FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation


Options
Execute

FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST (Galaxy Version 0.8.0)

Using reference database

silva123 16S

Select reference from the list

Also perform RDP assignation?

Yes [✓ No]

Taxonomy affiliation will be perform thanks to Blast. This option allow you to perform it also with RDP classifier (default No)

OTU seed sequence

17: FROGS Filters: sequences.fasta

OTU sequences (format: fasta).

Abundance file

18: FROGS Filters: abundance.biom

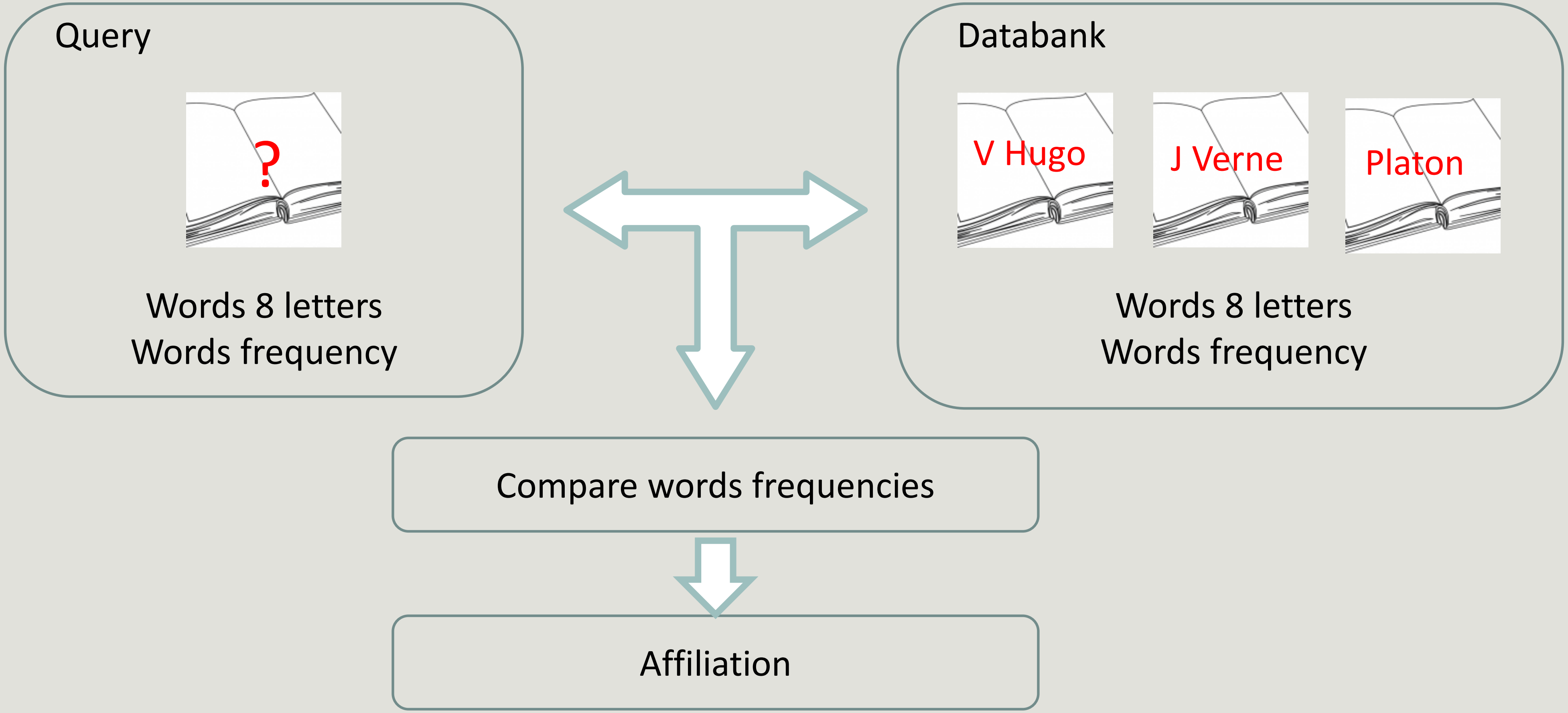
OTU abundances (format: BIOM).

Optional and not in our guideline

Who have already used RDP previously ?

Escape RDP explanation

How works RDP ?


?
V Hugo
J Verne
Platon

Query

Words 8 letters
Words frequency

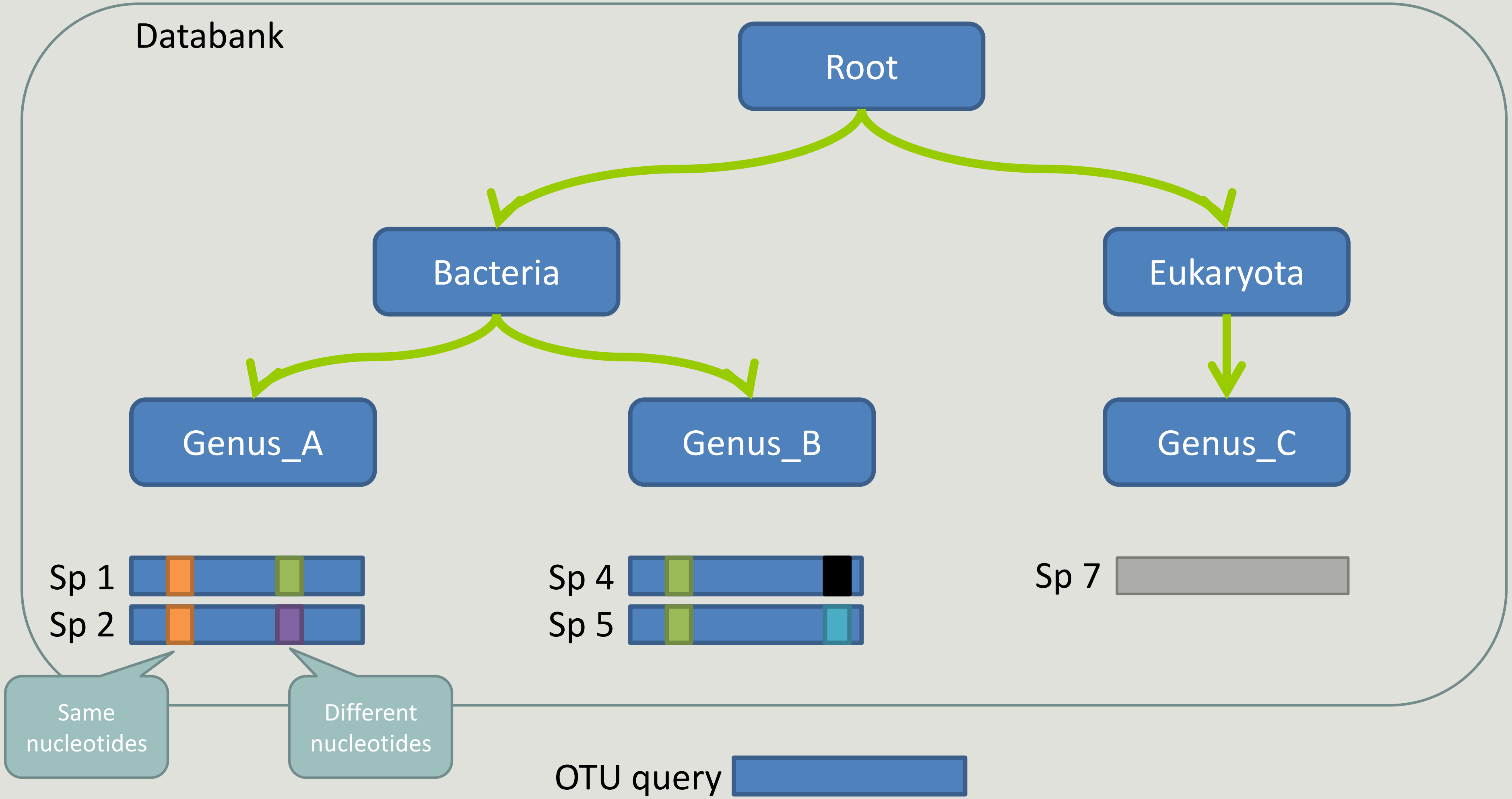
Databank

Words 8 letters
Words frequency

Compare words frequencies

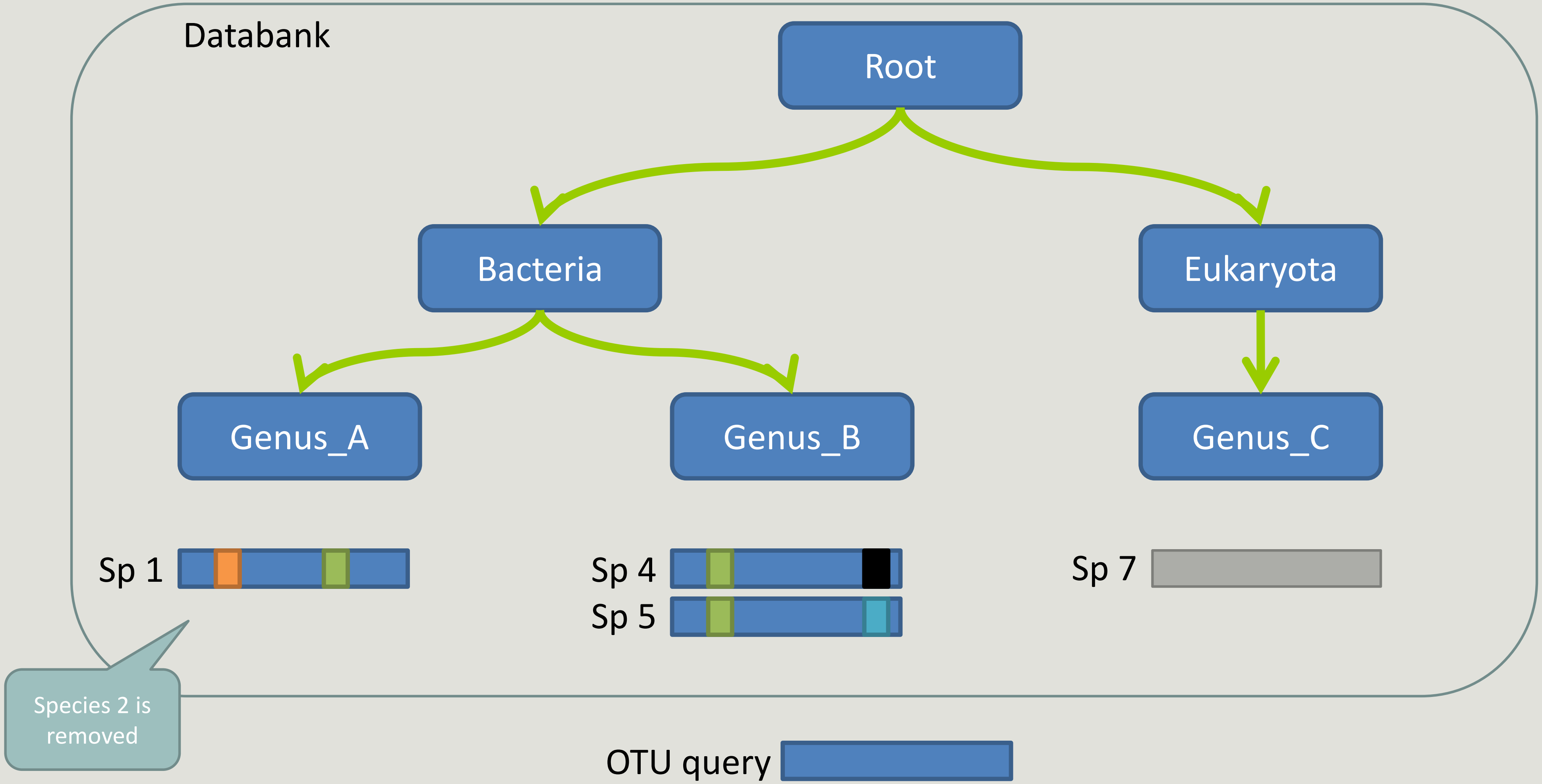
Affiliation

How works RDP ?


Databank
Root
Bacteria
Eukaryota
Genus_A
Genus_B
Genus_C
Sp 1
Sp 2
Sp 4
Sp 5
Sp 7
Same nucleotides
Different nucleotides
OTU query

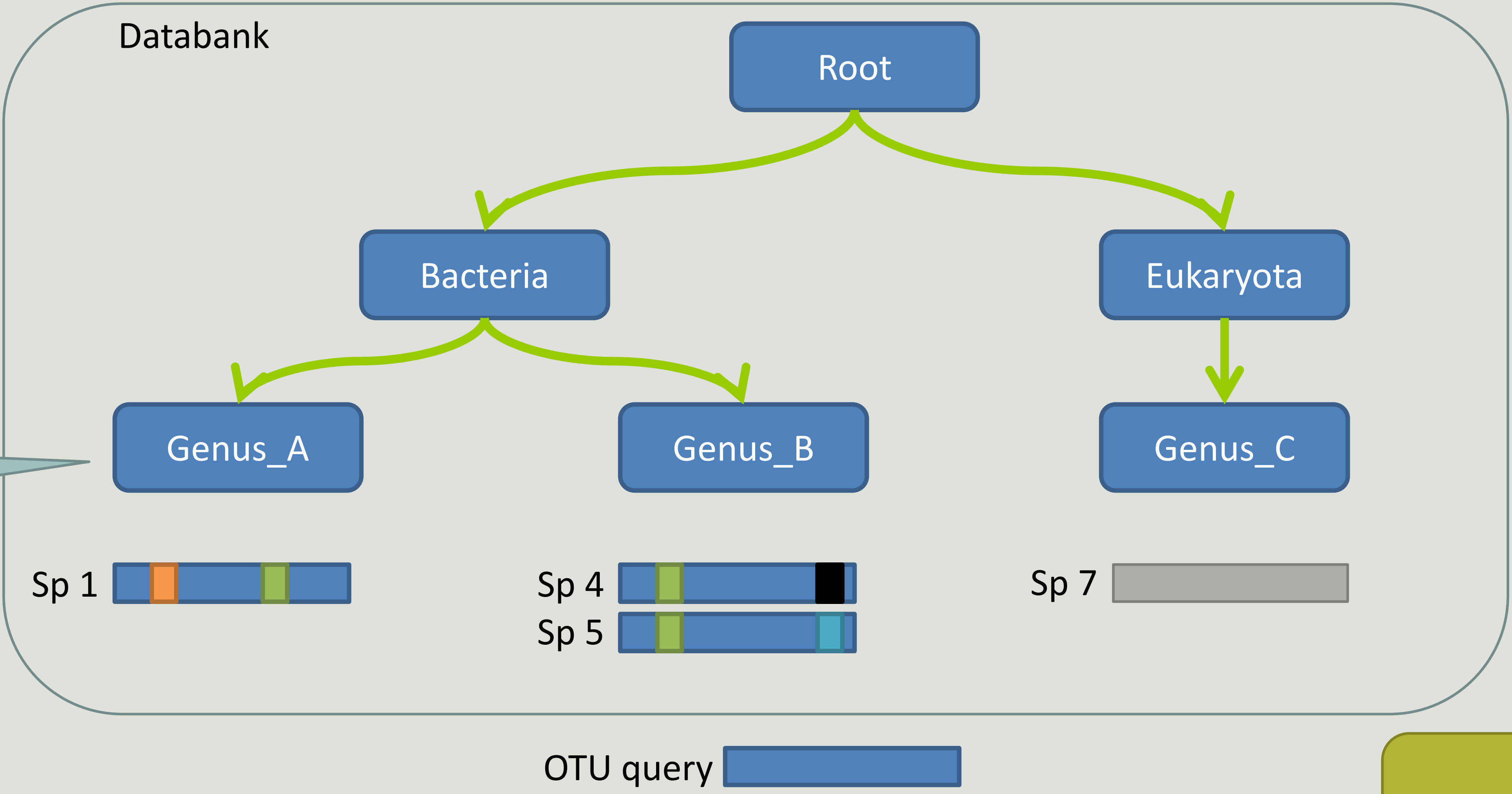
Result:
Bacteria(100) ; Genus_A(50) ; Sp1(25)

The dysfunctions of RDP ?


Databank
Root
Bacteria
Eukaryota
Genus_A
Genus_B
Genus_C
Sp 1
Sp 4
Sp 5
Sp 7
Species 2 is removed
OTU query

Result:
?

The dysfunctions of RDP n°1 ?


Databank
Root
Bacteria
Eukaryota
Genus_A
Genus_B
Genus_C
Sp 1
Sp 4
Sp 5
Sp 7
OTU query

Genus (50) was expected

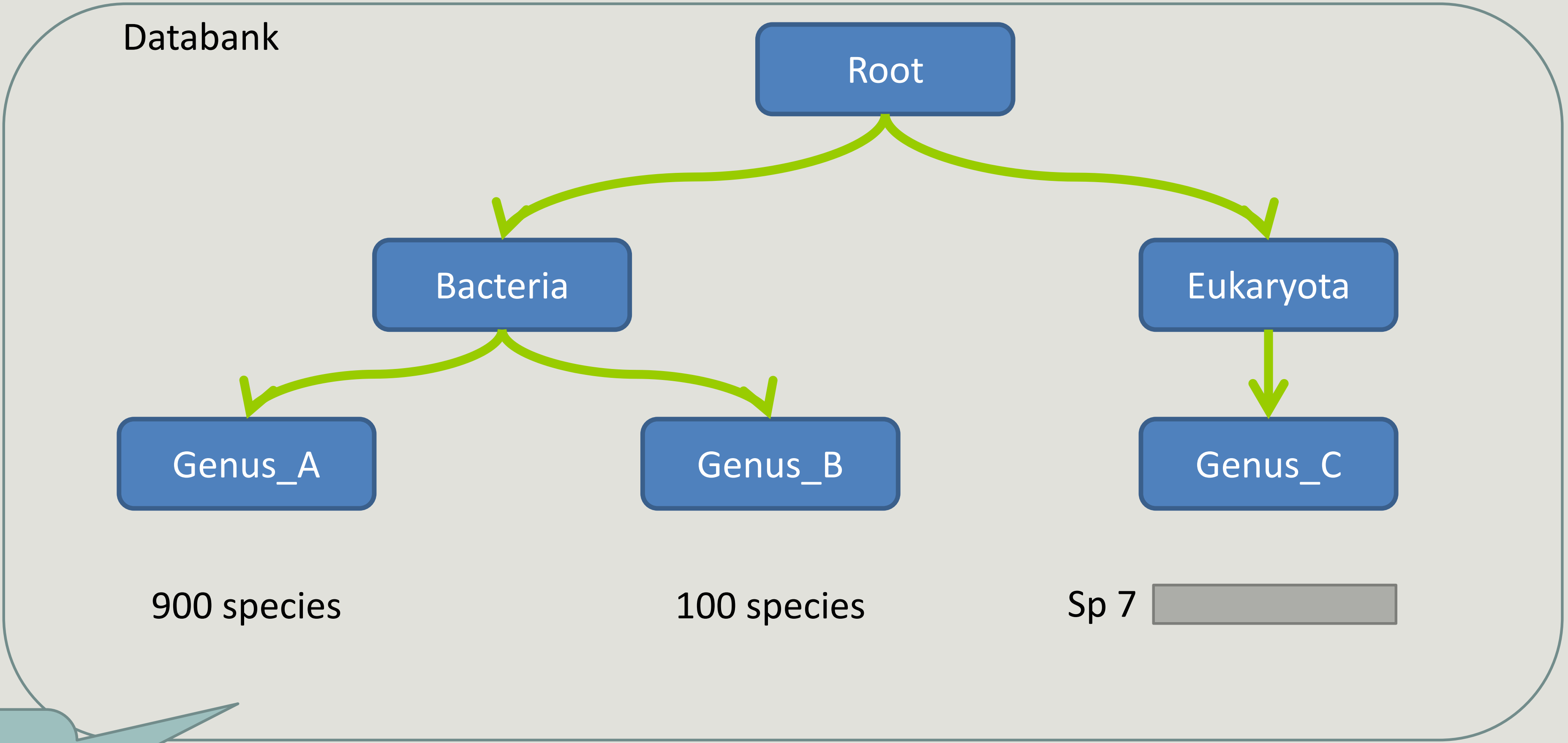
Genus (50) was expected.
If seq order was inversed in banks, so we got the second statistics.

Result:

Bacteria(100); Genus_A(33); sp1(33) OR Bacteria(100); Genus_B(66); sp5(33)

Order dependent

The dysfunctions of RDP n°2 ?


Databank
Root
Bacteria
Eukaryota
Genus_A
Genus_B
Genus_C
900 species
100 species
Sp 7

OTU query

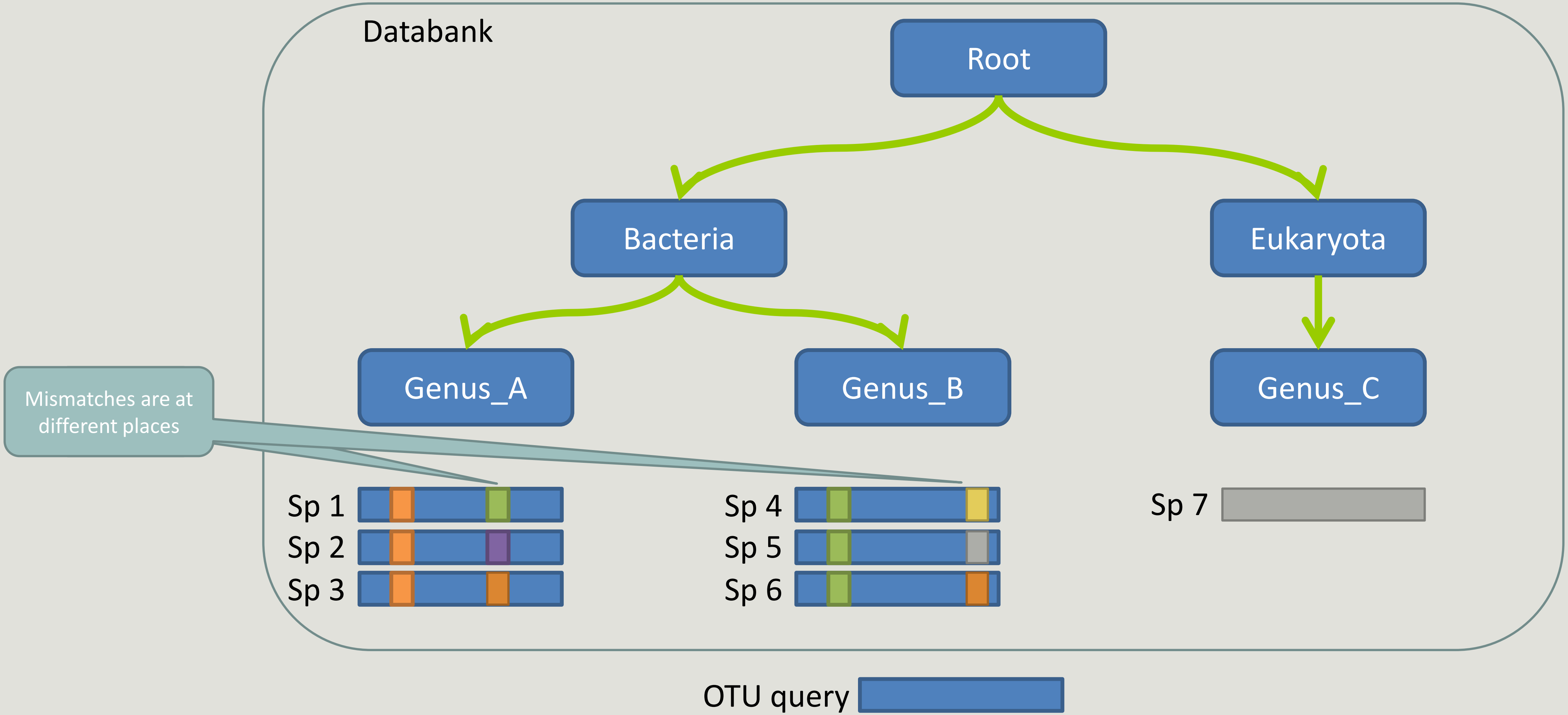
Many species in one genus and little in the other: So, RDP can give very different results

Influenced by heterogeneity in last ranks

Result:

Bacteria(100); Genus_A(90); spX(0.1) OR Bacteria(100); Genus_B(10); spX(0.1)

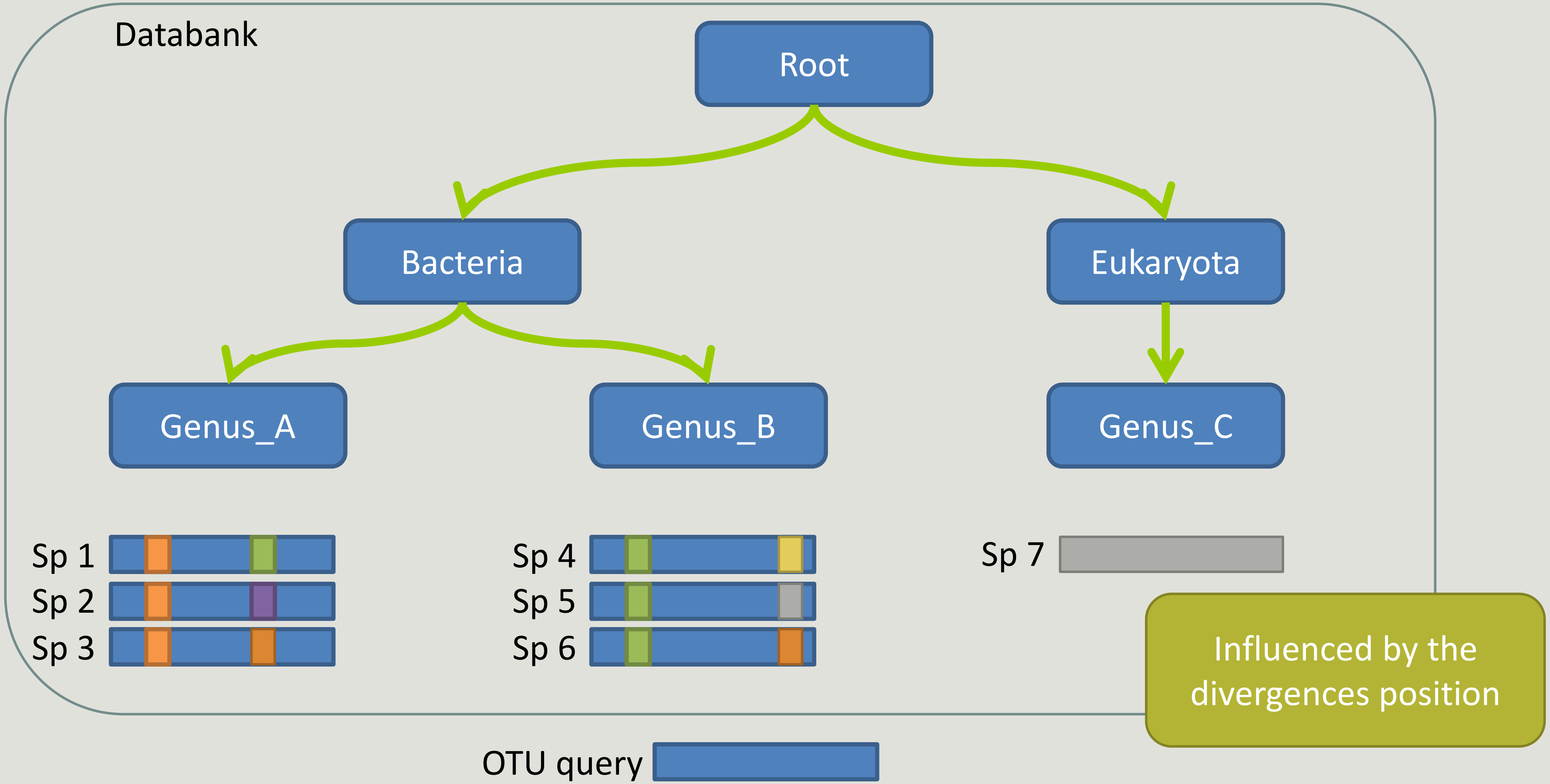
The dysfunctions of RDP n°3 ?


Databank
Root
Bacteria
Eukaryota
Genus_A
Genus_B
Genus_C
Sp 1
Sp 2
Sp 3
Sp 4
Sp 5
Sp 6
Sp 7
OTU query

Mismatches are at different places

Result:
?

The dysfunctions of RDP n°3 ?


Databank
Root
Bacteria
Eukaryota
Genus_A
Genus_B
Genus_C
Sp 1
Sp 2
Sp 3
Sp 4
Sp 5
Sp 6
Sp 7
OTU query

Influenced by the divergences position

Si le mismatch se fait sur un mot très "significatif" dans le profil de k-mers, RDP ne tombera que rarement sur l'espèce lors du bootstrap. Avec une même distance d'édition (2 mismatchs) on peut donc avoir une grande différence de bootstrap pour peu que le mot affecté soit important dans le profil.

Divergence on the composition of microbial communities at the different taxonomic ranks

RDPClassifier

NCBI blastn+

Reliable ?

Taxonomic ranks	Average divergence of the affiliations of the 10 samples (%) 500setA	Average divergence of the affiliations of the 10 samples (%) 100setA
Kingdom	0.00	0.00
Phylum	0.46	0.41
Class	0.64	0.50
Order	0.94	0.68
Familly	1.18	0.78
Genus	1.76	1.30
Species	23.87	34.80

Identical V3-V4

solution

Report on abundance table, the multiple identical affiliations

Only one best hit

Taxonomic ranks	Average divergence of the affiliations of the 10 samples (%) 500setA	Average divergence of the affiliations of the 10 samples (%) 100setA
Kingdom	0.00	0.00
Phylum	0.46	0.41
Class	0.64	0.50
Order	0.94	0.68
Familly	1.18	0.78
Genus	1.76	1.30
Species	23.87	34.80

Multiple best hit

Taxonomic ranks	Median divergence of the affiliations of the 10 samples (%) 500setA	Median divergence of the affiliations of the 10 samples (%) 100setA
Kingdom	0.00	0.00
Phylum	0.46	0.41
Class	0.64	0.50
Order	0.93	0.68
Familly	1.17	0.78
Genus	1.60	1.00
Species	6.63	5.75

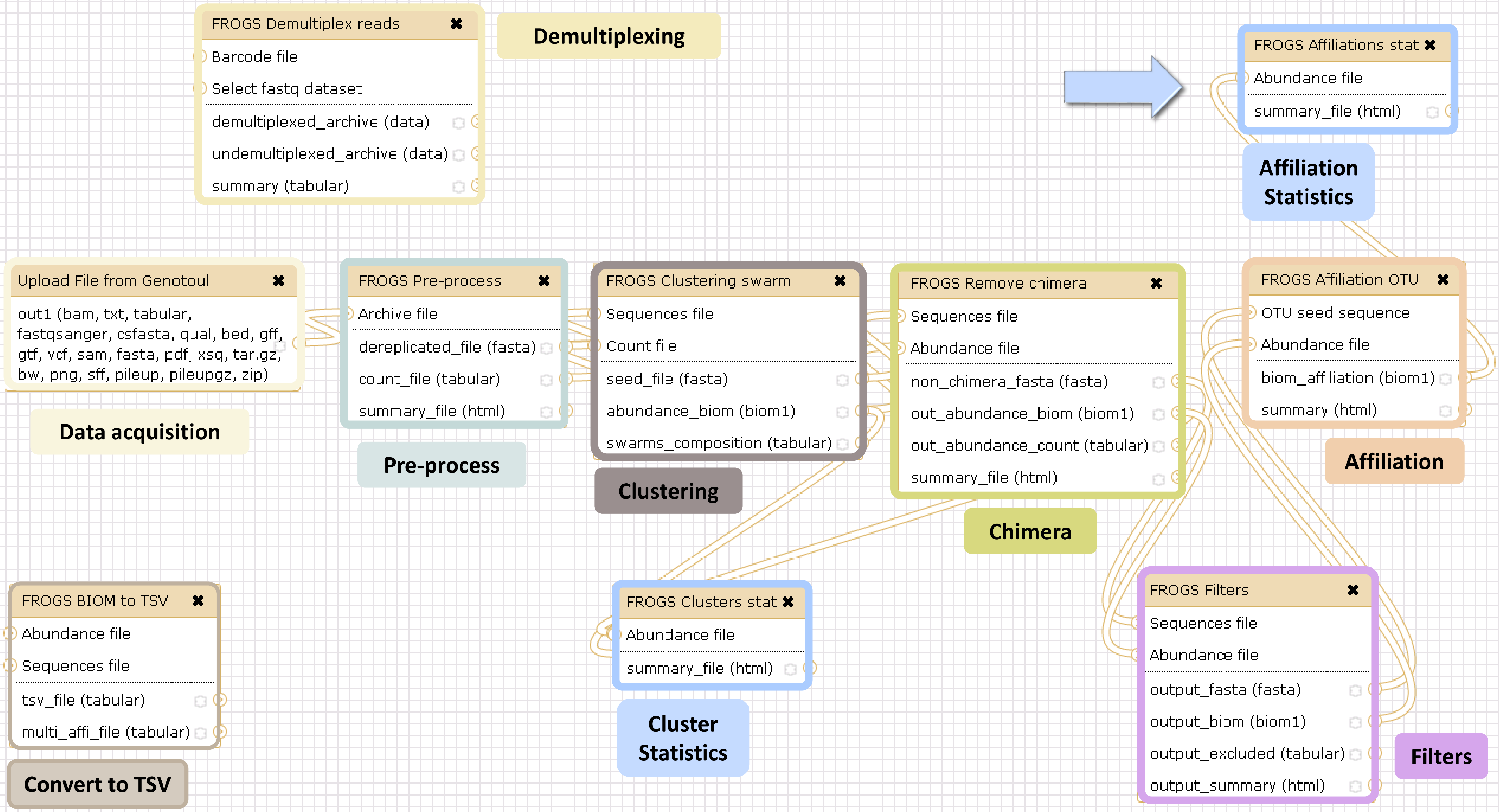
With the FROGS guideline

Taxonomic ranks	Median divergence of the affiliations of the 10 samples (%) 500setA filter: 0.005% - 505 OTUs	Median divergence of the affiliations of the 10 samples (%) 100setA filter: 0.005% - 100 OTUs
Kingdom	0.00	0.00
Phylum	0.38	0.38
Class	0.57	0.48
Order	0.81	0.64
Familly	1.08	0.74
Genus	1.43	0.76
Species	1.53	0.78

Careful: Multi hit blast table is non exhaustive !

- Chimera (multiple affiliation)
- V3V4 included in others
- Missed primers on some 16S during database building

Affiliation Stat



FROGS Demultiplex reads ✖
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)

Demultiplexing

FROGS Affiliations stat ✖
Abundance file
summary_file (html)

Affiliation Statistics

Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

Data acquisition

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

Pre-process

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

Clustering

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

Chimera

FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

Affiliation

FROGS BIOM to TSV ✖
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)

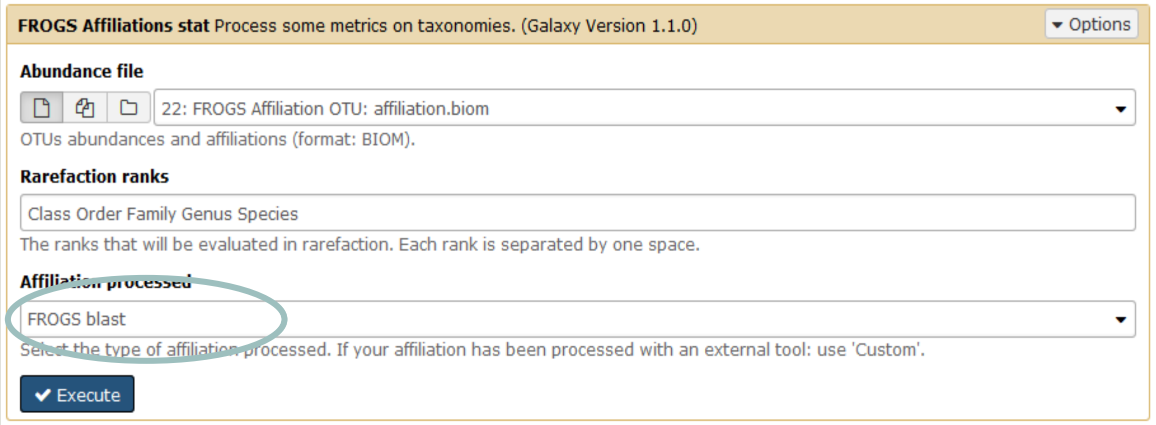
Convert to TSV

FROGS Clusters stat ✖
Abundance file
summary_file (html)

Cluster Statistics

FROGS Filters ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

Filters



FROGS Affiliations stat Process some metrics on taxonomies. (Galaxy Version 1.1.0)

Abundance file

22: FROGS Affiliation OTU: affiliation.biom

OTUs abundances and affiliations (format: BIOM).

Rarefaction ranks

Class Order Family Genus Species

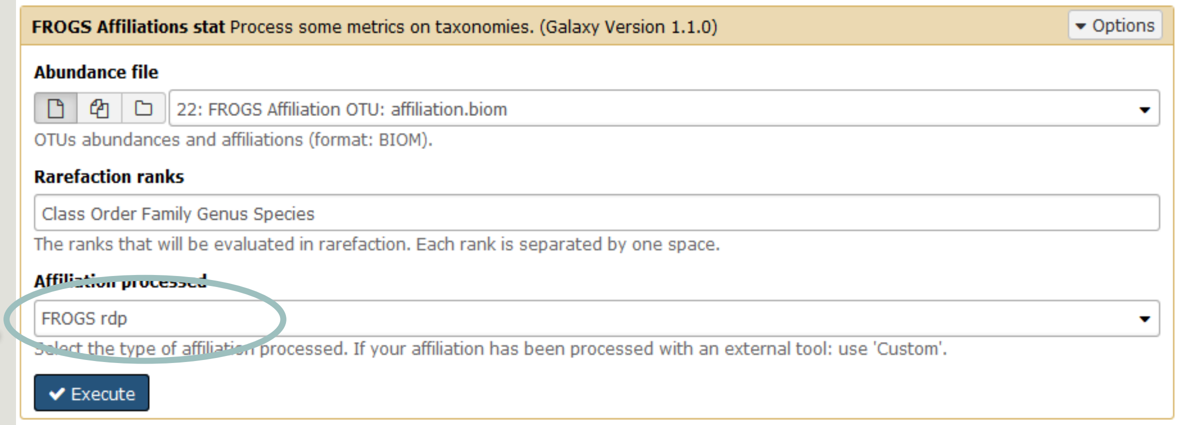
The ranks that will be evaluated in rarefaction. Each rank is separated by one space.

Affiliation processed

FROGS blast

Select the type of affiliation processed. If your affiliation has been processed with an external tool: use 'Custom'.

OR



FROGS Affiliations stat Process some metrics on taxonomies. (Galaxy Version 1.1.0)

Abundance file

22: FROGS Affiliation OTU: affiliation.biom

OTUs abundances and affiliations (format: BIOM).

Rarefaction ranks

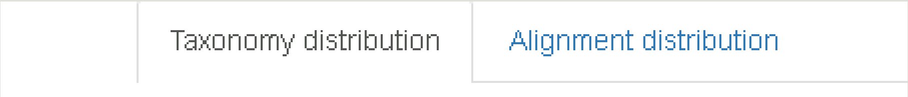
Class Order Family Genus Species

The ranks that will be evaluated in rarefaction. Each rank is separated by one space.

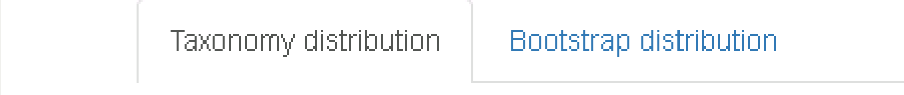
Affiliation processed

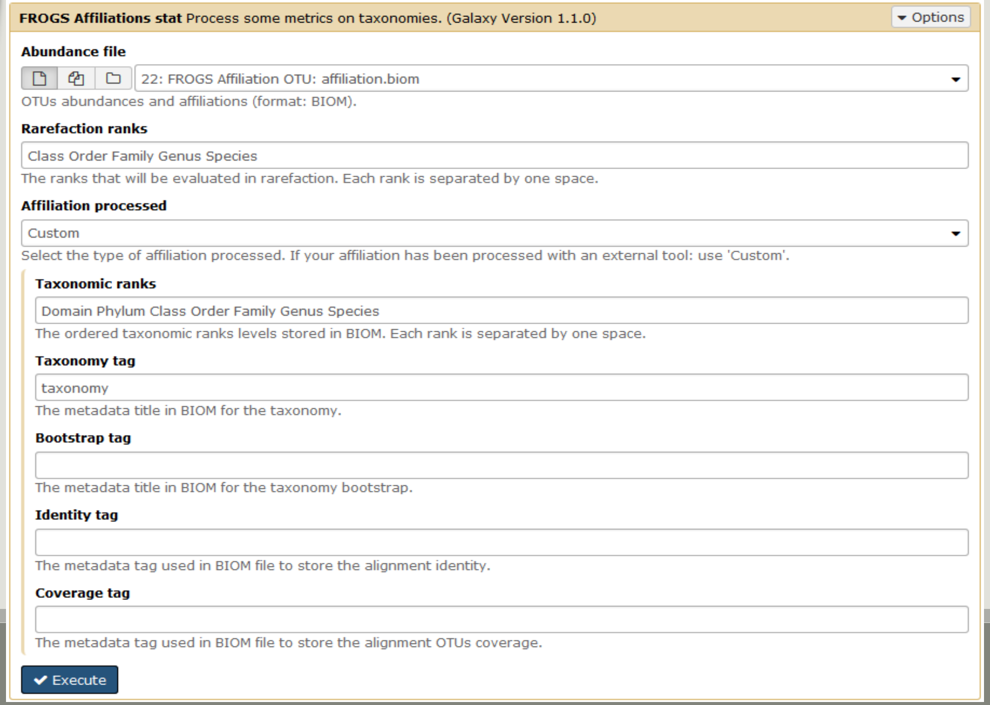
FROGS rdp

Select the type of affiliation processed. If your affiliation has been processed with an external tool: use 'Custom'.



OR





FROGS Affiliations stat Process some metrics on taxonomies. (Galaxy Version 1.1.0)

Abundance file

22: FROGS Affiliation OTU: affiliation.biom

OTUs abundances and affiliations (format: BIOM).

Rarefaction ranks

Class Order Family Genus Species

The ranks that will be evaluated in rarefaction. Each rank is separated by one space.

Affiliation processed

Custom

Select the type of affiliation processed. If your affiliation has been processed with an external tool: use 'Custom'.

Taxonomic ranks

Domain Phylum Class Order Family Genus Species

The ordered taxonomic ranks levels stored in BIOM. Each rank is separated by one space.

Taxonomy tag

taxonomy

The metadata title in BIOM for the taxonomy.

Bootstrap tag

The metadata title in BIOM for the taxonomy bootstrap.

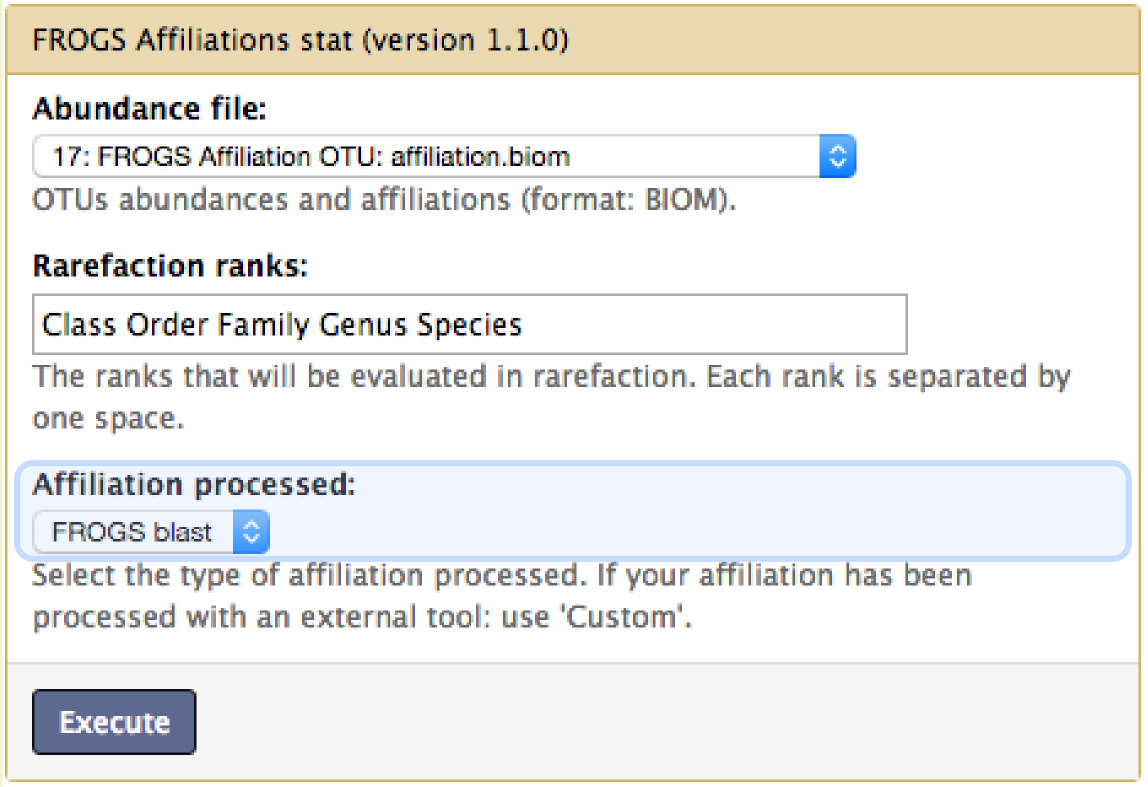
Identity tag

The metadata tag used in BIOM file to store the alignment identity.

Coverage tag

The metadata tag used in BIOM file to store the alignment OTUs coverage.

Exercise 7.2

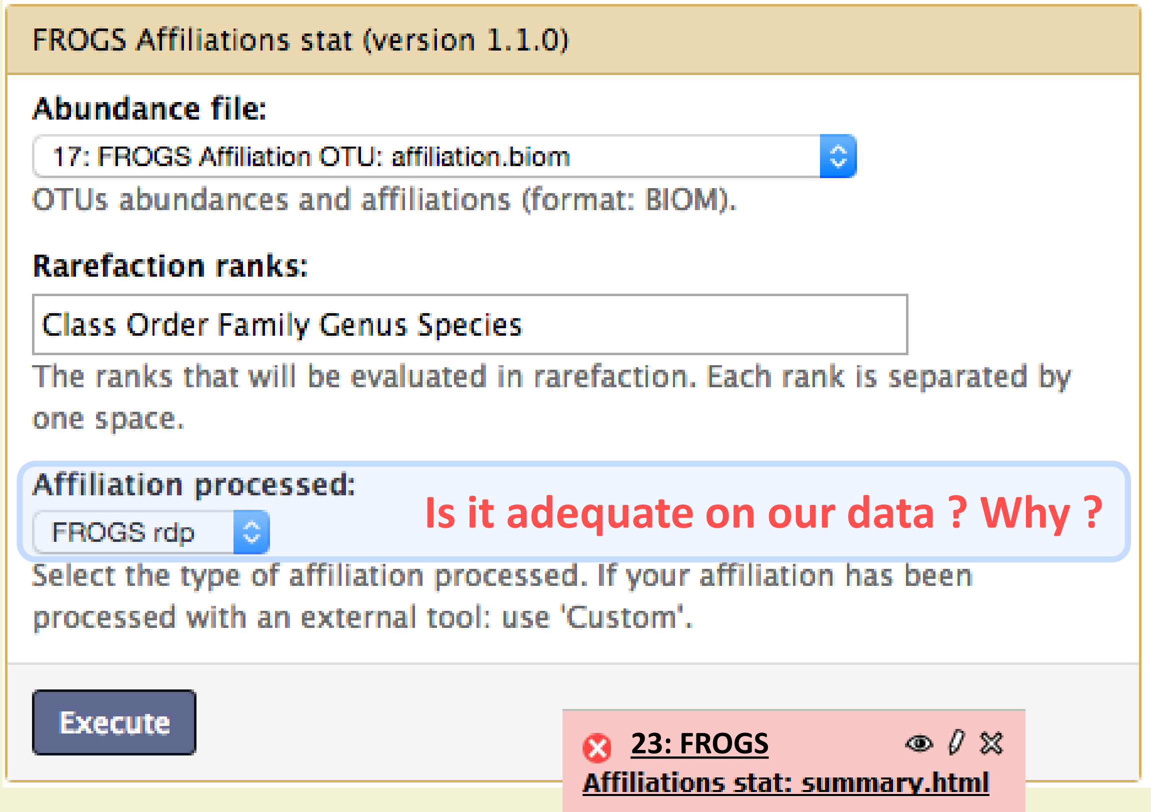


FROGS Affiliations stat (version 1.1.0)

Abundance file:
17: FROGS Affiliation OTU: affiliation.biom
OTUs abundances and affiliations (format: BIOM).

Rarefaction ranks:
Class Order Family Genus Species
The ranks that will be evaluated in rarefaction. Each rank is separated by one space.

Affiliation processed:
FROGS blast
Select the type of affiliation processed. If your affiliation has been processed with an external tool: use 'Custom'.



FROGS Affiliations stat (version 1.1.0)

Abundance file:
17: FROGS Affiliation OTU: affiliation.biom
OTUs abundances and affiliations (format: BIOM).

Rarefaction ranks:
Class Order Family Genus Species
The ranks that will be evaluated in rarefaction. Each rank is separated by one space.

Affiliation processed:
FROGS rdp
Select the type of affiliation processed. If your affiliation has been processed with an external tool: use 'Custom'.

23: FROGS Affiliations stat: summary.html

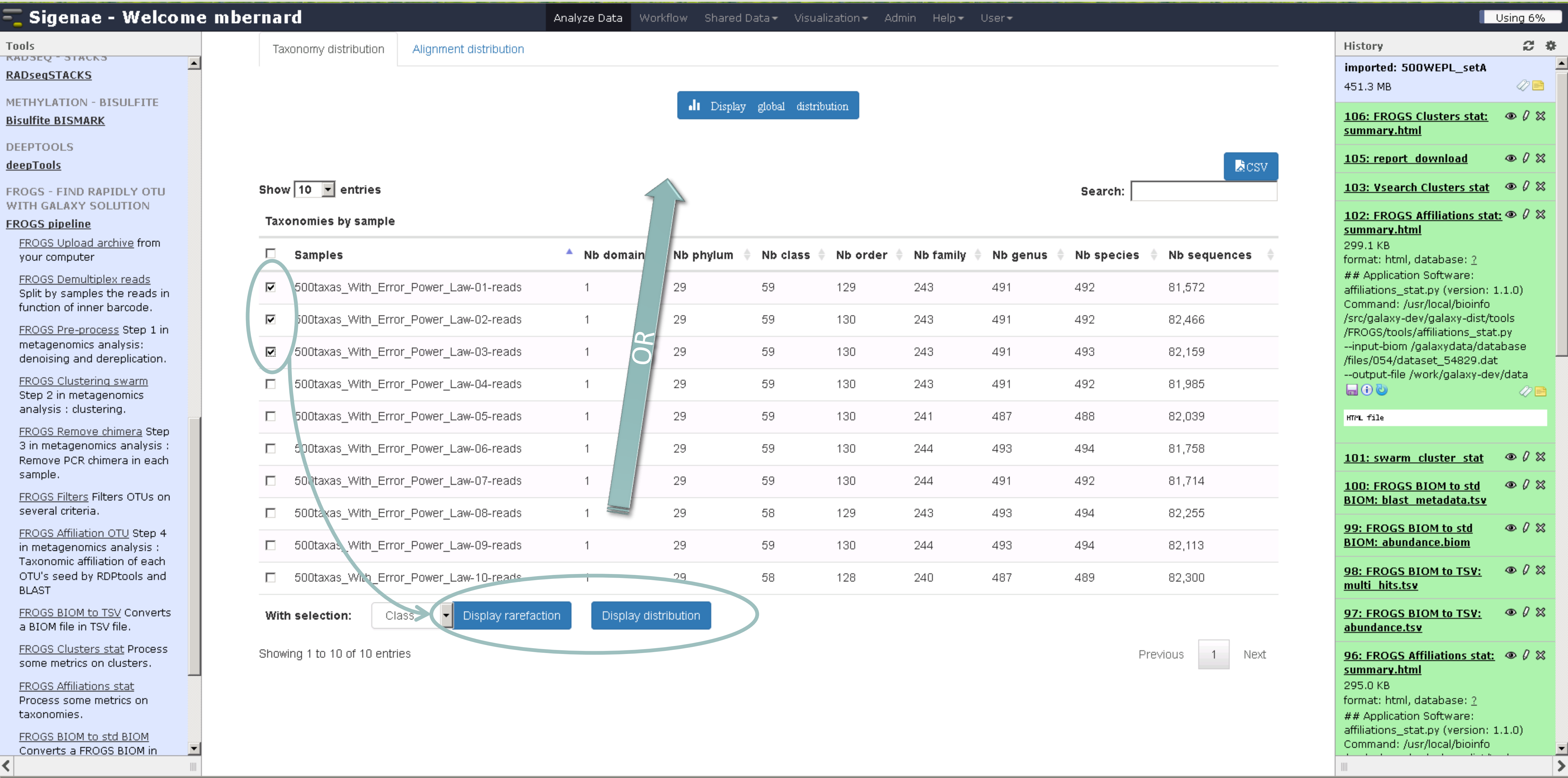
Is it adequate on our data ? Why ?

Exercise 7.2

→ objectives :

understand rarefaction curve and sunburst

1. Explore the Affiliation stat results on FROGS blast affiliation.
2. What kind of graphs can you generate? What do they mean?



Tools

RADSEQ - STACKS
RADseqSTACKS

METHYLATION - BISULFITE
Bisulfite BISMARK

DEEPTOOLS
deepTools

FROGS - FIND RAPIDLY OTU WITH GALAXY SOLUTION
FROGS pipeline
FROGS Upload archive from your computer
FROGS Demultiplex reads Split by samples the reads in function of inner barcode.
FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication.
FROGS Clustering swarm Step 2 in metagenomics analysis : clustering.
FROGS Remove chimera Step 3 in metagenomics analysis : Remove PCR chimera in each sample.
FROGS Filters Filters OTUs on several criteria.
FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST
FROGS BIOM to TSV Converts a BIOM file in TSV file.
FROGS Clusters stat Process some metrics on clusters.
FROGS Affiliations stat Process some metrics on taxonomies.
FROGS BIOM to std BIOM Converts a FROGS BIOM in

Taxonomy distribution | Alignment distribution

Display global distribution

CSV

Show 10 entries

Search:

Taxonomies by sample

☐	Samples	Nb domain	Nb phylum	Nb class	Nb order	Nb family	Nb genus	Nb species	Nb sequences
☑	500taxas_With_Error_Power_Law-01-reads	1	29	59	129	243	491	492	81,572
☑	500taxas_With_Error_Power_Law-02-reads	1	29	59	130	243	491	492	82,466
☑	500taxas_With_Error_Power_Law-03-reads	1	29	59	130	243	491	493	82,159
☐	500taxas_With_Error_Power_Law-04-reads	1	29	59	130	243	491	492	81,985
☐	500taxas_With_Error_Power_Law-05-reads	1	29	59	130	241	487	488	82,039
☐	500taxas_With_Error_Power_Law-06-reads	1	29	59	130	244	493	494	81,758
☐	500taxas_With_Error_Power_Law-07-reads	1	29	59	130	244	491	492	81,714
☐	500taxas_With_Error_Power_Law-08-reads	1	29	58	129	243	493	494	82,255
☐	500taxas_With_Error_Power_Law-09-reads	1	29	59	130	244	493	494	82,113
☐	500taxas_With_Error_Power_Law-10-reads	1	29	58	128	240	487	489	82,300

With selection: Class | Display rarefaction | Display distribution

Showing 1 to 10 of 10 entries

Previous 1 Next

History

imported: 500WEPL_setA
451.3 MB

106: FROGS Clusters stat: summary.html

105: report_download

103: Vsearch Clusters stat

102: FROGS Affiliations stat: summary.html
299.1 KB
format: html, database: ?
Application Software:
affiliations_stat.py (version: 1.1.0)
Command: /usr/local/bioinfo
/src/galaxy-dev/galaxy-dist/tools
/FROGS/tools/affiliations_stat.py
--input-biom /galaxydata/database
/files/054/dataset_54829.dat
--output-file /work/galaxy-dev/data

HTML file

101: swarm_cluster_stat

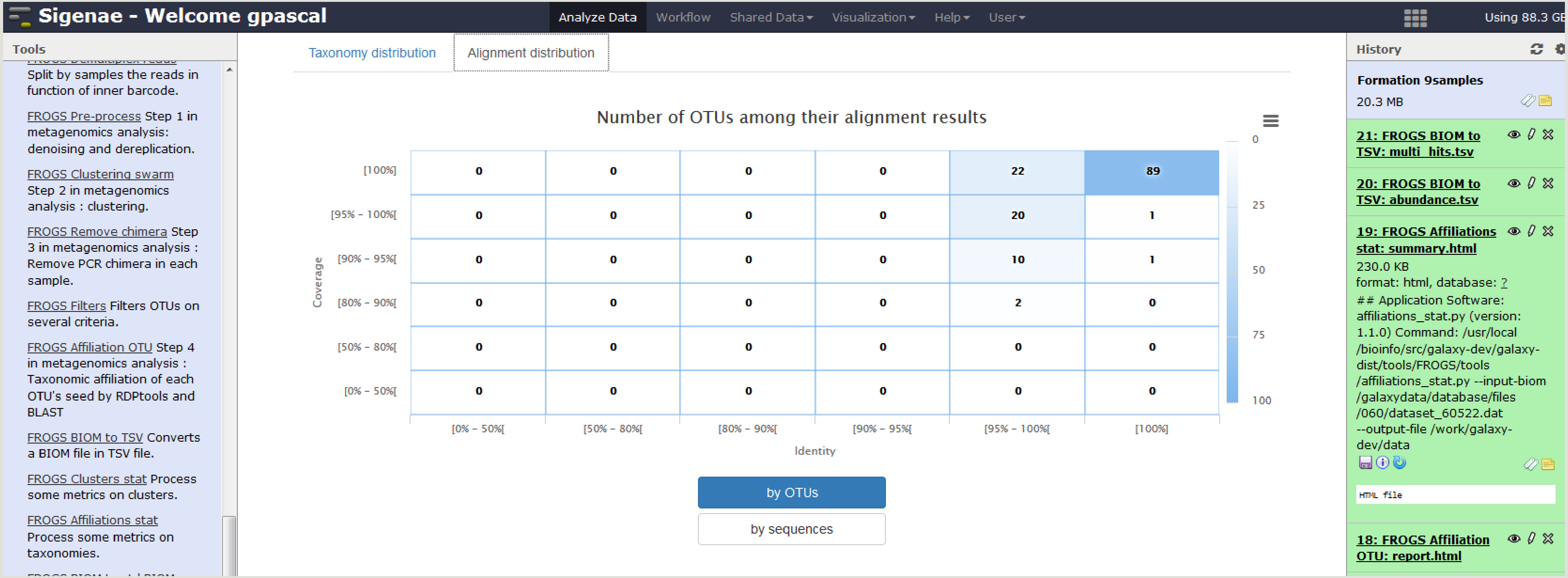
100: FROGS BIOM to std BIOM: blast_metadata.tsv

99: FROGS BIOM to std BIOM: abundance.biom

98: FROGS BIOM to TSV: multi_hits.tsv

97: FROGS BIOM to TSV: abundance.tsv

96: FROGS Affiliations stat: summary.html
295.0 KB
format: html, database: ?
Application Software:
affiliations_stat.py (version: 1.1.0)
Command: /usr/local/bioinfo


Sigenae - Welcome gpascal
Analyze Data
Workflow
Shared Data
Visualization
Help
User
Using 88.3 GB
Tools
History
Taxonomy distribution
Alignment distribution
by OTUs
by sequences

Split by samples the reads in function of inner barcode.

FROGS Pre-process Step 1 in metagenomics analysis: denoising and dereplication.

FROGS Clustering swarm Step 2 in metagenomics analysis : clustering.

FROGS Remove chimera Step 3 in metagenomics analysis : Remove PCR chimera in each sample.

FROGS Filters Filters OTUs on several criteria.

FROGS Affiliation OTU Step 4 in metagenomics analysis : Taxonomic affiliation of each OTU's seed by RDPtools and BLAST

FROGS BIOM to TSV Converts a BIOM file in TSV file.

FROGS Clusters stat Process some metrics on clusters.

FROGS Affiliations stat Process some metrics on taxonomies.

Number of OTUs among their alignment results

Coverage \ Identity	[0% – 50%[	[50% – 80%[	[80% – 90%[	[90% – 95%[	[95% – 100%[	[100%]
[100%]	0	0	0	0	22	89
[95% – 100%[	0	0	0	0	20	1
[90% – 95%[	0	0	0	0	10	1
[80% – 90%[	0	0	0	0	2	0
[50% – 80%[	0	0	0	0	0	0
[0% – 50%[	0	0	0	0	0	0

0
25
50
75
100

Formation 9samples
20.3 MB

21: FROGS BIOM to TSV: multi_hits.tsv

20: FROGS BIOM to TSV: abundance.tsv

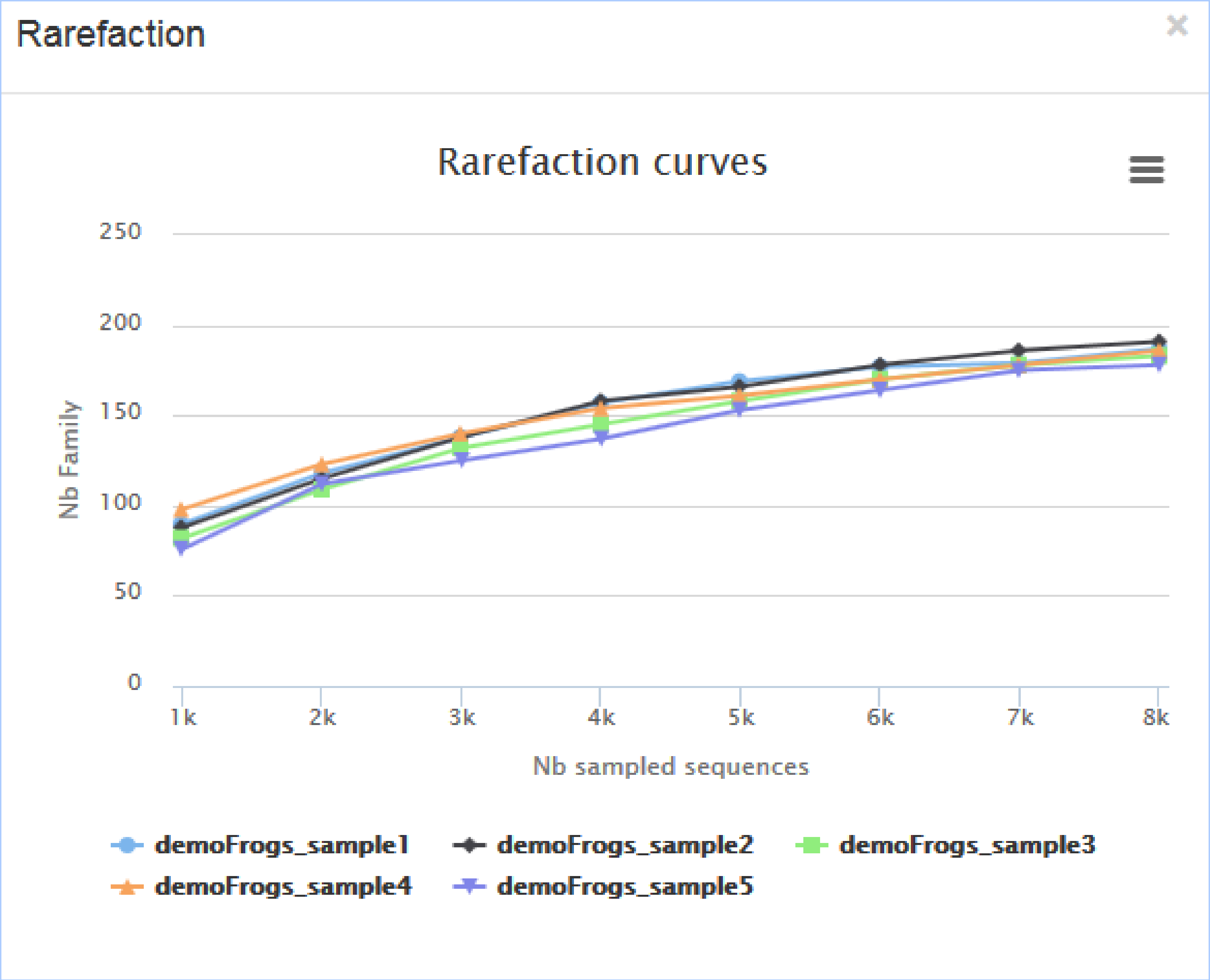
19: FROGS Affiliations stat: summary.html
230.0 KB
format: html, database: ?
Application Software: affiliations_stat.py (version: 1.1.0) Command: /usr/local /bioinfo/src/galaxy-dev/galaxy-dist/tools/FROGS/tools /affiliations_stat.py --input-biom /galaxydata/database/files /060/dataset_60522.dat --output-file /work/galaxy-dev/data

HTML file

18: FROGS Affiliation OTU: report.html

Available only after AFFILIATION TOOL

Samples size ~8500 sequences


Rarefaction
Rarefaction curves
Nb Family
250
200
150
100
50
0
1k
2k
3k
4k
5k
6k
7k
8k
Nb sampled sequences
demoFrogs_sample1
demoFrogs_sample2
demoFrogs_sample3
demoFrogs_sample4
demoFrogs_sample5

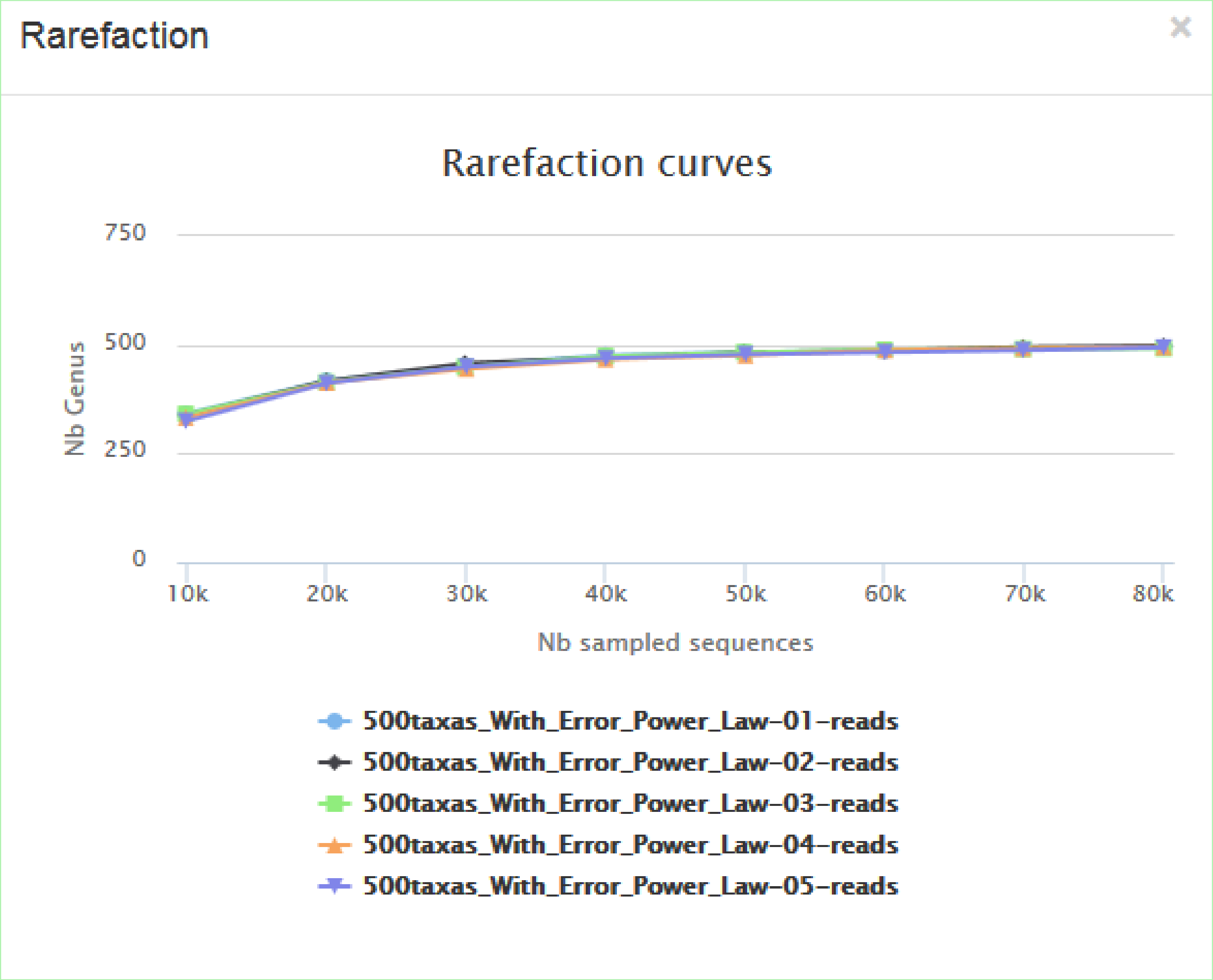
The curve continues to rise

The number of sequences per sample is not large enough to cover all of the bacterial families

Rarefaction tab

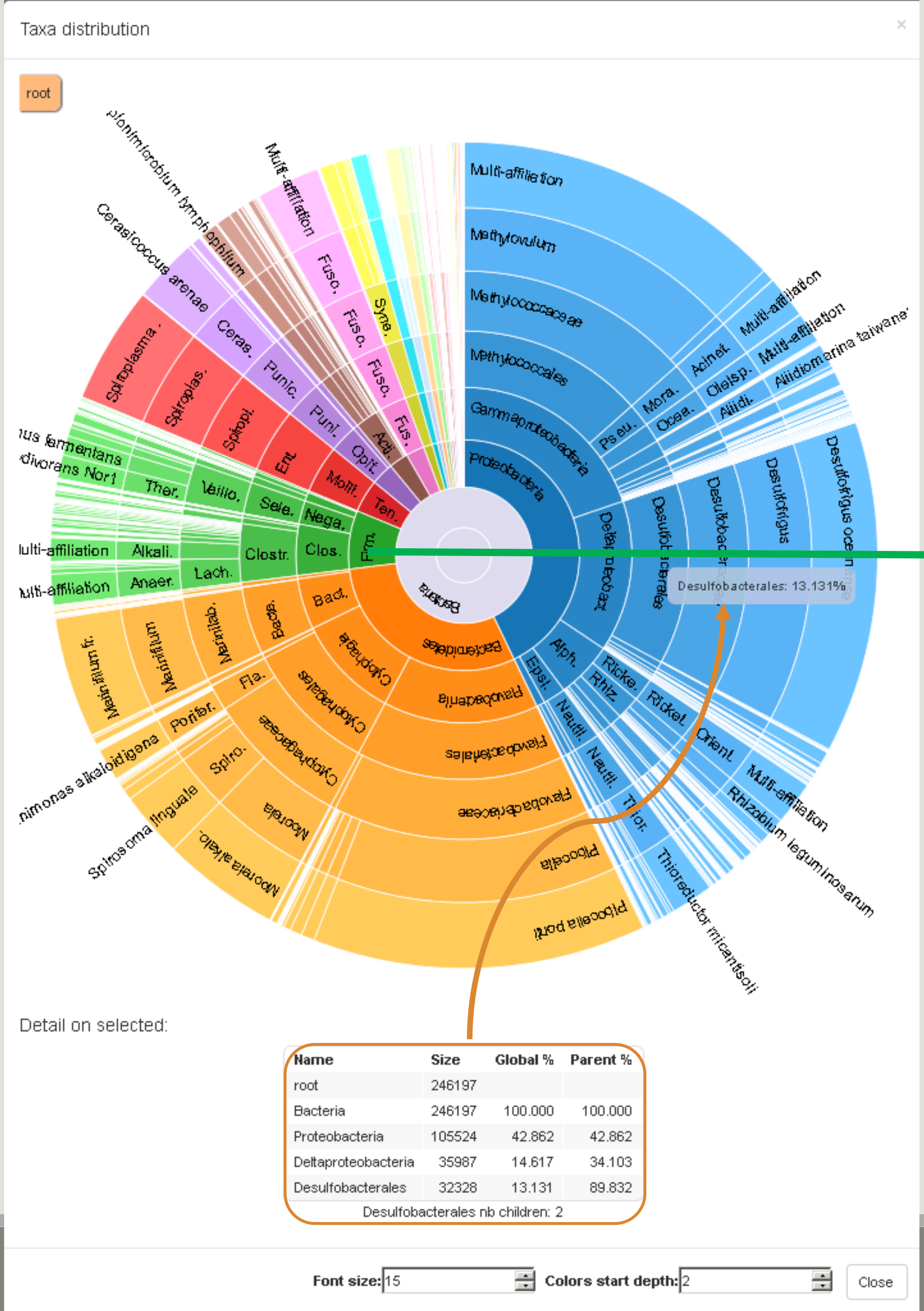
Available only after AFFILIATION TOOL

Samples size ~85 000 sequences



The curve slows to rise with ~50 000 sequences

With 60 000 sequences, we catch almost all genus of bacteria



root
Multi-affiliation
Methylovulum
Methylococcaceae
Methylococcales
Gammaproteobacteria
Proteobacteria
Deltaproteobacteria
Desulfobacterales
Desulfobacterales
Desulfofrigus
Desulfofrigus oceanense
Alphaproteobacteria
Bacteroidetes
Flavobacteriia
Flavobacteriales
Flavobacteriaceae
Ploceella
Ploceella pori
Cytophagia
Cytophagales
Cytophagaceae
Spiroplasma
Thioreductor micantisoli
Rhizobium leguminosarum
Aliidiomarina taiwanensis
Spirosoma linguale
Bacteria

Taxa distribution

root

Desulfobacterales: 13.131%

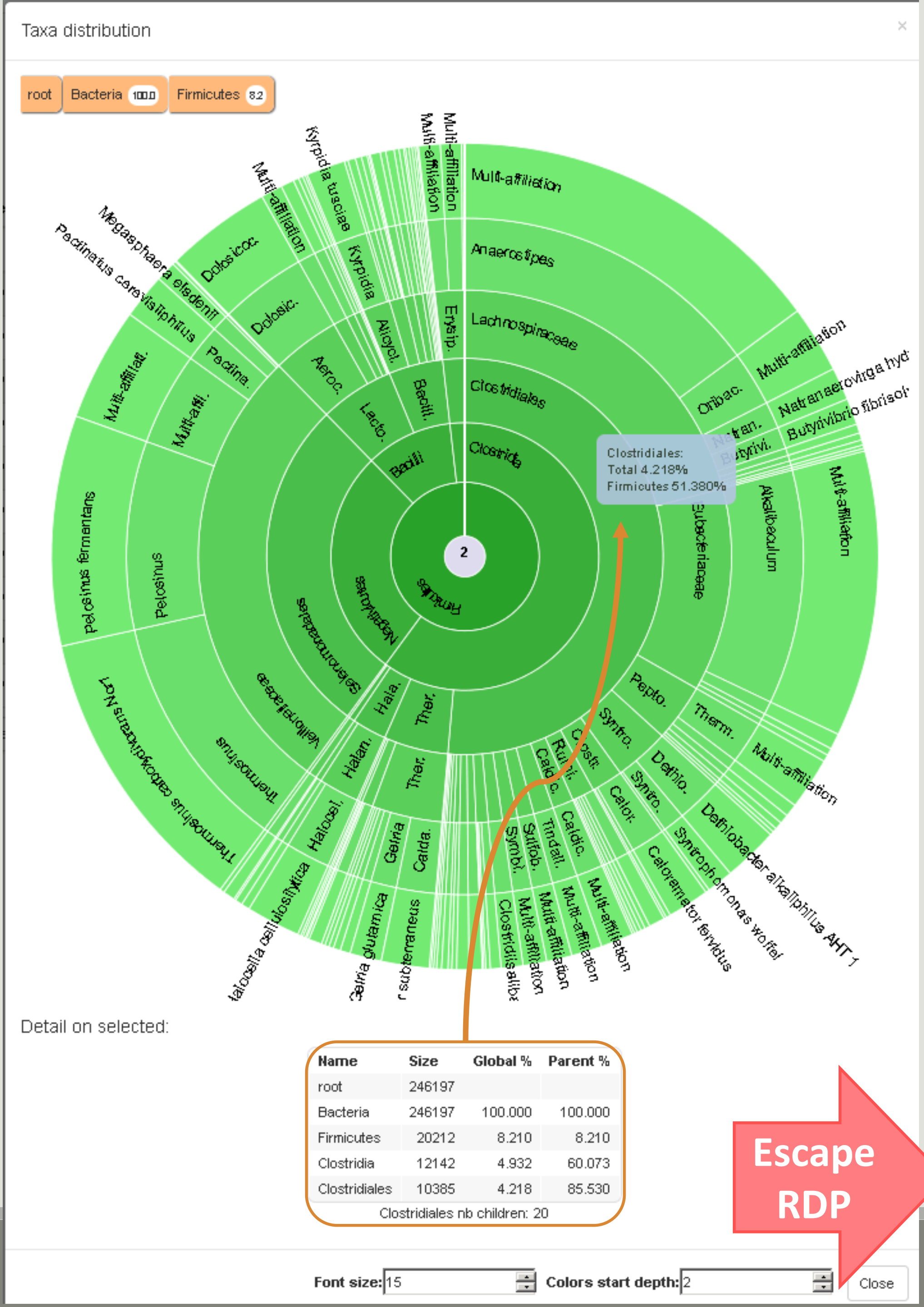
Detail on selected:

Name	Size	Global %	Parent %
root	246197		
Bacteria	246197	100.000	100.000
Proteobacteria	105524	42.862	42.862
Deltaproteobacteria	35987	14.617	34.103
Desulfobacterales	32328	13.131	89.832

Desulfobacterales nb children: 2

Font size: 15 Colors start depth: 2 Close

Zoom in on firmicutes



Multi-affiliation
Anaerostipes
Lachnospiraceae
Clostridiales
Clostridia
Bacilli
Lacto.
Erysip.
Kyrpidia
Kyrpidia tusciae
Dolosicoccus
Megasphaera elsdenii
Pectinatus cerevisiiphilus
Pelosinus fermentans
Pelosinus
Selenomonadales
Veillonellaceae
Negativicutes
Thermosinus
Thermosinus carboxydivorans Nor1
Firmicutes
Peptococcaceae
Alkalibaculum
Oribac.
Natranaerovirga hydrolytica
Butyrivibrio fibrisolvens
Dethiobacter alkaliphilus AHT 1
Syntrophomonas wolfei
Caloramator fervidus
Halobacteroides
Halocella cellulosilytica
2

Taxa distribution

root Bacteria 100.0 Firmicutes 8.2

Clostridiales:
Total 4.218%
Firmicutes 51.380%

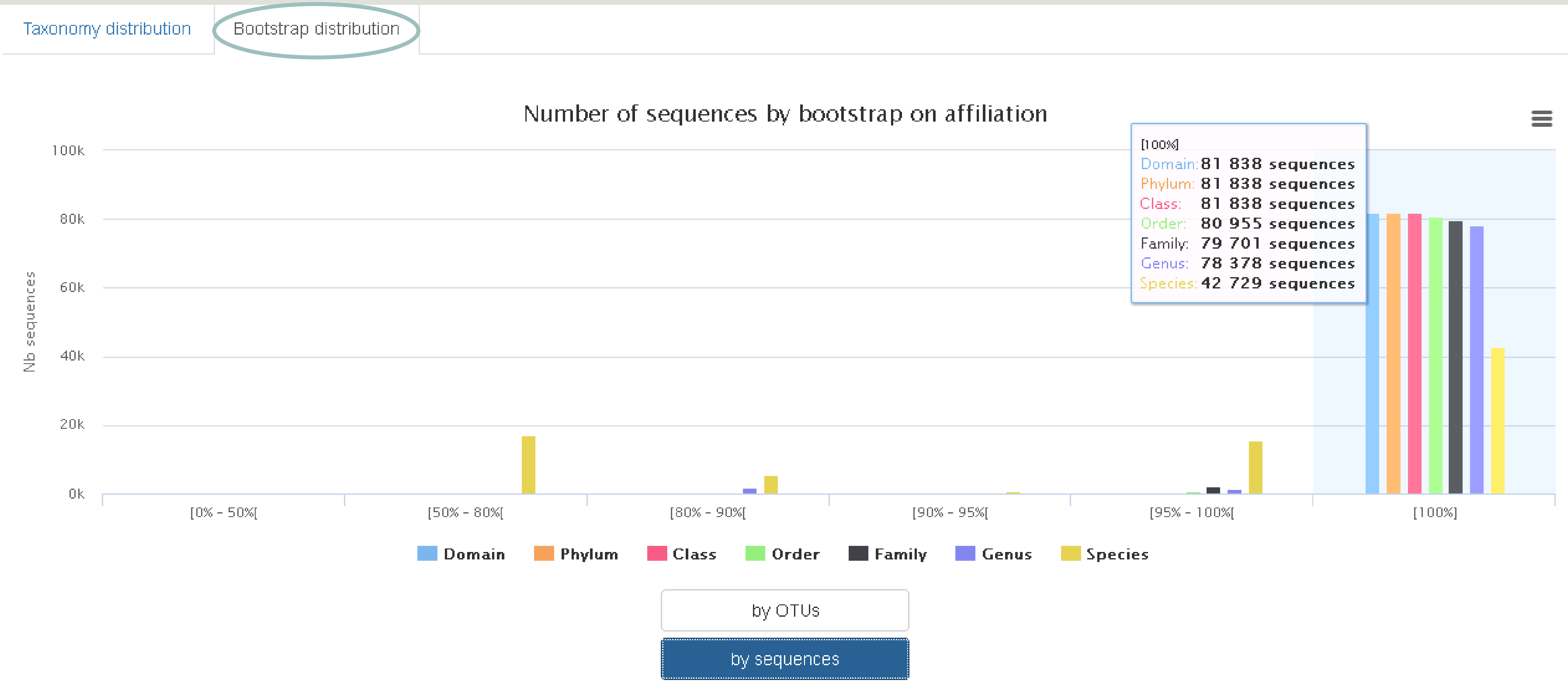
Detail on selected:

Name	Size	Global %	Parent %
root	246197		
Bacteria	246197	100.000	100.000
Firmicutes	20212	8.210	8.210
Clostridia	12142	4.932	60.073
Clostridiales	10385	4.218	85.530

Clostridiales nb children: 20

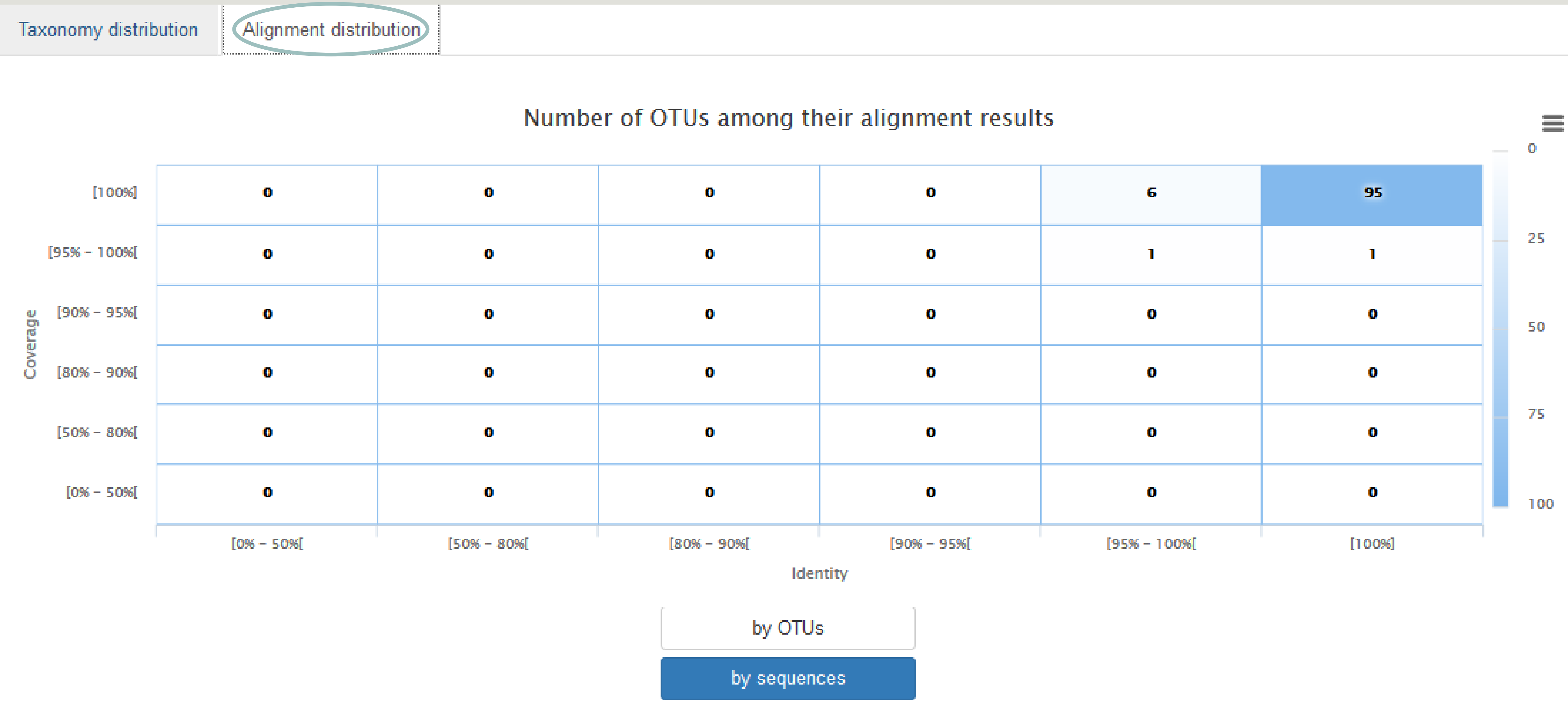
Font size: 15 Colors start depth: 2 Close

Escape RDP



Taxonomy distribution
Bootstrap distribution
Number of sequences by bootstrap on affiliation
Nb sequences
100k
80k
60k
40k
20k
0k
[0% - 50%[
[50% - 80%[
[80% - 90%[
[90% - 95%[
[95% - 100%[
[100%]
Domain
Phylum
Class
Order
Family
Genus
Species
by OTUs
by sequences

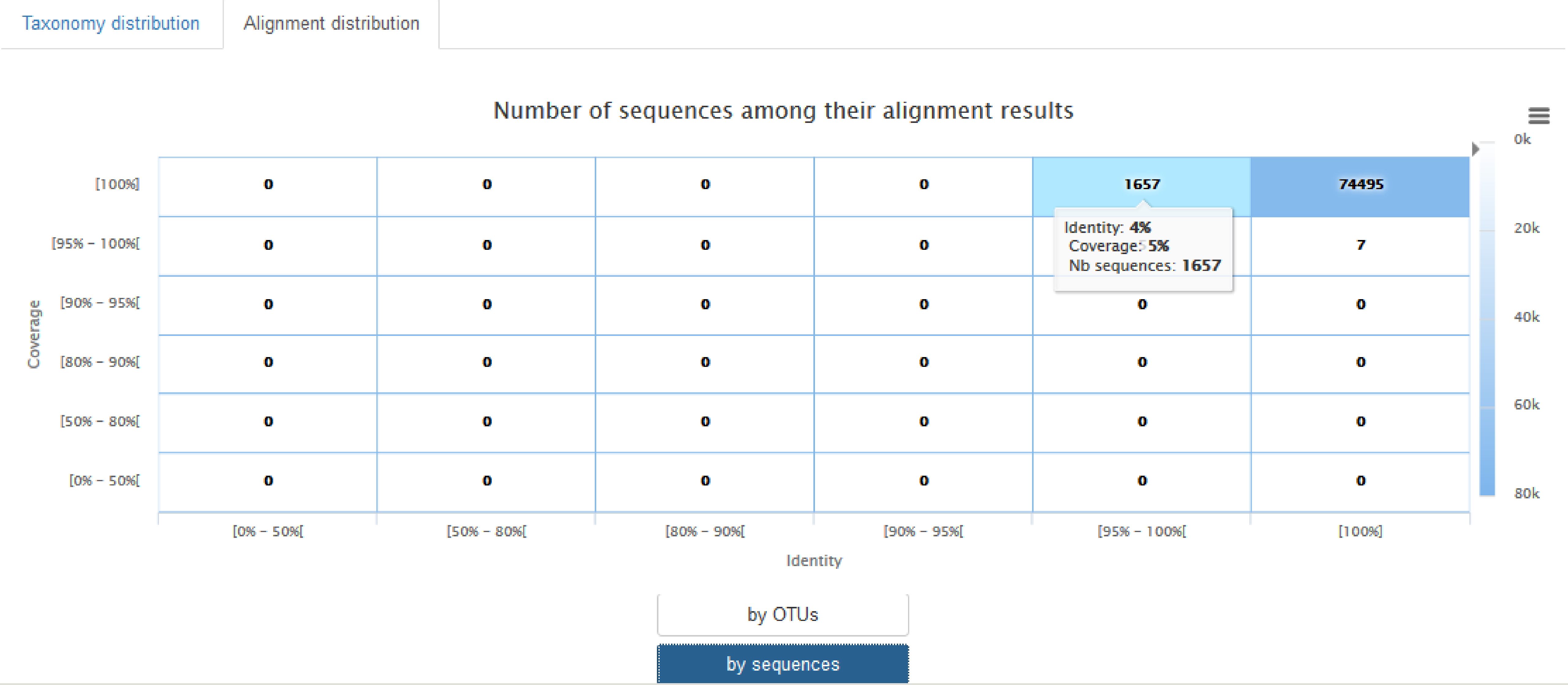
[100%]
Domain: 81 838 sequences
Phylum: 81 838 sequences
Class: 81 838 sequences
Order: 80 955 sequences
Family: 79 701 sequences
Genus: 78 378 sequences
Species: 42 729 sequences


Taxonomy distribution
Alignment distribution

Number of OTUs among their alignment results

	[0% – 50%[	[50% – 80%[	[80% – 90%[	[90% – 95%[	[95% – 100%[	[100%]
[100%]	0	0	0	0	6	95
[95% – 100%[	0	0	0	0	1	1
[90% – 95%[	0	0	0	0	0	0
[80% – 90%[	0	0	0	0	0	0
[50% – 80%[	0	0	0	0	0	0
[0% – 50%[	0	0	0	0	0	0

Coverage
Identity
0
25
50
75
100
by OTUs
by sequences

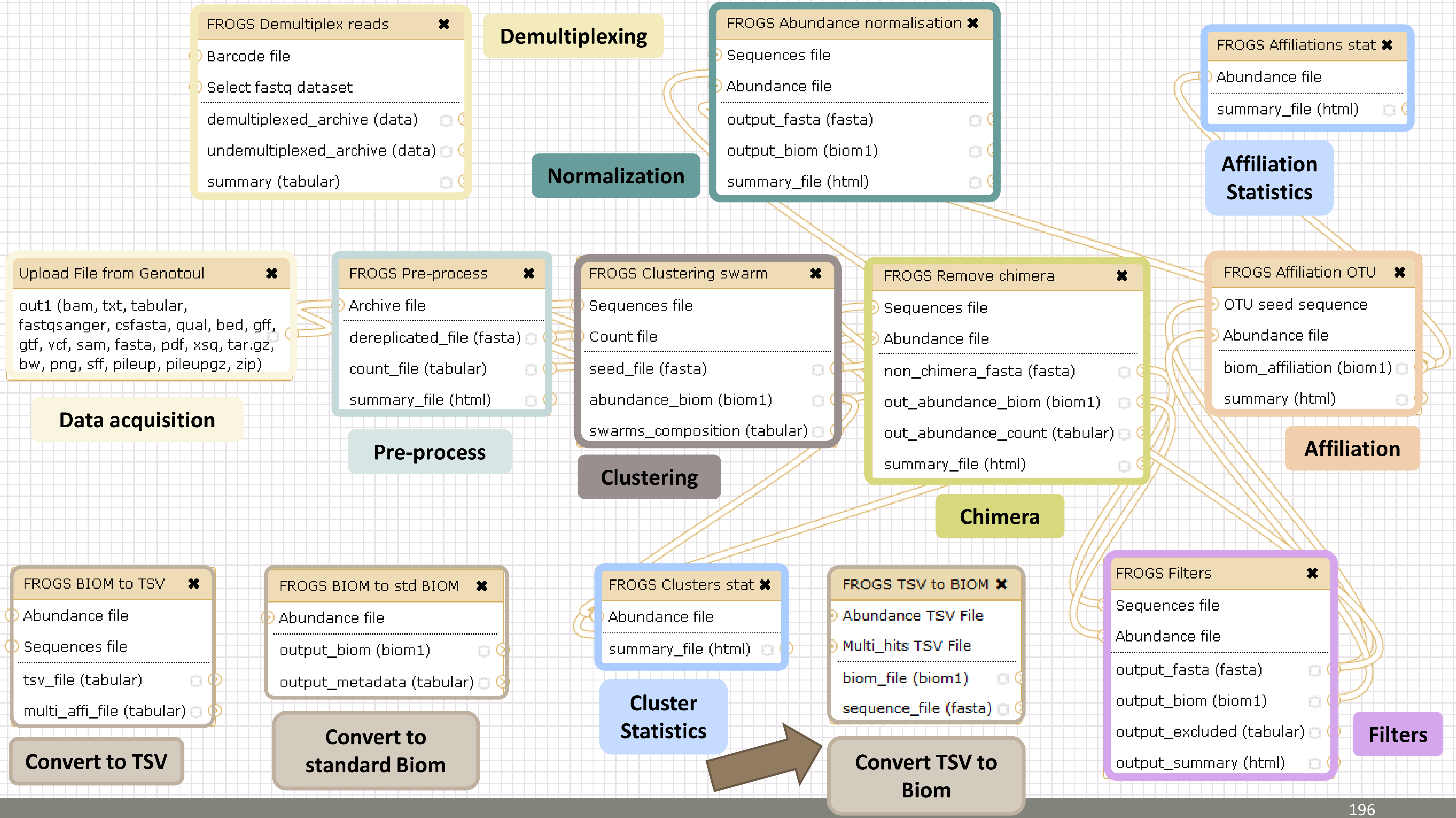

Taxonomy distribution
Alignment distribution
0k
20k
40k
60k
80k
Coverage
Identity
by OTUs
by sequences

Number of sequences among their alignment results

	[0% - 50%[	[50% - 80%[	[80% - 90%[	[90% - 95%[	[95% - 100%[	[100%]
[100%]	0	0	0	0	1657	74495
[95% - 100%[	0	0	0	0	[illegible]	7
[90% - 95%[	0	0	0	0	0	0
[80% - 90%[	0	0	0	0	0	0
[50% - 80%[	0	0	0	0	0	0
[0% - 50%[	0	0	0	0	0	0

Identity: 4%
Coverage: 5%
Nb sequences: 1657

TSV to BIOM


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
FROGS Abundance normalisation
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
summary_file (html)
FROGS Affiliations stat
Abundance file
summary_file (html)
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
FROGS BIOM to TSV
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)
FROGS BIOM to std BIOM
Abundance file
output_biom (biom1)
output_metadata (tabular)
FROGS Clusters stat
Abundance file
summary_file (html)
FROGS TSV to BIOM
Abundance TSV File
Multi_hits TSV File
biom_file (biom1)
sequence_file (fasta)
FROGS Filters
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

Demultiplexing

Normalization

Affiliation Statistics

Data acquisition

Pre-process

Clustering

Chimera

Affiliation

Convert to TSV

Convert to standard Biom

Cluster Statistics

Convert TSV to Biom

Filters

TSV to BIOM

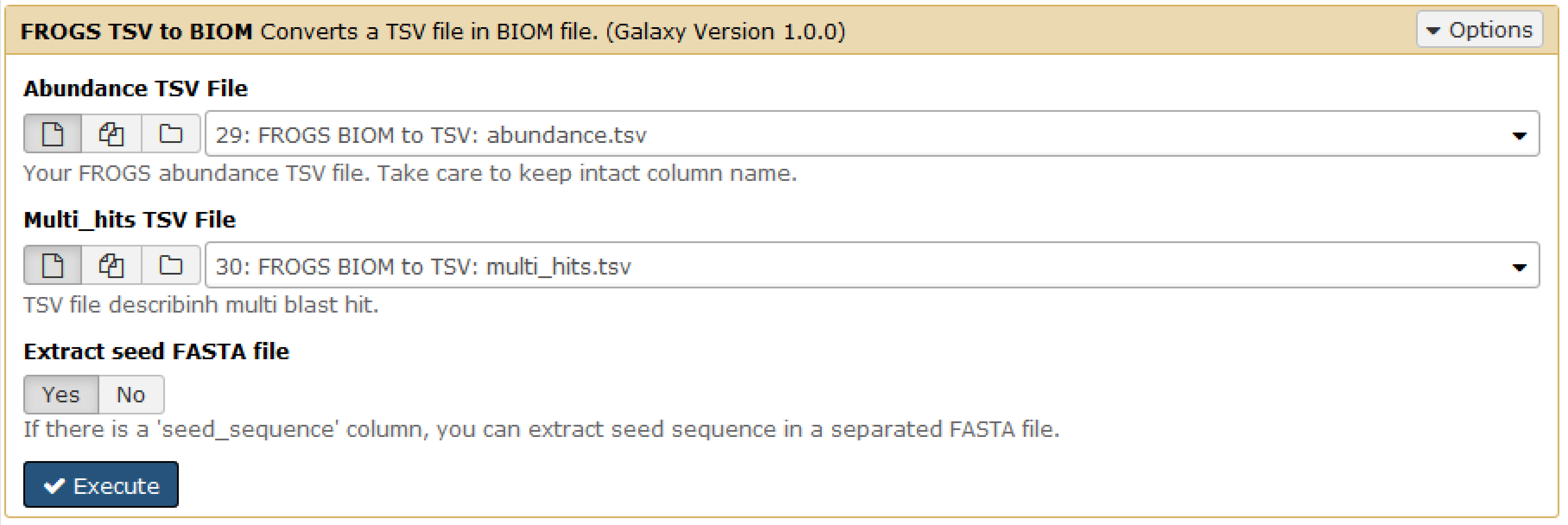
After modifying your abundance TSV file you can again:

- generate rarefaction curve
- sunburst

Careful :

- do not modify column name
- do not remove column
- take care to choose a taxonomy available in your multi_hit TSV file
- if deleting line from multi_hit, take care to not remove a complete cluster without removing all "multi tags" in you abundance TSV file.
- if you want to rename a taxon level (ex : genus "Ruminiclostridium 5;" to genus "Ruminiclostridium;"), do not forget to modify also your multi_hit TSV file.

TSV to BIOM



FROGS TSV to BIOM Converts a TSV file in BIOM file. (Galaxy Version 1.0.0)

Abundance TSV File

29: FROGS BIOM to TSV: abundance.tsv

Your FROGS abundance TSV file. Take care to keep intact column name.

Multi_hits TSV File

30: FROGS BIOM to TSV: multi_hits.tsv

TSV file describinh multi blast hit.

Extract seed FASTA file

[✓ Yes] No

If there is a 'seed_sequence' column, you can extract seed sequence in a separated FASTA file.

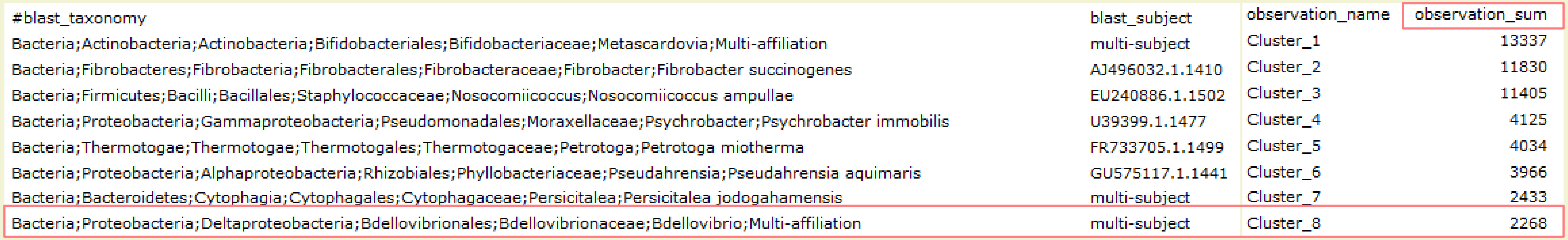
Your Turn! – 8

PLAY WITH TSV_TO_BIOM

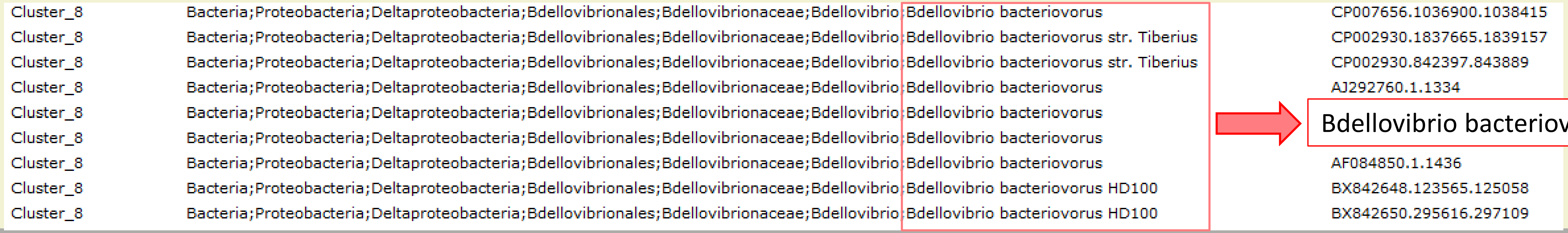
Exercise 8

→ objectives : Play with multi-affiliation and TSV_to_BIOM

1. Observe in Multi_hit.tsv and abundance.tsv cluster_8 annotation



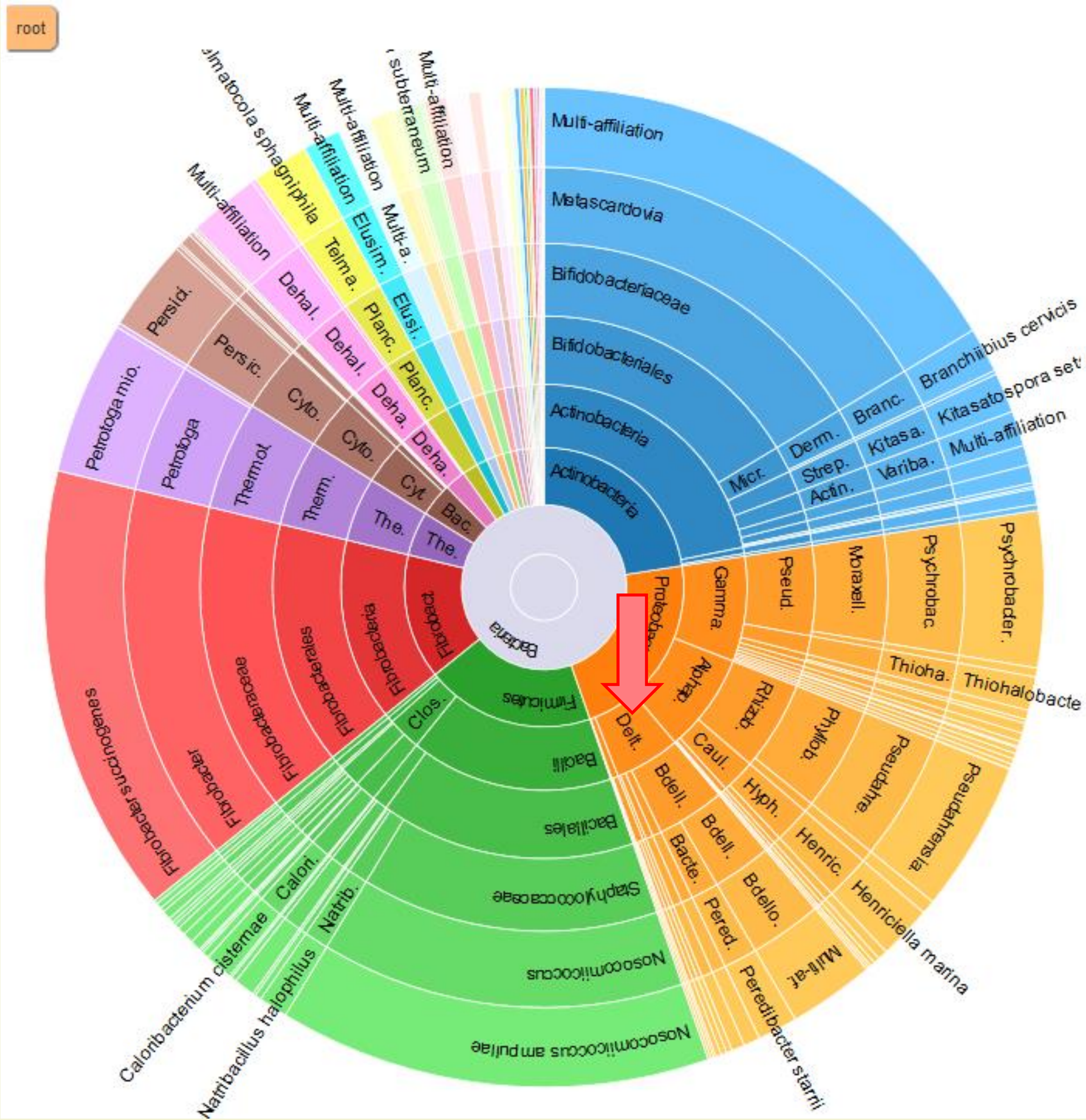
#blast_taxonomy	blast_subject	observation_name	observation_sum
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Metascardovia;Multi-affiliation	multi-subject	Cluster_1	13337
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter;Fibrobacter succinogenes	AJ496032.1.1410	Cluster_2	11830
Bacteria;Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Nosocomiicoccus;Nosocomiicoccus ampullae	EU240886.1.1502	Cluster_3	11405
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;Psychrobacter immobilis	U39399.1.1477	Cluster_4	4125
Bacteria;Thermotogae;Thermotogae;Thermotogales;Thermotogaceae;Petrotoga;Petrotoga miotherma	FR733705.1.1499	Cluster_5	4034
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia;Pseudahrensia aquimaris	GU575117.1.1441	Cluster_6	3966
Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;Persicitalea;Persicitalea jodogahamensis	multi-subject	Cluster_7	2433
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio;Multi-affiliation	multi-subject	Cluster_8	2268

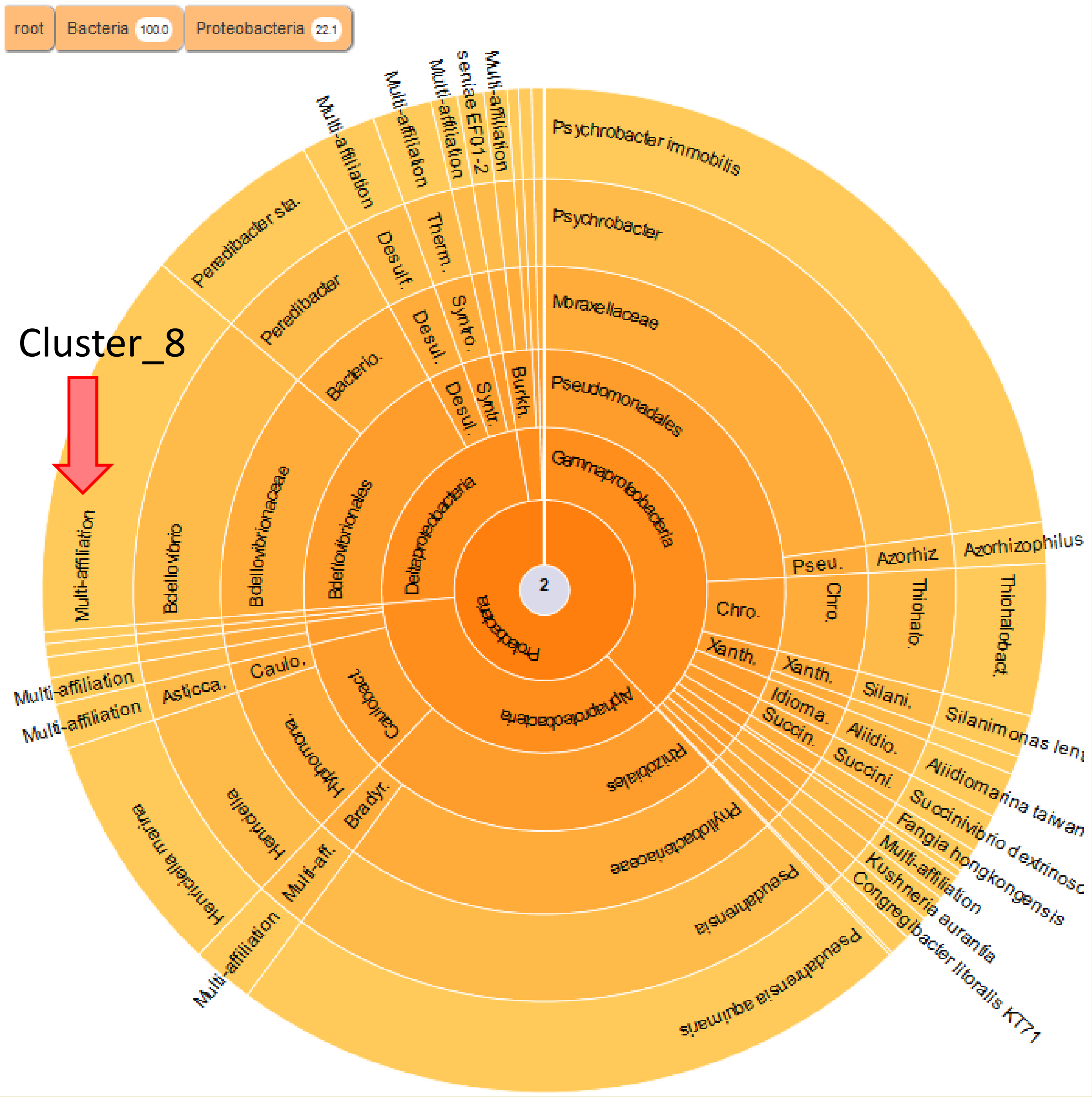


Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus	CP007656.1036900.1038415
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus str. Tiberius	CP002930.1837665.1839157
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus str. Tiberius	CP002930.842397.843889
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus	AJ292760.1.1334
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus	
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus	
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus	AF084850.1.1436
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus HD100	BX842648.123565.125058
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus HD100	BX842650.295616.297109

→ Bdellovibrio bacteriovorus

2. Observe le diversity diagramm





Cluster_8

Exercise 8

3. How to change affiliation of cluster 8 ????

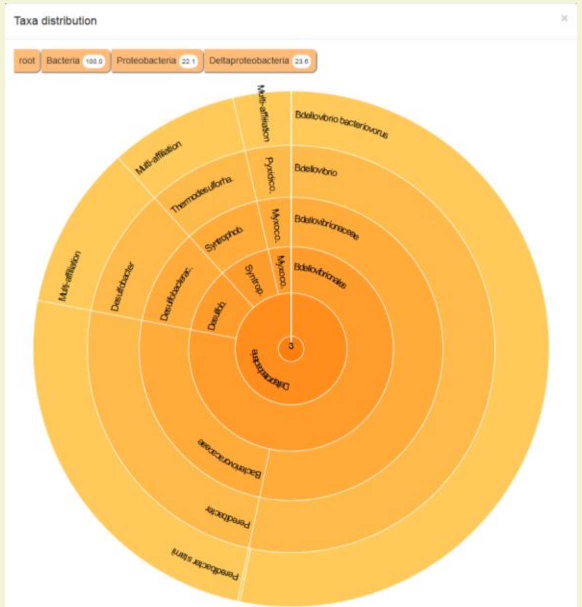
Exercise 8

4. Modify multi_hit.tsv and keep only :

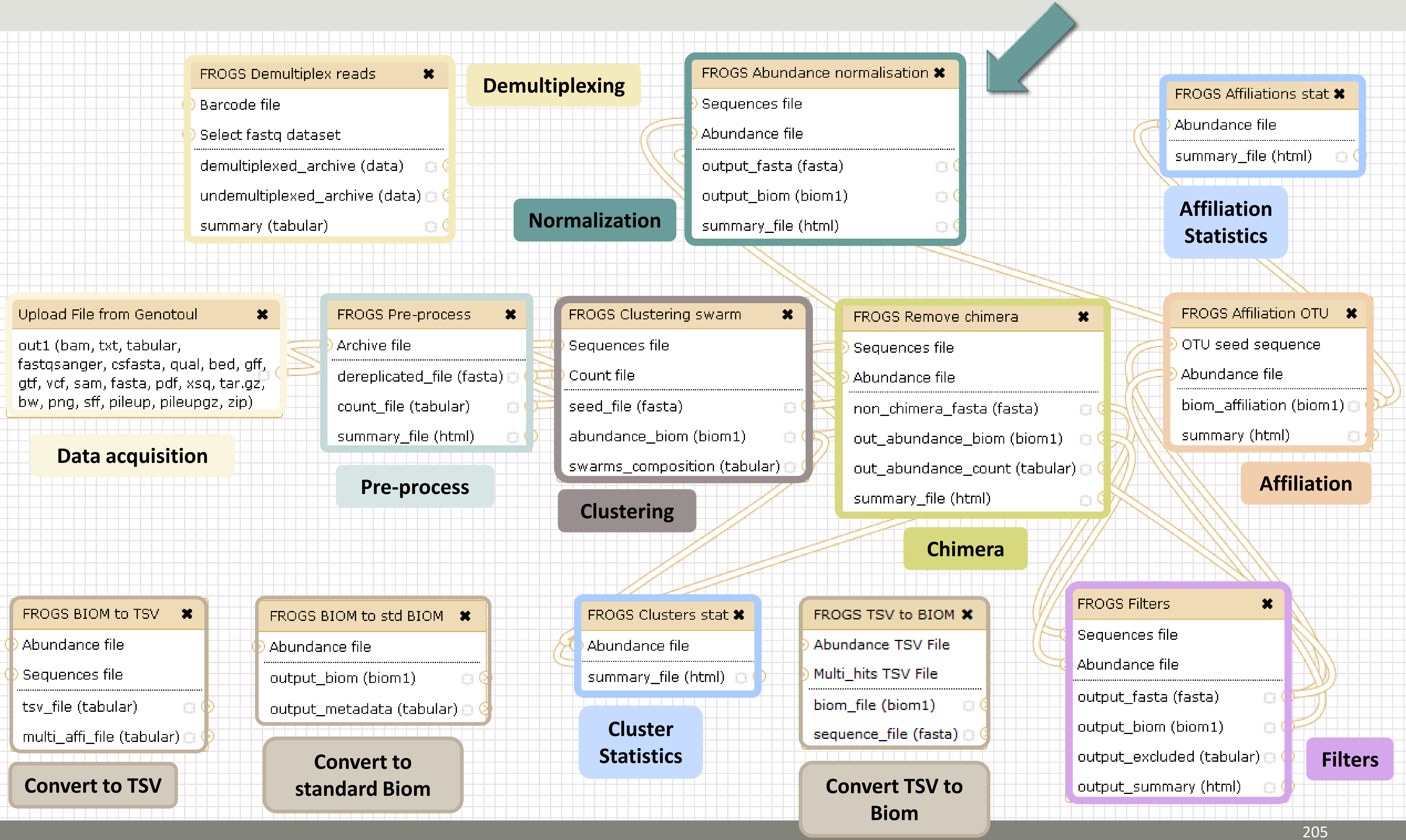
```
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio;Bdellovibrio bacteriovorus	CP007656.1036900.1038415
```

Careful, <u>no quotes </u>around text !!!

5. Upload the new multihit file.
6. Create a new biom with a TSV_to_BIOM tool
7. Launch again the affilation_stat tool on this new biom
8. Observe the diversity diagram


Taxa distribution
root
Bacteria 100.0
Proteobacteria 22.1
Deltaproteobacteria 23.6
Multi-affiliation
Bdellovibrio bacteriovorus
Bdellovibrio
Bdellovibrionaceae
Bdellovibrionales
Multi-affiliation
Multi-affiliation
Deltaproteobacteria

Normalization


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Demultiplexing
FROGS Abundance normalisation
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
summary_file (html)
Normalization
FROGS Affiliations stat
Abundance file
summary_file (html)
Affiliation Statistics
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
Data acquisition
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
Pre-process
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
Clustering
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
Chimera
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
Affiliation
FROGS BIOM to TSV
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)
Convert to TSV
FROGS BIOM to std BIOM
Abundance file
output_biom (biom1)
output_metadata (tabular)
Convert to standard Biom
FROGS Clusters stat
Abundance file
summary_file (html)
Cluster Statistics
FROGS TSV to BIOM
Abundance TSV File
Multi_hits TSV File
biom_file (biom1)
sequence_file (fasta)
Convert TSV to Biom
FROGS Filters
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)
Filters

Normalization

Conserve a predefined number of sequence per sample:

- update Biom abundance file
- update seed fasta file

May be used when :

  - Low sequencing sample
  - Required for some statistical methods to compare the samples in pairs

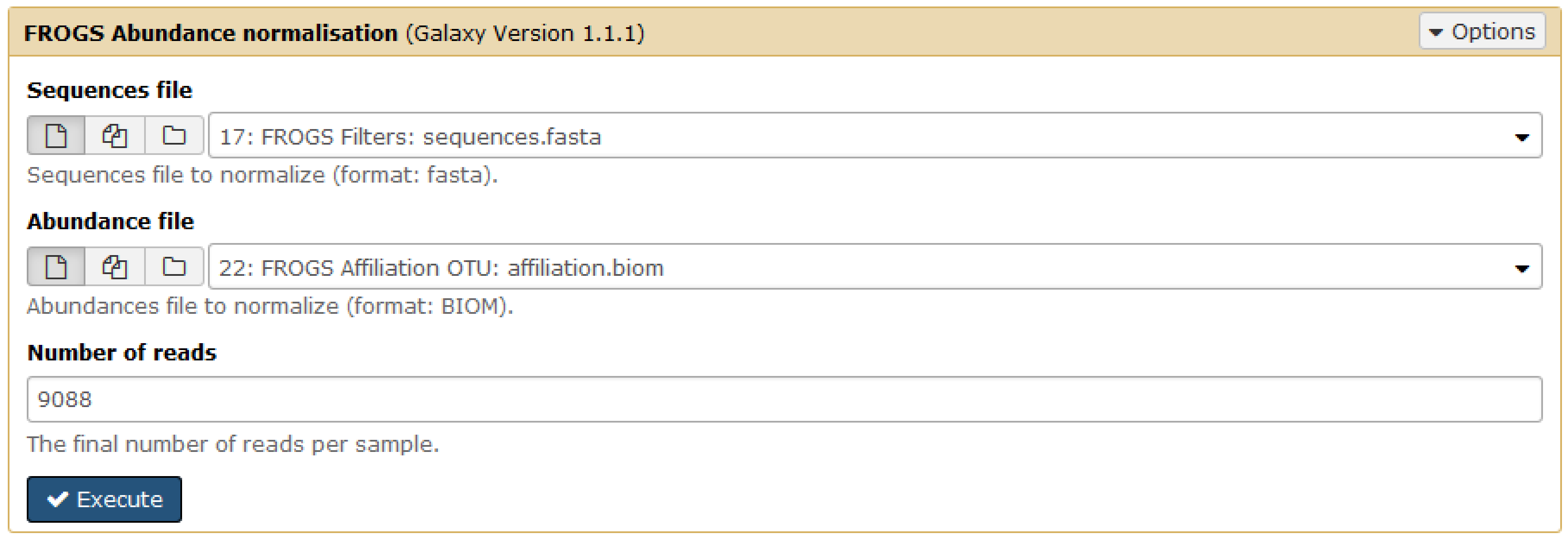
Your Turn! – 9

LAUNCH NORMALIZATION TOOL

Exercise 9

Launch Normalization Tool

1. What is the smallest sequenced samples ?
2. Normalize your data from Affiliation based on this number of sequence
3. Explore the report HTML result.
4. Try other threshold and explore the report HTML result
   What do you remark ?



FROGS Abundance normalisation (Galaxy Version 1.1.1)

Sequences file

17: FROGS Filters: sequences.fasta

Sequences file to normalize (format: fasta).

Abundance file

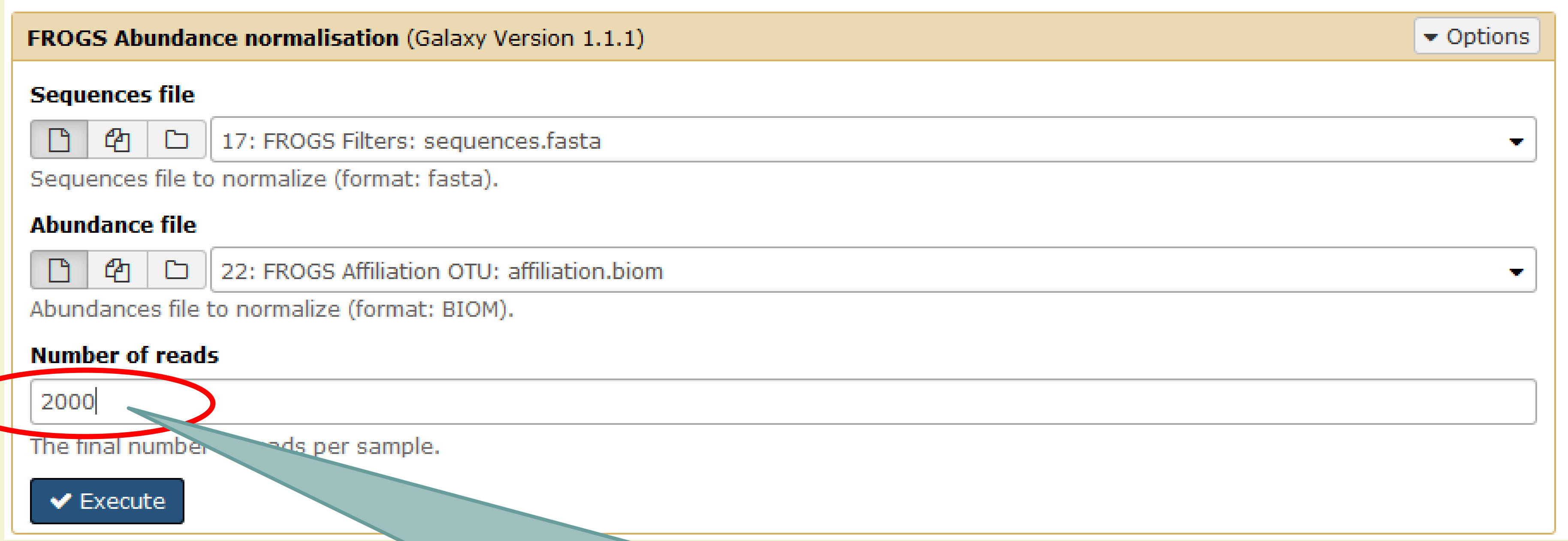
22: FROGS Affiliation OTU: affiliation.biom

Abundances file to normalize (format: BIOM).

Number of reads

9088

The final number of reads per sample.



FROGS Abundance normalisation (Galaxy Version 1.1.1)

Sequences file

17: FROGS Filters: sequences.fasta

Sequences file to normalize (format: fasta).

Abundance file

22: FROGS Affiliation OTU: affiliation.biom

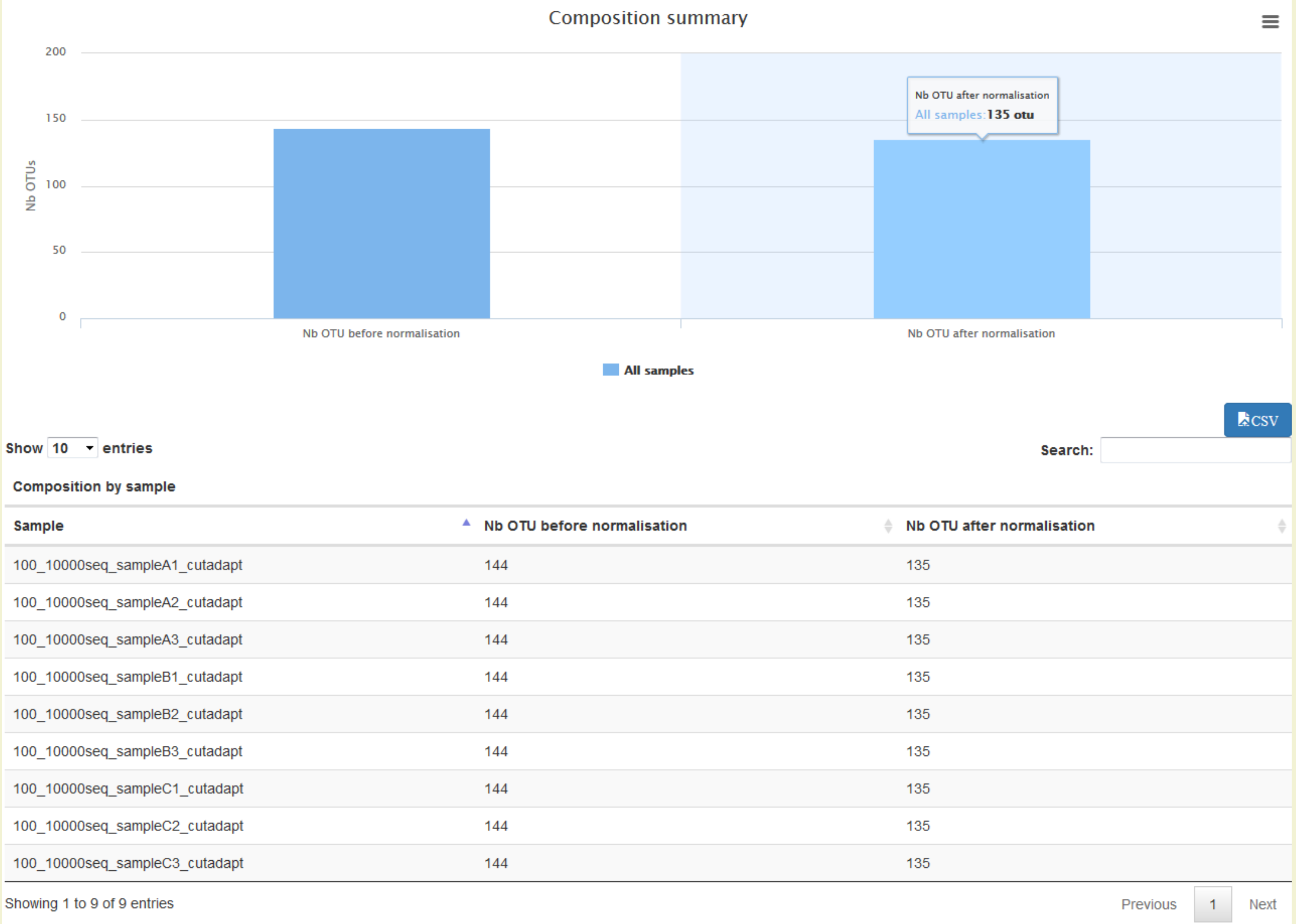
Abundances file to normalize (format: BIOM).

Number of reads

2000

The final numbe ds per sample.

Or, this number can be chosen according to the rarefaction curve. For example, we can choose the smallest number of sequences that still retain all the genus.


Composition summary
200
150
100
50
0
Nb OTUs
Nb OTU after normalisation
All samples: 135 otu
Nb OTU before normalisation
Nb OTU after normalisation
All samples
CSV
Show 10 entries
Search:

Composition by sample

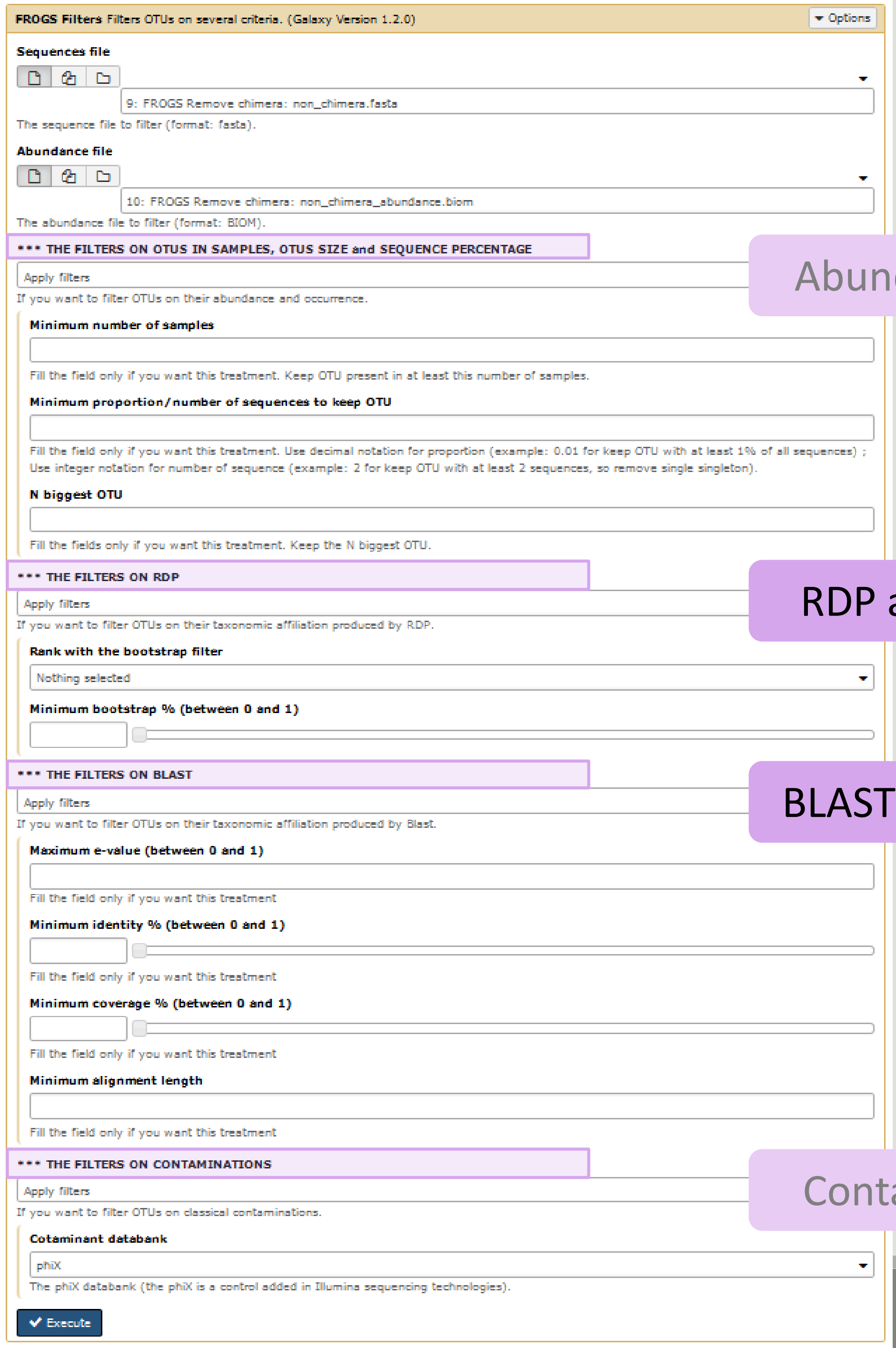
Sample	Nb OTU before normalisation	Nb OTU after normalisation
100_10000seq_sampleA1_cutadapt	144	135
100_10000seq_sampleA2_cutadapt	144	135
100_10000seq_sampleA3_cutadapt	144	135
100_10000seq_sampleB1_cutadapt	144	135
100_10000seq_sampleB2_cutadapt	144	135
100_10000seq_sampleB3_cutadapt	144	135
100_10000seq_sampleC1_cutadapt	144	135
100_10000seq_sampleC2_cutadapt	144	135
100_10000seq_sampleC3_cutadapt	144	135

Showing 1 to 9 of 9 entries

Previous
1
Next

Filters on affiliations

Do not forget, with filter tool we can filter the data based on their affiliation



FROGS Filters Filters OTUs on several criteria. (Galaxy Version 1.2.0)

Sequences file

9: FROGS Remove chimera: non_chimera.fasta

The sequence file to filter (format: fasta).

Abundance file

10: FROGS Remove chimera: non_chimera_abundance.biom

The abundance file to filter (format: BIOM).

***** THE FILTERS ON OTUS IN SAMPLES, OTUS SIZE and SEQUENCE PERCENTAGE**

Apply filters

If you want to filter OTUs on their abundance and occurrence.

Minimum number of samples

Fill the field only if you want this treatment. Keep OTU present in at least this number of samples.

Minimum proportion/number of sequences to keep OTU

Fill the field only if you want this treatment. Use decimal notation for proportion (example: 0.01 for keep OTU with at least 1% of all sequences) ; Use integer notation for number of sequence (example: 2 for keep OTU with at least 2 sequences, so remove single singleton).

N biggest OTU

Fill the fields only if you want this treatment. Keep the N biggest OTU.

***** THE FILTERS ON RDP**

Apply filters

If you want to filter OTUs on their taxonomic affiliation produced by RDP.

Rank with the bootstrap filter

Nothing selected

Minimum bootstrap % (between 0 and 1)

***** THE FILTERS ON BLAST**

Apply filters

If you want to filter OTUs on their taxonomic affiliation produced by Blast.

Maximum e-value (between 0 and 1)

Fill the field only if you want this treatment

Minimum identity % (between 0 and 1)

Fill the field only if you want this treatment

Minimum coverage % (between 0 and 1)

Fill the field only if you want this treatment

Minimum alignment length

Fill the field only if you want this treatment

***** THE FILTERS ON CONTAMINATIONS**

Apply filters

If you want to filter OTUs on classical contaminations.

Cotaminant databank

phiX

The phiX databank (the phiX is a control added in Illumina sequencing technologies).

Abundance filters

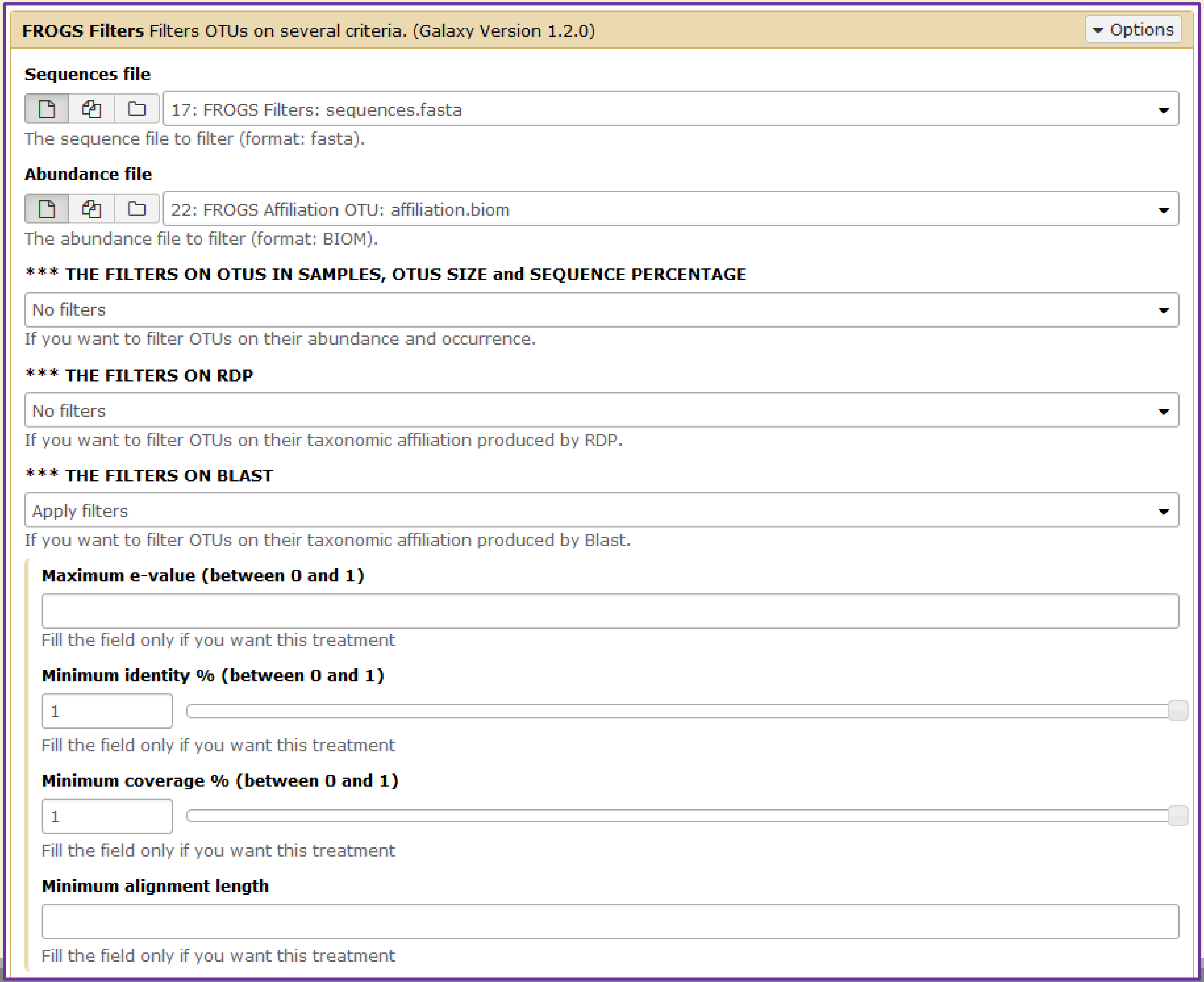
RDP affiliation filters

BLAST affiliation filters

Contamination filter

Exercise 10

1. Apply filters to keep only data with perfect alignment.
2. How many clusters have you keep ?



FROGS Filters Filters OTUs on several criteria. (Galaxy Version 1.2.0)

Sequences file

17: FROGS Filters: sequences.fasta

The sequence file to filter (format: fasta).

Abundance file

22: FROGS Affiliation OTU: affiliation.biom

The abundance file to filter (format: BIOM).

***** THE FILTERS ON OTUS IN SAMPLES, OTUS SIZE and SEQUENCE PERCENTAGE**

No filters

If you want to filter OTUs on their abundance and occurrence.

***** THE FILTERS ON RDP**

No filters

If you want to filter OTUs on their taxonomic affiliation produced by RDP.

***** THE FILTERS ON BLAST**

Apply filters

If you want to filter OTUs on their taxonomic affiliation produced by Blast.

Maximum e-value (between 0 and 1)

Fill the field only if you want this treatment

Minimum identity % (between 0 and 1)

1

Fill the field only if you want this treatment

Minimum coverage % (between 0 and 1)

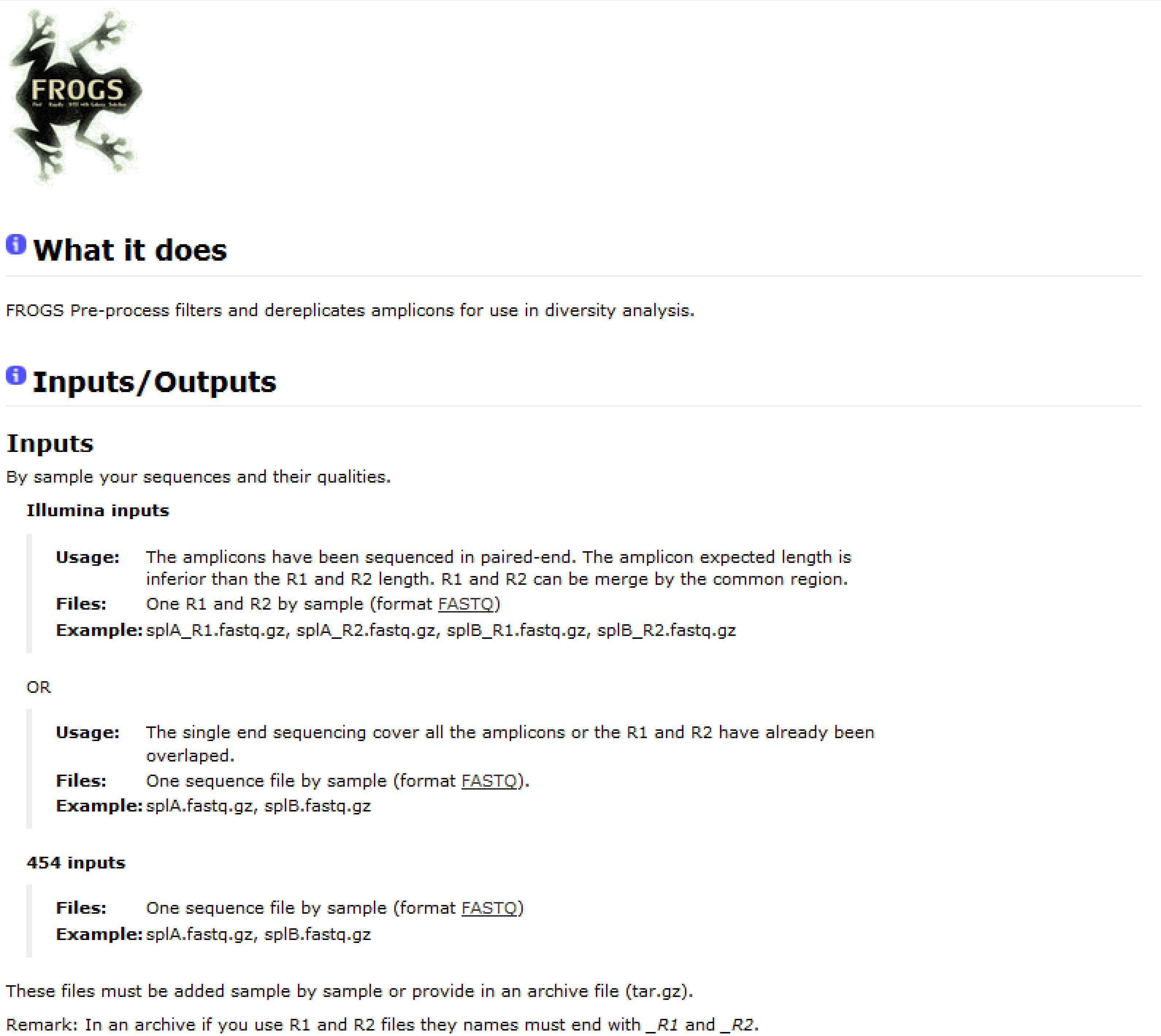
1

Fill the field only if you want this treatment

Minimum alignment length

Fill the field only if you want this treatment

Tool descriptions



What it does

FROGS Pre-process filters and dereplicates amplicons for use in diversity analysis.

Inputs/Outputs

Inputs

By sample your sequences and their qualities.

Illumina inputs

> **Usage:** The amplicons have been sequenced in paired-end. The amplicon expected length is inferior than the R1 and R2 length. R1 and R2 can be merge by the common region.
> **Files:** One R1 and R2 by sample (format FASTQ)
> **Example:** splA_R1.fastq.gz, splA_R2.fastq.gz, splB_R1.fastq.gz, splB_R2.fastq.gz

OR

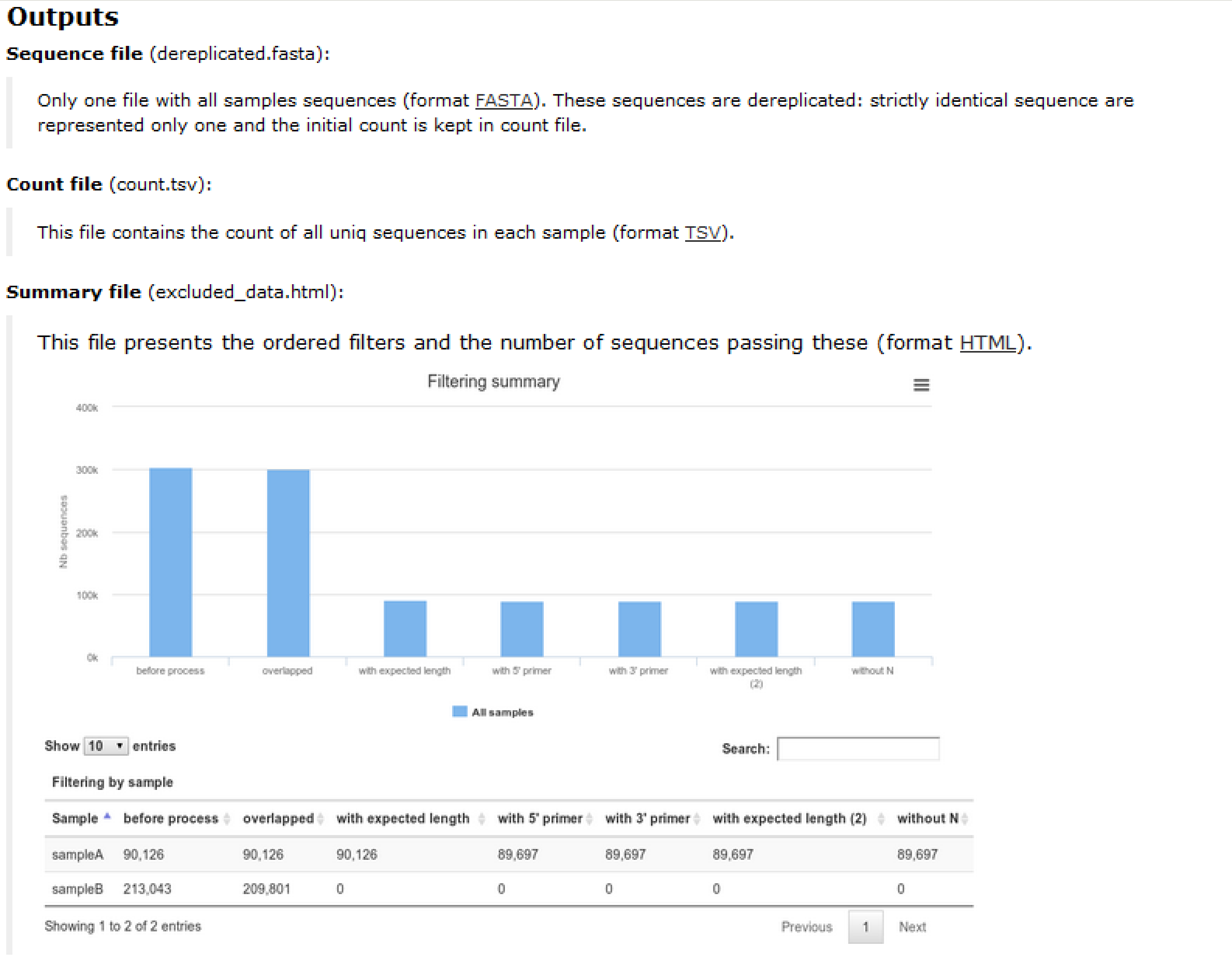
> **Usage:** The single end sequencing cover all the amplicons or the R1 and R2 have already been overlaped.
> **Files:** One sequence file by sample (format FASTQ).
> **Example:** splA.fastq.gz, splB.fastq.gz

454 inputs

> **Files:** One sequence file by sample (format FASTQ)
> **Example:** splA.fastq.gz, splB.fastq.gz

These files must be added sample by sample or provide in an archive file (tar.gz).

Remark: In an archive if you use R1 and R2 files they names must end with _*R1* and _*R2*.



Outputs

Sequence file (dereplicated.fasta):

> Only one file with all samples sequences (format FASTA). These sequences are dereplicated: strictly identical sequence are represented only one and the initial count is kept in count file.

Count file (count.tsv):

> This file contains the count of all uniq sequences in each sample (format TSV).

Summary file (excluded_data.html):

> This file presents the ordered filters and the number of sequences passing these (format HTML).

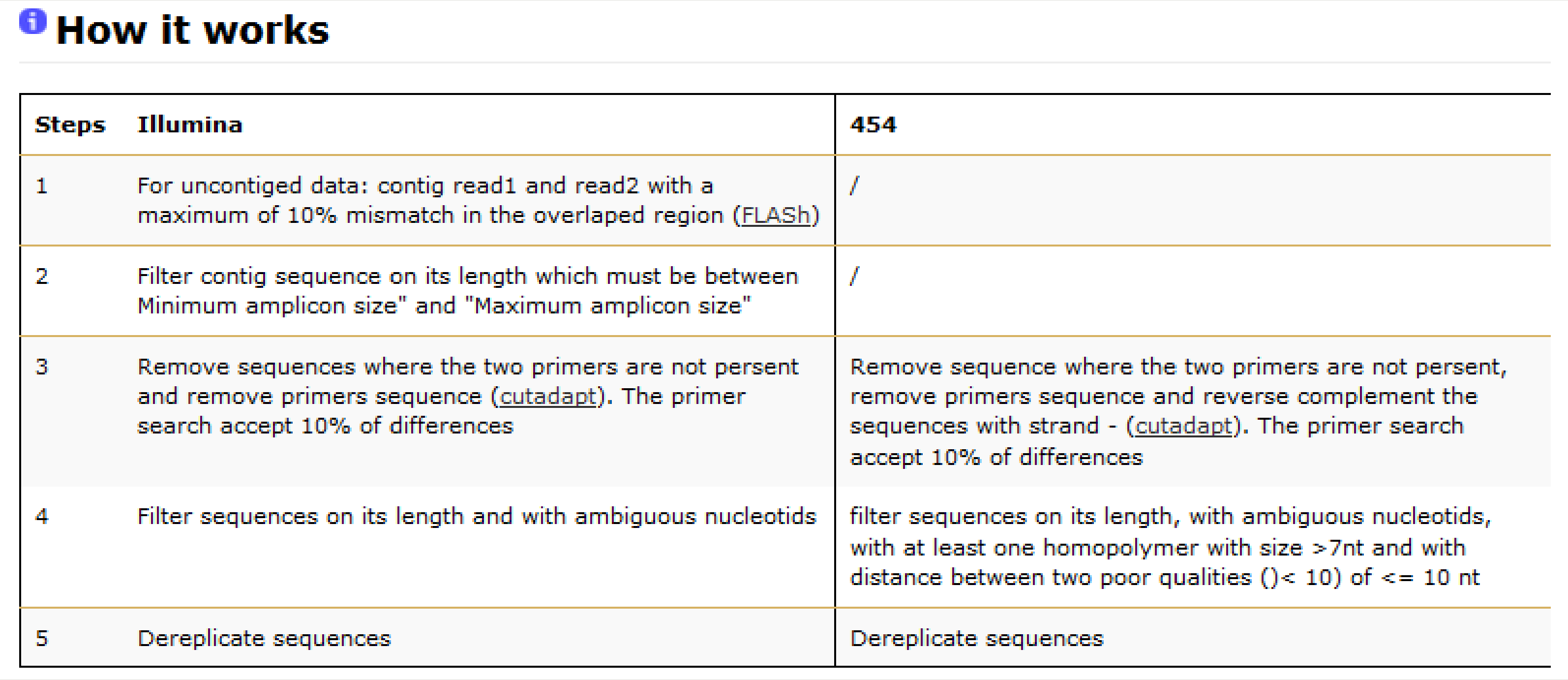
Filtering summary
≡
400k
300k
200k
100k
0k
Nb sequences
before process
overlapped
with expected length
with 5' primer
with 3' primer
with expected length (2)
without N
All samples
Show 10 ▾ entries
Search:

Filtering by sample

Sample ▲	before process	overlapped	with expected length	with 5' primer	with 3' primer	with expected length (2)	without N
sampleA	90,126	90,126	90,126	89,697	89,697	89,697	89,697
sampleB	213,043	209,801	0	0	0	0	0

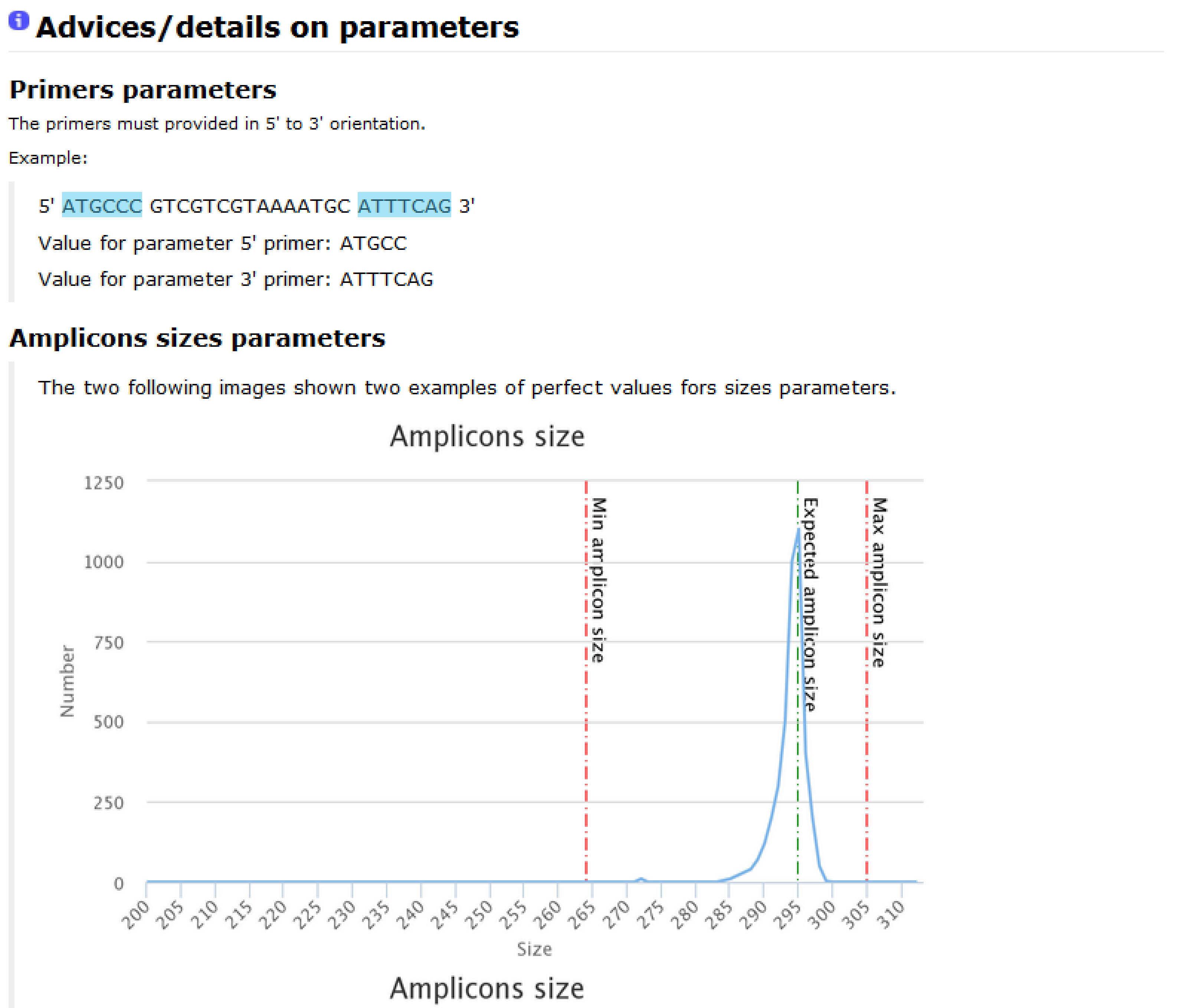
Showing 1 to 2 of 2 entries

Previous
1
Next



How it works

Steps	Illumina	454
1	For uncontiged data: contig read1 and read2 with a maximum of 10% mismatch in the overlaped region (FLASh)	/
2	Filter contig sequence on its length which must be between Minimum amplicon size" and "Maximum amplicon size"	/
3	Remove sequences where the two primers are not persent and remove primers sequence (cutadapt). The primer search accept 10% of differences	Remove sequence where the two primers are not persent, remove primers sequence and reverse complement the sequences with strand - (cutadapt). The primer search accept 10% of differences
4	Filter sequences on its length and with ambiguous nucleotids	filter sequences on its length, with ambiguous nucleotids, with at least one homopolymer with size >7nt and with distance between two poor qualities ()< 10) of <= 10 nt
5	Dereplicate sequences	Dereplicate sequences



Advices/details on parameters

Primers parameters

The primers must provided in 5' to 3' orientation.

Example:

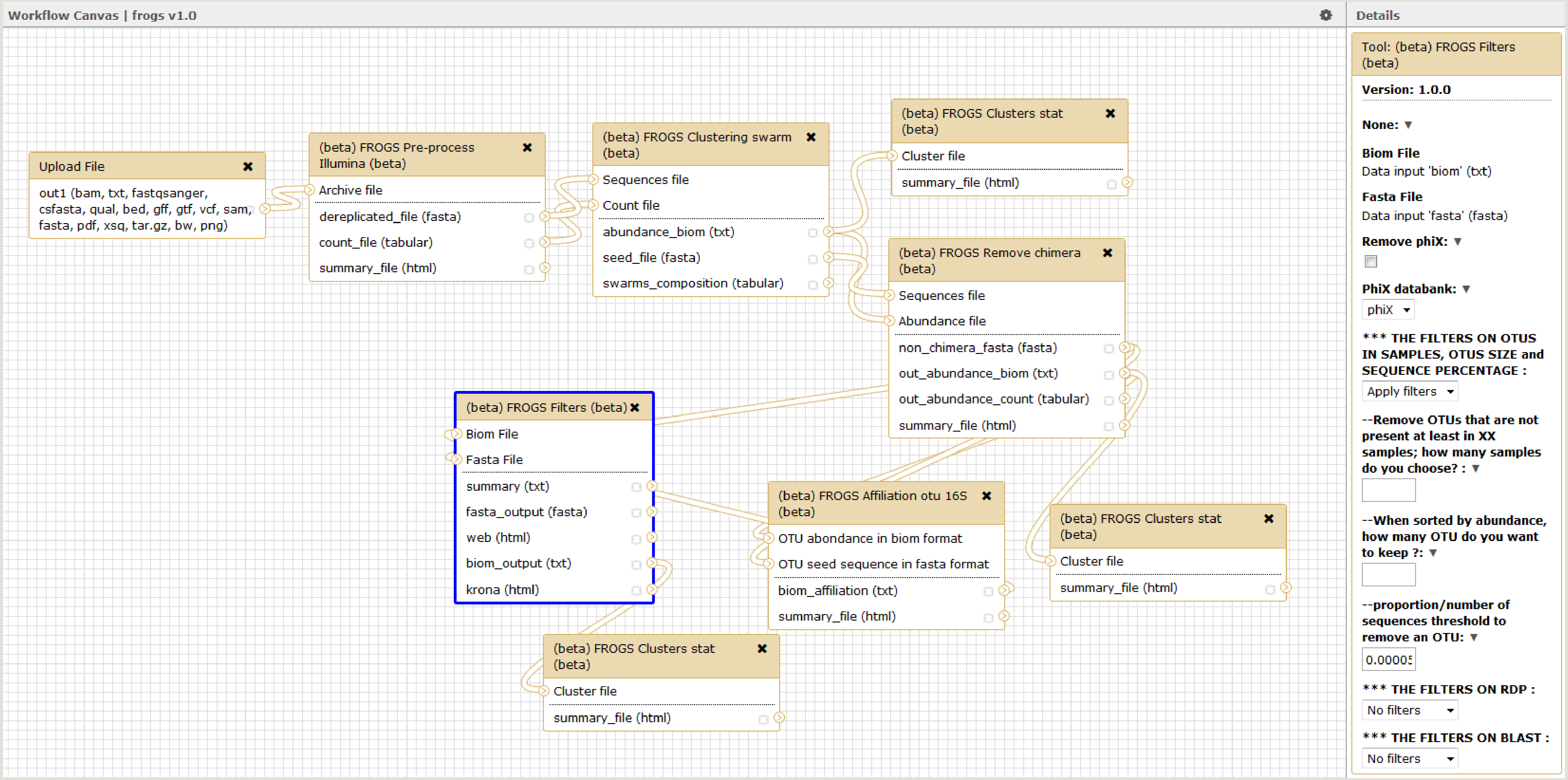
> 5' ATGCCC GTCGTCGTAAAATGC ATTTCAG 3'
>
> Value for parameter 5' primer: ATGCC
>
> Value for parameter 3' primer: ATTTCAG

Amplicons sizes parameters

> The two following images shown two examples of perfect values fors sizes parameters.

Amplicons size
1250
1000
750
500
250
0
Number
Min amplicon size
Expected amplicon size
Max amplicon size
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
Size
Amplicons size

Workflow creation


Workflow Canvas | frogs v1.0
Upload File
out1 (bam, txt, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png)
(beta) FROGS Pre-process Illumina (beta)
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
(beta) FROGS Clustering swarm (beta)
Sequences file
Count file
abundance_biom (txt)
seed_file (fasta)
swarms_composition (tabular)
(beta) FROGS Clusters stat (beta)
Cluster file
summary_file (html)
(beta) FROGS Remove chimera (beta)
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (txt)
out_abundance_count (tabular)
summary_file (html)
(beta) FROGS Filters (beta)
Biom File
Fasta File
summary (txt)
fasta_output (fasta)
web (html)
biom_output (txt)
krona (html)
(beta) FROGS Affiliation otu 16S (beta)
OTU abondance in biom format
OTU seed sequence in fasta format
biom_affiliation (txt)
summary_file (html)
(beta) FROGS Clusters stat (beta)
Cluster file
summary_file (html)
(beta) FROGS Clusters stat (beta)
Cluster file
summary_file (html)
Details

Tool: (beta) FROGS Filters (beta)

Version: 1.0.0

None: ▼

Biom File
Data input 'biom' (txt)

Fasta File
Data input 'fasta' (fasta)

Remove phiX: ▼
☐

PhiX databank: ▼
phiX

***** THE FILTERS ON OTUS IN SAMPLES, OTUS SIZE and SEQUENCE PERCENTAGE :**
Apply filters

--Remove OTUs that are not present at least in XX samples; how many samples do you choose? : ▼

--When sorted by abundance, how many OTU do you want to keep ?: ▼

--proportion/number of sequences threshold to remove an OTU: ▼
0.0000

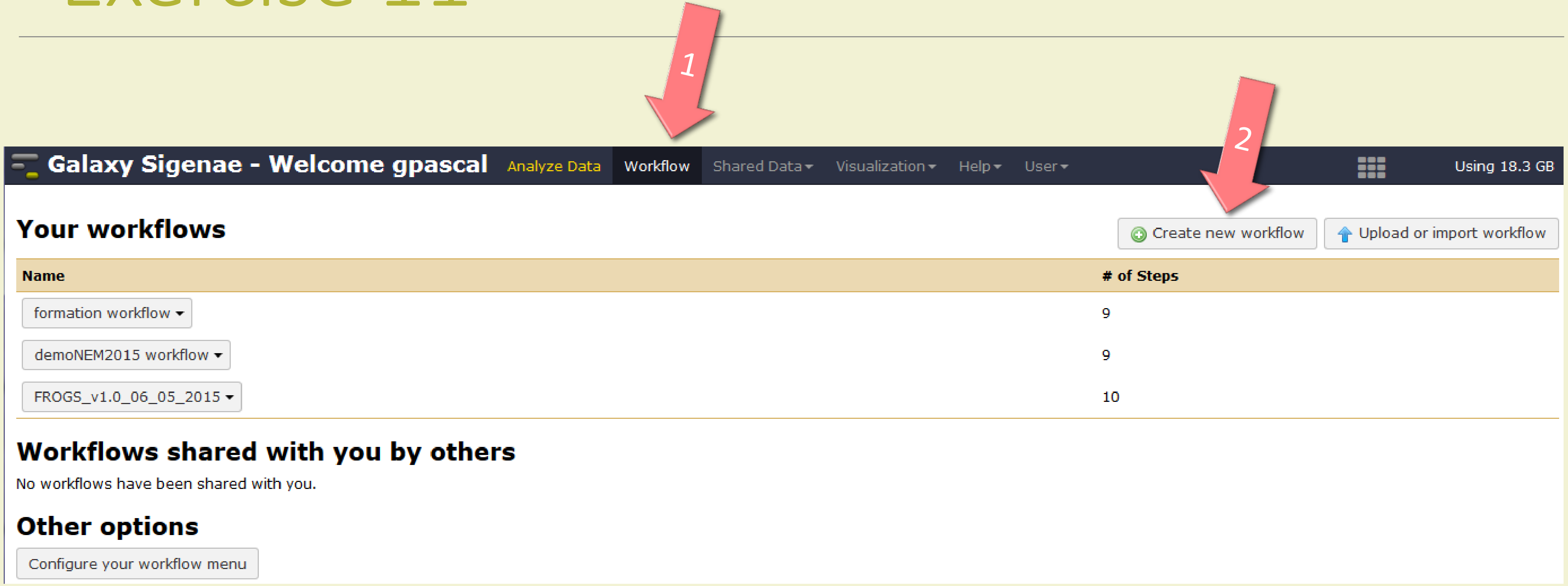
***** THE FILTERS ON RDP :**
No filters

***** THE FILTERS ON BLAST :**
No filters

Your Turn! – 11

CREATE YOUR OWN WORKFLOW !

Exercise 11


1
2
Galaxy Sigenae - Welcome gpascal
Analyze Data
Workflow
Shared Data
Visualization
Help
User
Using 18.3 GB
Create new workflow
Upload or import workflow
formation workflow
demoNEM2015 workflow
FROGS_v1.0_06_05_2015
Configure your workflow menu

Your workflows

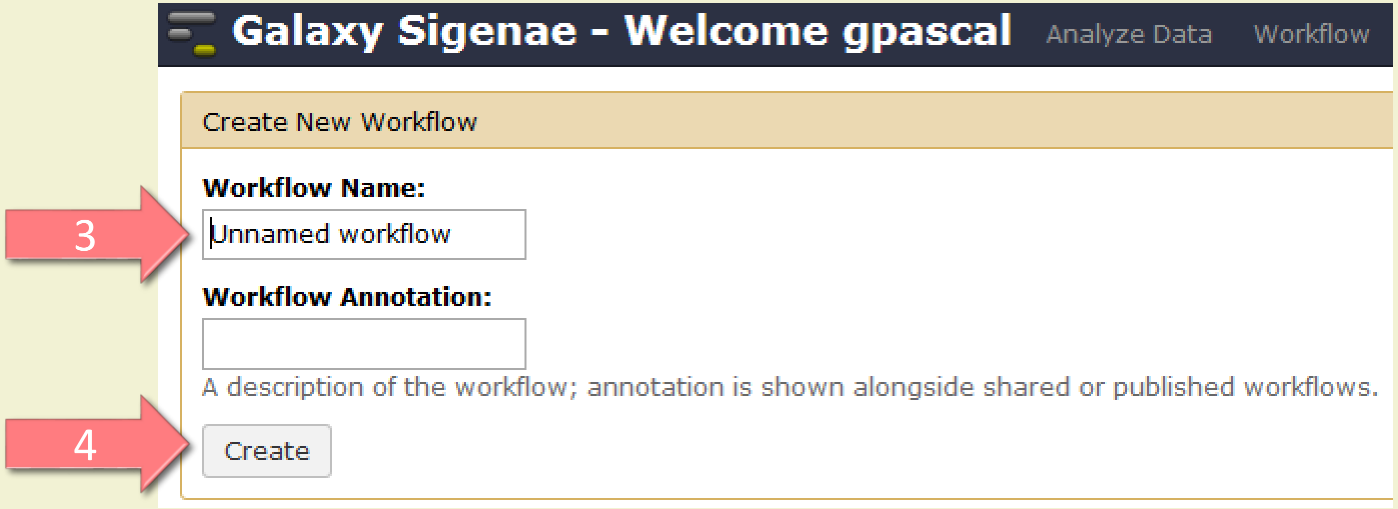
Name	# of Steps
formation workflow	9
demoNEM2015 workflow	9
FROGS_v1.0_06_05_2015	10

Workflows shared with you by others

No workflows have been shared with you.

Other options

Exercise 11


Galaxy Sigenae - Welcome gpascal
Analyze Data
Workflow
3
4
Create

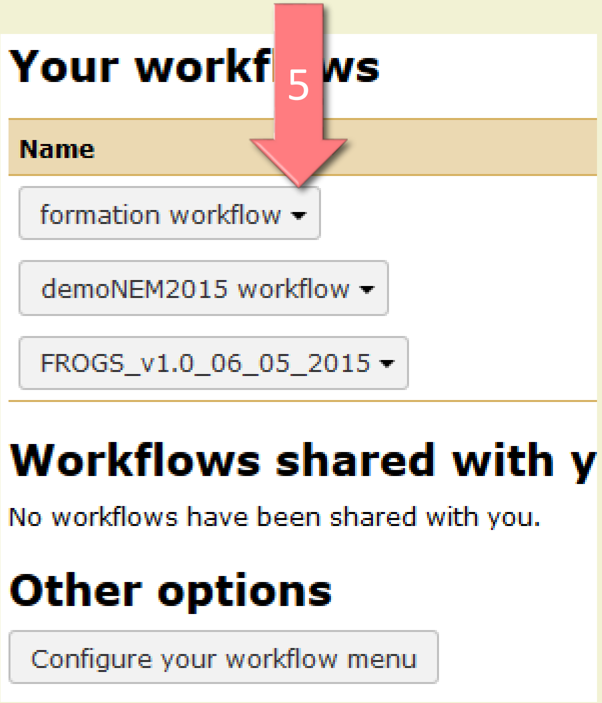
Create New Workflow

Workflow Name: Unnamed workflow

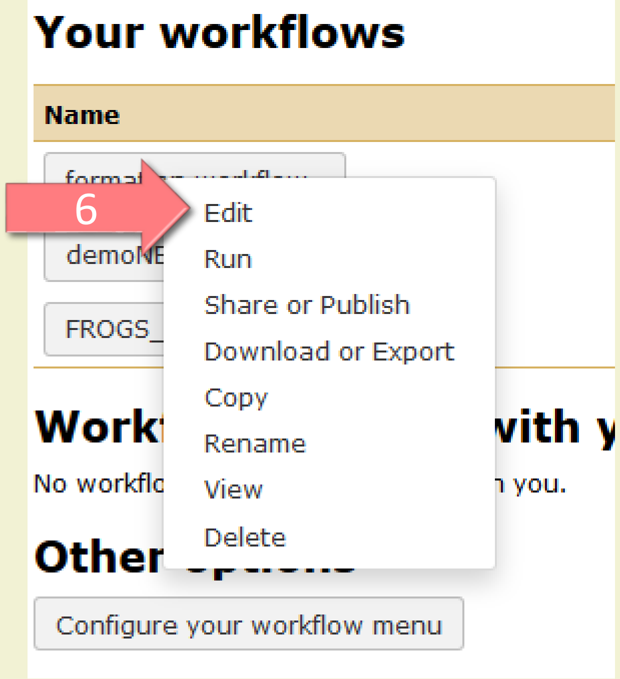
Workflow Annotation:

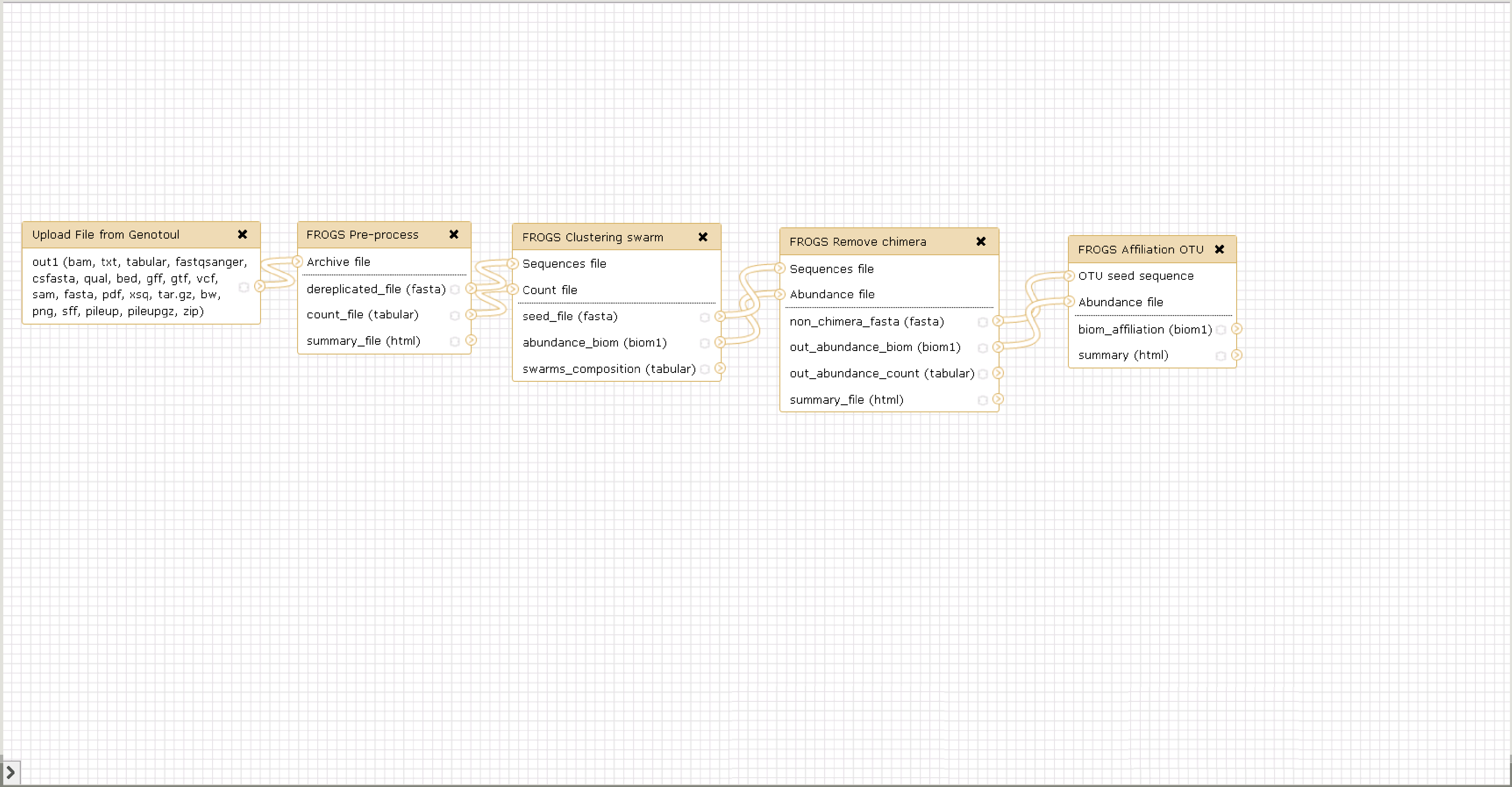
A description of the workflow; annotation is shown alongside shared or published workflows.

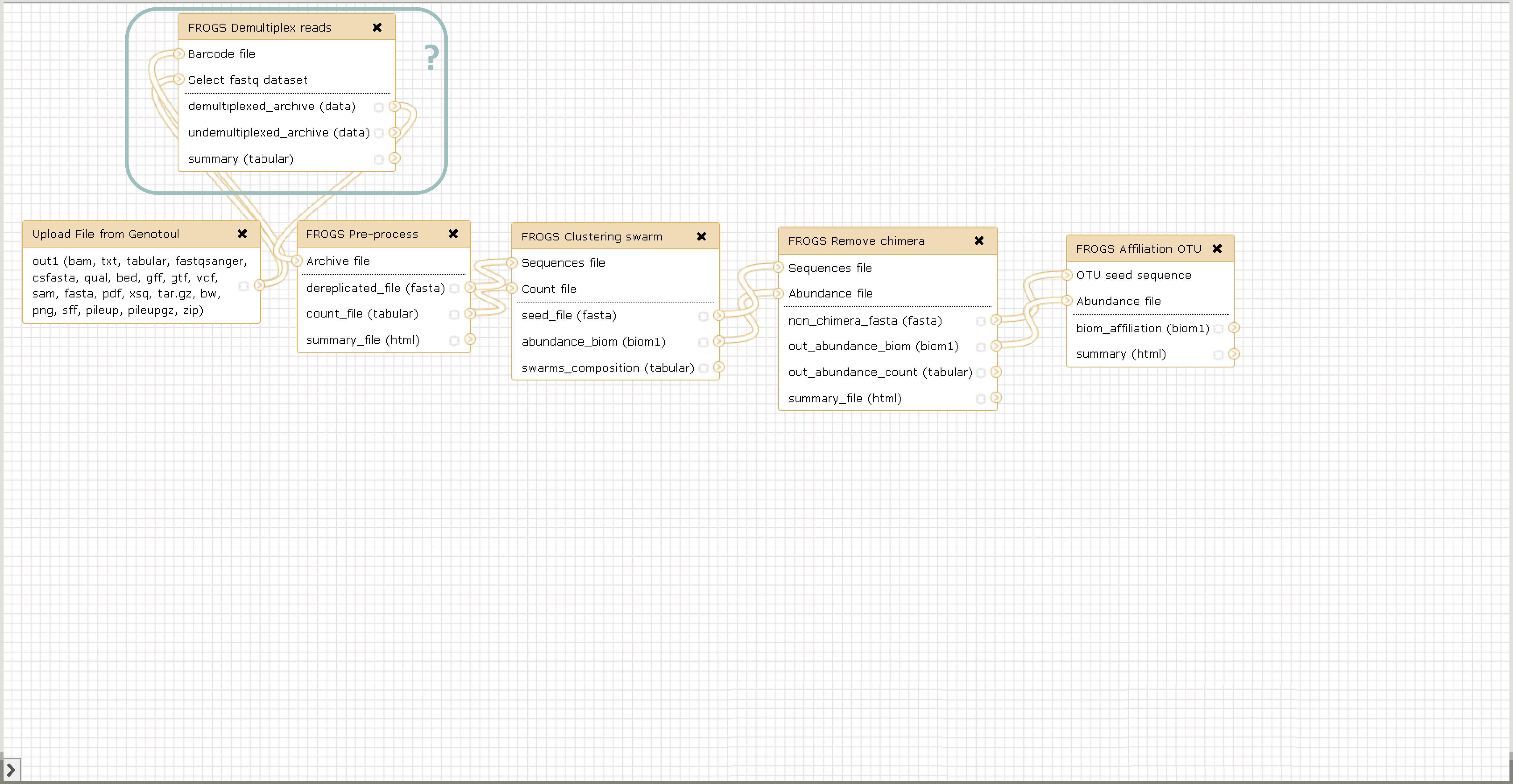
Exercise 11


5
Your workflows
Name
formation workflow
demoNEM2015 workflow
FROGS_v1.0_06_05_2015
Workflows shared with y
Other options
Configure your workflow menu

No workflows have been shared with you.


Your workflows
Name
6
Edit
Run
Share or Publish
Download or Export
Copy
Rename
View
Delete
Configure your workflow menu


Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)



FROGS Demultiplex reads ✖

Barcode file

Select fastq dataset

demultiplexed_archive (data)

undemultiplexed_archive (data)

summary (tabular)

Upload File from Genotoul ✖

out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

FROGS Pre-process ✖

Archive file

dereplicated_file (fasta)

count_file (tabular)

summary_file (html)

FROGS Clustering swarm ✖

Sequences file

Count file

seed_file (fasta)

abundance_biom (biom1)

swarms_composition (tabular)

FROGS Remove chimera ✖

Sequences file

Abundance file

non_chimera_fasta (fasta)

out_abundance_biom (biom1)

out_abundance_count (tabular)

summary_file (html)

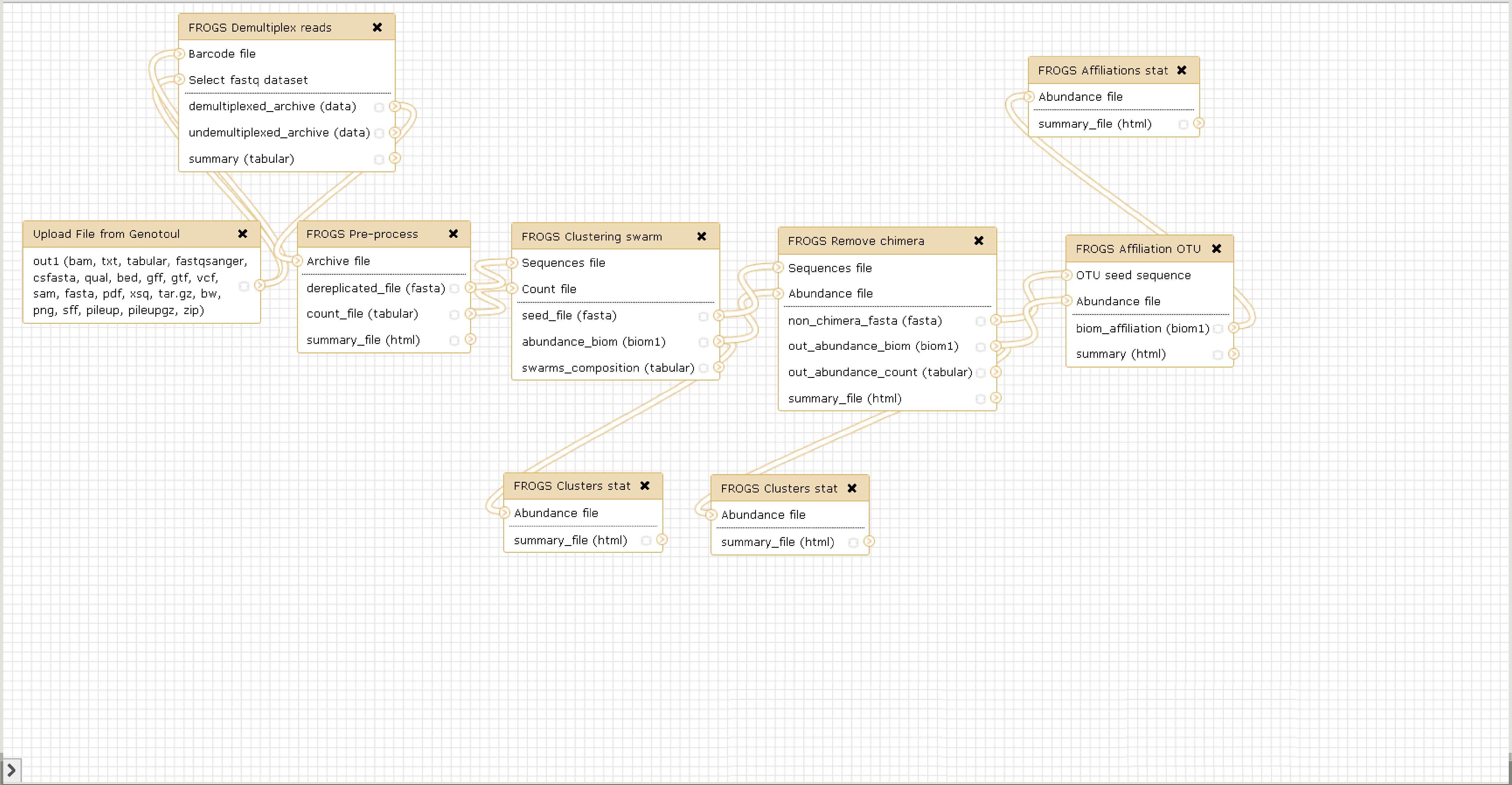
FROGS Affiliation OTU ✖

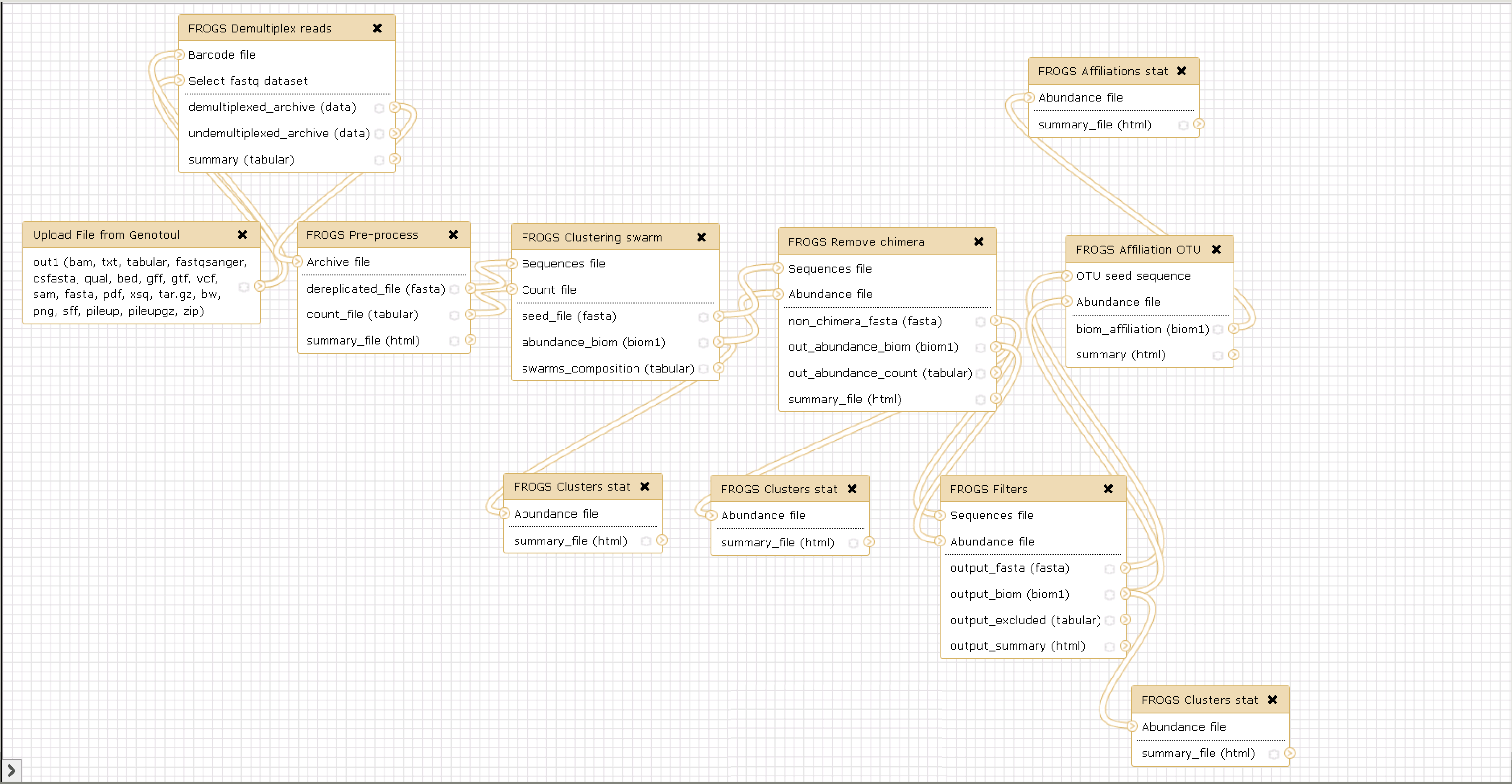
OTU seed sequence

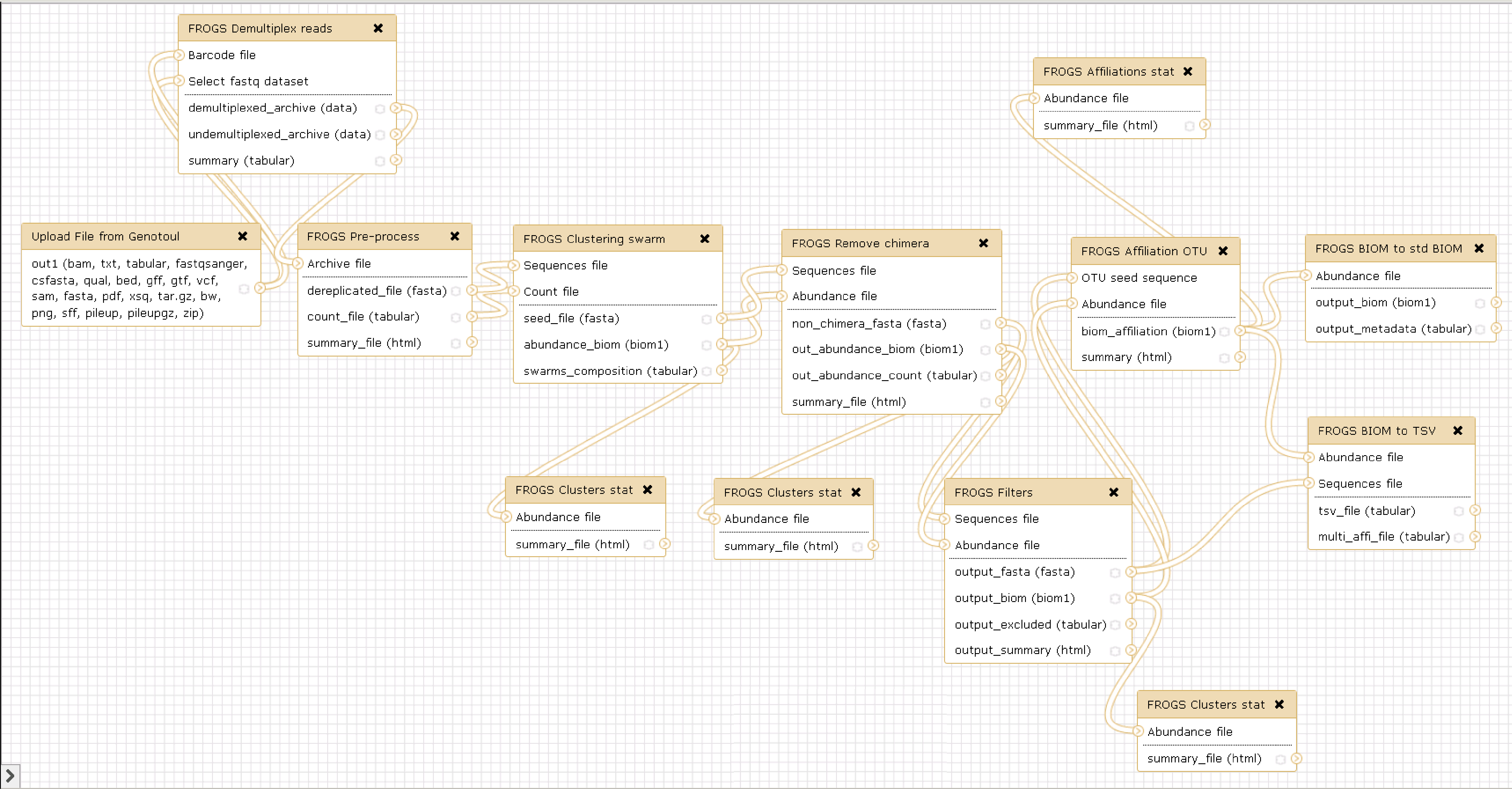
Abundance file

biom_affiliation (biom1)

summary (html)


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliations stat
Abundance file
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
FROGS Clusters stat
Abundance file
summary_file (html)
FROGS Clusters stat
Abundance file
summary_file (html)


FROGS Demultiplex reads
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)
FROGS Affiliations stat
Abundance file
summary_file (html)
Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
FROGS Clusters stat
Abundance file
summary_file (html)
FROGS Clusters stat
Abundance file
summary_file (html)
FROGS Filters
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)
FROGS Clusters stat
Abundance file
summary_file (html)



FROGS Demultiplex reads ✖
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)

Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

FROGS Clusters stat ✖
Abundance file
summary_file (html)

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

FROGS Clusters stat ✖
Abundance file
summary_file (html)

FROGS Affiliations stat ✖
Abundance file
summary_file (html)

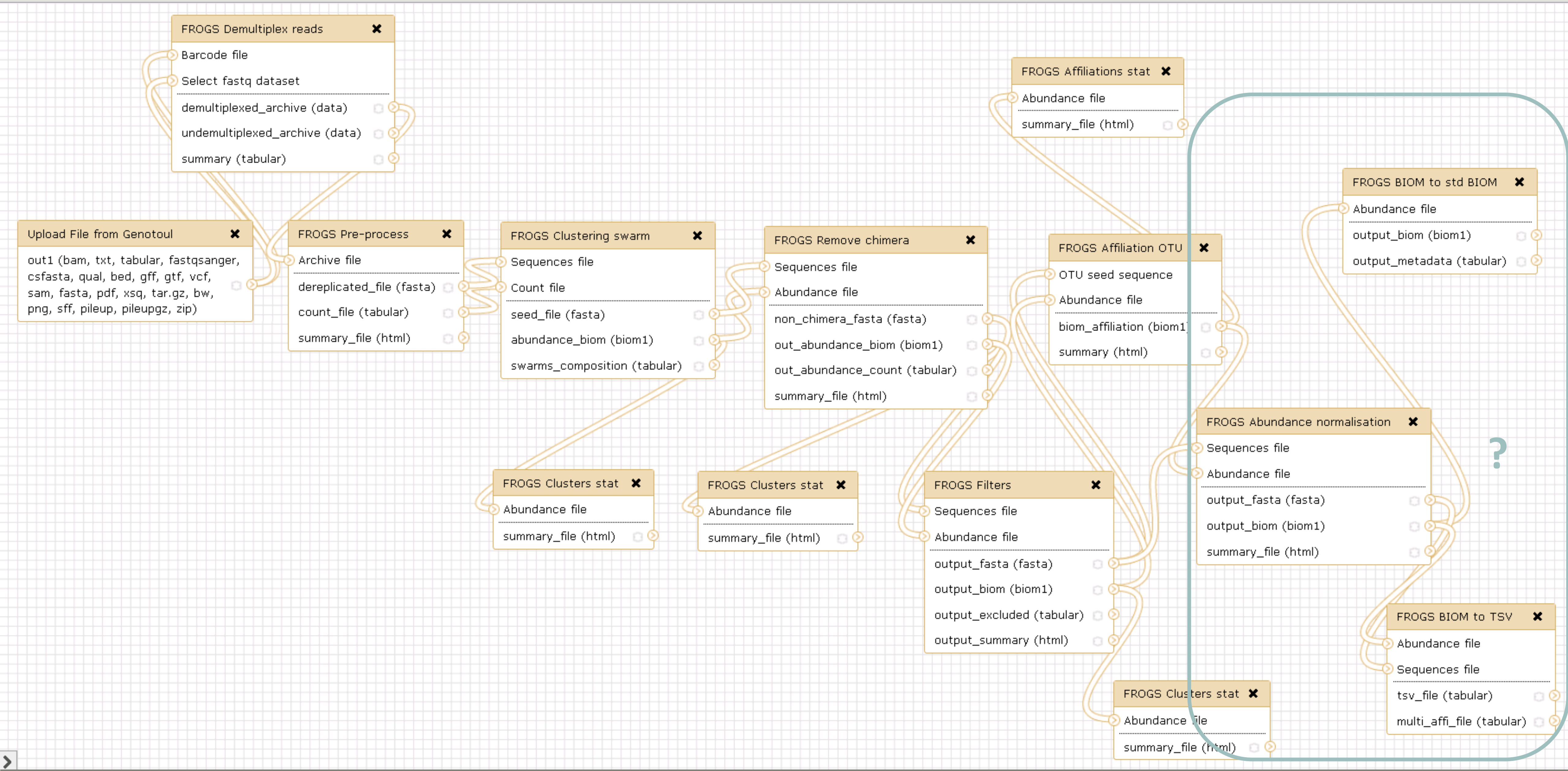
FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

FROGS Filters ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

FROGS BIOM to std BIOM ✖
Abundance file
output_biom (biom1)
output_metadata (tabular)

FROGS BIOM to TSV ✖
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)

FROGS Clusters stat ✖
Abundance file
summary_file (html)



FROGS Demultiplex reads ✖
Barcode file
Select fastq dataset
demultiplexed_archive (data)
undemultiplexed_archive (data)
summary (tabular)

Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

FROGS Clusters stat ✖
Abundance file
summary_file (html)

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

FROGS Clusters stat ✖
Abundance file
summary_file (html)

FROGS Affiliations stat ✖
Abundance file
summary_file (html)

FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

FROGS Filters ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)

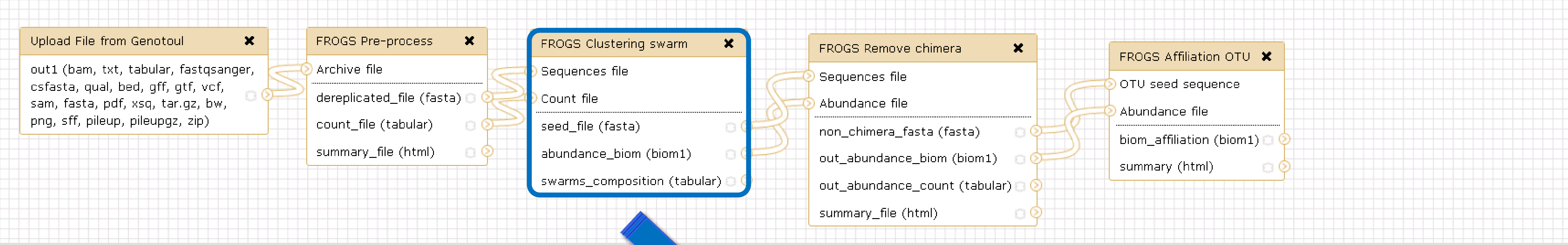
FROGS Clusters stat ✖
Abundance file
summary_file (html)

FROGS BIOM to std BIOM ✖
Abundance file
output_biom (biom1)
output_metadata (tabular)

FROGS Abundance normalisation ✖
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
summary_file (html)

FROGS BIOM to TSV ✖
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)

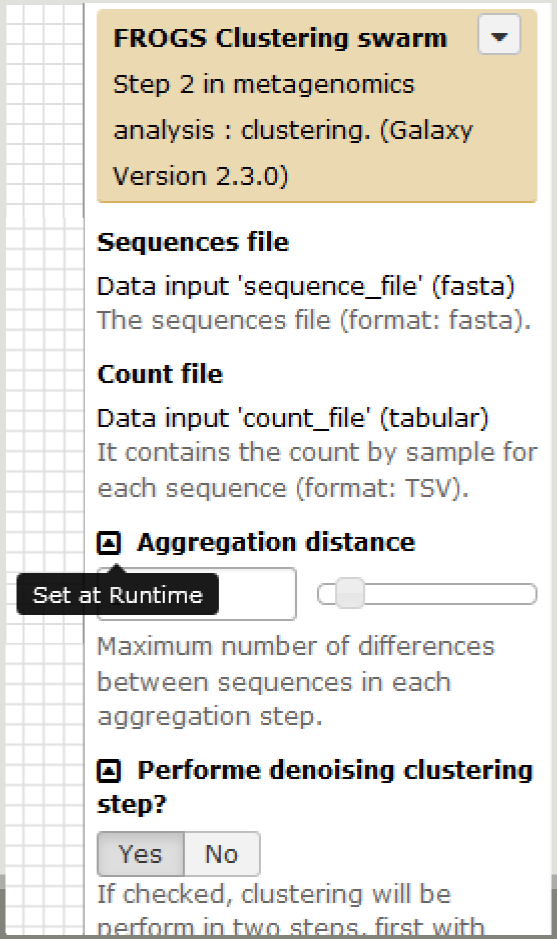
?


Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

For each tool, think to:

- Fixe parameter ?

?



FROGS Clustering swarm

Step 2 in metagenomics analysis : clustering. (Galaxy Version 2.3.0)

Sequences file

Data input 'sequence_file' (fasta)

The sequences file (format: fasta).

Count file

Data input 'count_file' (tabular)

It contains the count by sample for each sequence (format: TSV).

Aggregation distance

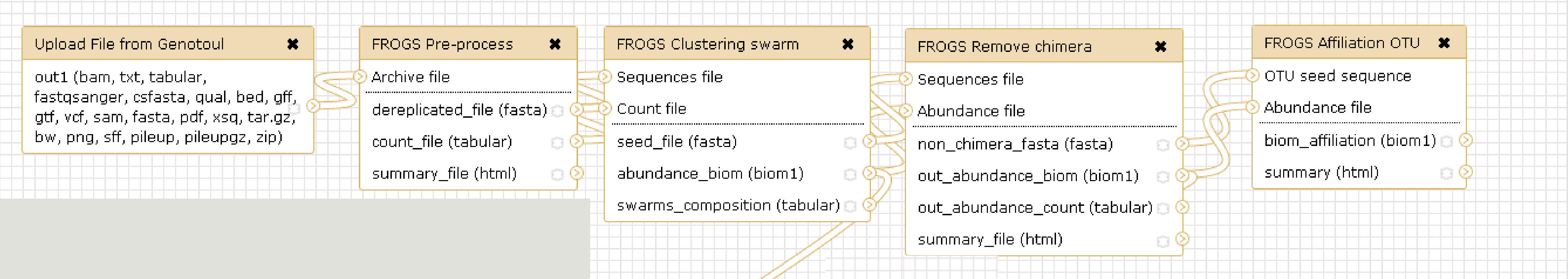
Maximum number of differences between sequences in each aggregation step.

Performe denoising clustering step?

Yes No

If checked, clustering will be perform in two steps, first with

Set at Runtime



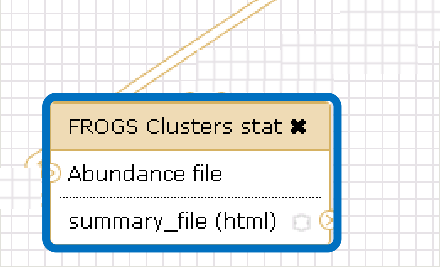
Upload File from Genotoul ✖
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)

FROGS Pre-process ✖
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)

FROGS Clustering swarm ✖
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)

FROGS Remove chimera ✖
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)

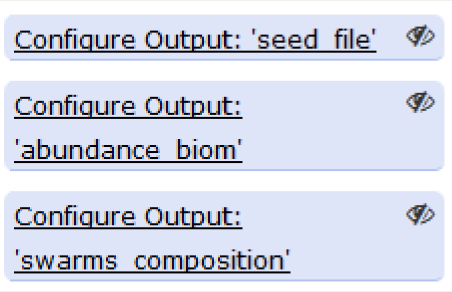
FROGS Affiliation OTU ✖
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)



FROGS Clusters stat ✖
Abundance file
summary_file (html)

For each tool, think to:

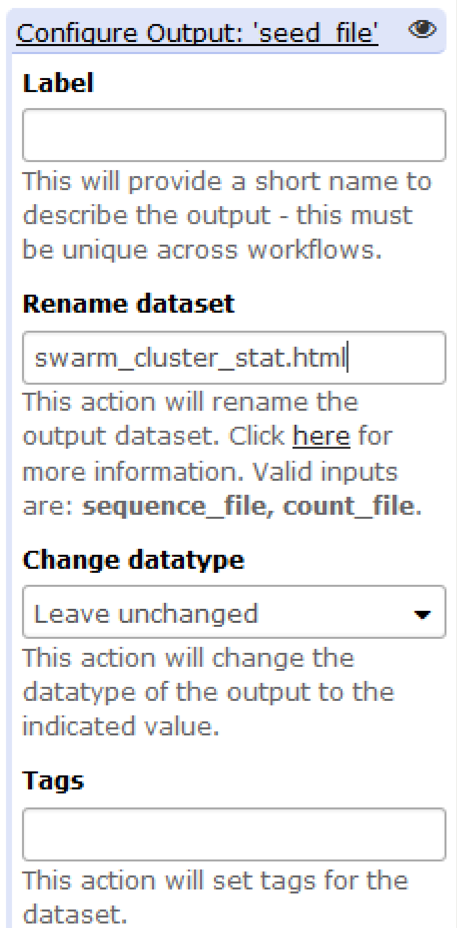
- Fixe parameter ?
- Automatically rename output files



Configure Output: 'seed_file'

Configure Output: 'abundance_biom'

Configure Output: 'swarms_composition'



Configure Output: 'seed_file'

Label

This will provide a short name to describe the output - this must be unique across workflows.

Rename dataset

swarm_cluster_stat.html

This action will rename the output dataset. Click here for more information. Valid inputs are: **sequence_file, count_file**.

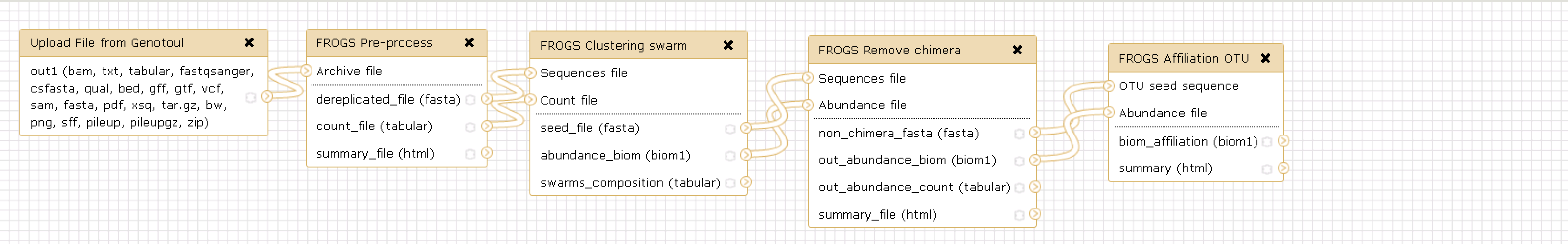
Change datatype

Leave unchanged

This action will change the datatype of the output to the indicated value.

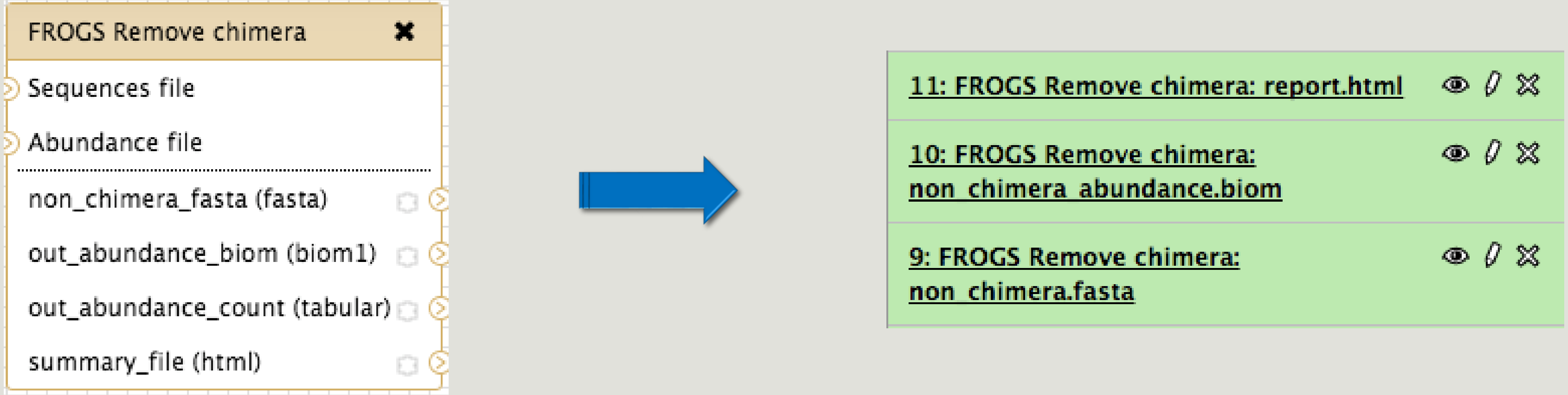
Tags

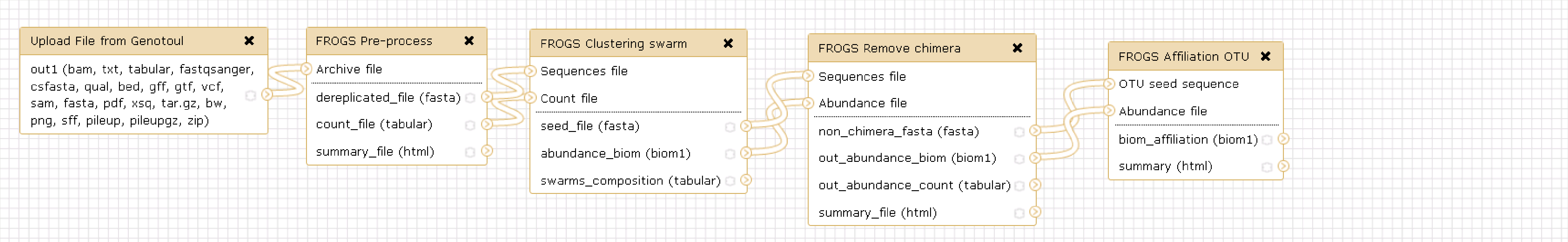
This action will set tags for the dataset.


Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
Count file
seed_file (fasta)
abundance_biom (biom1)
swarms_composition (tabular)
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

For each tool, think to:

- Fixe parameter ?
- Automatically rename output files
- Hide intermediate files ?


FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
11: FROGS Remove chimera: report.html
10: FROGS Remove chimera: non_chimera_abundance.biom
9: FROGS Remove chimera: non_chimera.fasta



Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
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FROGS Remove chimera
Sequences file
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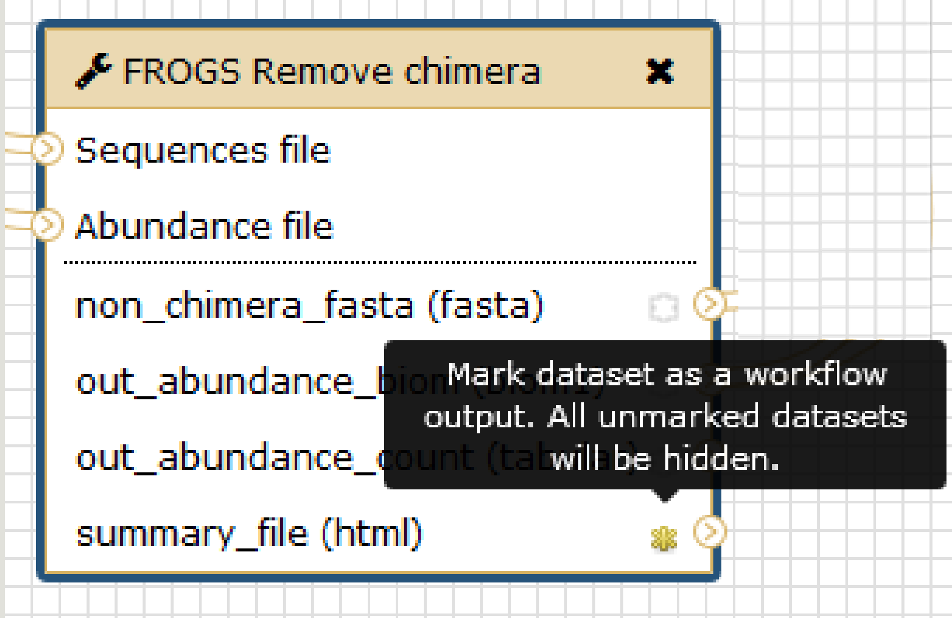
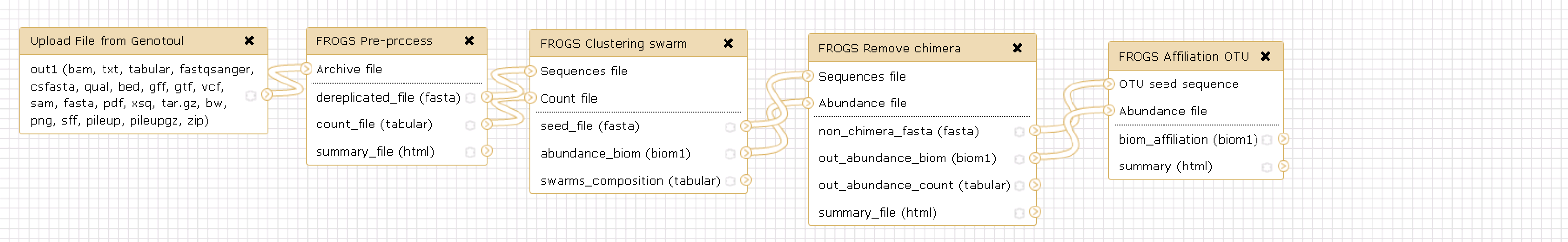
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

For each tool, think to:

- Fixe parameter ?
- Automatically rename output files
- Hide intermediate files ?



FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
Mark dataset as a workflow output. All unmarked datasets will be hidden.


Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
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FROGS Clustering swarm
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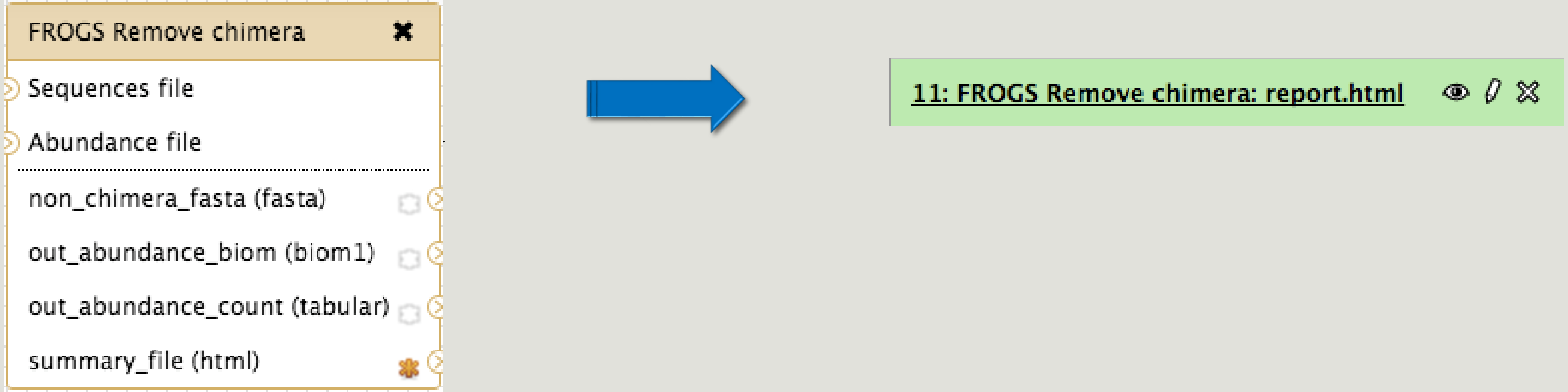
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)

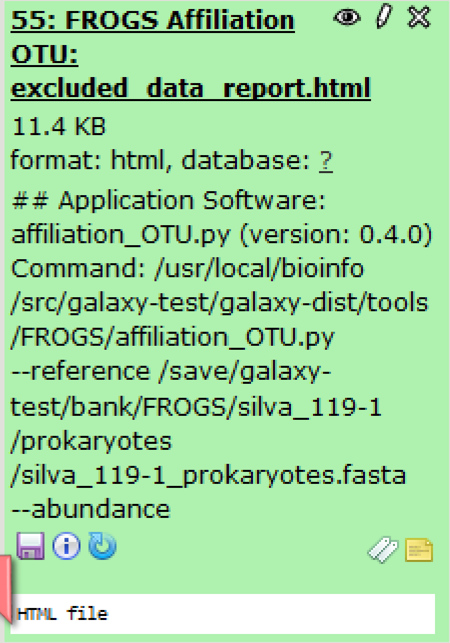
For each tool, think to:

- Fixe parameter ?
- Automatically rename output files
- Hide intermediate files ?


FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
11: FROGS Remove chimera: report.html

Download your data

You have to download one per one your files



55: FROGS Affiliation OTU: excluded_data_report.html

11.4 KB

format: html, database: ?

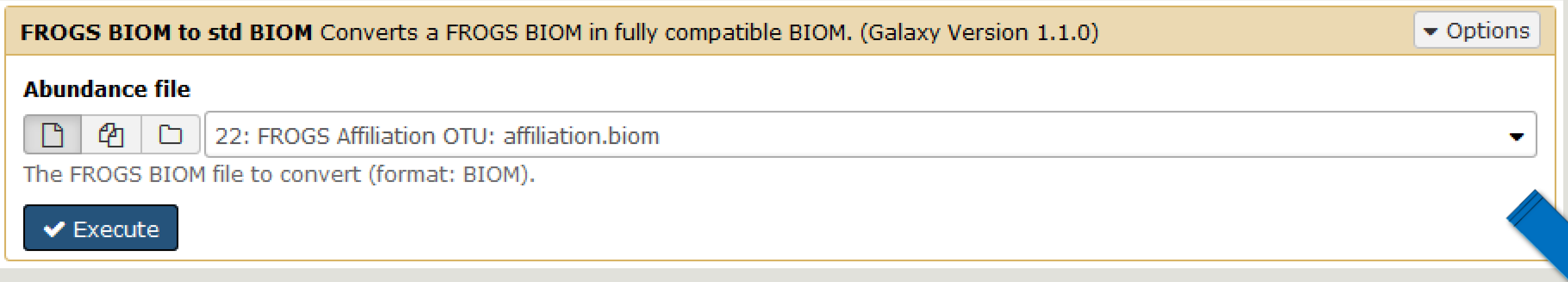
Application Software: affiliation_OTU.py (version: 0.4.0) Command: /usr/local/bioinfo /src/galaxy-test/galaxy-dist/tools /FROGS/affiliation_OTU.py --reference /save/galaxy-test/bank/FROGS/silva_119-1 /prokaryotes /silva_119-1_prokaryotes.fasta --abundance

HTML file

FROGS BIOM to Standard BIOM

FROGS biom to standard Biom

This step is required to run R

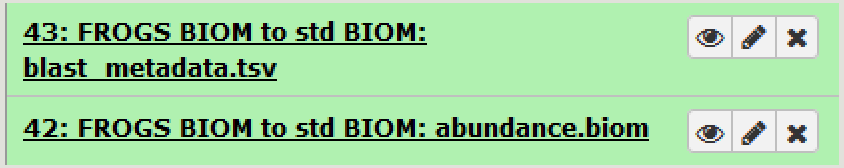


FROGS BIOM to std BIOM Converts a FROGS BIOM in fully compatible BIOM. (Galaxy Version 1.1.0)

Abundance file

22: FROGS Affiliation OTU: affiliation.biom

The FROGS BIOM file to convert (format: BIOM).



43: FROGS BIOM to std BIOM: blast_metadata.tsv

42: FROGS BIOM to std BIOM: abundance.biom

Some figures

Some figures - Fast

NB SEQ	TIME with complete pipeline without Filters
50 000	40 min
400 000	4 hrs
3 500 000	2 days
10 000 000	5 days

Speed on real datasets

9 600 000 sequences of a complete MiSeq run

Preprocess : 9 300 000 sequences ~ 15 min

Swarm clustering : 680 000 clusters ~ 10 hrs

Chimera removal : 556 700 non-chimeric cl. ~ 15 min

Filtering*: 556 200 OTUs

*Filter OTU abundances at 0.005%

PhiX removal ~ 8 min

RDP affiliation ~ 25 min

Blast affiliation ~ 5 min

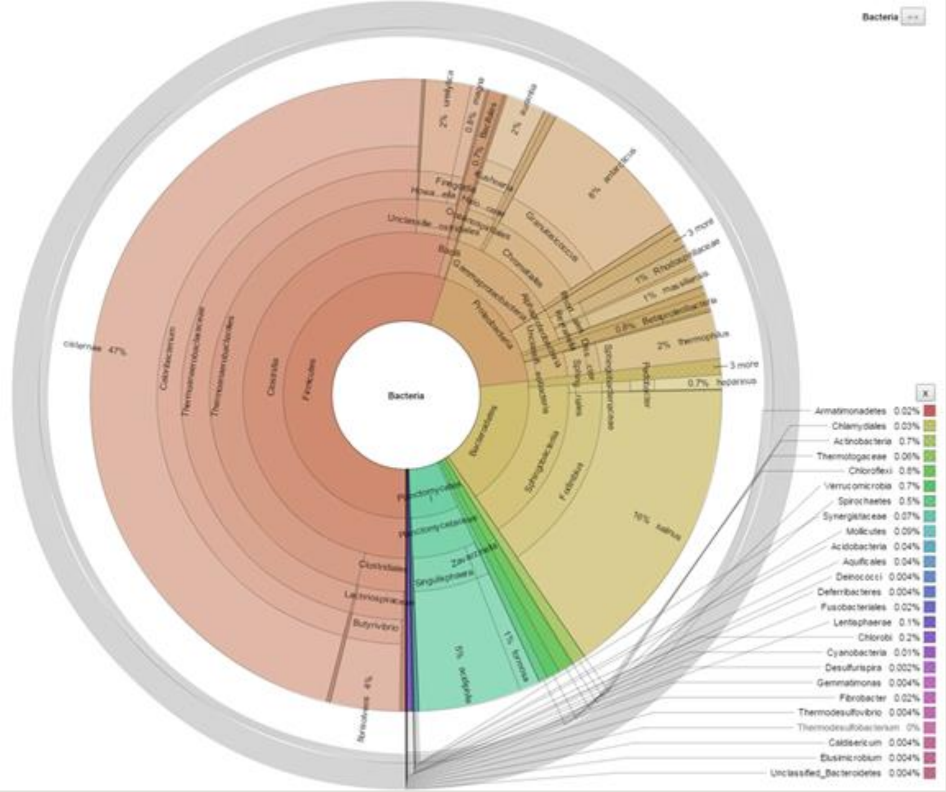
FROGS : 500 OTUs

~ 11 hours

Simulated datasets, for testing FROGS' Accuracy

- 500 species, covering all bacterial phyla
- Power Law distribution of the species abundances
- Error rate calibrated with real sequencing runs
- 20% chimeras
- 10 samples of 100 000 sequences each (1M sequences)

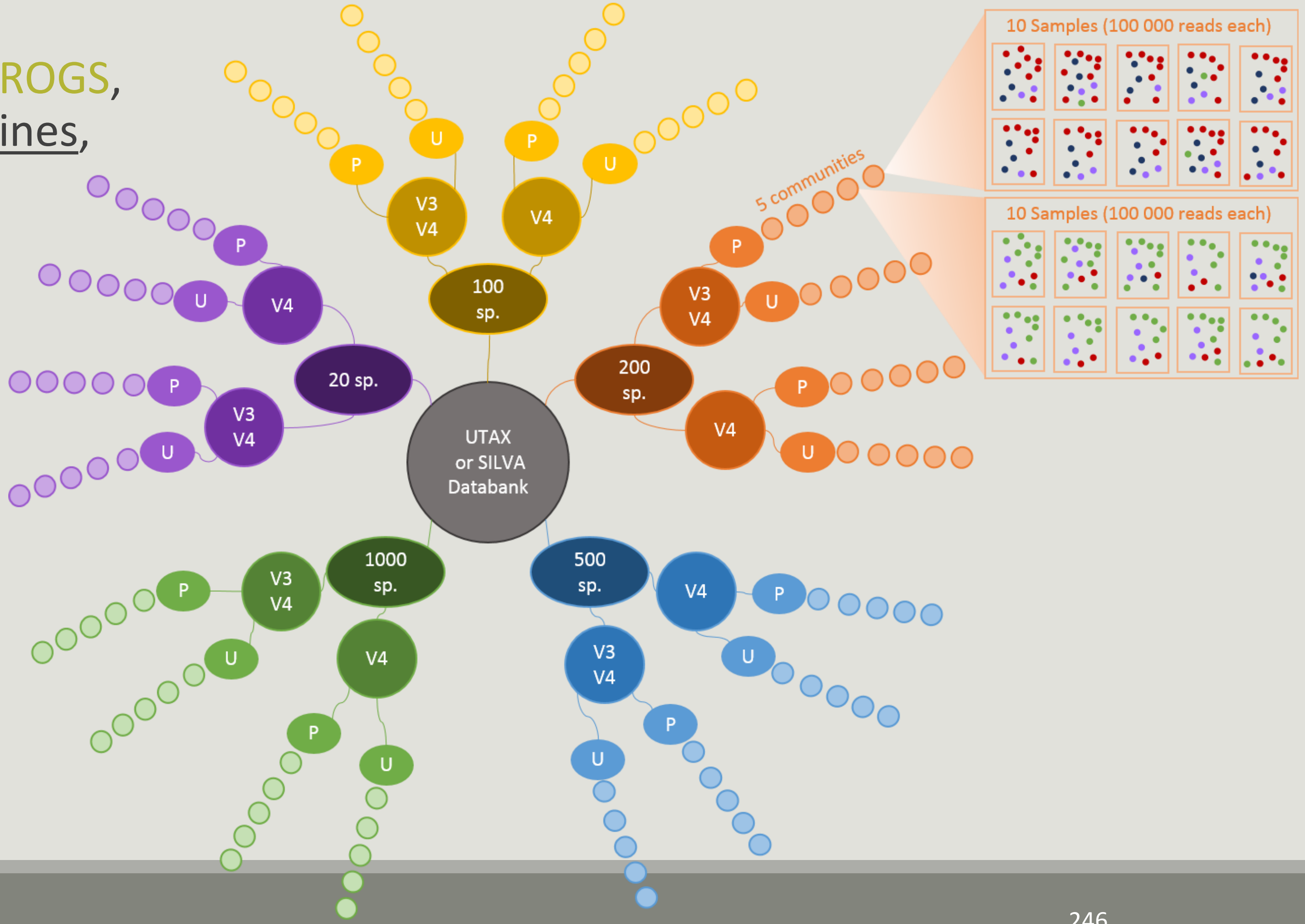
Simulated dataset : 1M sequences
↓
SWARM : 109 000 clusters
↓
VSEARCH: 21 000 clusters
↓
filters : 0.005% **505 OTUs**



FROGS' Accuracy

- 1.10^{+8} synthetic sequences were treated with FROGS, UPARSE and MOTHUR, QIIME, with their guidelines, to compare their performances
- 20, 100, 200, 500 or 1000 different species
- power law or a uniform distribution
- 5 to 20% of chimera

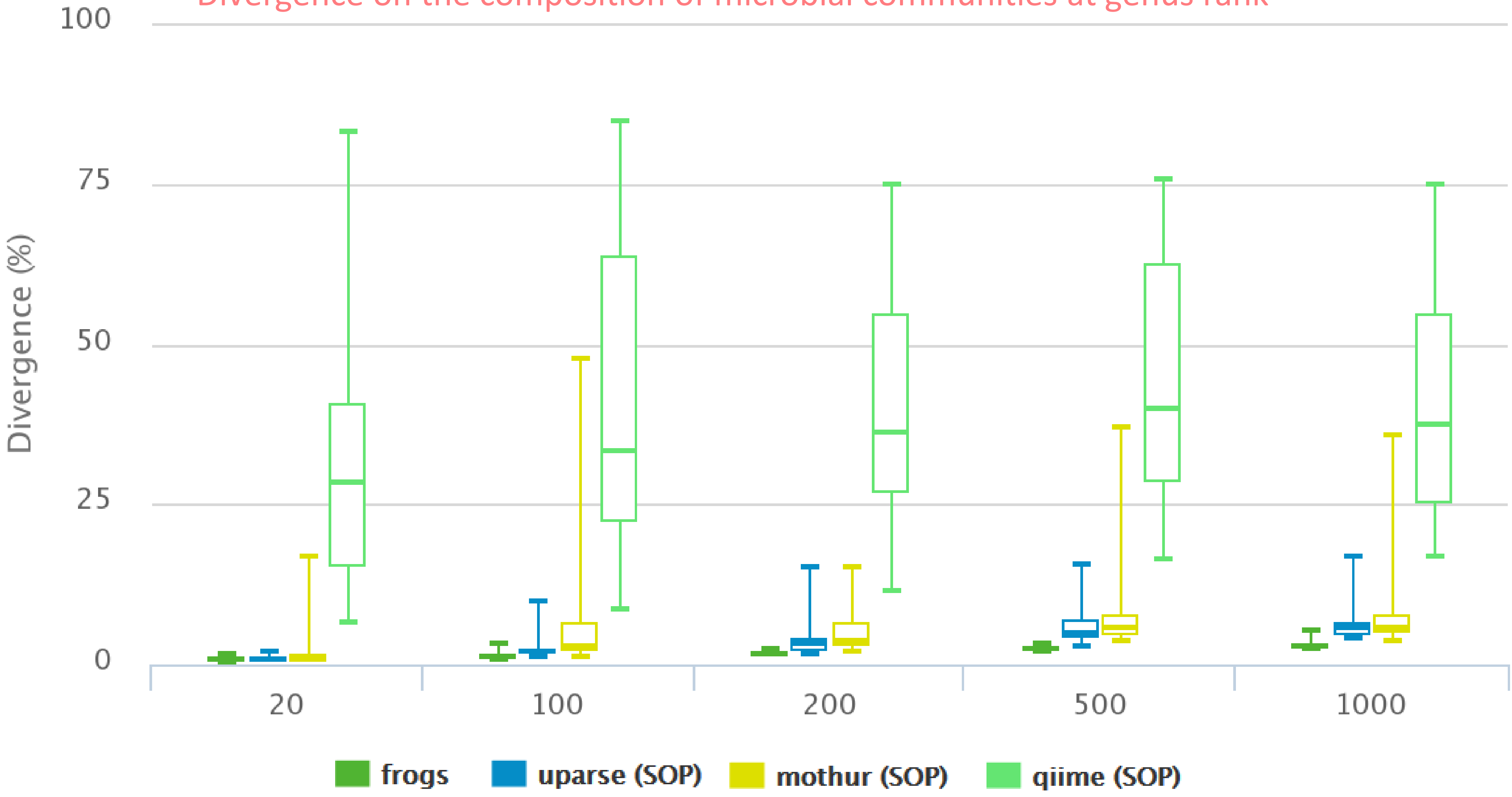
→ Divergence on the composition of microbial communities at the different taxonomic ranks

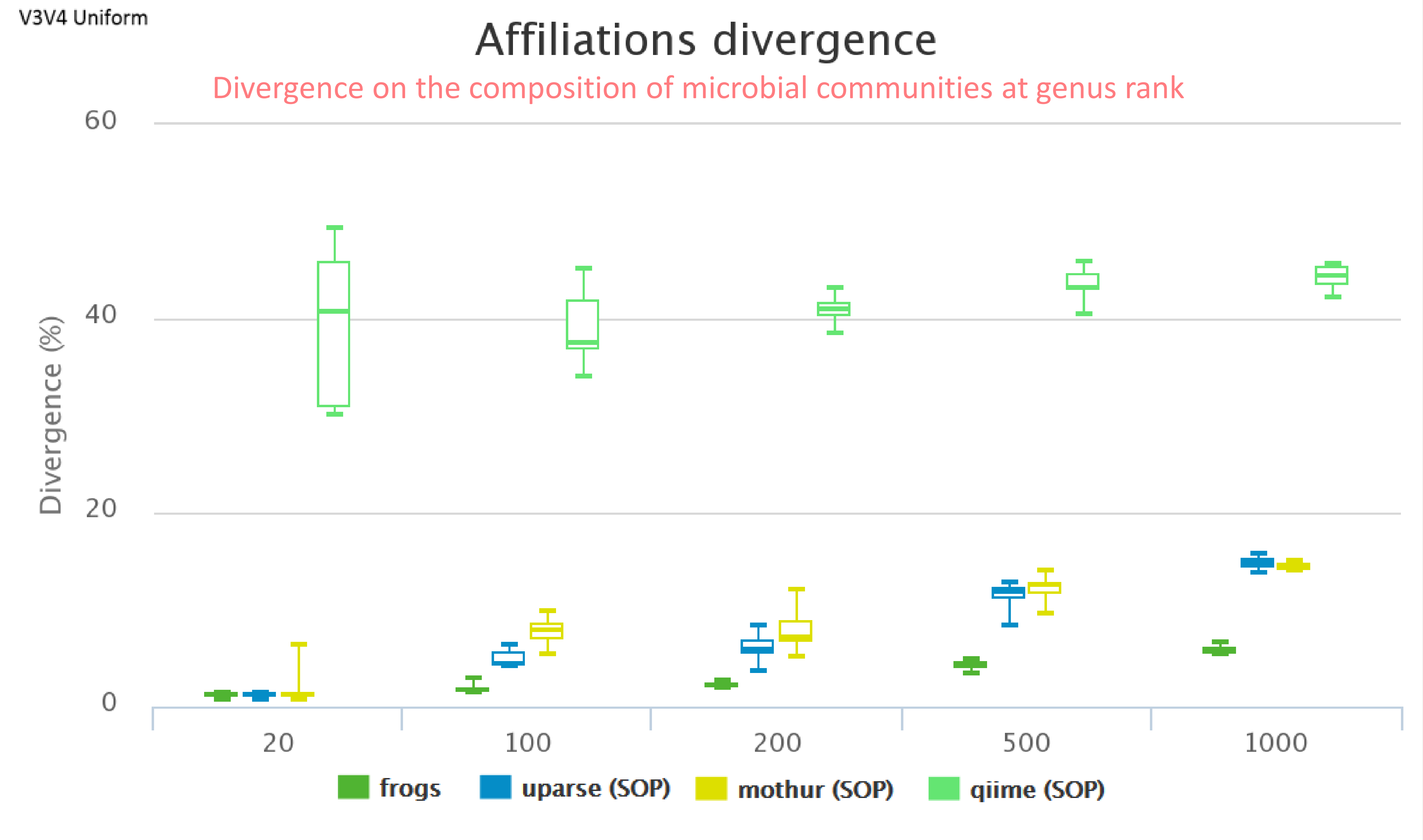

UTAX
or SILVA
Databank
20 sp.
100 sp.
200 sp.
500 sp.
1000 sp.
V3 V4
V4
P
U
5 communities
10 Samples (100 000 reads each)
10 Samples (100 000 reads each)

V3V4 Power Law

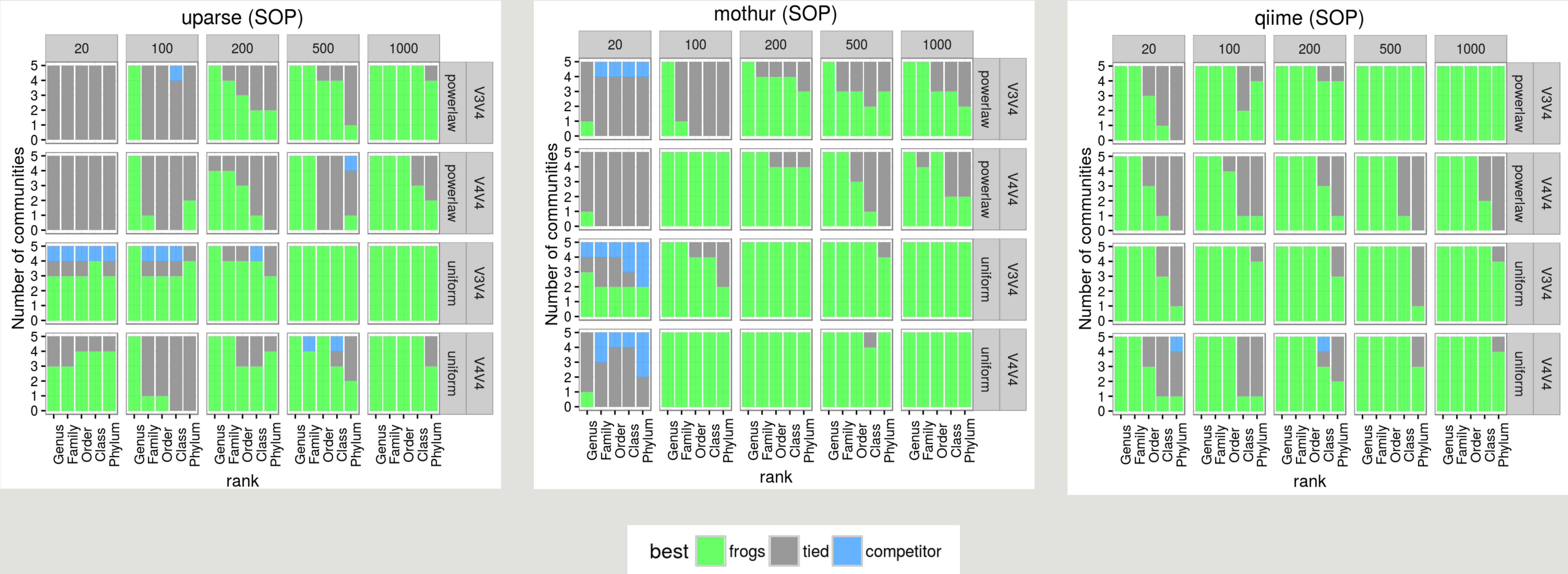
Affiliations divergence

Divergence on the composition of microbial communities at genus rank




V3V4 Uniform
Affiliations divergence
Divergence on the composition of microbial communities at genus rank
Divergence (%)
60
40
20
0
20
100
200
500
1000
frogs
uparse (SOP)
mothur (SOP)
qiime (SOP)

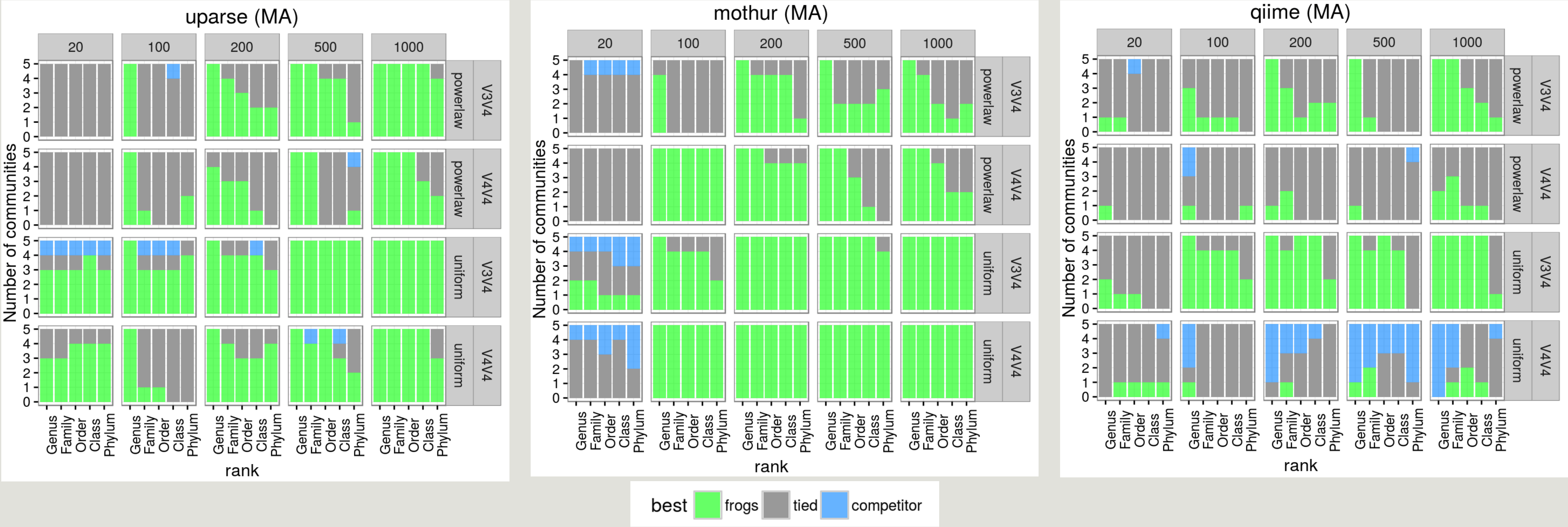
The results of non-parametric paired tests (signed rank test) of Affiliation divergence on simulated data from UTAX



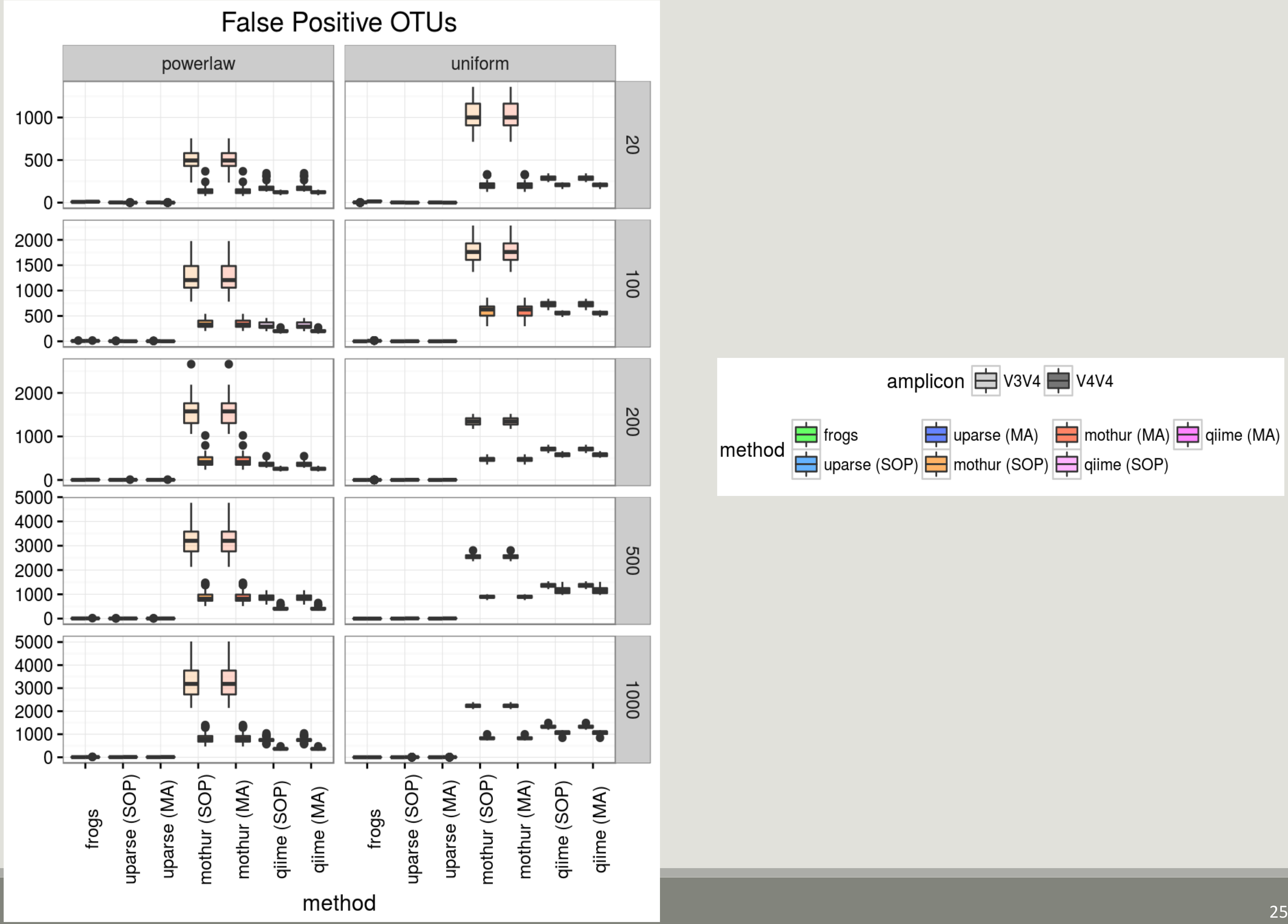
FROGS performs as well as or better than UPARSE, MOTHUR and QIIME in most settings. The only condition in which FROGS does worse than UPARSE and MOTHUR is small community size (20).

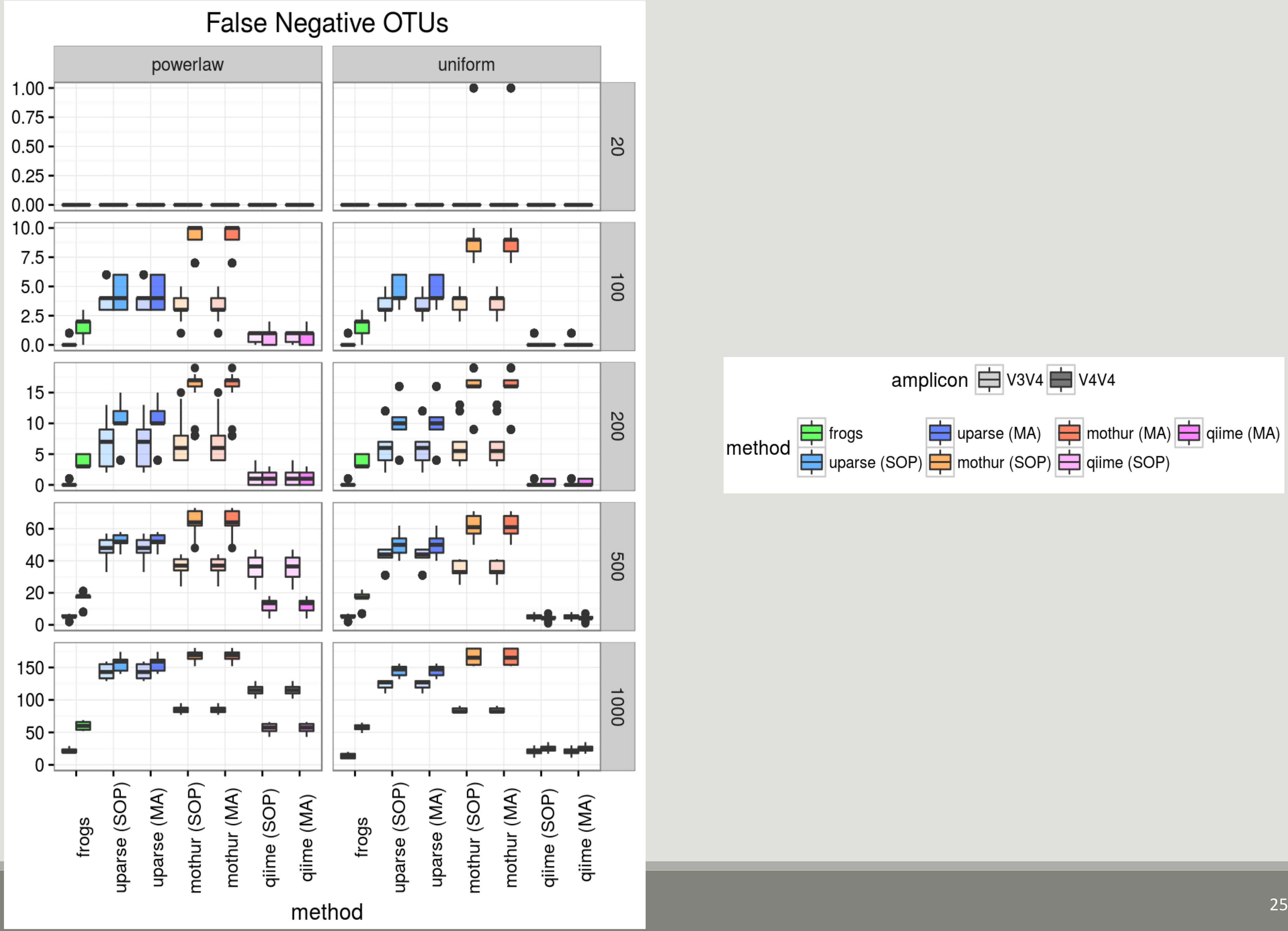
The results of non-parametric paired tests (signed rank test) of Affiliation divergence on simulated data from UTAX

With FROGS multi-Affiliation



QIIME (MA) with large communities (size > 200) with uniform abundance using the V4 region is better than FROGS. The differences, although significant, are small in that case: less than 2 percentage points in all cases and most marked at the Genus level where the divergences of both FROGS and QIIME (MA) are already quite moderate (6~10%).


False Positive OTUs
powerlaw
uniform
20
100
200
500
1000
0
500
1000
1500
2000
3000
4000
5000
frogs
uparse (SOP)
uparse (MA)
mothur (SOP)
mothur (MA)
qiime (SOP)
qiime (MA)
method
amplicon
V3V4
V4V4
method
frogs
uparse (MA)
mothur (MA)
qiime (MA)
uparse (SOP)
mothur (SOP)
qiime (SOP)


False Negative OTUs
powerlaw
uniform
20
100
200
500
1000
1.00
0.75
0.50
0.25
0.00
10.0
7.5
5.0
2.5
0.0
15
10
5
0
60
40
20
0
150
100
50
0
frogs
uparse (SOP)
uparse (MA)
mothur (SOP)
mothur (MA)
qiime (SOP)
qiime (MA)
method
amplicon
V3V4
V4V4
method
frogs
uparse (SOP)
uparse (MA)
mothur (SOP)
mothur (MA)
qiime (SOP)
qiime (MA)

Conclusions

Why Use FROGS ?

- User-friendly
- Fast
- 454 data and Illumina data
  - sequencing methods change but same tool
  - easier for comparisons
- Clustering without global threshold and independent of sequence order
- New chimera removal method (Vsearch + cross-validation)
- Filters tool
- Multi-affiliation with 2 taxonomy affiliation procedures
- Cluster Stat and Affiliation Stat tools
- A lot of graphics
- Independant tools

How to cite FROGS

In waiting for the publication:

Pipeline FROGS on http://sigenae-workbench.toulouse.inra.fr/

Github: https://github.com/geraldinepascal/FROGS.git

Poster FROGS: Escudie F., Auer L., Bernard M., Cauquil L., Vidal K., Maman S., Mariadassou M., Combes S., Hernadez-Raquet G., Pascal G., 2016. FROGS: Find Rapidly OTU with Galaxy Solution. In: ISME-2016 Montreal, CANADA,

http://bioinfo.genotoul.fr/wp-content/uploads/FROGS_ISME2016_poster.pdf

To contact

FROGS:

frogs@toulouse.inra.fr

Galaxy:

sigenae-support@listes.inra.fr

Newsletter – demande d’abonnement:

mailto:sympa@listes.inra.fr?subject=sub%20frogs-newsletter

frogs-newsletter-request@listes.inra.fr

Next training sessions

3rd to 6th July 2017 4 days

0.5 Galaxy day
2 FROGS days
1.5 Statistics phyloseq day (under R)