Training on Galaxy: Metagenomics

December 2016 – Hantagulumic session

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"?

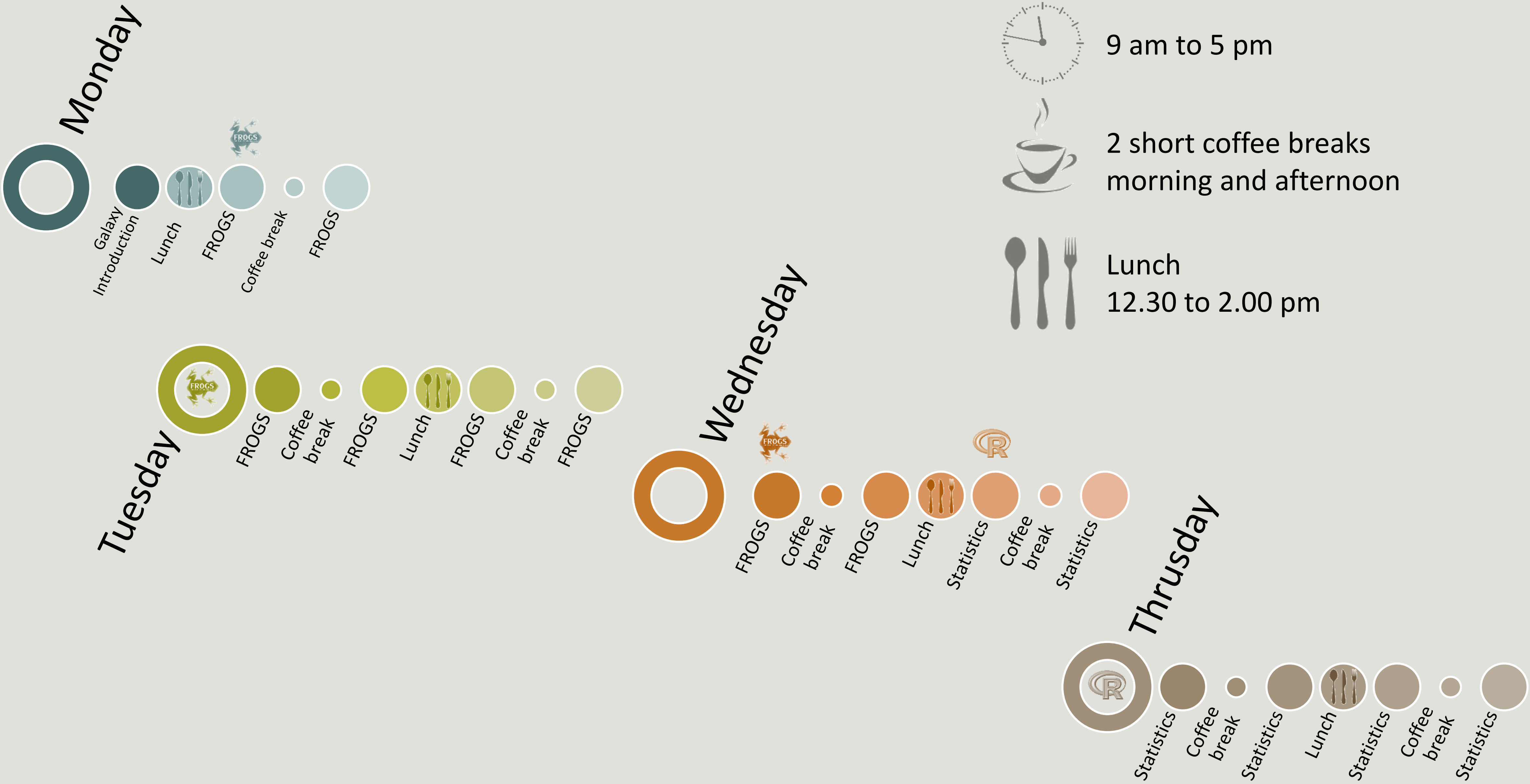
454 / MiSeq ?

Your background ?

9 am to 5 pm

2 short coffee breaks
morning and afternoon

Lunch
12.30 to 2.00 pm



Monday

Galaxy Introduction

Lunch

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

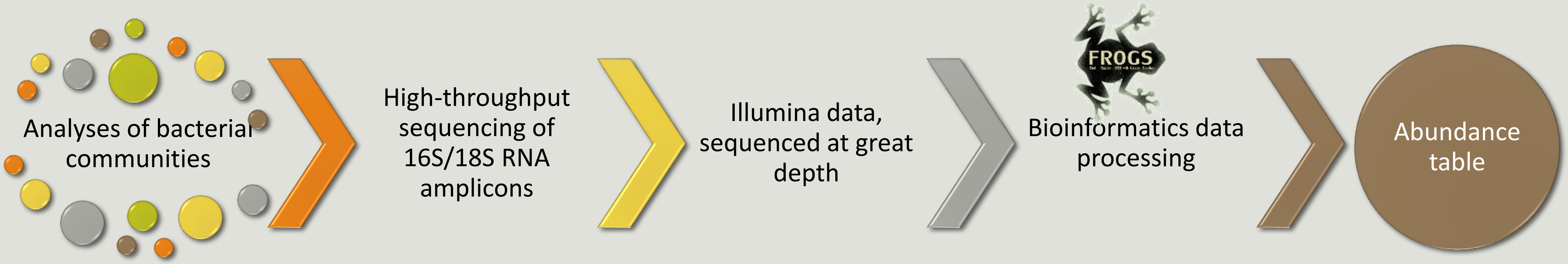
Coffee break

Statistics

Overview

- Objectives
- Material: data + FROGS
- Data upload into galaxy environment (done during Galaxy Introduction)
- 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

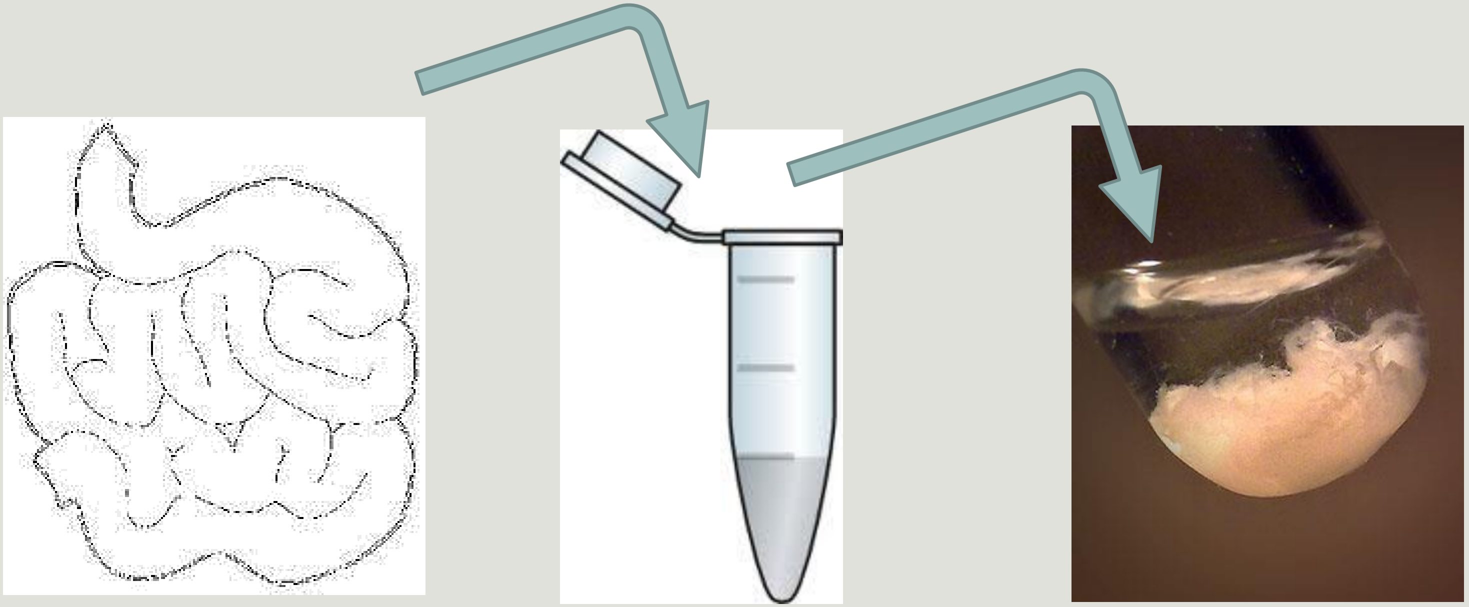
The current processing pipelines struggle to run in a reasonable time.

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

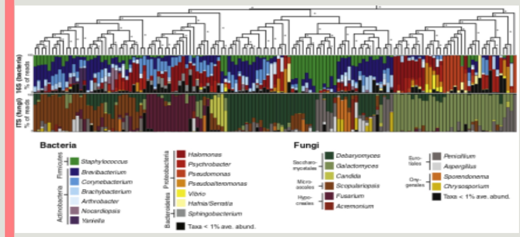
Metagenomics

Metatranscriptomics

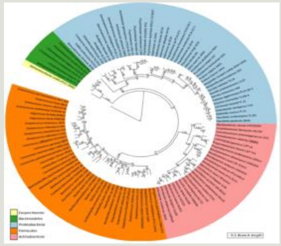
Amplicon sequencing

Shotgun sequencing

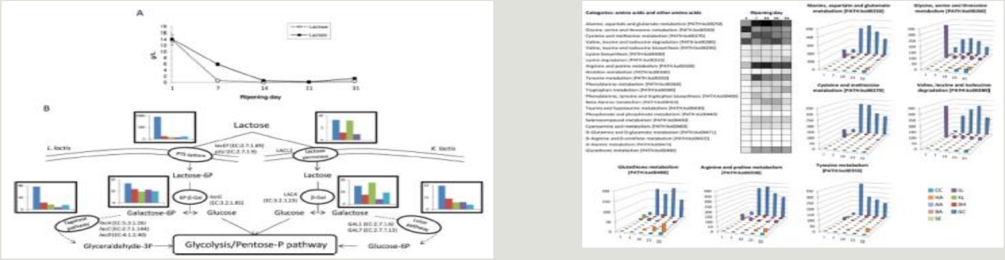
RNA sequencing



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

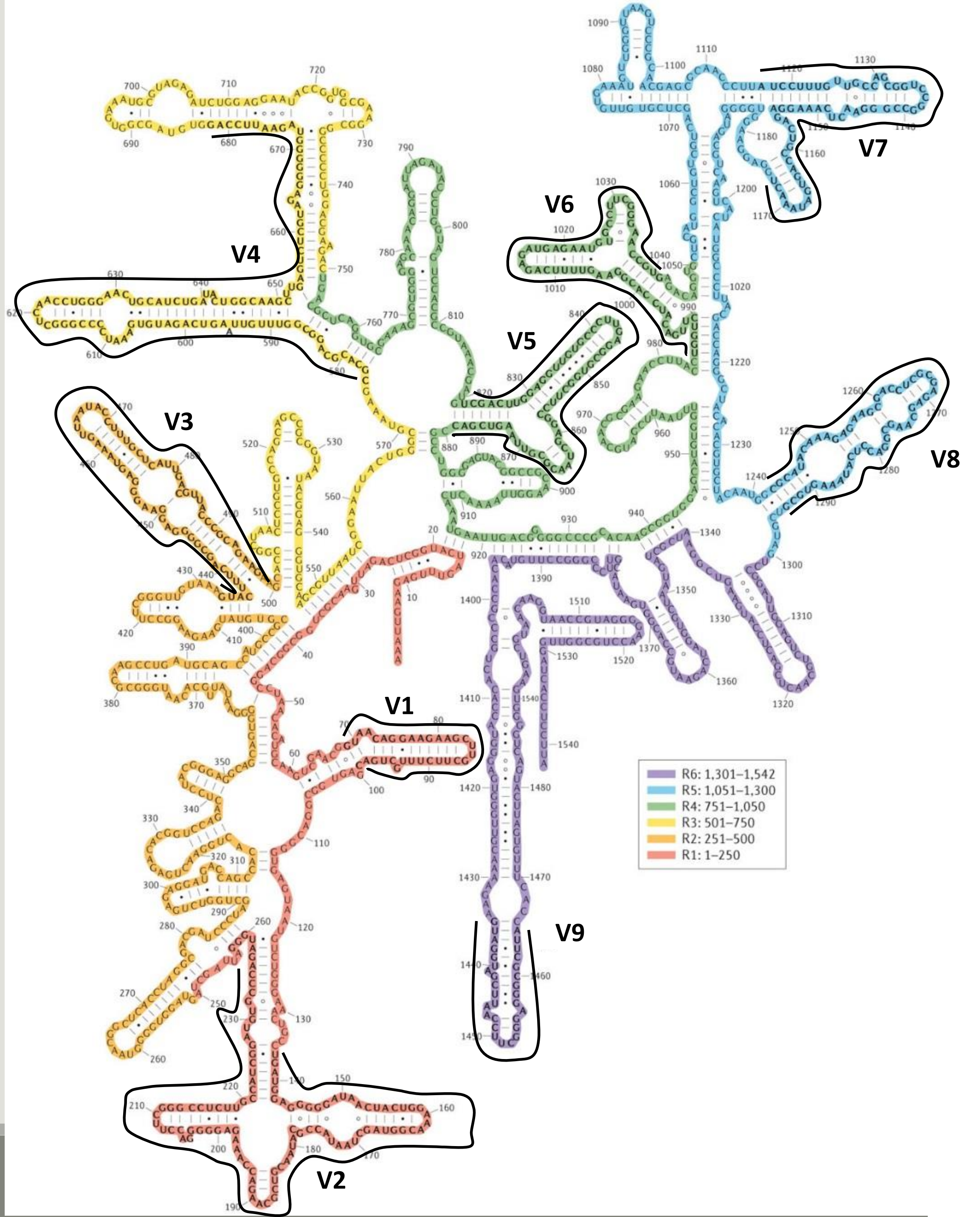
Gene encoding a ribosomal RNA : non-coding RNA (not translated), part of the small subunit of the ribosome which is responsible for the translation of mRNA in proteins

Not submitted to lateral gene transfer

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

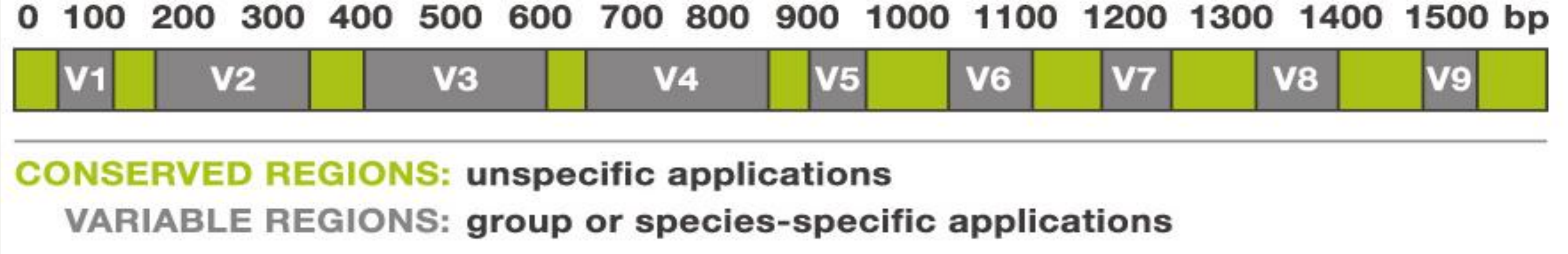
Secondary structure of the 16S rRNA of *Escherichia coli*

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

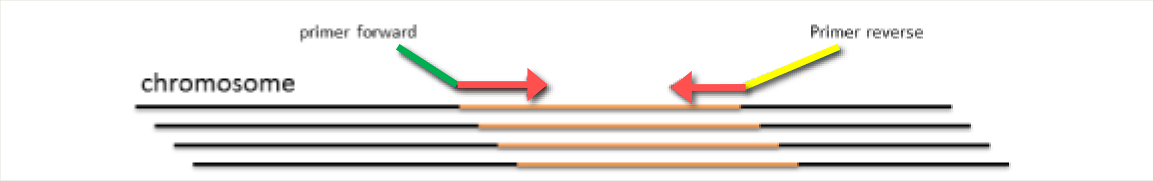


CONSERVED REGIONS: unspecific applications

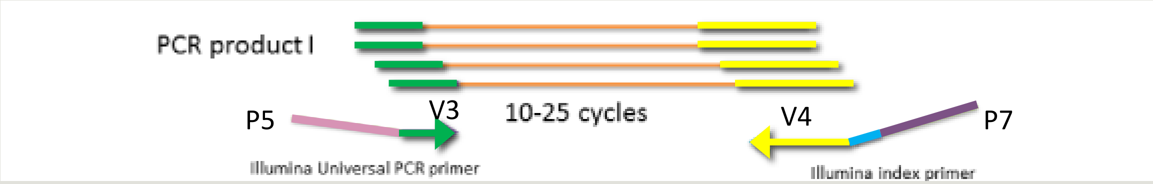
VARIABLE REGIONS: group or species-specific applications

Steps for Illumina sequencing

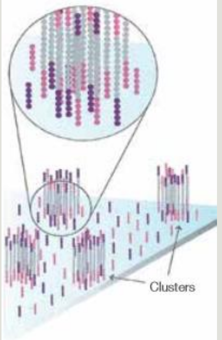
- 1st step : one PCR



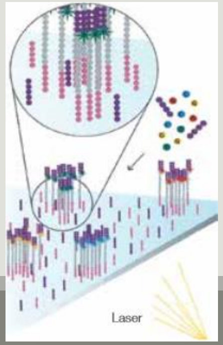
- 2nd step: one PCR



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



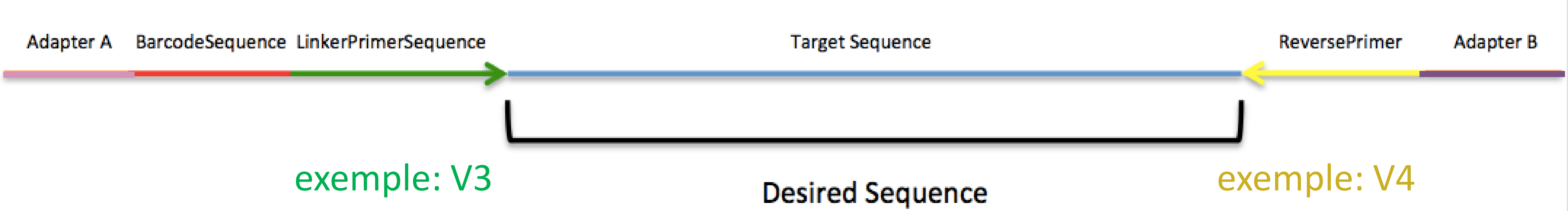
- 4th step: sequencing



Amplification and sequencing

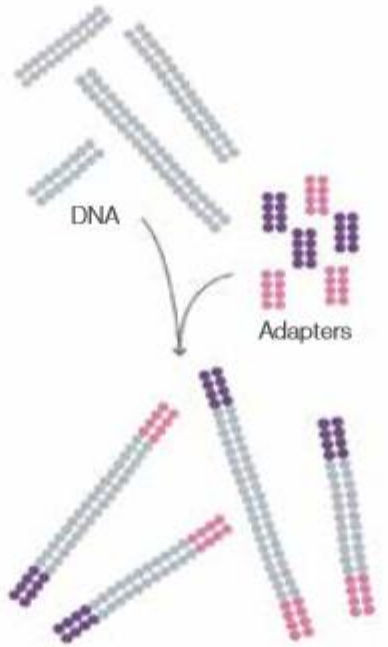
« Universal » primer sets are used for **PCR amplification** of the phylogenetic biomarker

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)



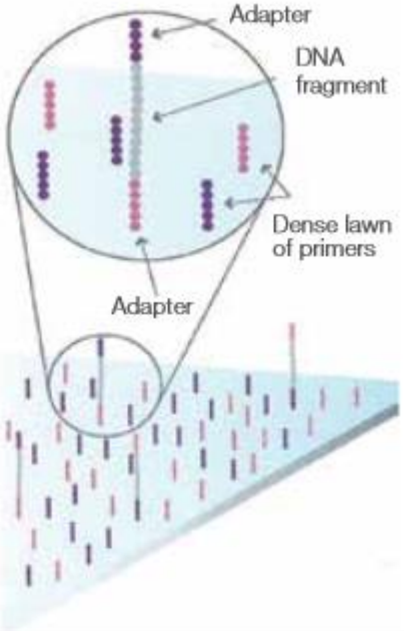
Cluster generation

Prepare Genomic DNA Sample



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

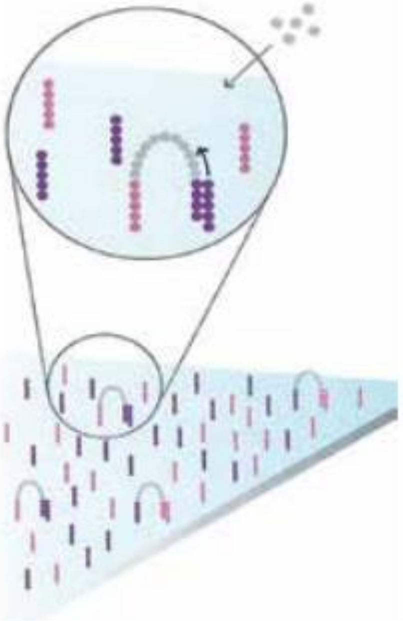
Attach DNA to Surface



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

Attach DNA to surface

Bridge Amplification

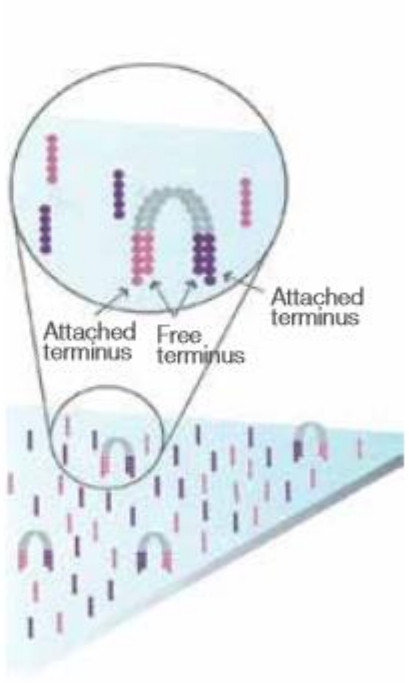


Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

Bridge amplification

Cluster generation

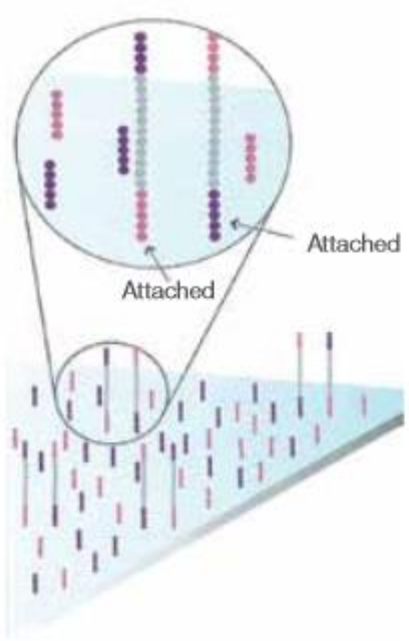
Fragments Become Double Stranded



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

Fragments become double stranded

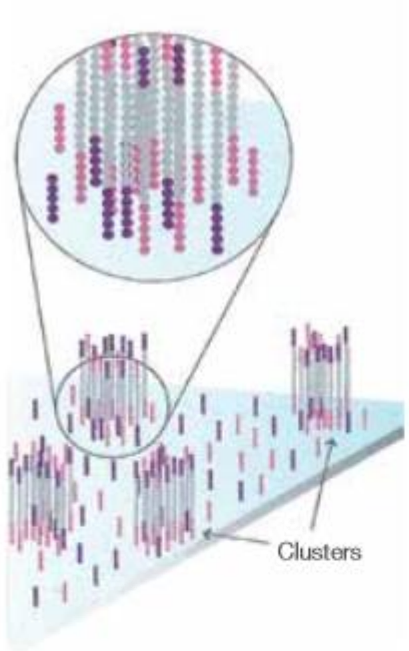
Denature the Double-Stranded Molecules



Denaturation leaves single-stranded templates anchored to the substrate.

Denature the double-stranded molecule

Complete Amplification

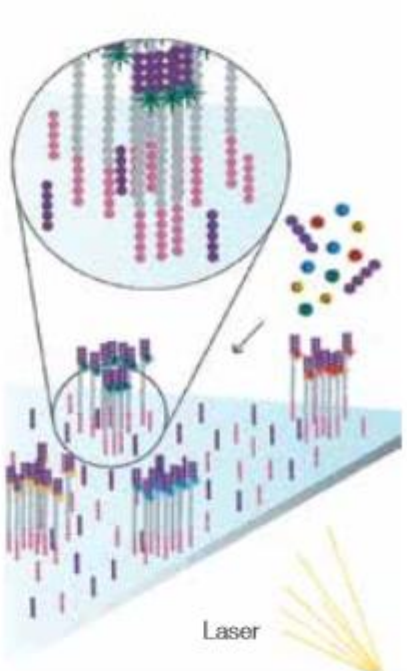


Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

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

Sequencing by synthesis

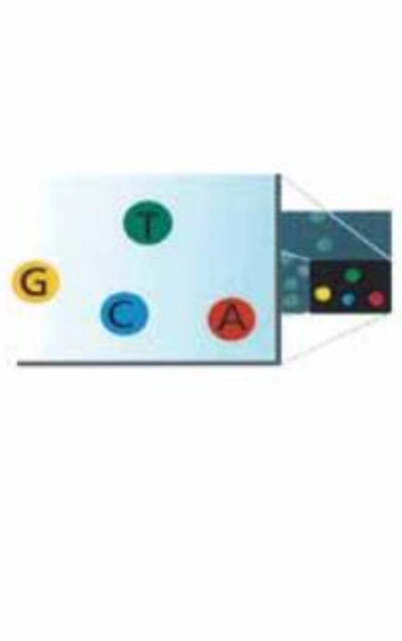
Determine First Base



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

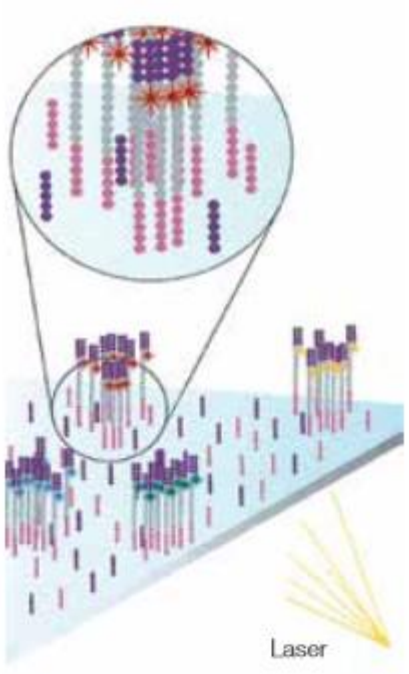
Light signal is more strong in cluster

Image First Base



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

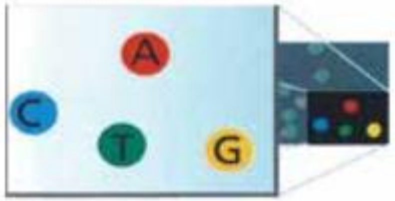
Determine Second Base



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

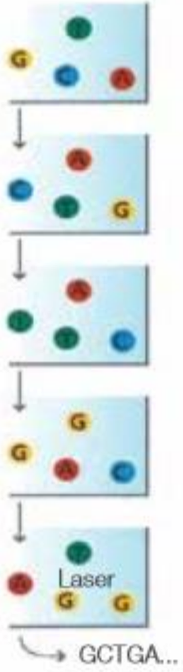
Sequencing by synthesis

Image Second Chemistry Cycle



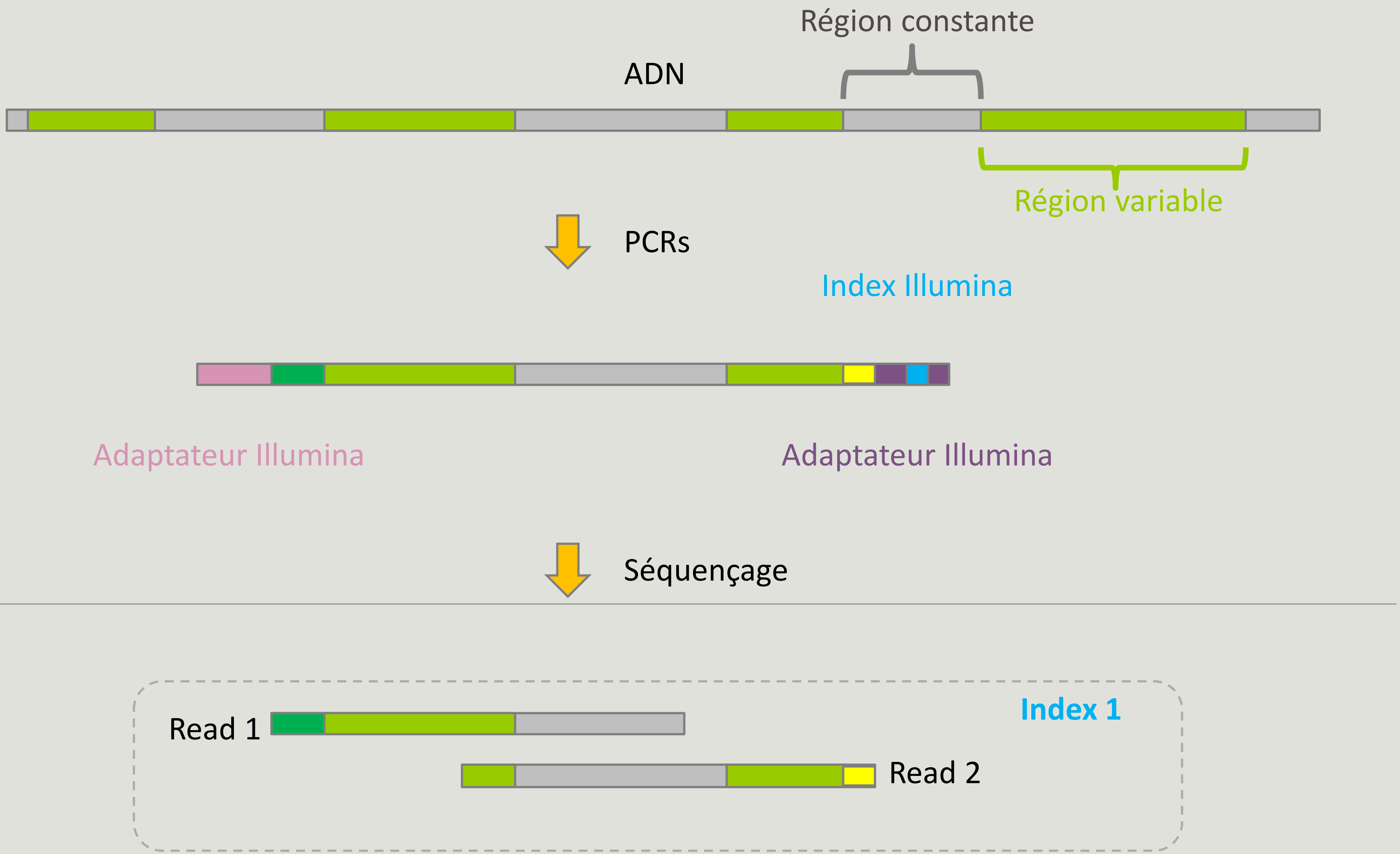
After laser excitation, the image is captured as before, and the identity of the second base is recorded.

Sequencing Over Multiple Chemistry Cycles

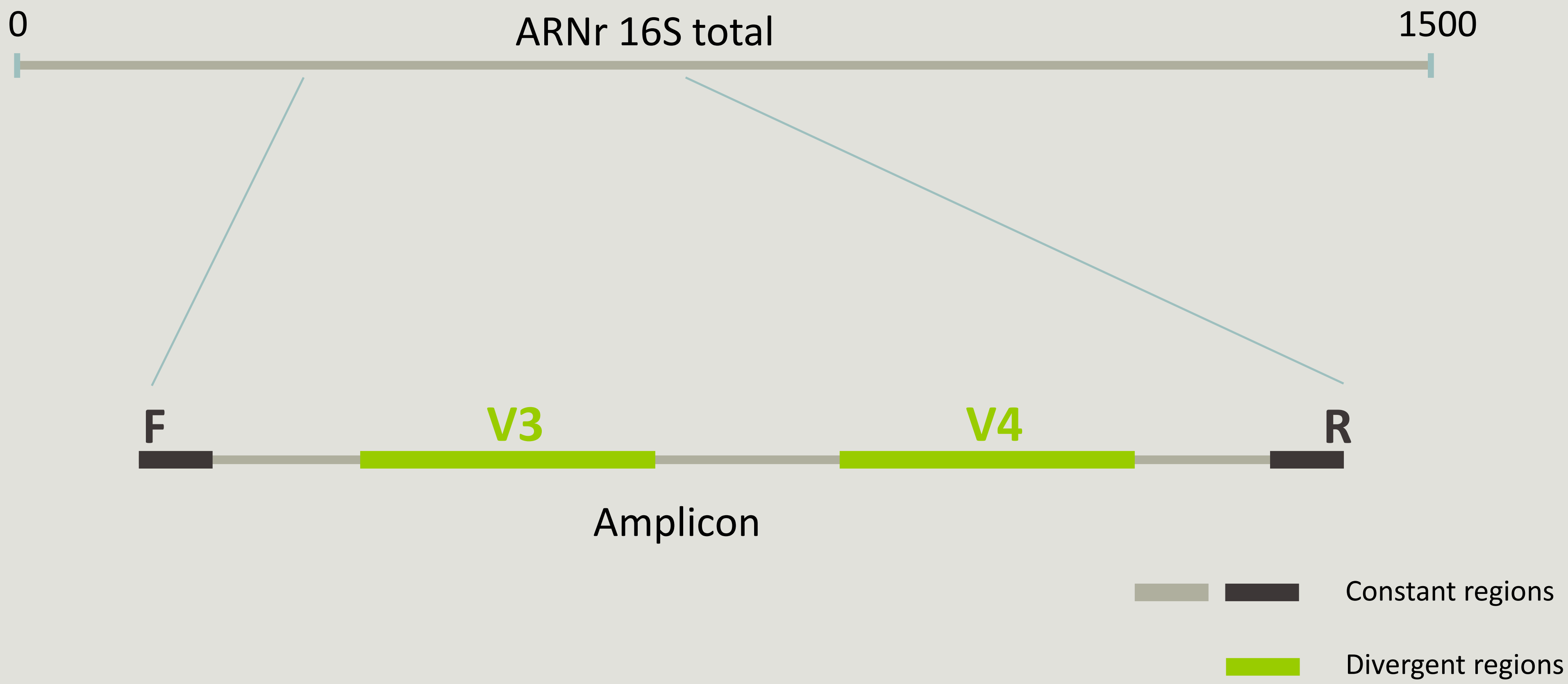


The sequencing cycles are repeated to determine the sequence of bases in a fragment, one base at a time.

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



Amplification and sequencing

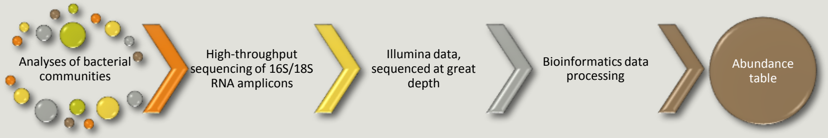
Sequencing is generally perform on **Roche-454** or **Illumina MiSeq** platforms.

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.

Methods


Analyses of bacterial communities
High-throughput sequencing of 16S/18S RNA amplicons
Illumina data, sequenced at great depth
Bioinformatics data processing
Abundance table

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

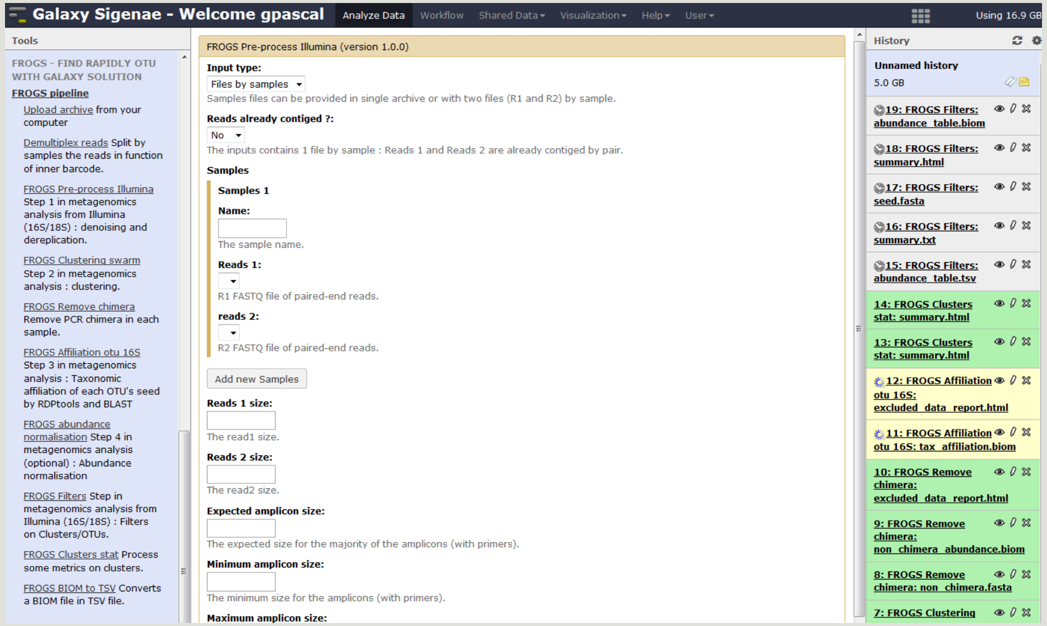
Independent modules

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.

Add new Samples

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).

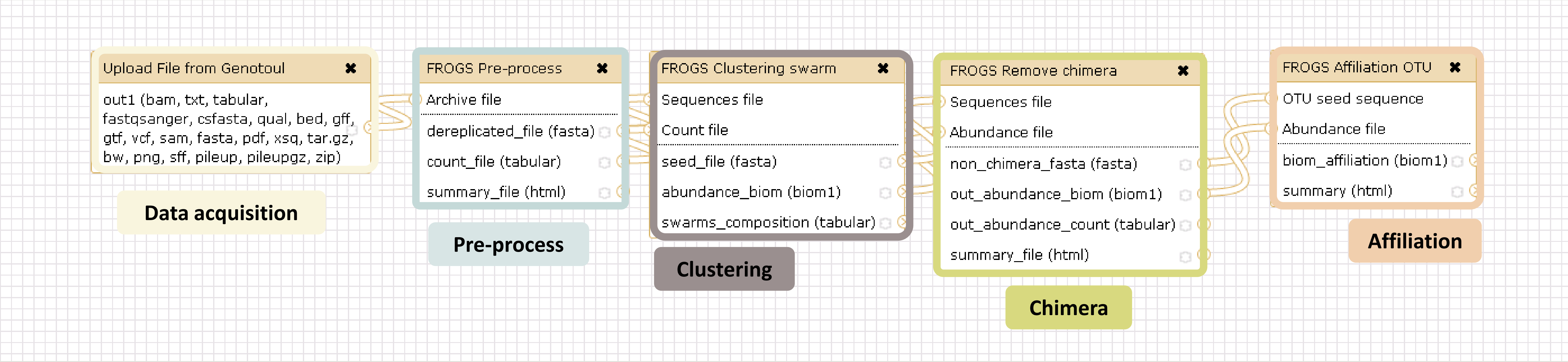
Minimum amplicon size:
The minimum size for the amplicons (with primers).

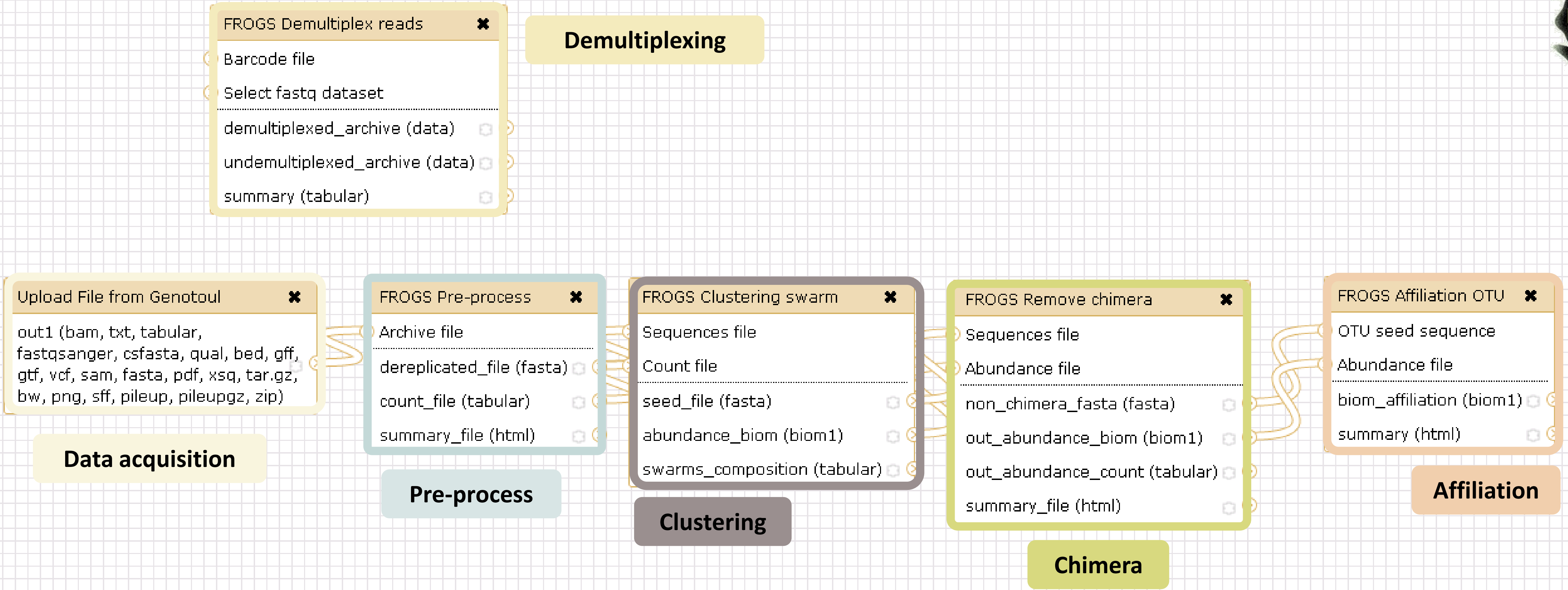
Maximum amplicon size:

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





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 ✖

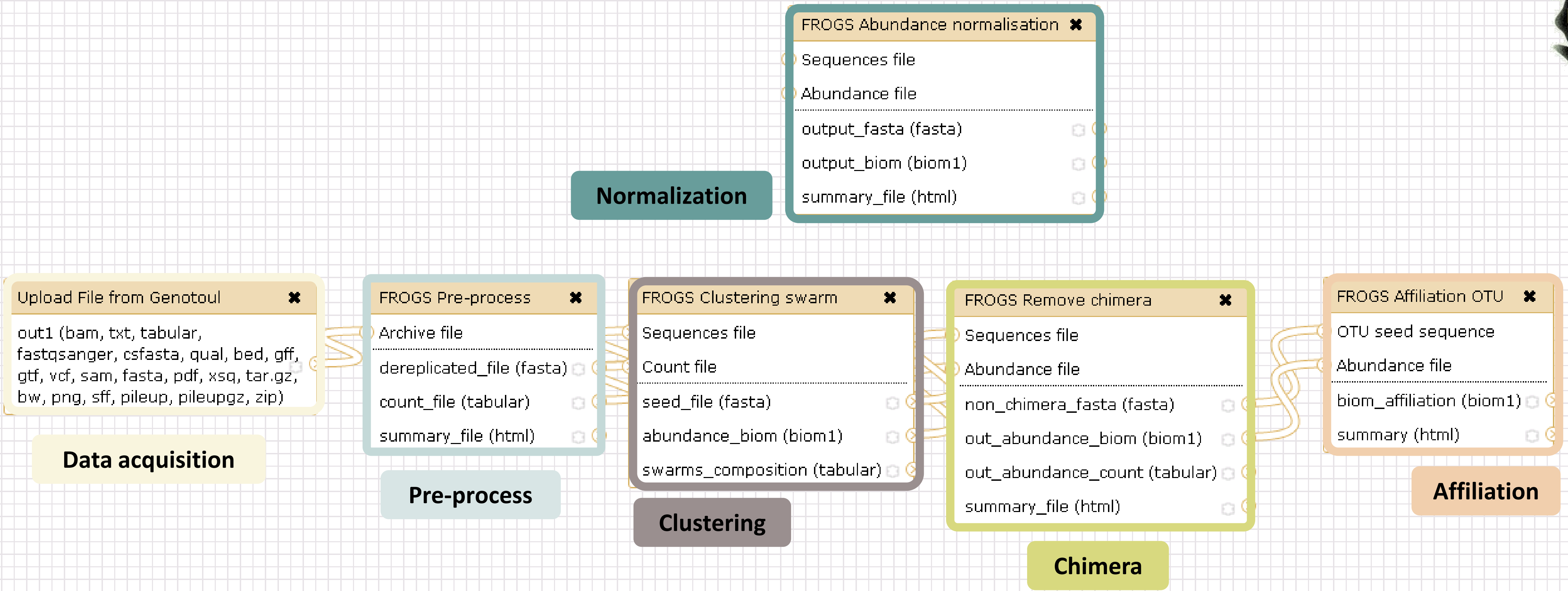
OTU seed sequence

Abundance file

biom_affiliation (biom1)

summary (html)

Affiliation



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

Normalization

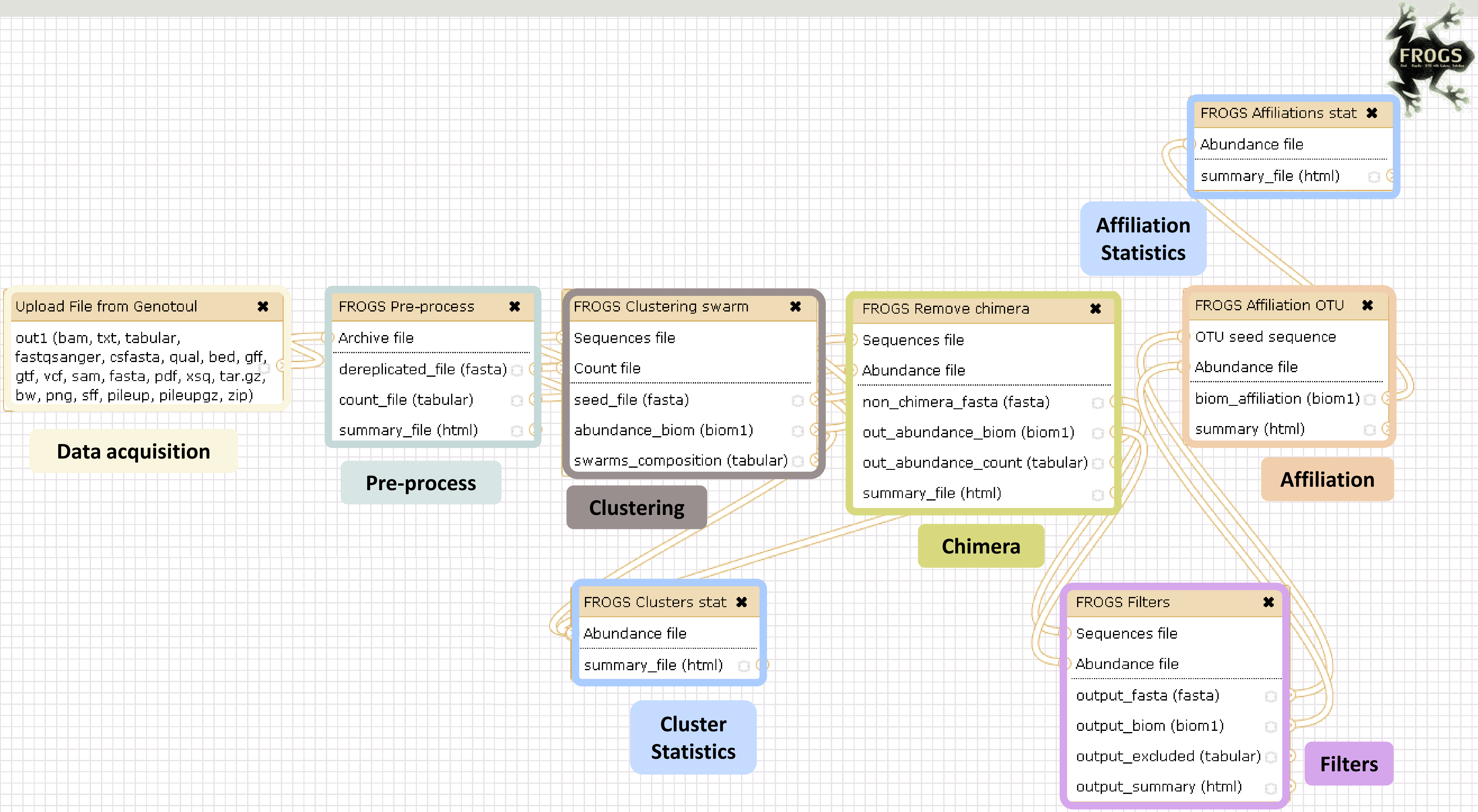
Data acquisition

Pre-process

Clustering

Chimera

Affiliation


FROGS
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)
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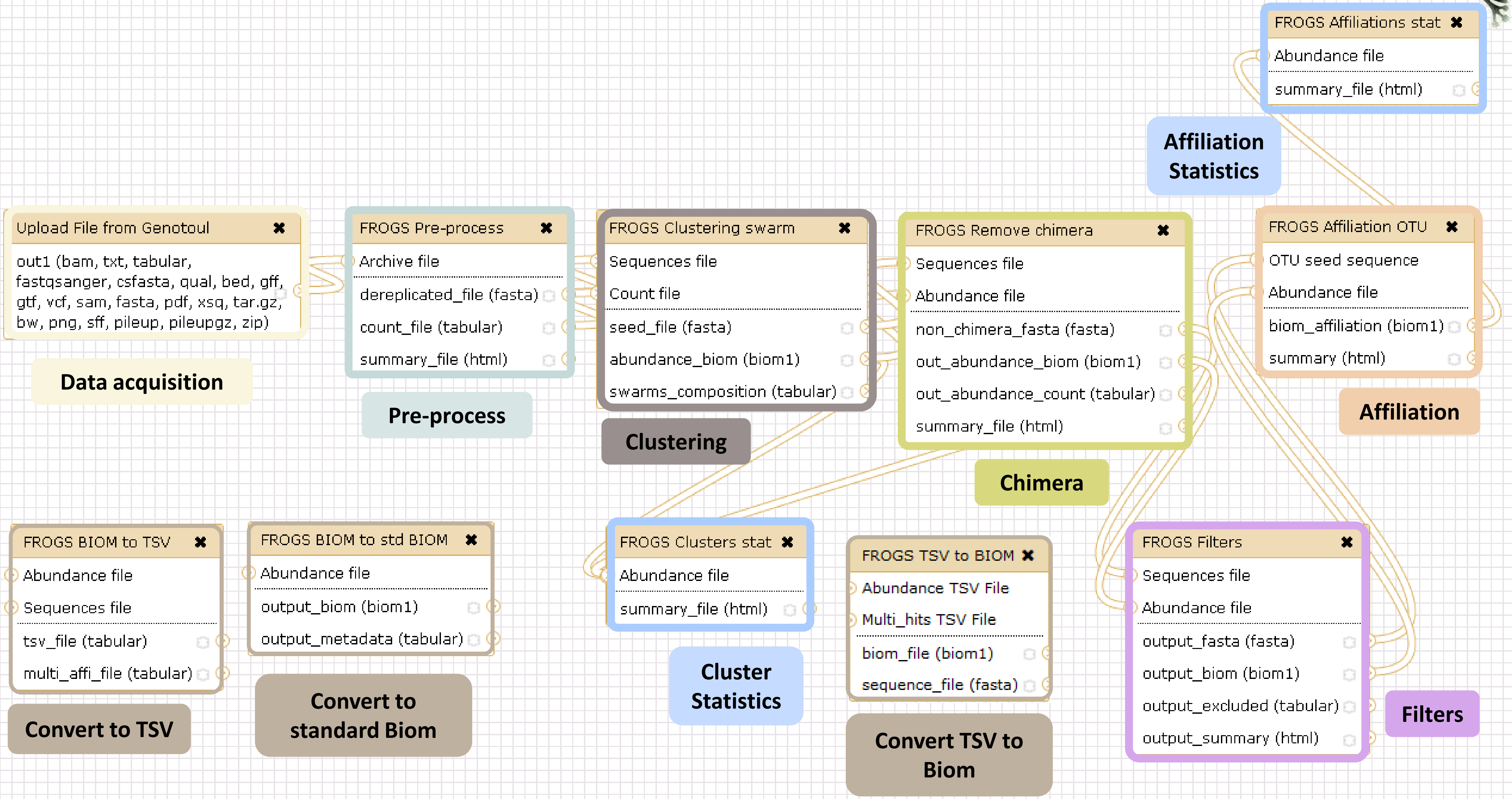
FROGS Remove chimera
Sequences file
Abundance file
non_chimera_fasta (fasta)
out_abundance_biom (biom1)
out_abundance_count (tabular)
summary_file (html)
Chimera
FROGS Affiliation OTU
OTU seed sequence
Abundance file
biom_affiliation (biom1)
summary (html)
Affiliation
FROGS Clusters stat
Abundance file
summary_file (html)
Cluster Statistics
FROGS Filters
Sequences file
Abundance file
output_fasta (fasta)
output_biom (biom1)
output_excluded (tabular)
output_summary (html)
Filters



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)

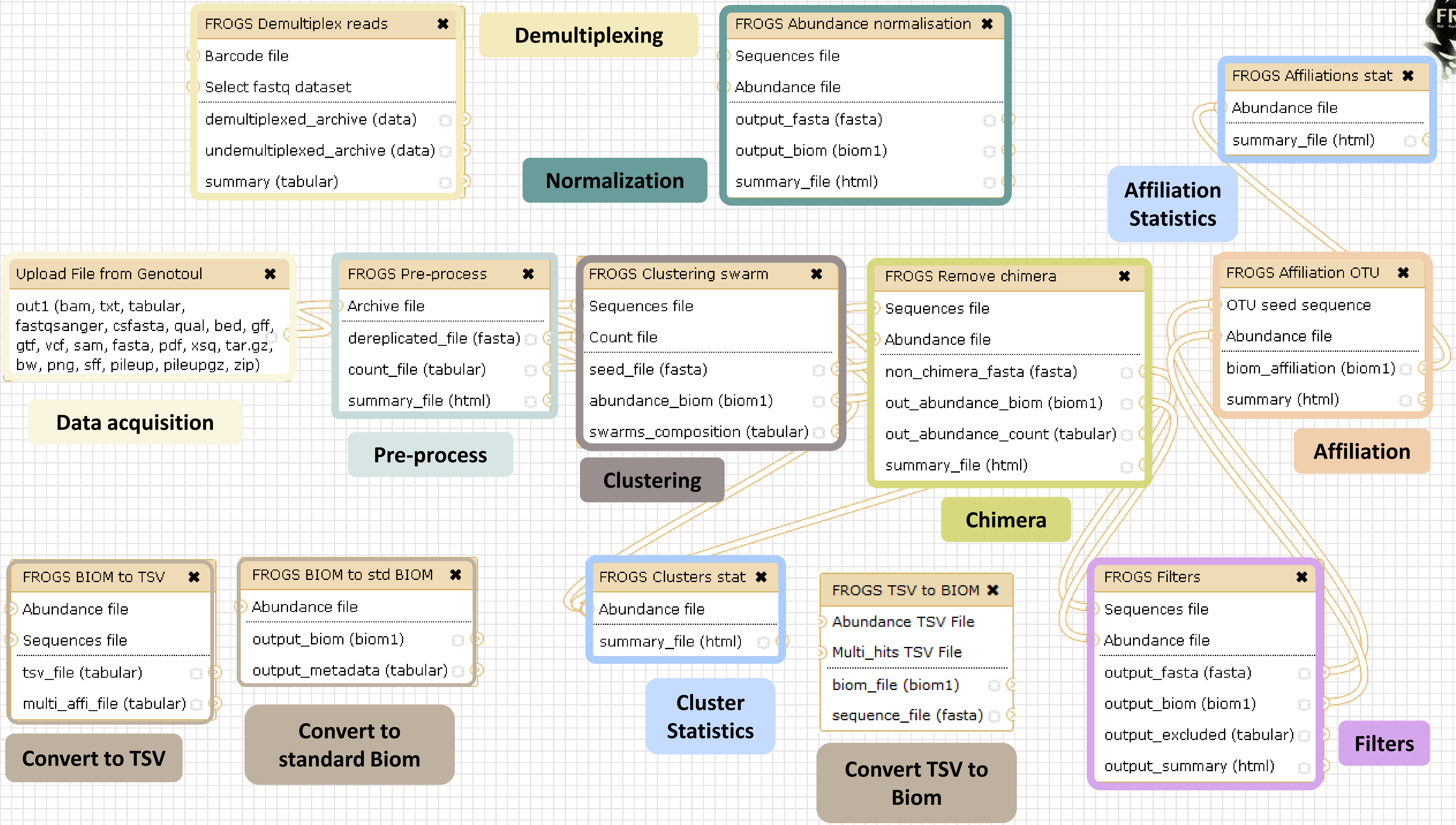
Cluster Statistics

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)

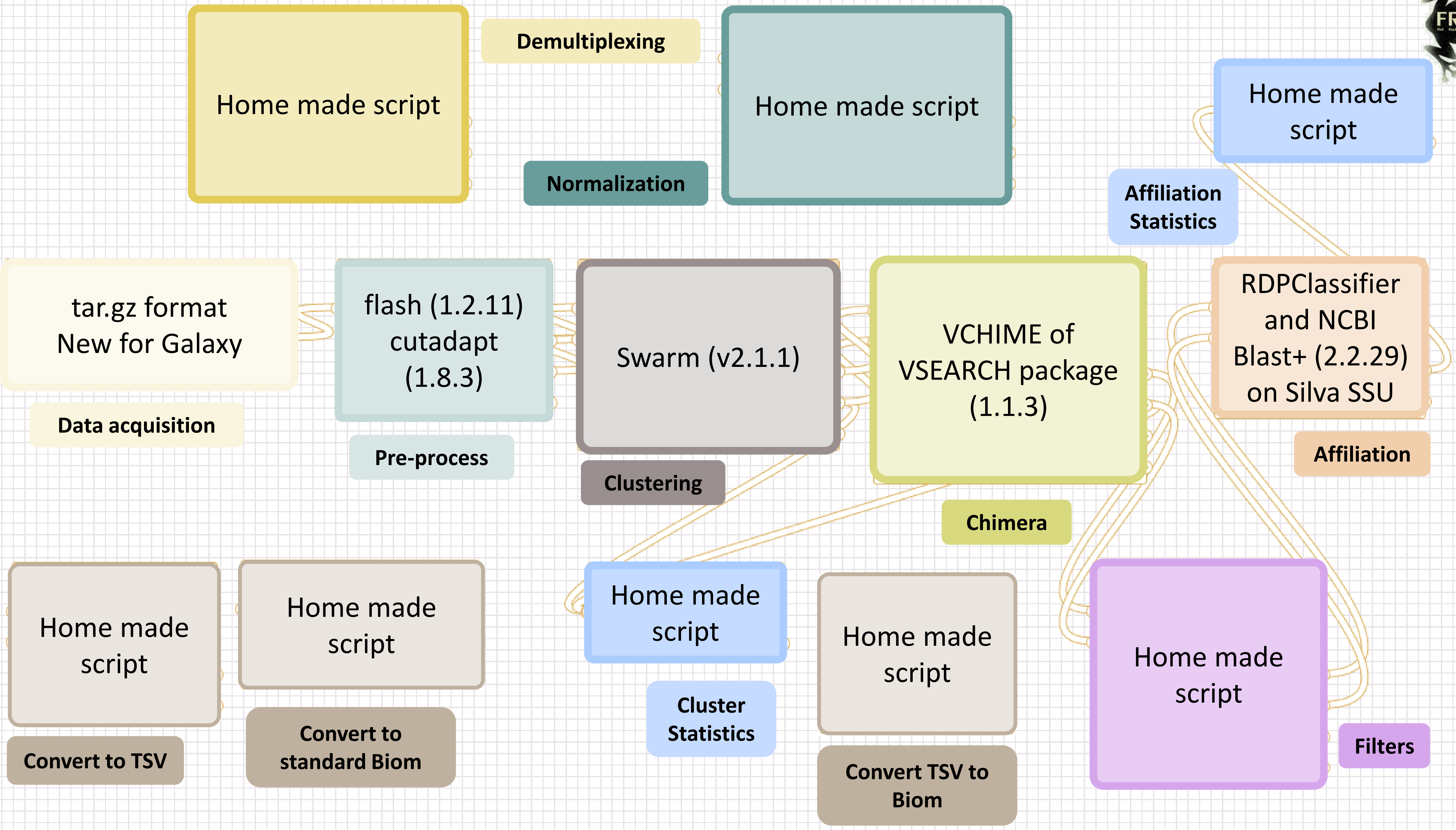
Cluster Statistics

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



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

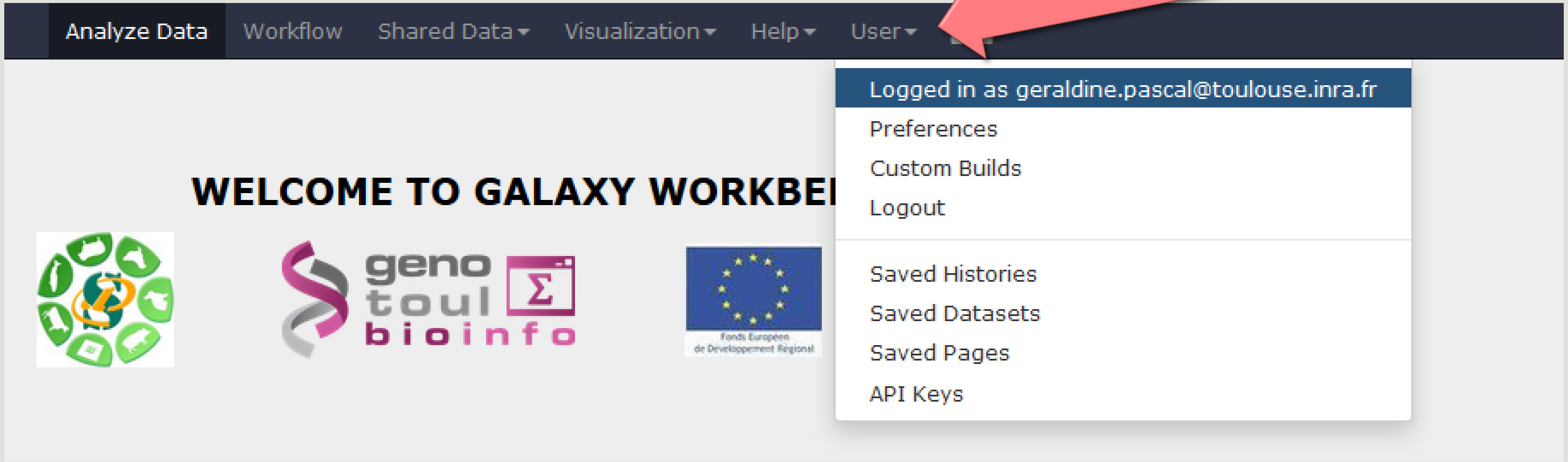
Filters

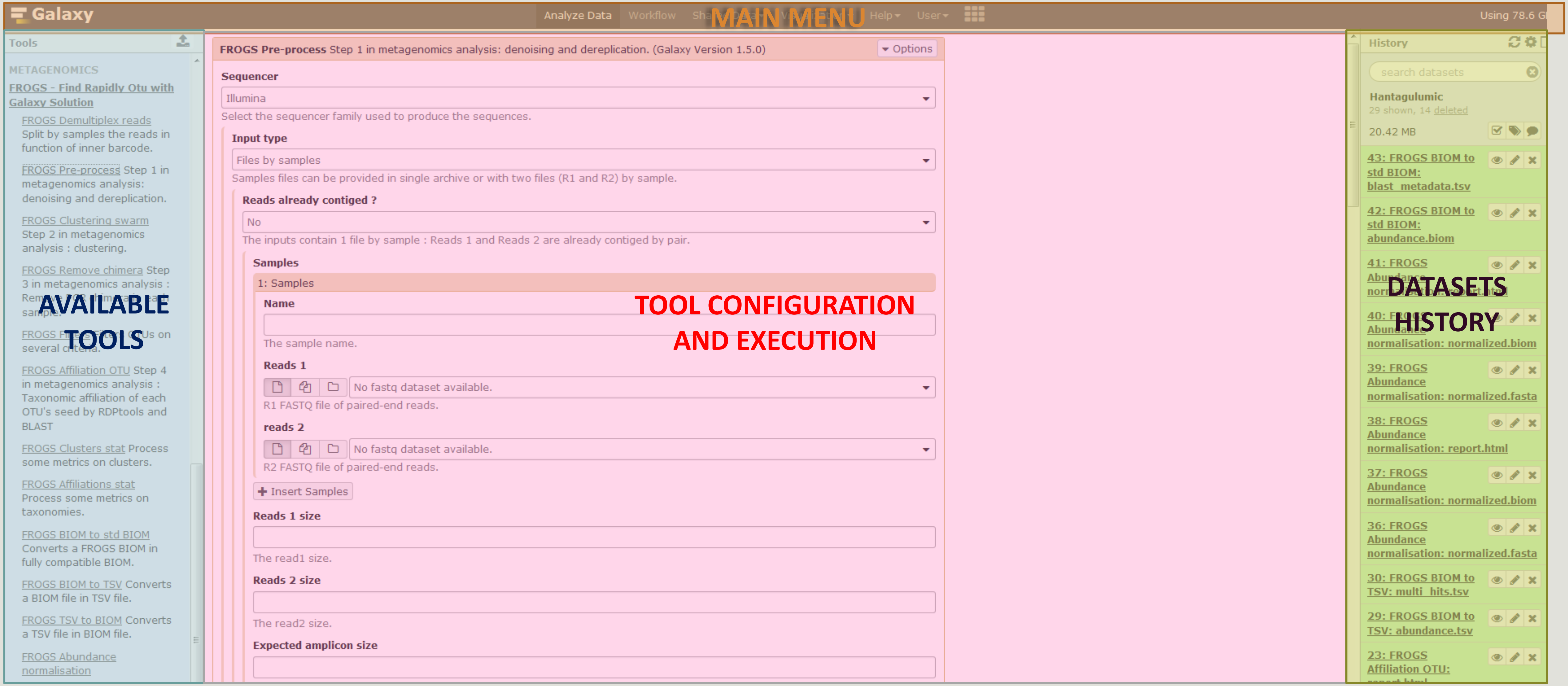
Together go to visit FROGS

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

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

Enter your email adress and password from GenoToul





MAIN MENU

AVAILABLE TOOLS

TOOL CONFIGURATION AND EXECUTION

DATASETS HISTORY

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 : Rem[illegible] sample.

FROGS F[illegible]Us 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

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 Abun[illegible] nor[illegible]

40: F[illegible] Abundance 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: report.html

Demultiplexing

Pre-process

Clustering

Chimera

Filters

Affiliation

Cluster Stat

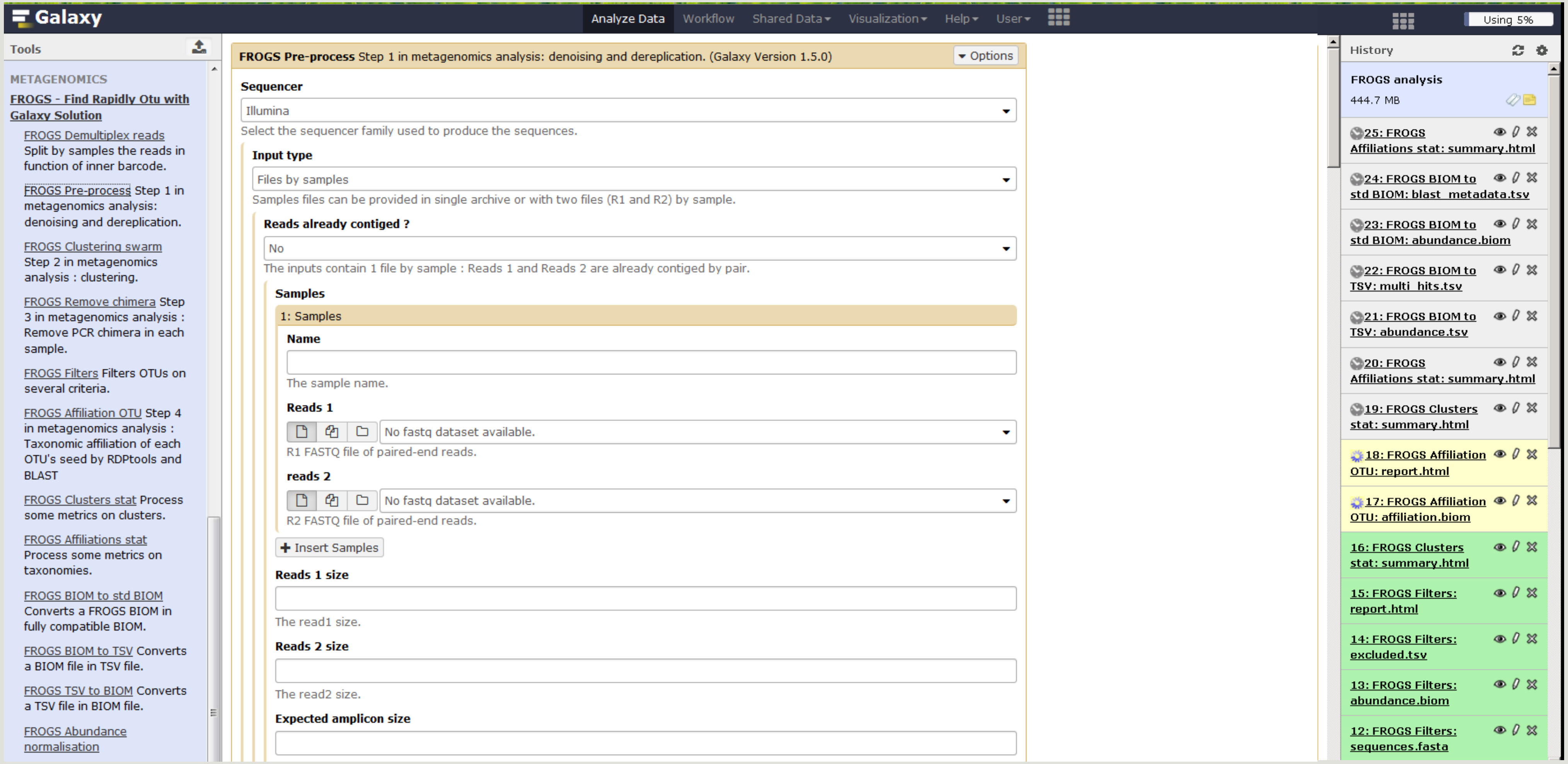
Affiliation Stat

Biom to std Biom

Biom to TSV

TSV to Biom

Normalization



Tools

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) ▾ Options

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

History

FROGS analysis

444.7 MB

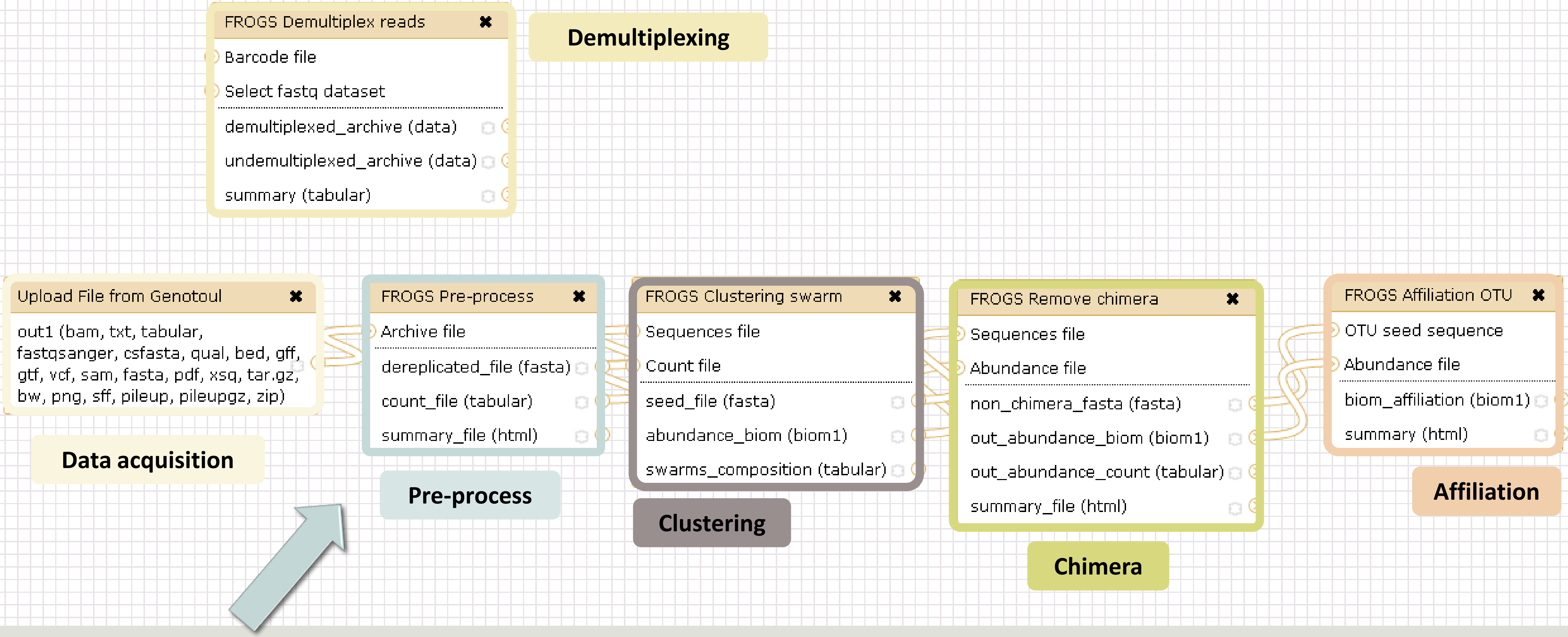
- 25: FROGS Affiliations stat: summary.html
- 24: FROGS BIOM to std BIOM: blast_metadata.tsv
- 23: FROGS BIOM to std BIOM: abundance.biom
- 22: FROGS BIOM to TSV: multi_hits.tsv
- 21: FROGS BIOM to TSV: abundance.tsv
- 20: FROGS Affiliations stat: summary.html
- 19: FROGS Clusters stat: summary.html
- 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

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 ✖

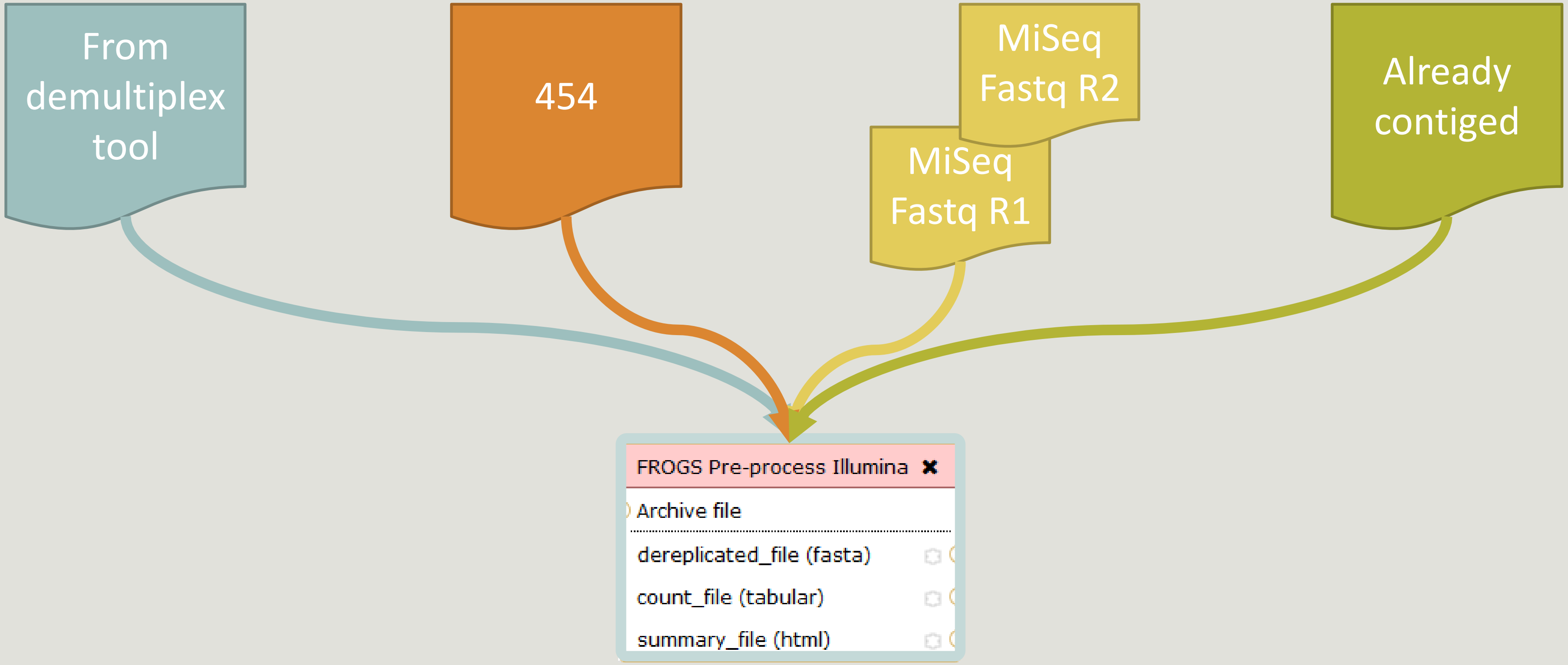
OTU seed sequence

Abundance file

biom_affiliation (biom1)

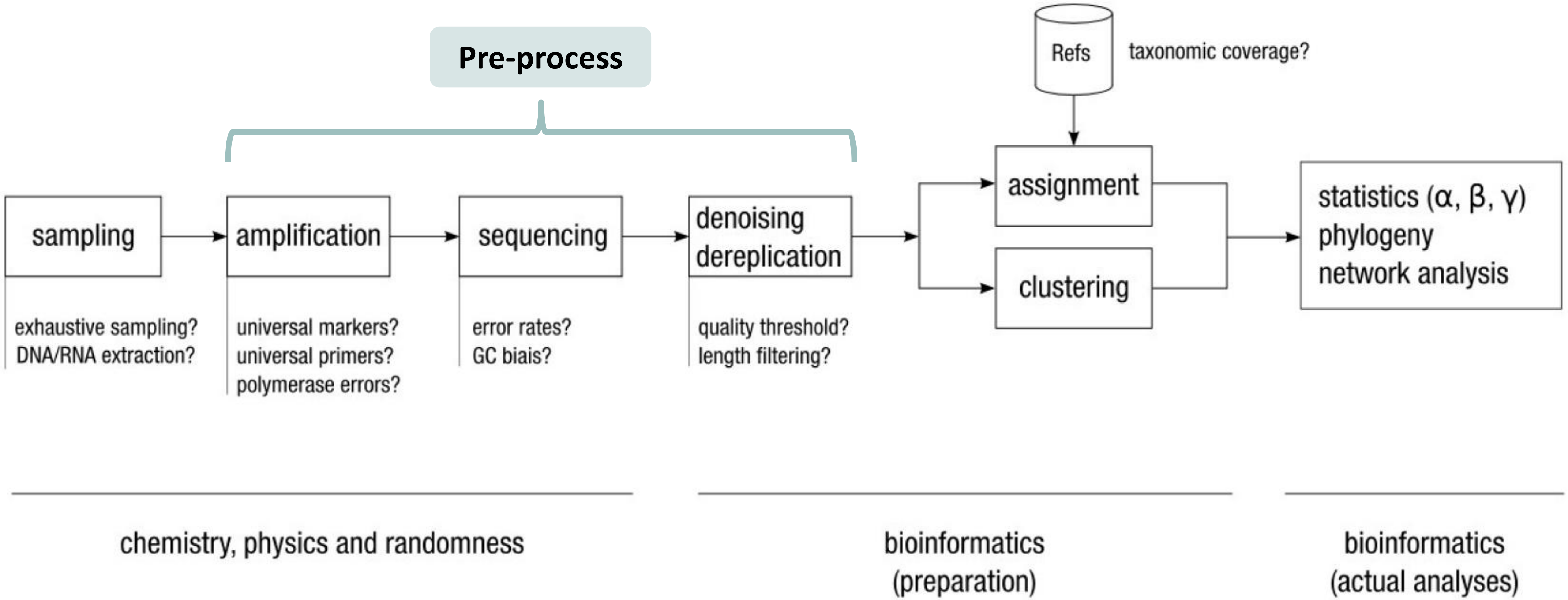
summary (html)

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

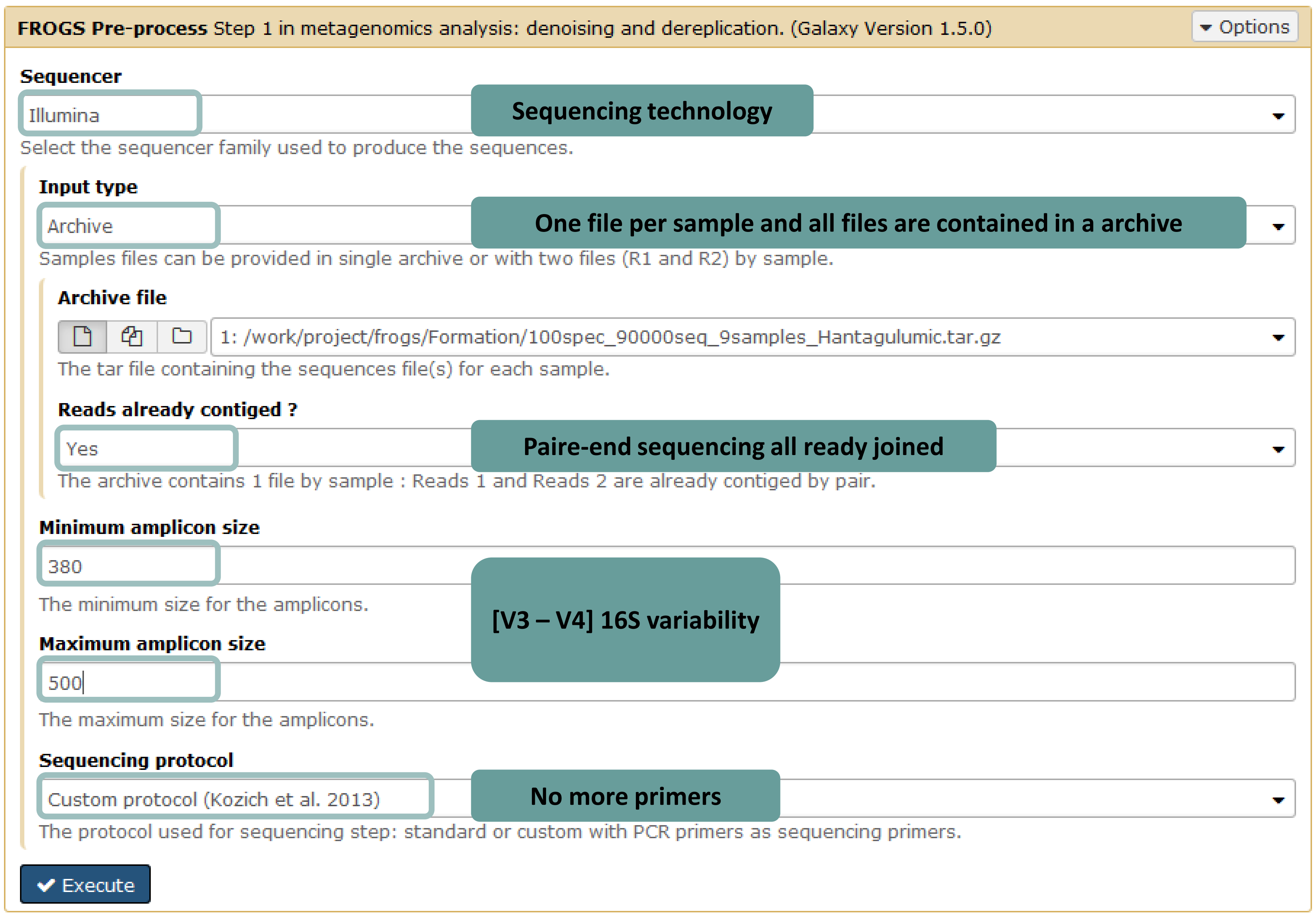


Pre-process

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

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



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

Sequencer

Illumina ▾

Sequencing technology

Select the sequencer family used to produce the sequences.

Input type

Archive ▾

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

Paire-end sequencing all ready joined

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.

[V3 – V4] 16S variability

Maximum amplicon size

500

The maximum size for the amplicons.

Sequencing protocol

Custom protocol (Kozich et al. 2013) ▾

No more primers

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

Pre-process

Your turn! - 1

GO TO EXERCISES 3

Exercise 1

Go to« MiSeq contiged » history

Launch the pre-process tool on that data set

→ objective : understand the parameters

→ objective: understand output files

Exercise 1

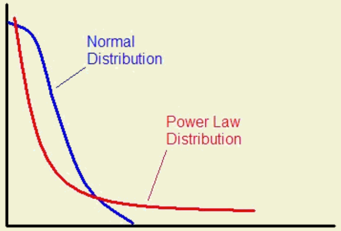
3 samples are **technically replicated** 3 times : 9 samples of 10 000 sequences each.

100_10000seq_sampleA1.fastq
100_10000seq_sampleA2.fastq
100_10000seq_sampleA3.fastq

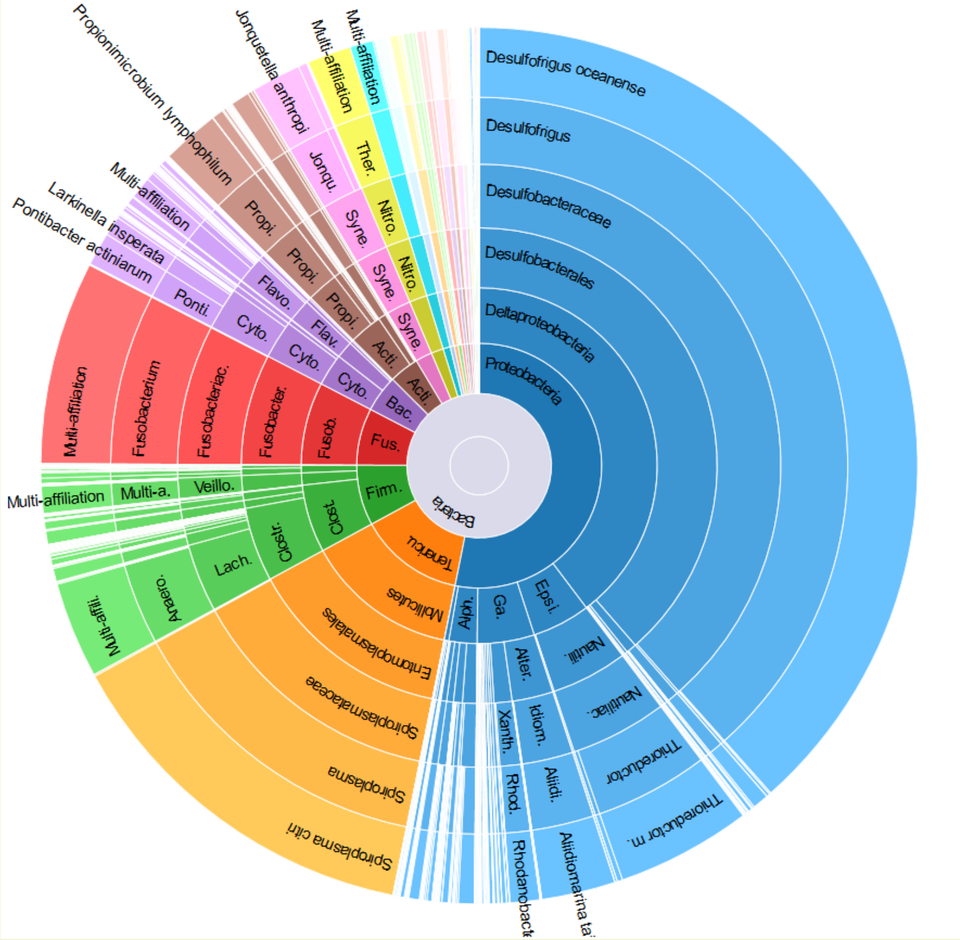
100_10000seq_sampleB1.fastq
100_10000seq_sampleB2.fastq
100_10000seq_sampleB3.fastq

100_10000seq_sampleC1.fastq
100_10000seq_sampleC2.fastq
100_10000seq_sampleC3.fastq

Exercise 1



- 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)



Exercise 1

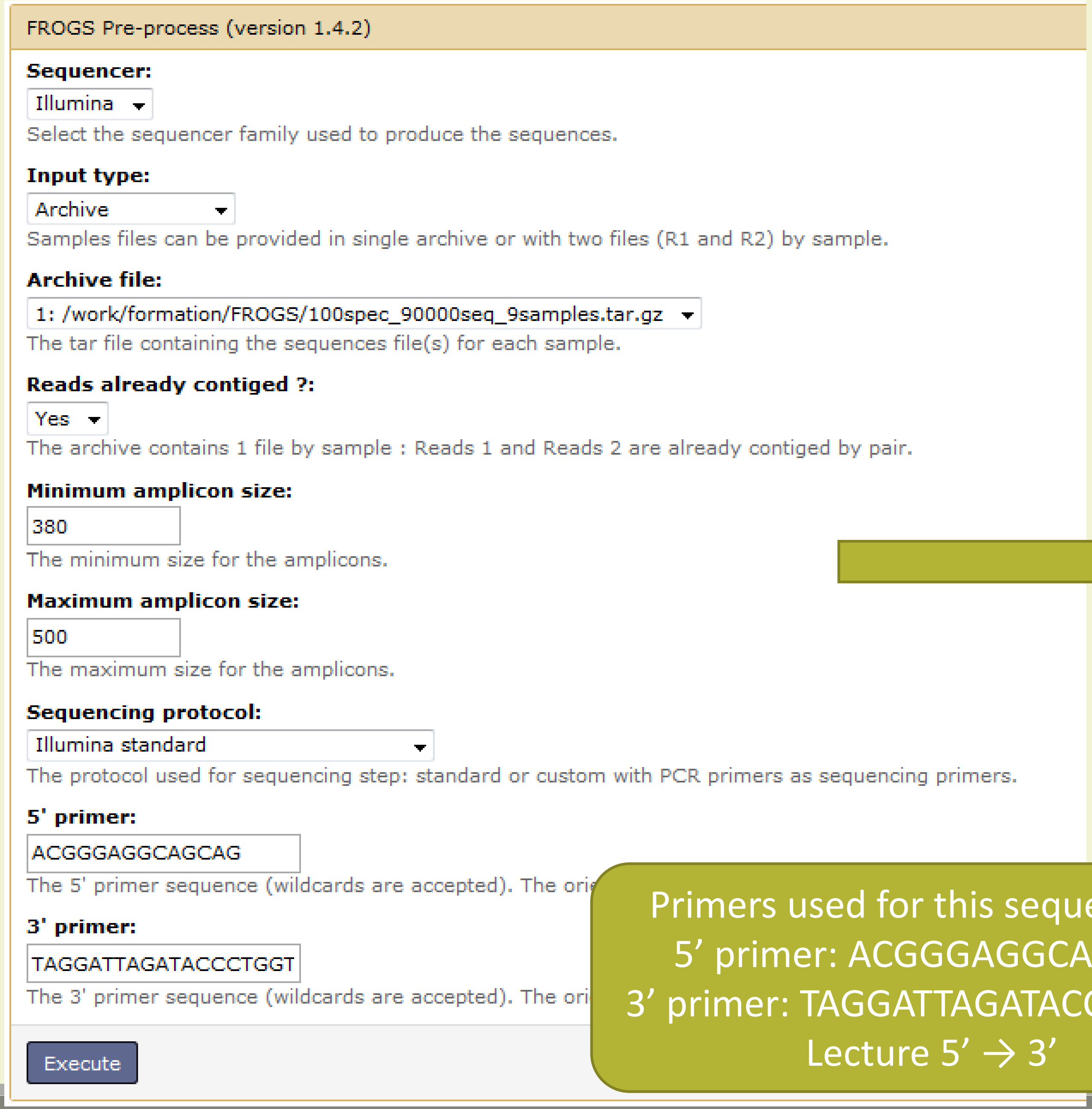
“Grinder (v 0.5.3) (Angly et al., 2012) was used to simulate the PCR amplification of full-length (V3-V4) sequences from reference databases. The reference database of size 100 were generated from the LTP SSU bank (version 115) (Yarza et al., 2008) by

(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.”



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.

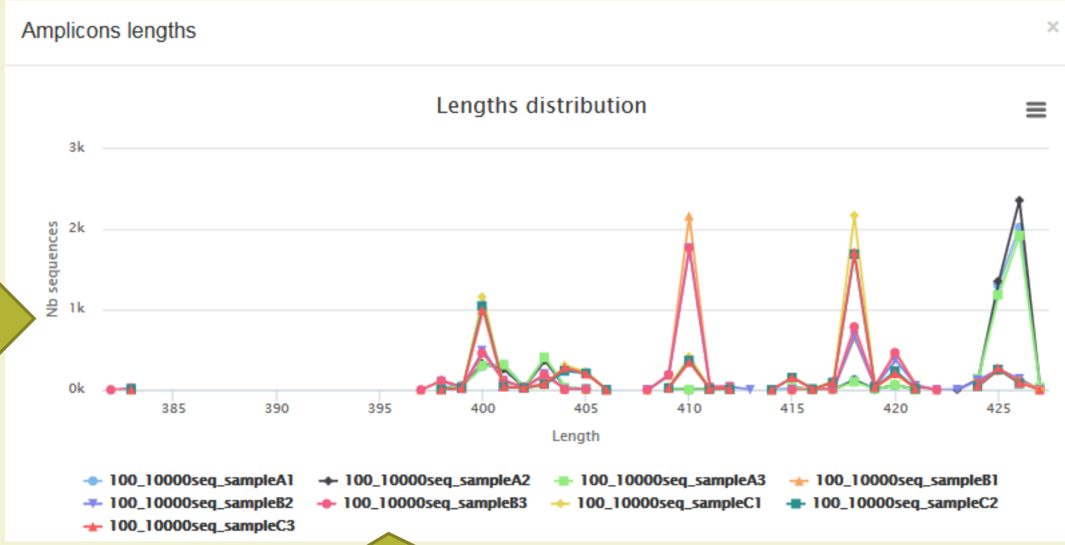
Minimum amplicon size:
380
The minimum size for the amplicons.

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



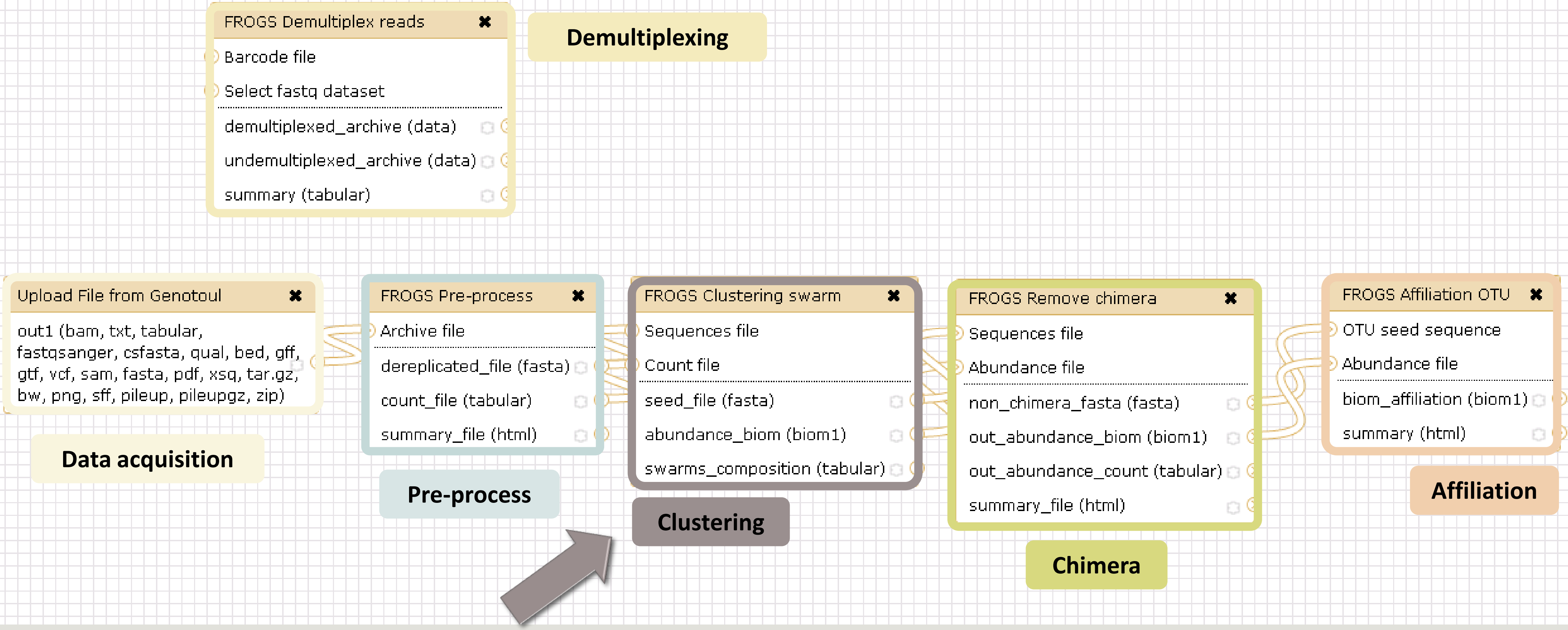
Click on legend

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

Exercise 1 - Questions

1. What is the length of your reads before preprocessing ?
2. Do you understand how enter your primers ?
3. What is the « FROGS Pre-process: dereplicated.fasta » file ?
4. What is the « FROGS Pre-process: count.tsv » file ?
5. Explore the file « FROGS Pre-process: report.html »
6. *Who loose a lot of sequences ?*
7. How many sequences are there in the input file ?
8. How many sequences did not have the 5’ primer?
9. How many sequences still are after pre-processing the data?
10. How much time did it take to pre-process the data ?
11. 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)

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

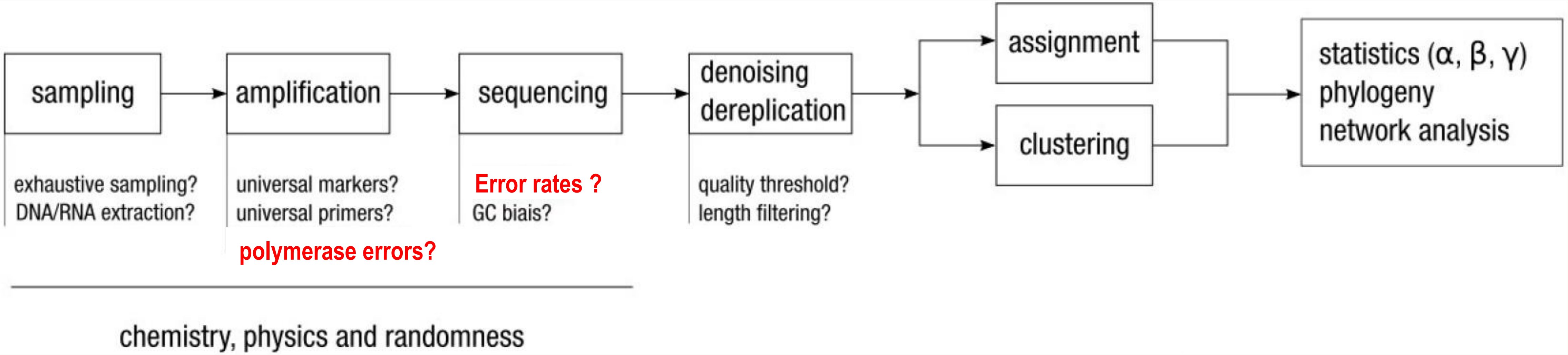
biom_affiliation (biom1)

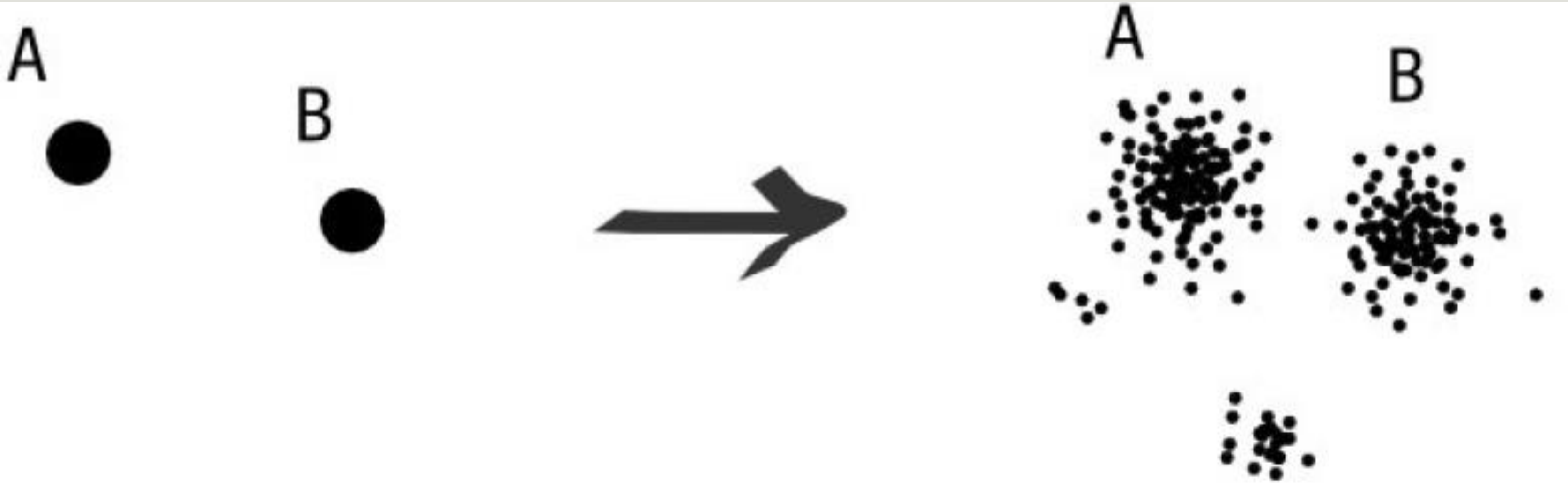
summary (html)

Affiliation

Why do we need clustering ?

Amplication and sequencing and are not perfect processes

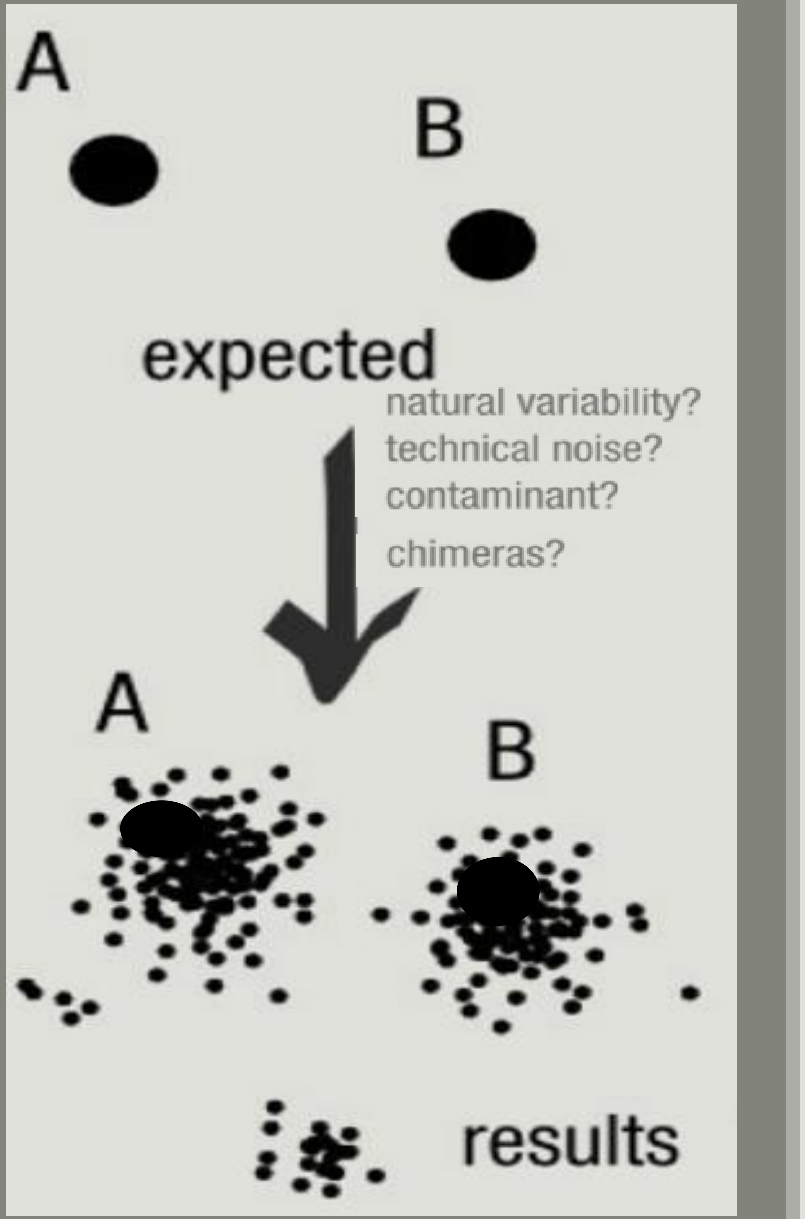




Expected

Results

Natural variability ?
Technical noise?
Contaminant?
Chimeras?



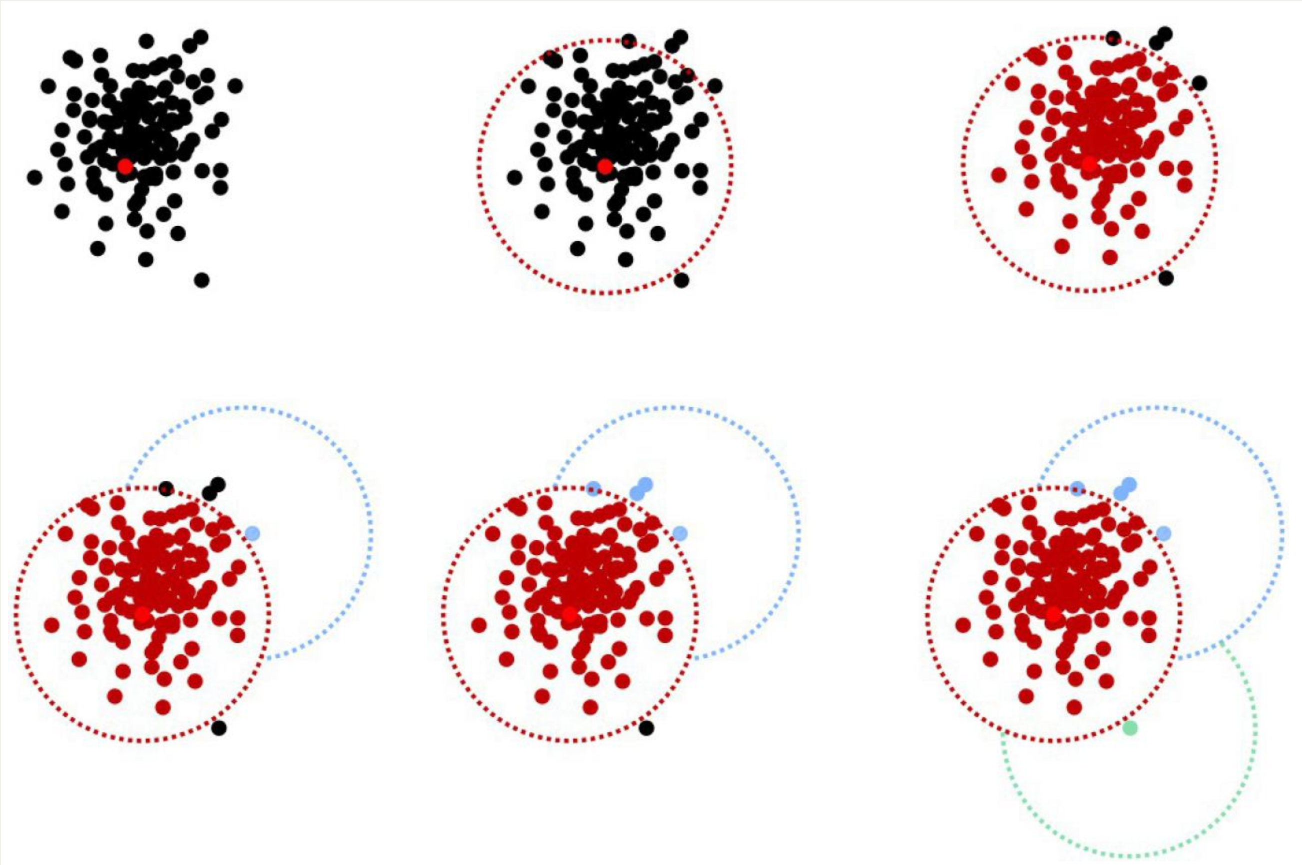
To have the best accuracy:

Method: All against all

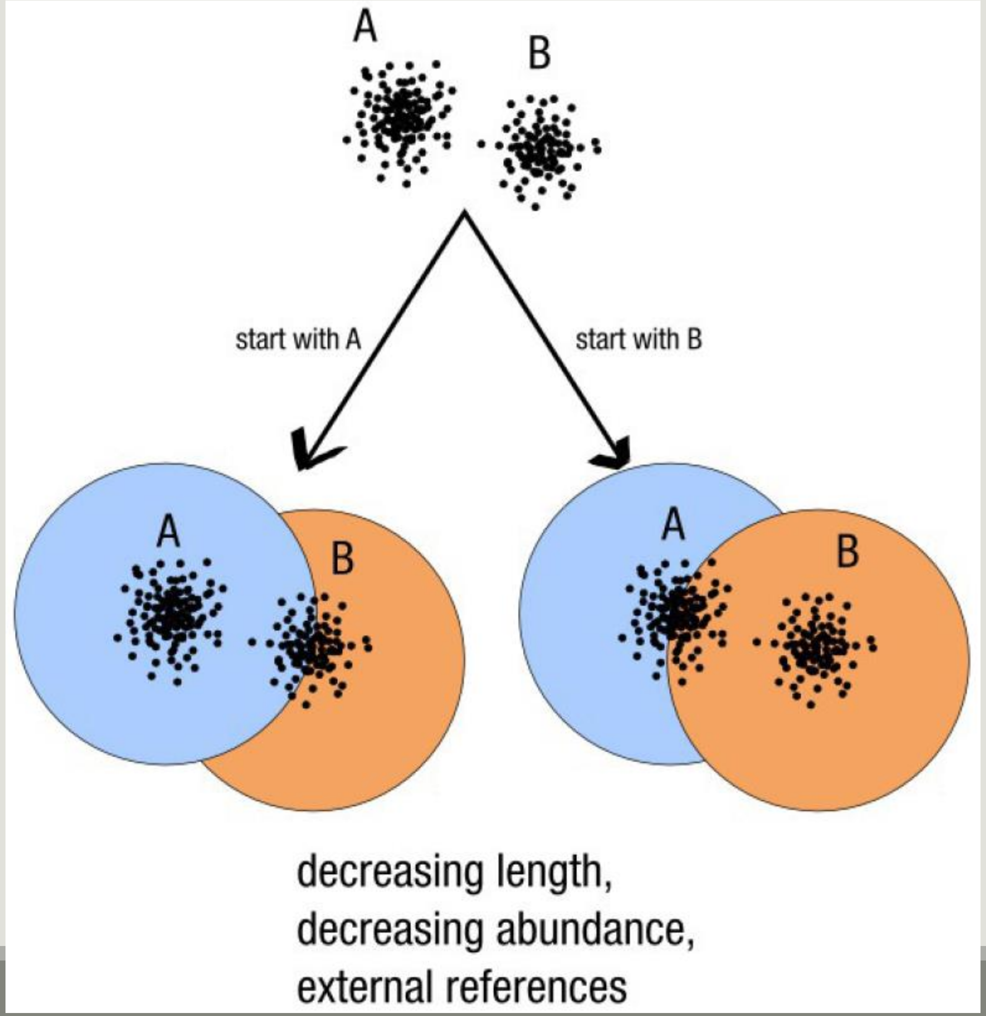
- Very accurate
- Requires a lot of memory and/or time

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

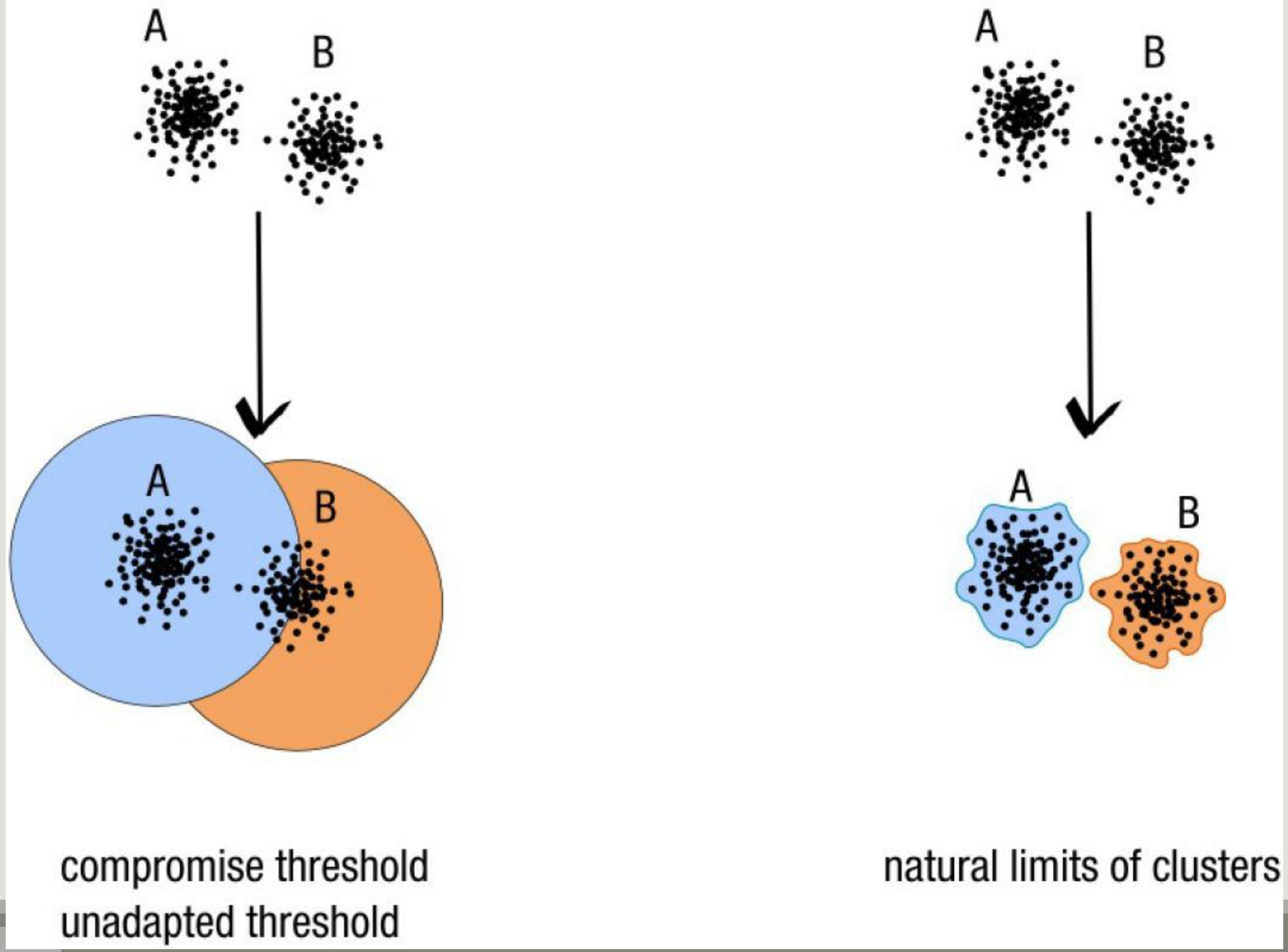
How traditional clustering works ?



Input order dependent results



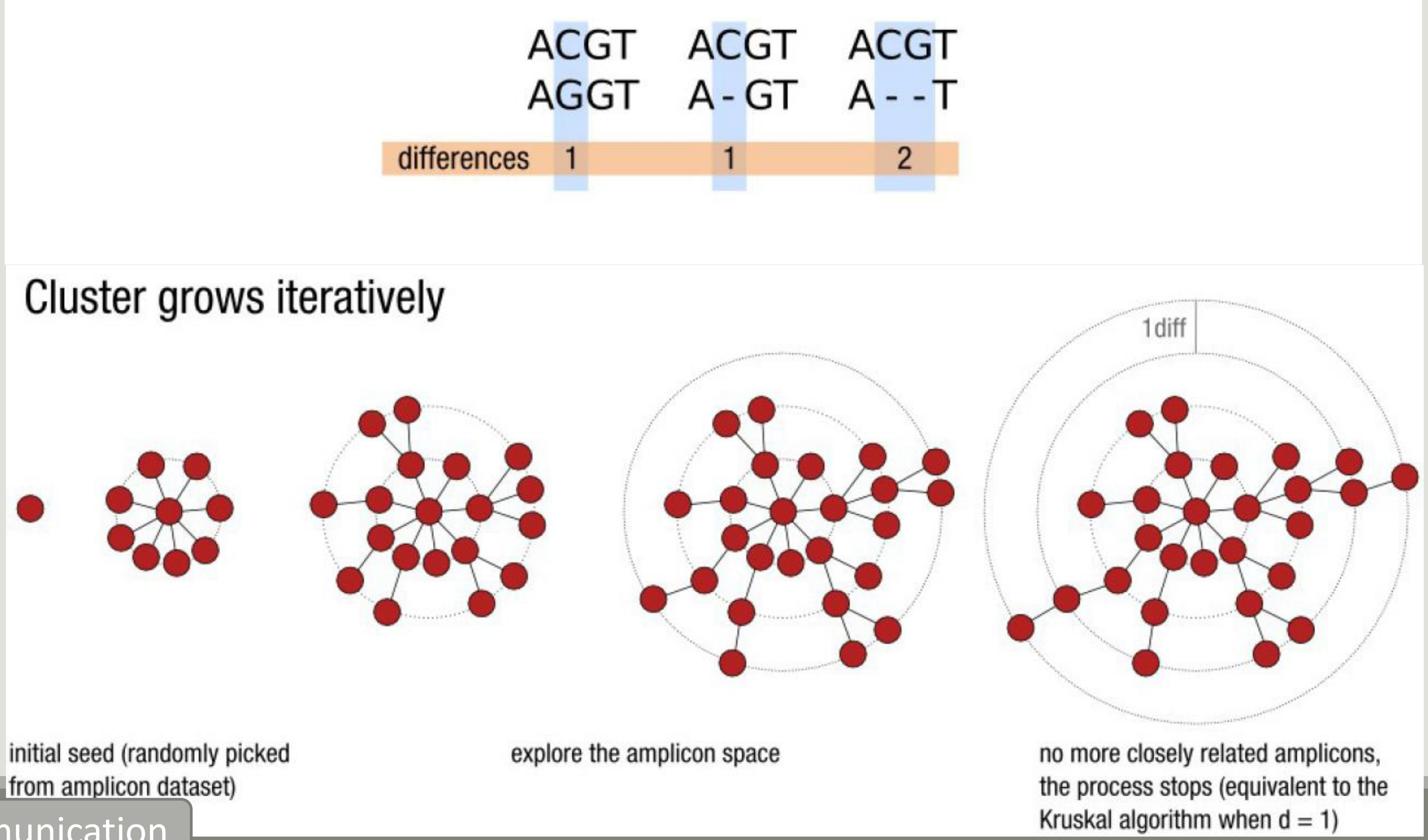
Single a priori clustering threshold



compromise threshold
unadapted threshold

natural limits of clusters

Swarm clustering method



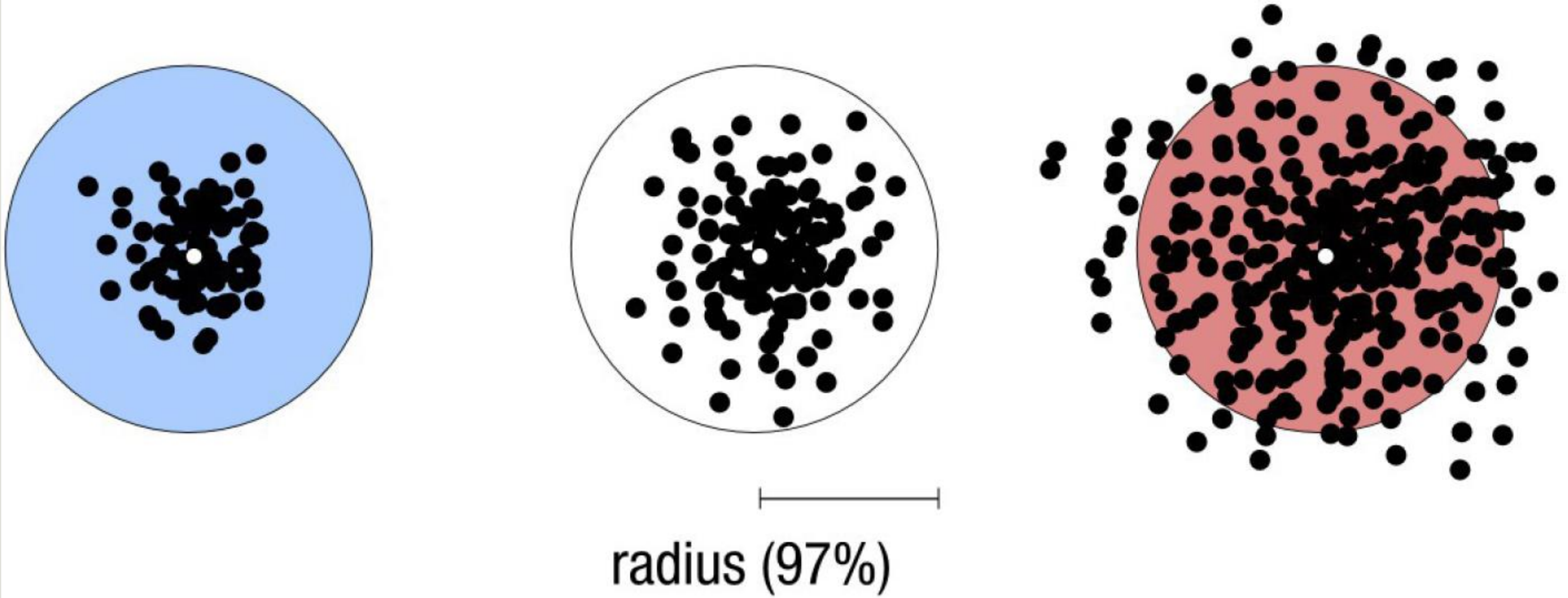
initial seed (randomly picked from amplicon dataset)

explore the amplicon space

no more closely related amplicons, the process stops (equivalent to the Kruskal algorithm when d = 1)

Fréderic Mahé communication

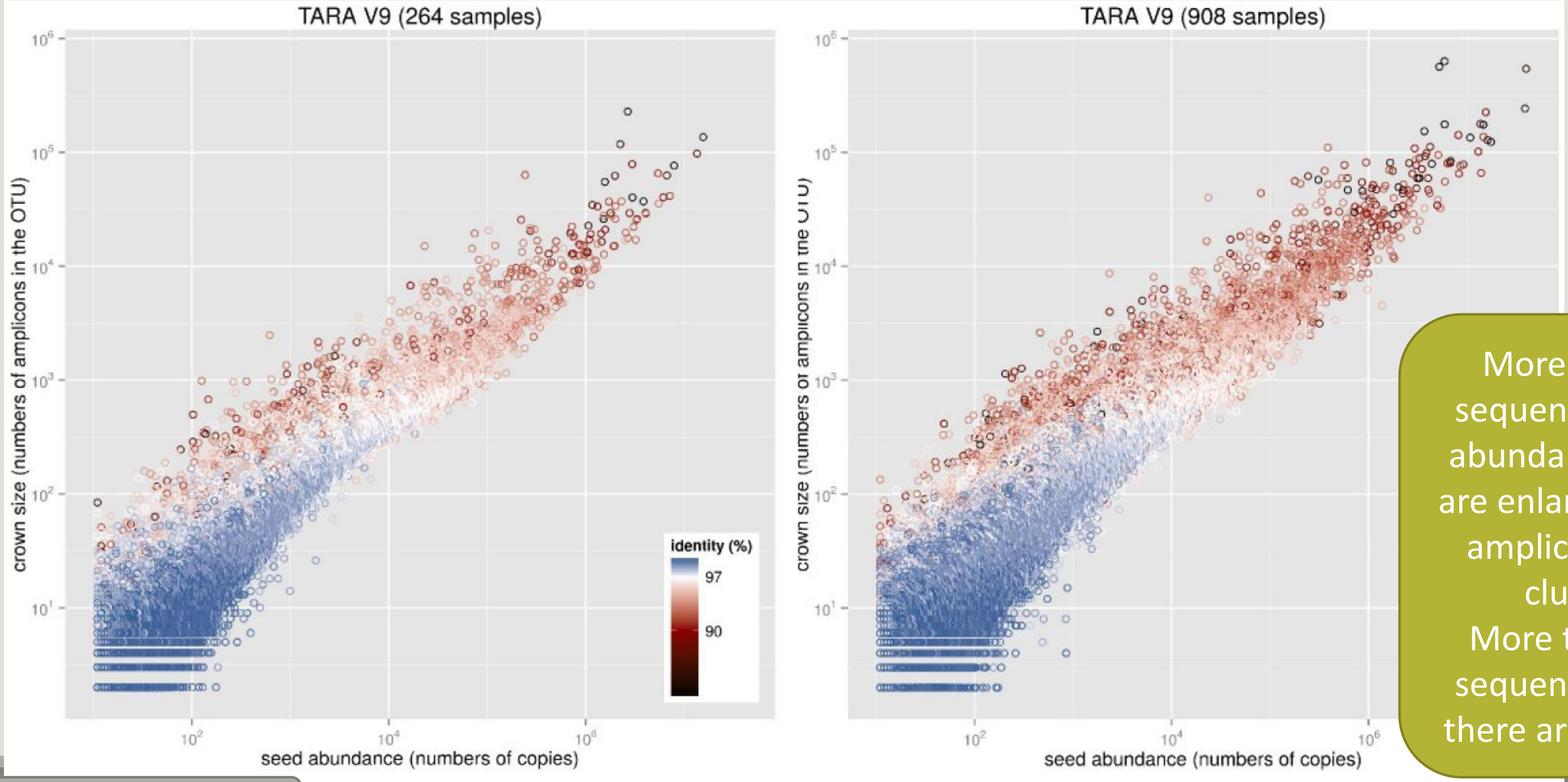
Comparison Swarm and 3% clusterings



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

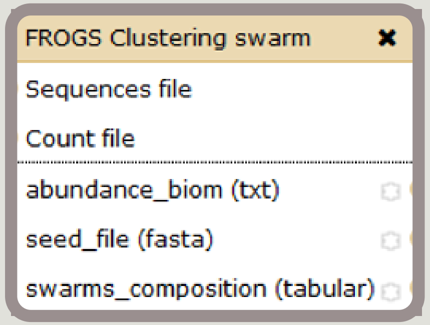
A robust and fast clustering method for amplicon-based studies.

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

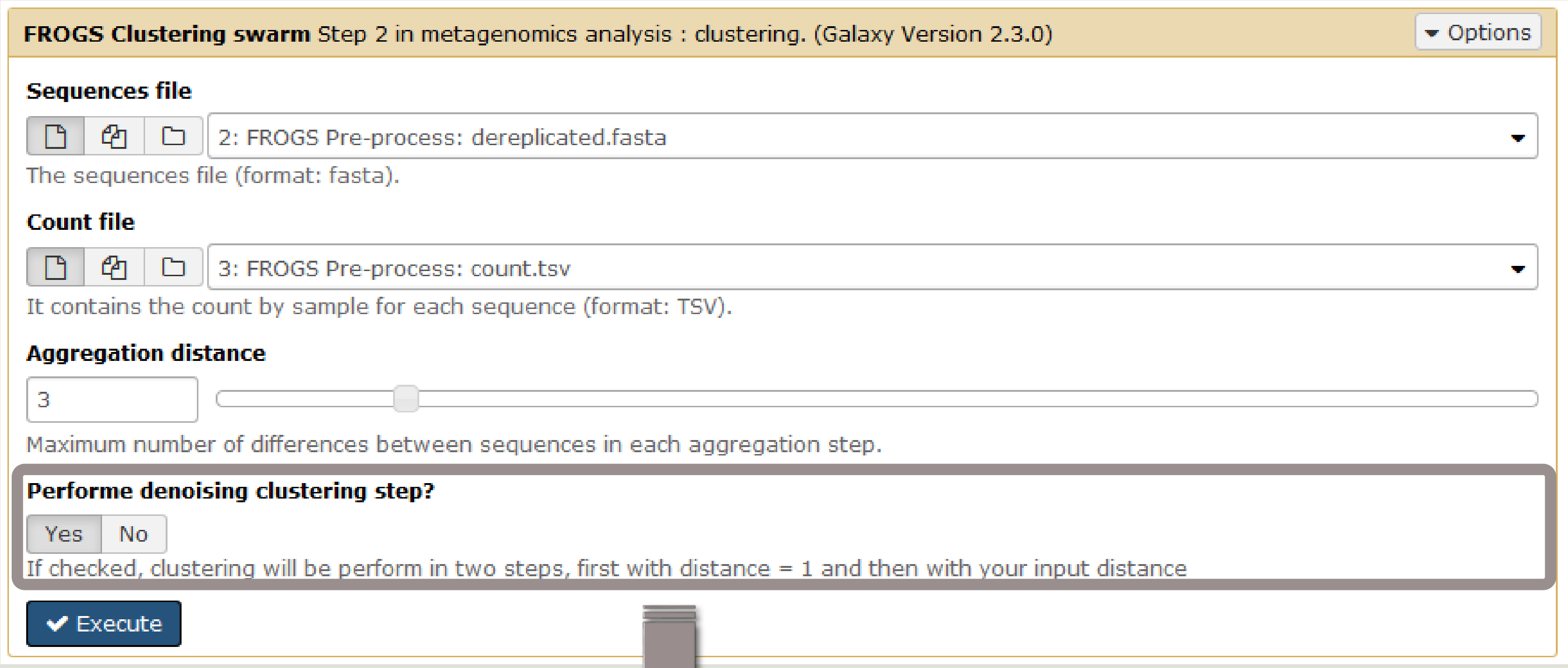
Count file

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:

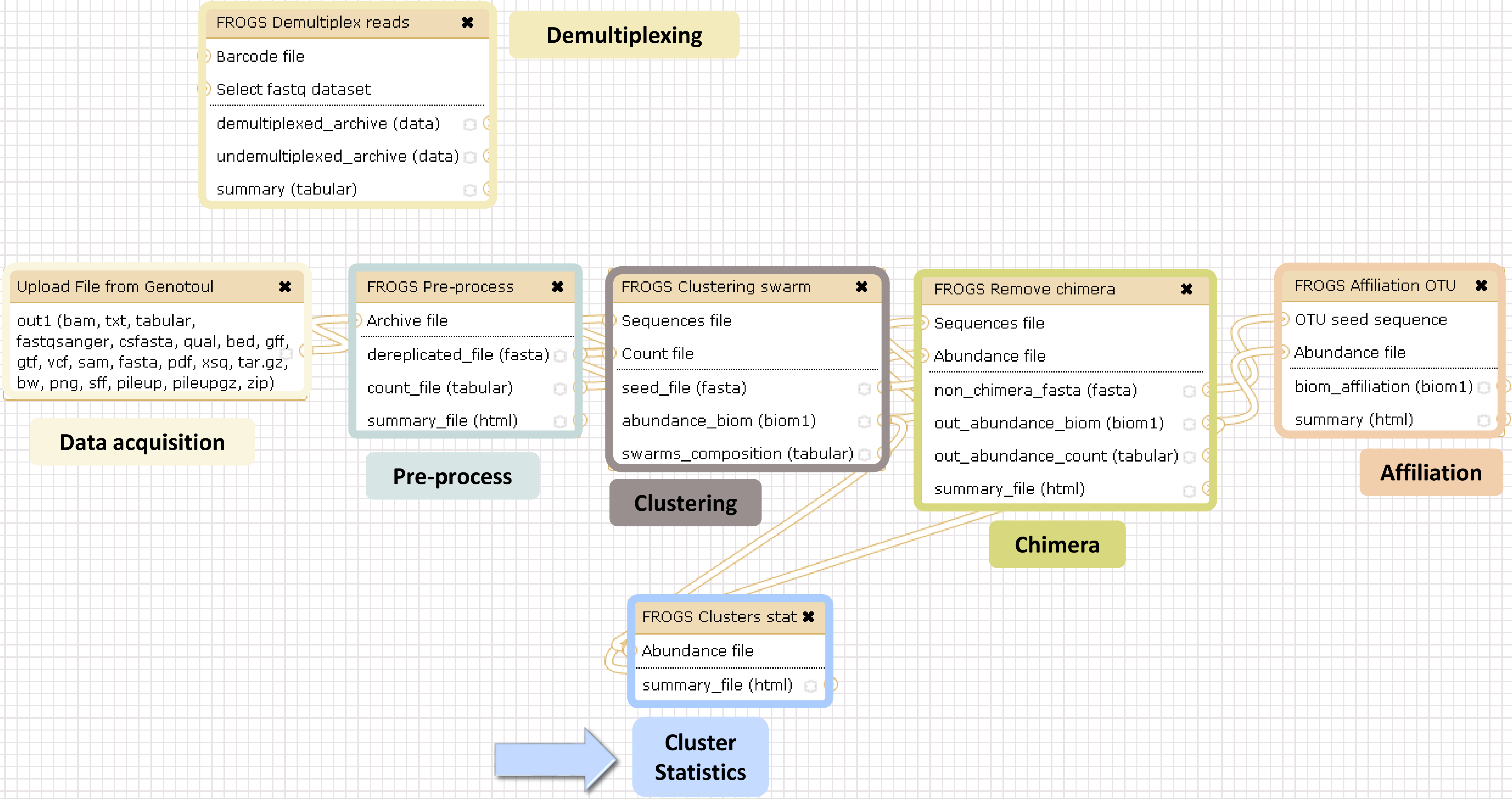
Swarm with d = 3 on the seeds of first Swarm
quadratic complexity

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)

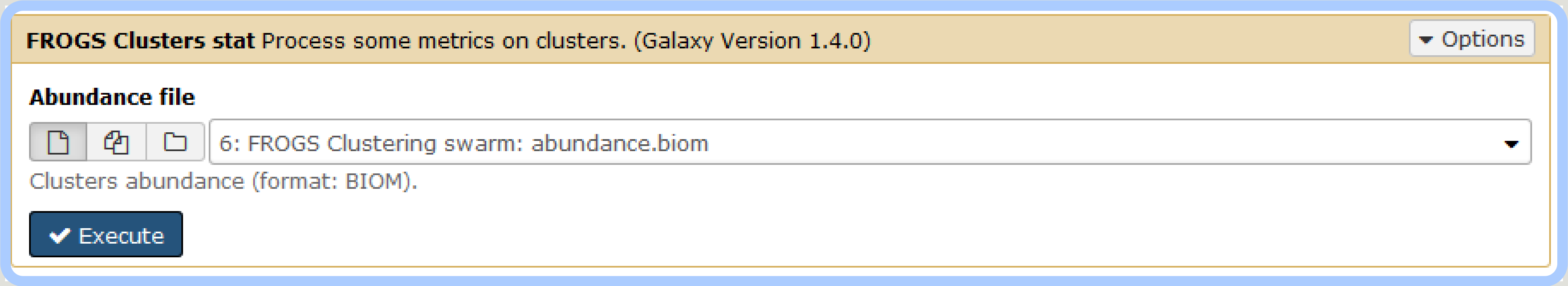
Affiliation

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! - 2

LAUNCH CLUSTERING AND CLUSTERSTAT TOOLS

Exercise 2

Go to « MiSeq contiged » history

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

→ objectives :

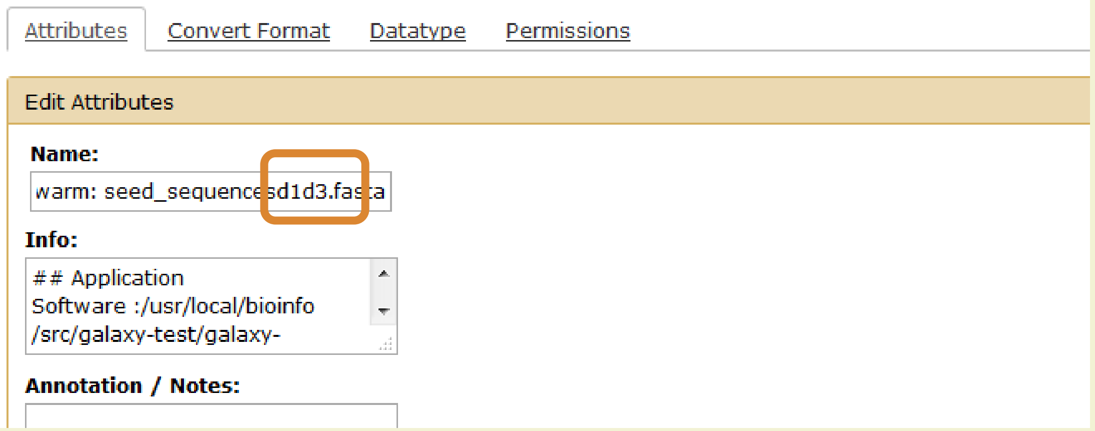
- understand the denoising efficiency
- understand the ClusterStat utility

Exercise 2

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

Exercise 2

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



Edit Attributes

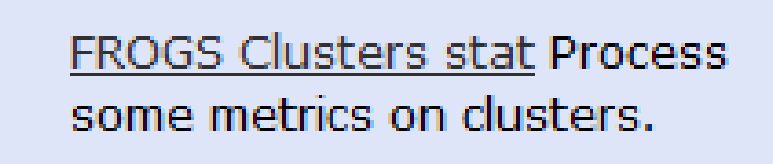
Name:

warm: seed_sequencesd1d3.fasta

Info:

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

Annotation / Notes:



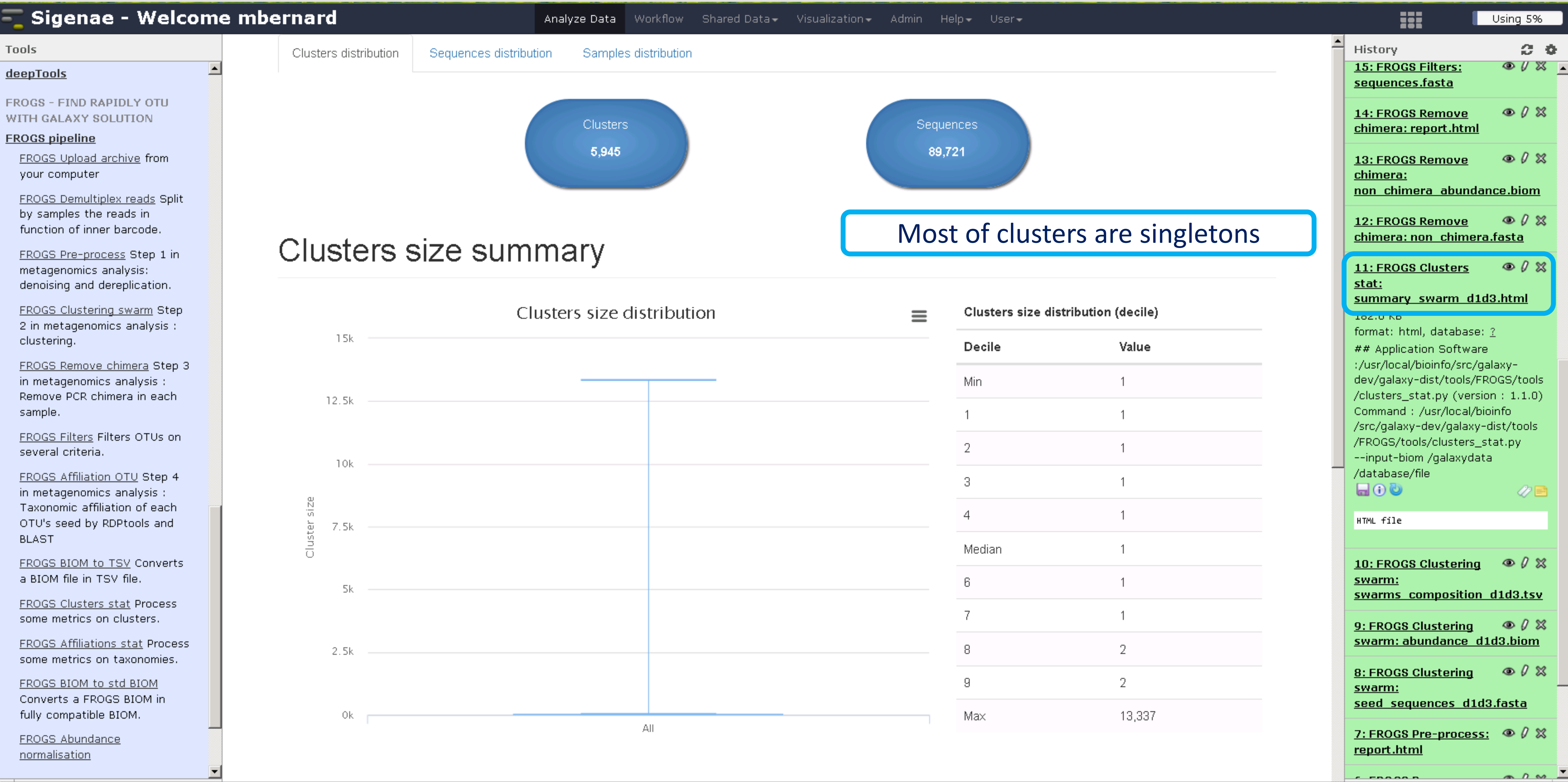
FROGS Clusters stat Process some metrics on clusters.

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

Exercise 2

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).



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 distribution | Sequences distribution | Samples distribution

Clusters 5,945

Sequences 89,721

Most of clusters are singletons

Clusters size summary

Clusters size distribution

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
```

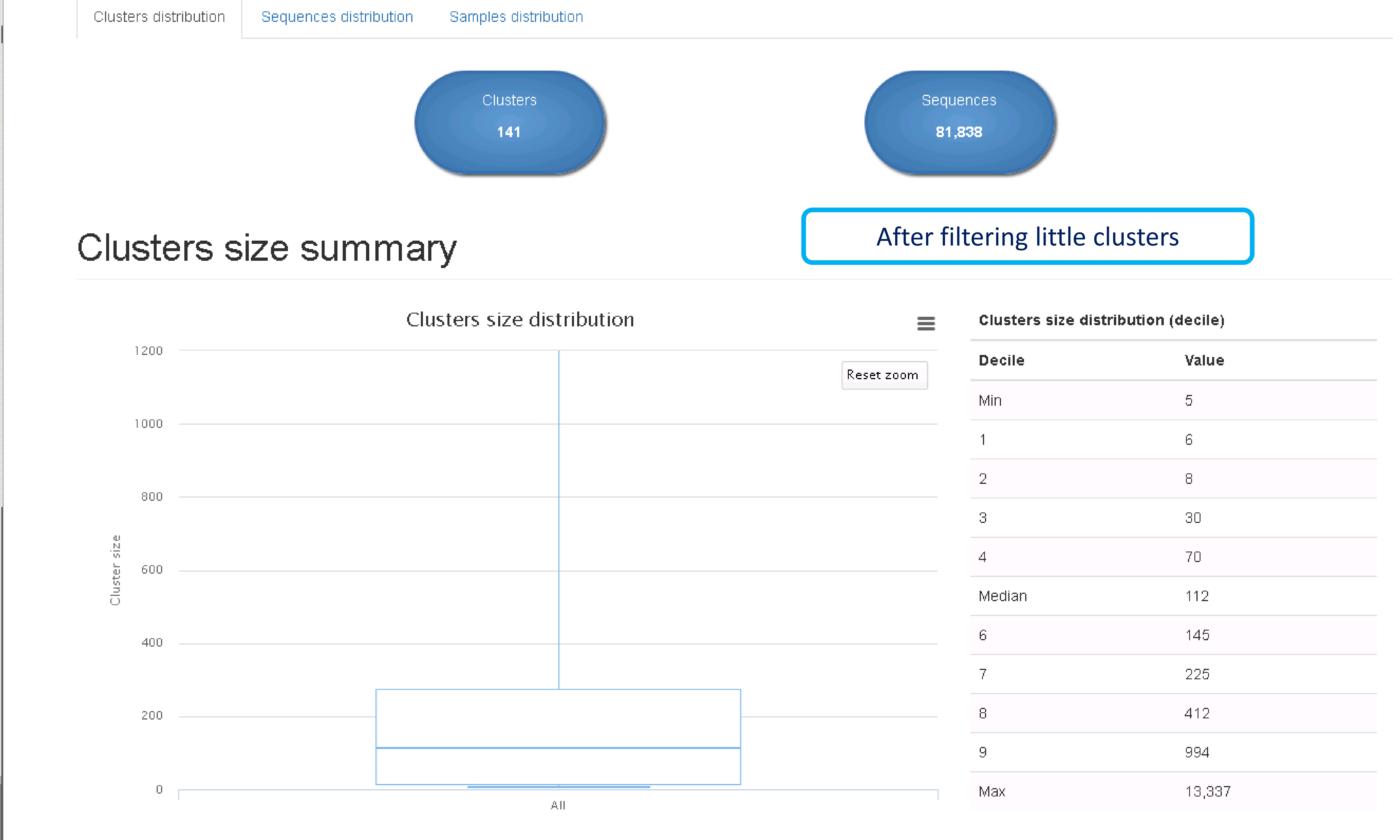
HTML file

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

141

Sequences

81,838

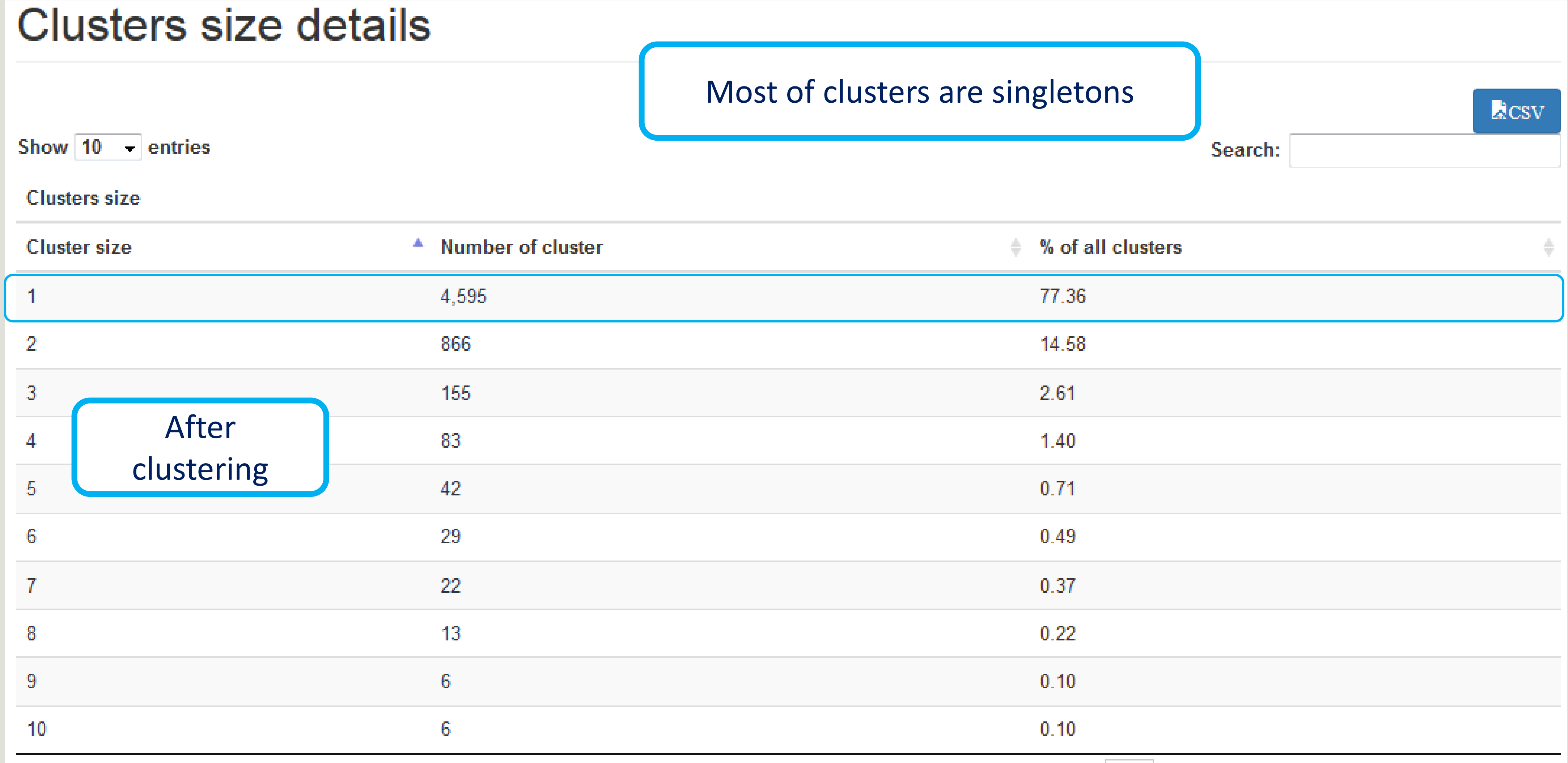
After filtering little clusters

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



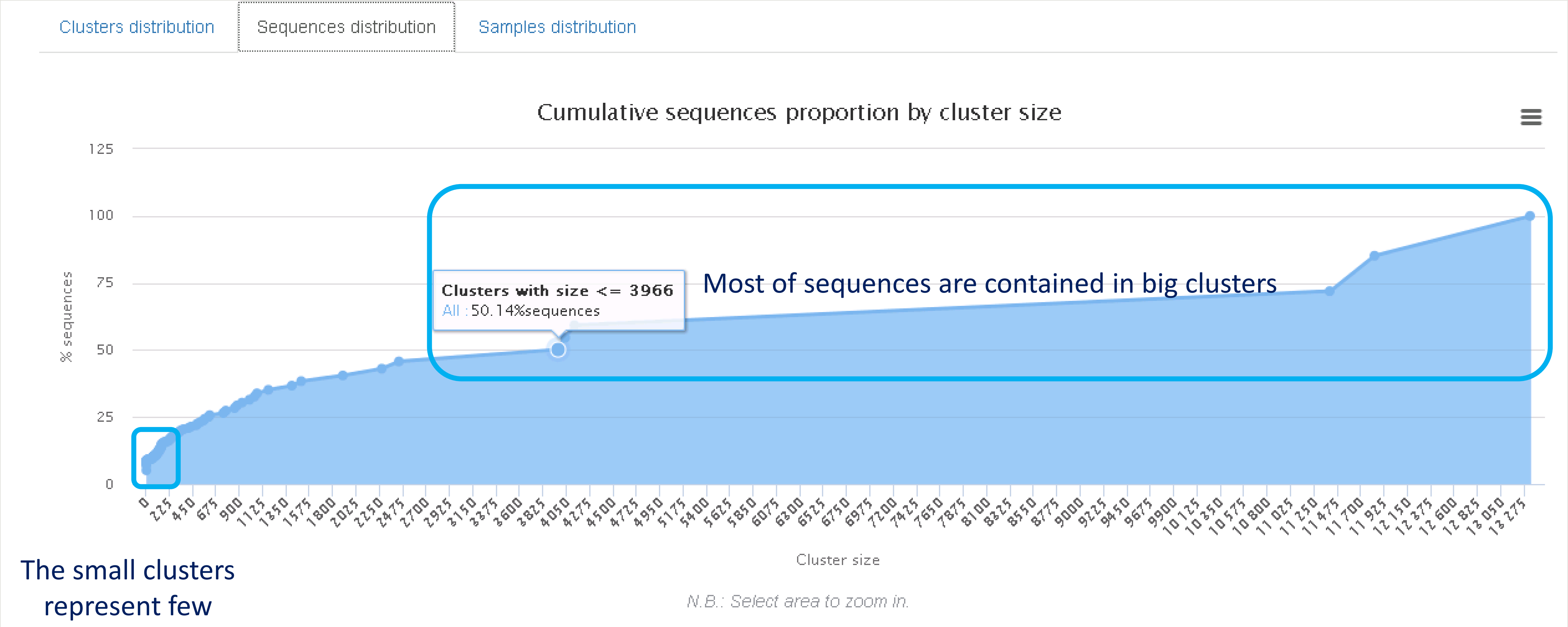
Clusters size details

Most of clusters are singletons

Clusters size

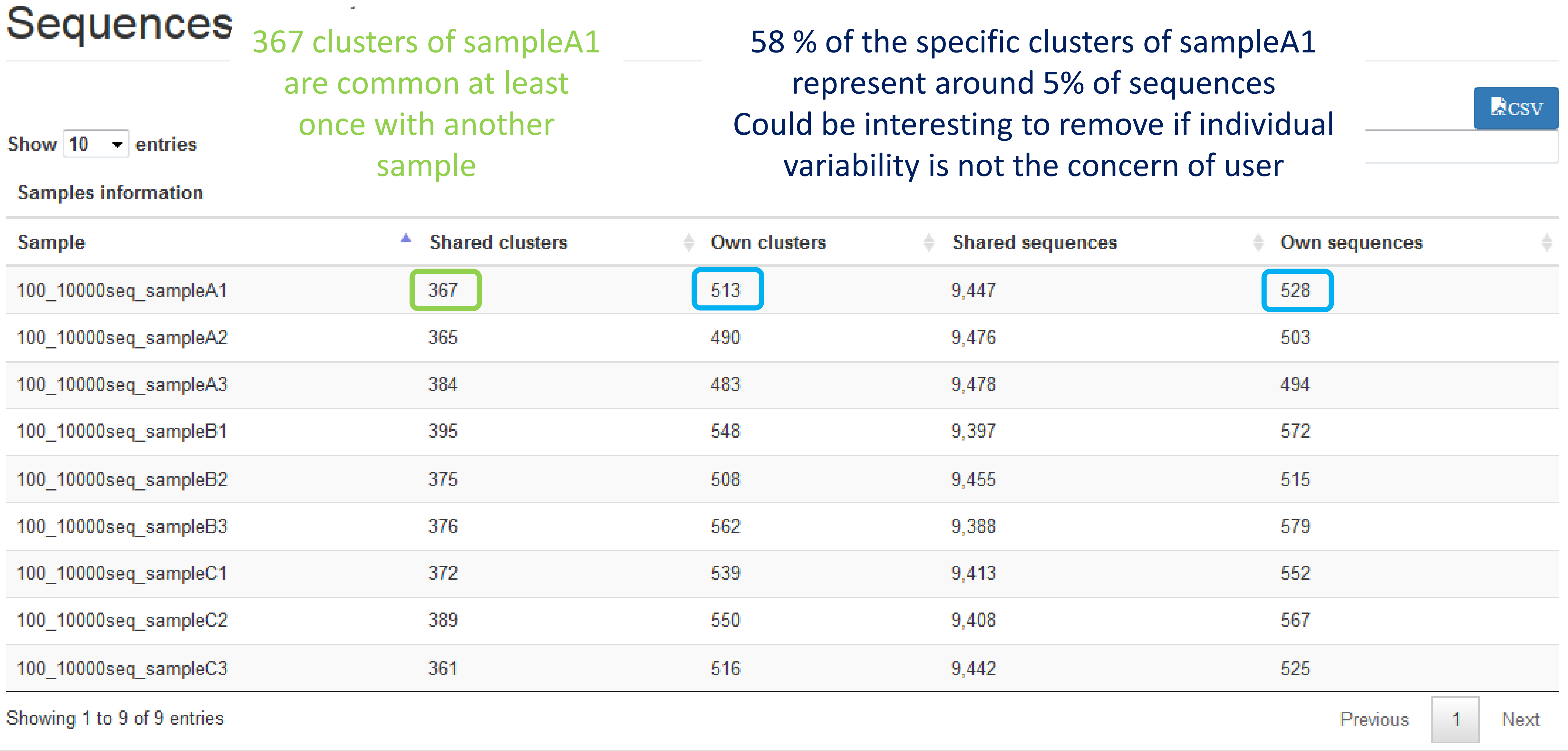
Cluster size	Number of cluster	% of all clusters
1	4,595	77.36
2	866	14.58
3	155	2.61
4	83	1.40
5	42	0.71
6	29	0.49
7	22	0.37
8	13	0.22
9	6	0.10
10	6	0.10

After clustering



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

Samples information

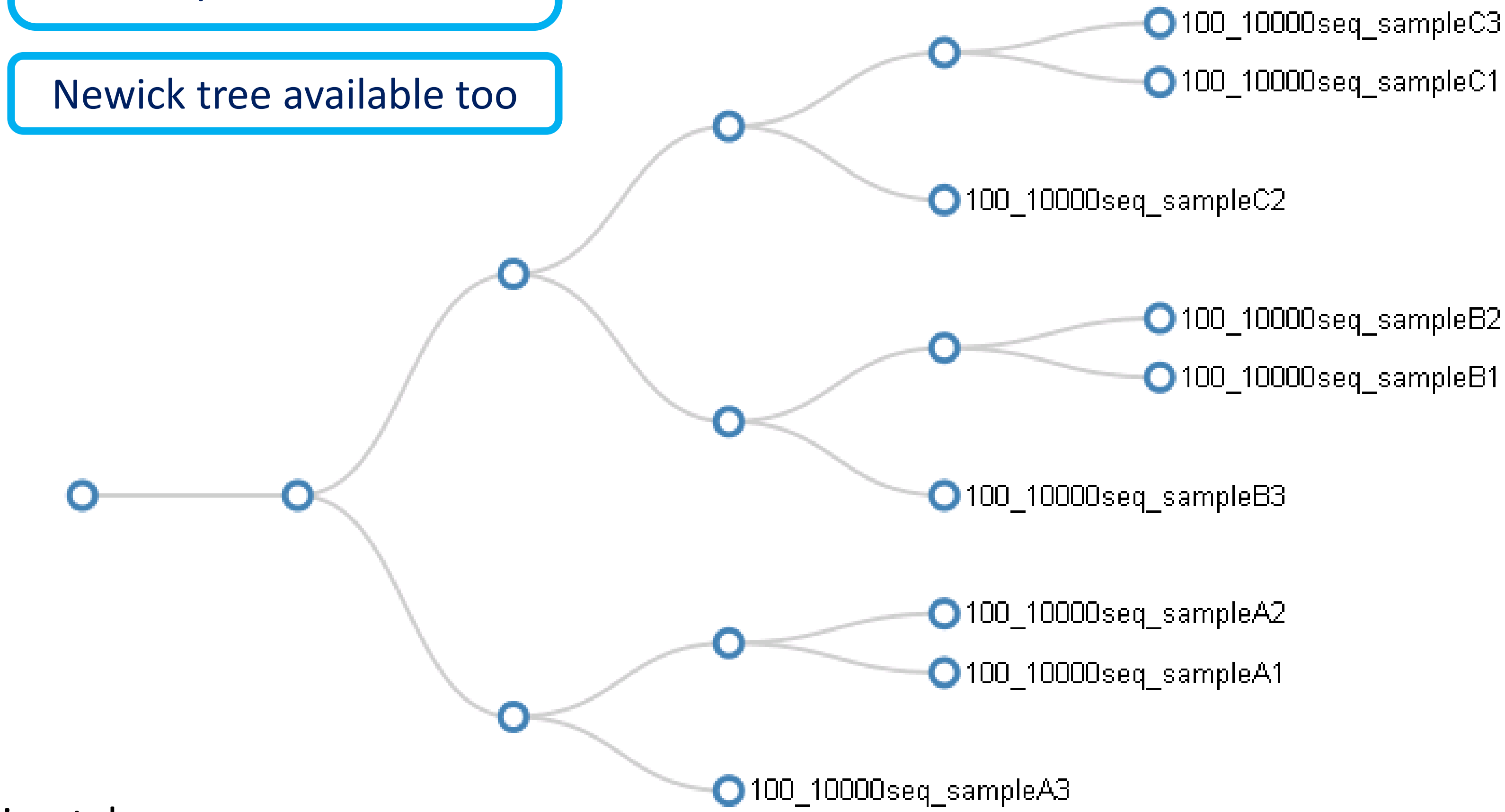
Sample	Shared clusters	Own clusters	Shared sequences	Own sequences
100_10000seq_sampleA1	367	513	9,447	528
100_10000seq_sampleA2	365	490	9,476	503
100_10000seq_sampleA3	384	483	9,478	494
100_10000seq_sampleB1	395	548	9,397	572
100_10000seq_sampleB2	375	508	9,455	515
100_10000seq_sampleB3	376	562	9,388	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

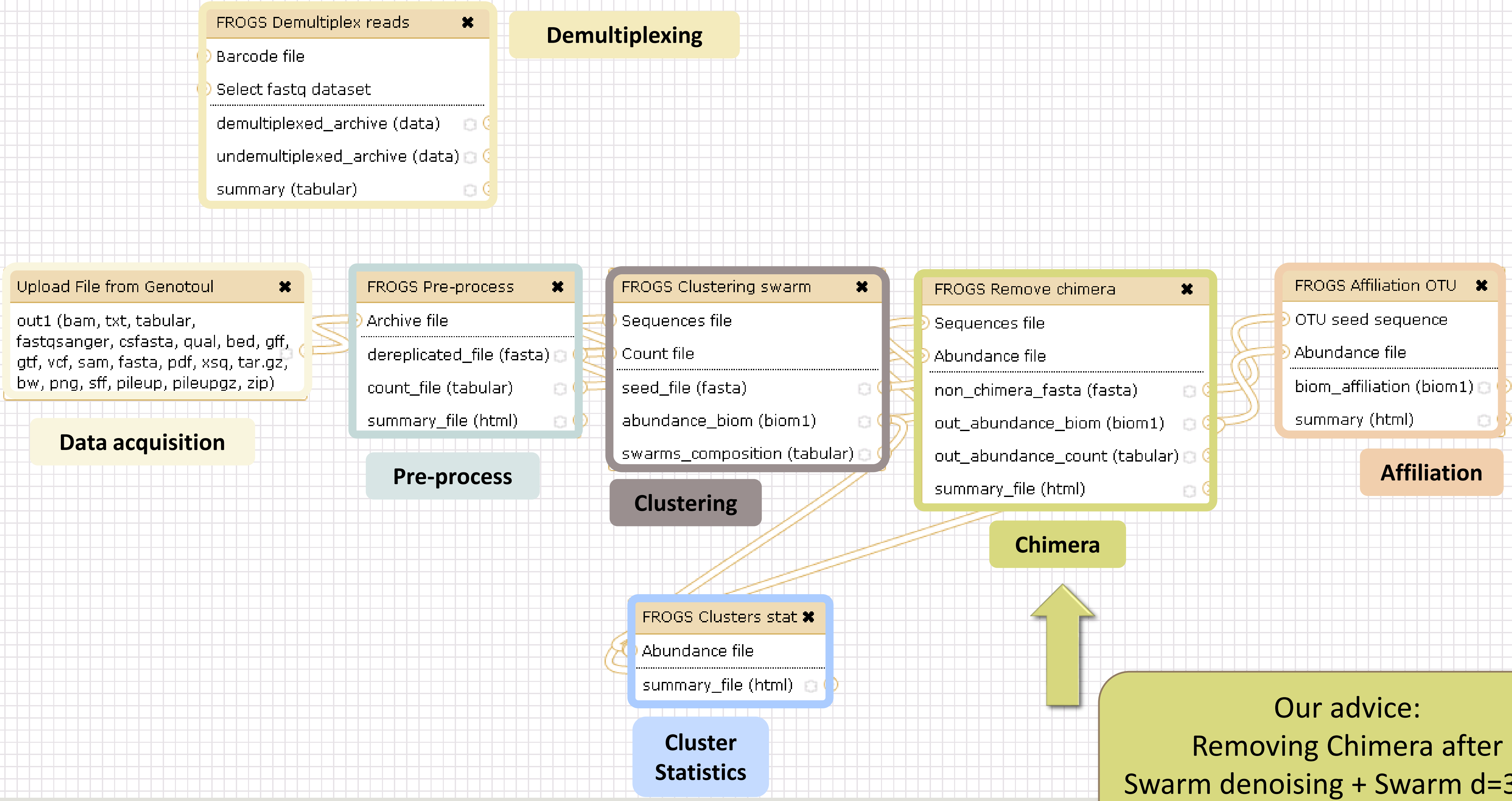
Hierarchical classification on Bray Curtis distance

Newick tree available too



Samples distribution tab

Chimera removal 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)

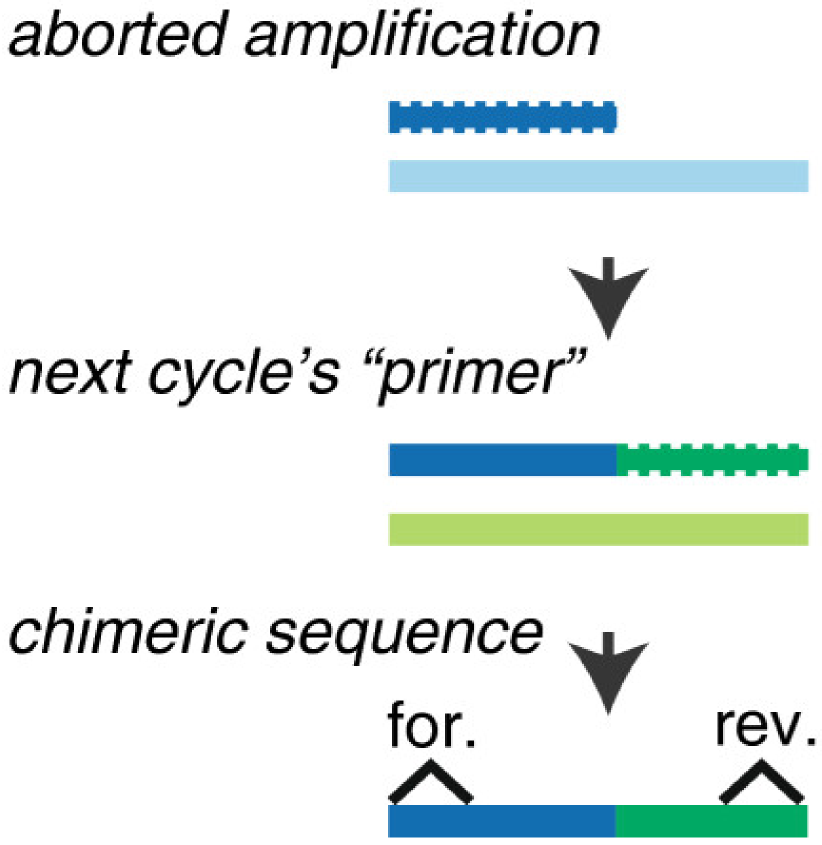
Cluster Statistics

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.

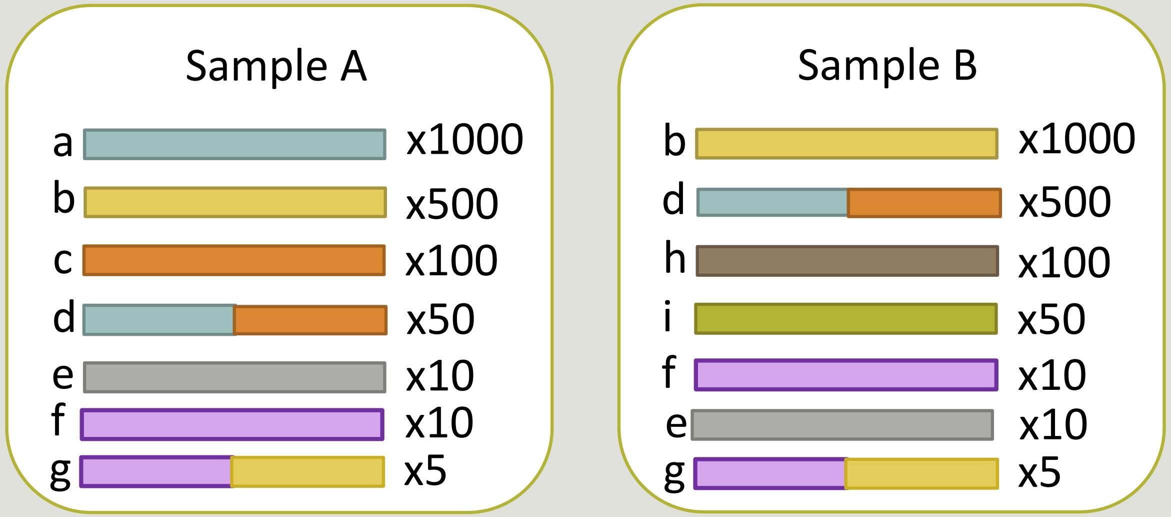
Chimera: from 5 to 45% of reads (Schloss 2011)



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



“ **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! - 3

LAUNCH THE REMOVE CHIMERA TOOL

Exercise 3

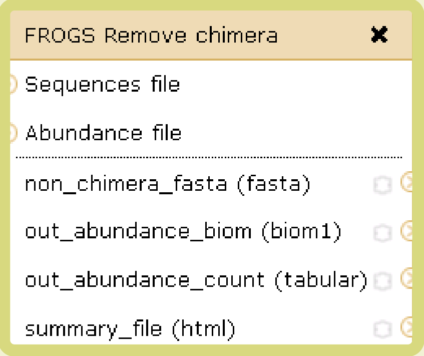
Go to « MiSeq contiged » history

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 clusters and chimeras


FROGS Remove chimera

Sequences file

Abundance file

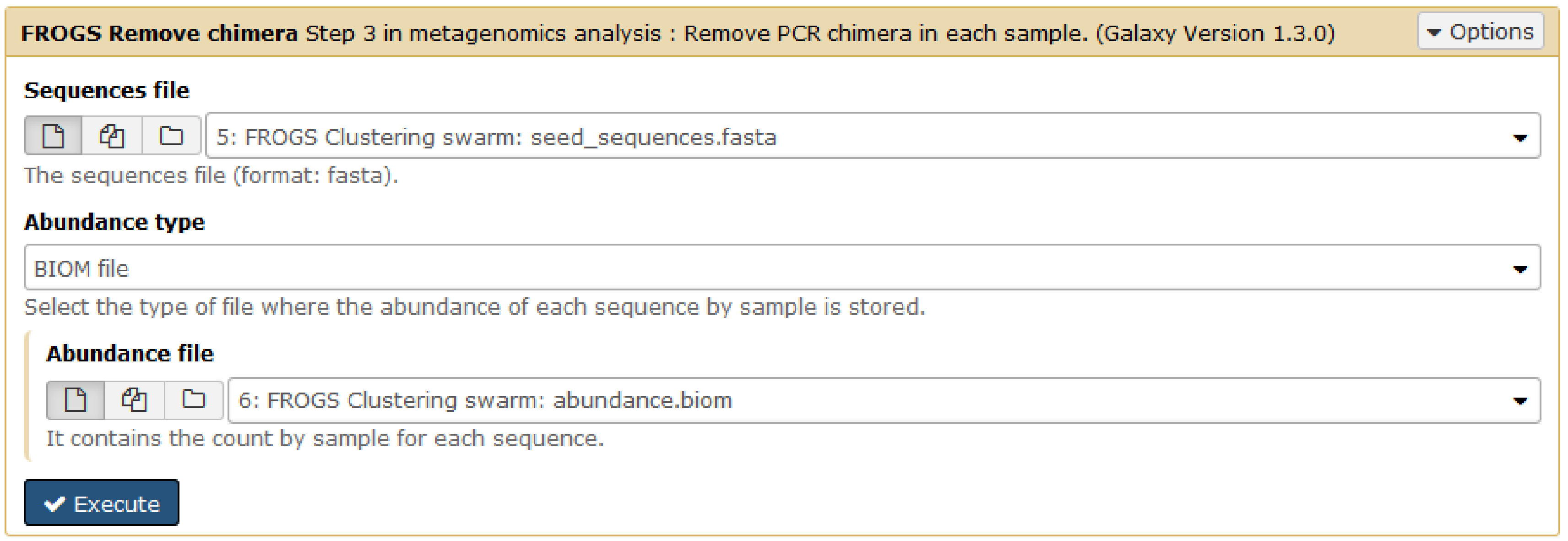
non_chimera_fasta (fasta)

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.

Exercise 3

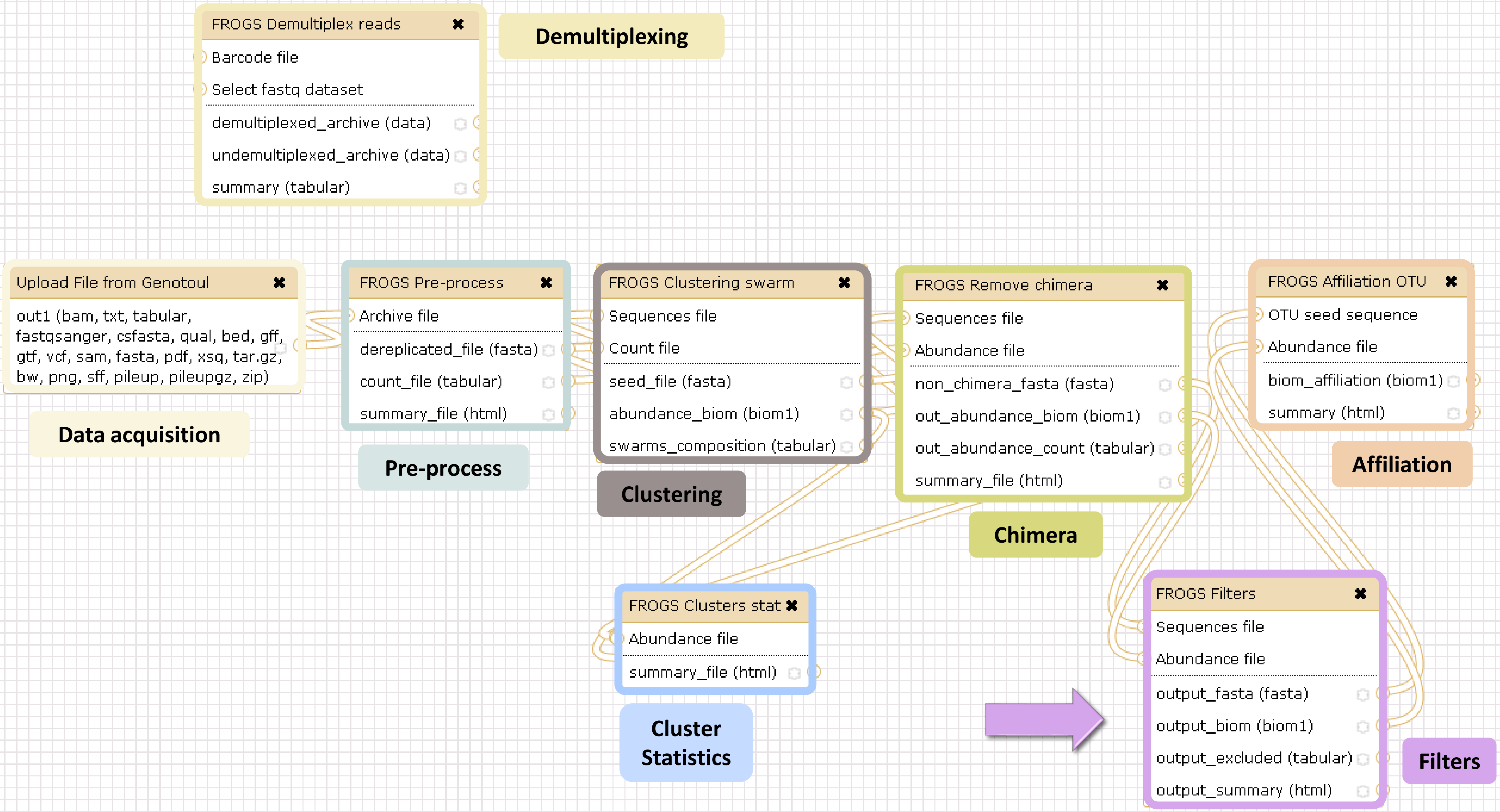
1. Understand the « FROGS remove chimera : report.html»
   a. How many clusters are kept after chimera removal?
   b. How many sequences that represent ? So what abundance?
   c. What do you conclude ?

Exercise 3

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 clusters are mainly false positives, our data would be much more exact if we remove them

Filters tool



Demultiplexing

Data acquisition

Pre-process

Clustering

Chimera

Affiliation

Cluster Statistics

Filters

Affiliation runs long time

Advise:

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

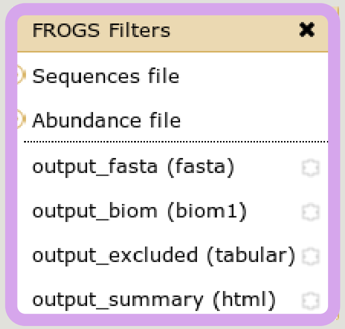
You will gain time !

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)

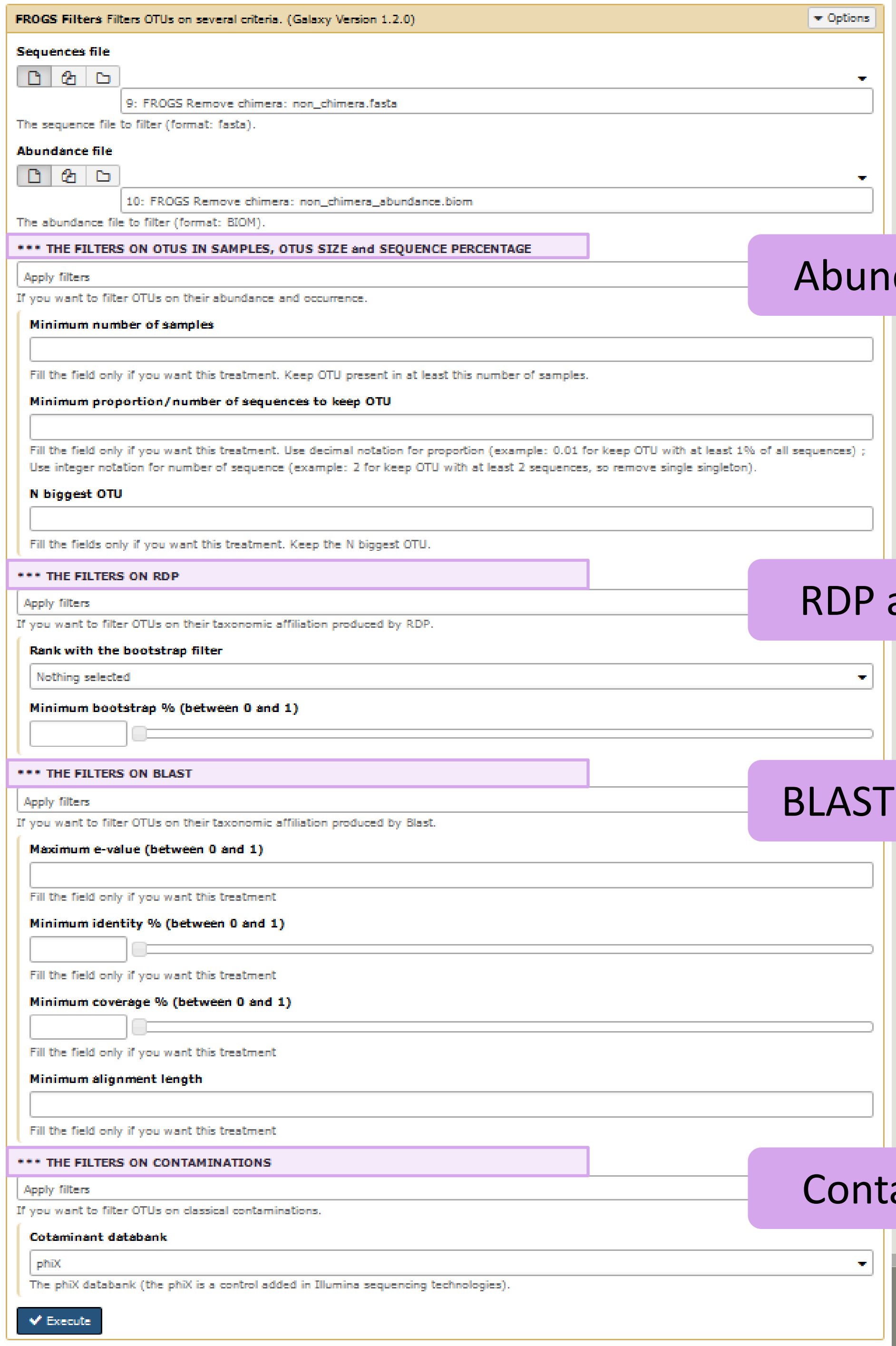
output_biom (biom1)

output_excluded (tabular)

output_summary (html)

Filters

4 filter sections



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

▾ Options

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.

Abundance filters

***** 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)

RDP affiliation filters

***** 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

BLAST affiliation filters

***** THE FILTERS ON CONTAMINATIONS**

Apply filters

If you want to filter OTUs on classical contaminations.

Cotaminant databank

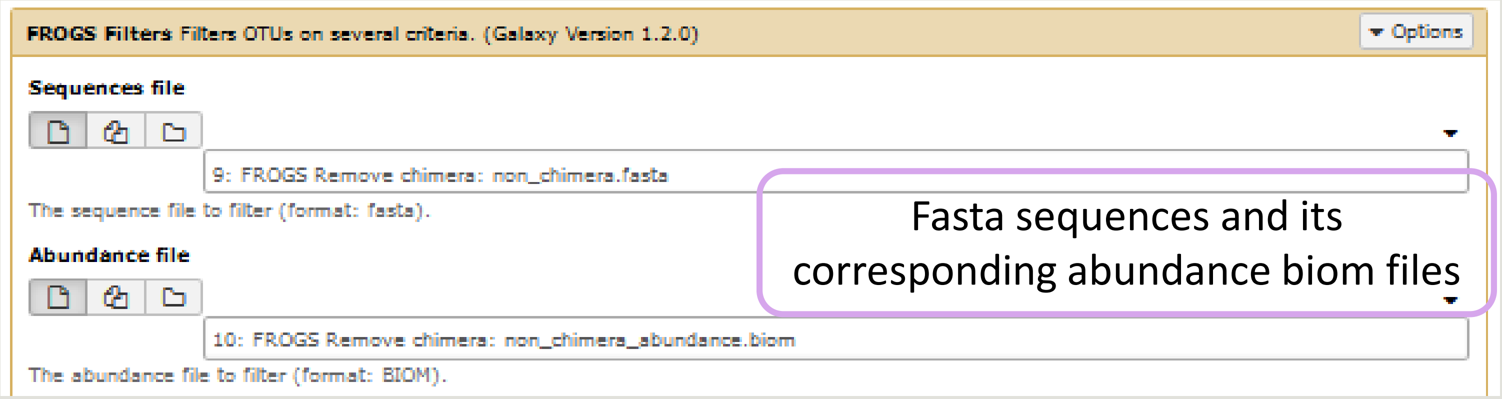
phiX

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

✓ Execute

Contamination filter

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).

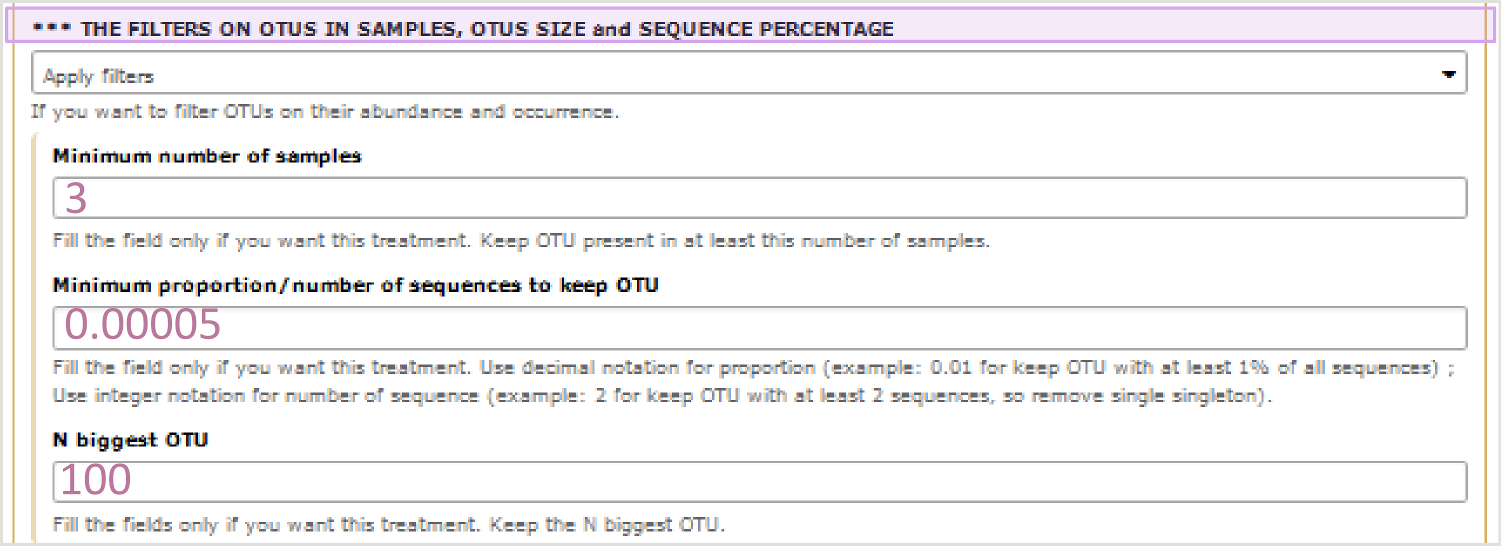
Abundance file

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

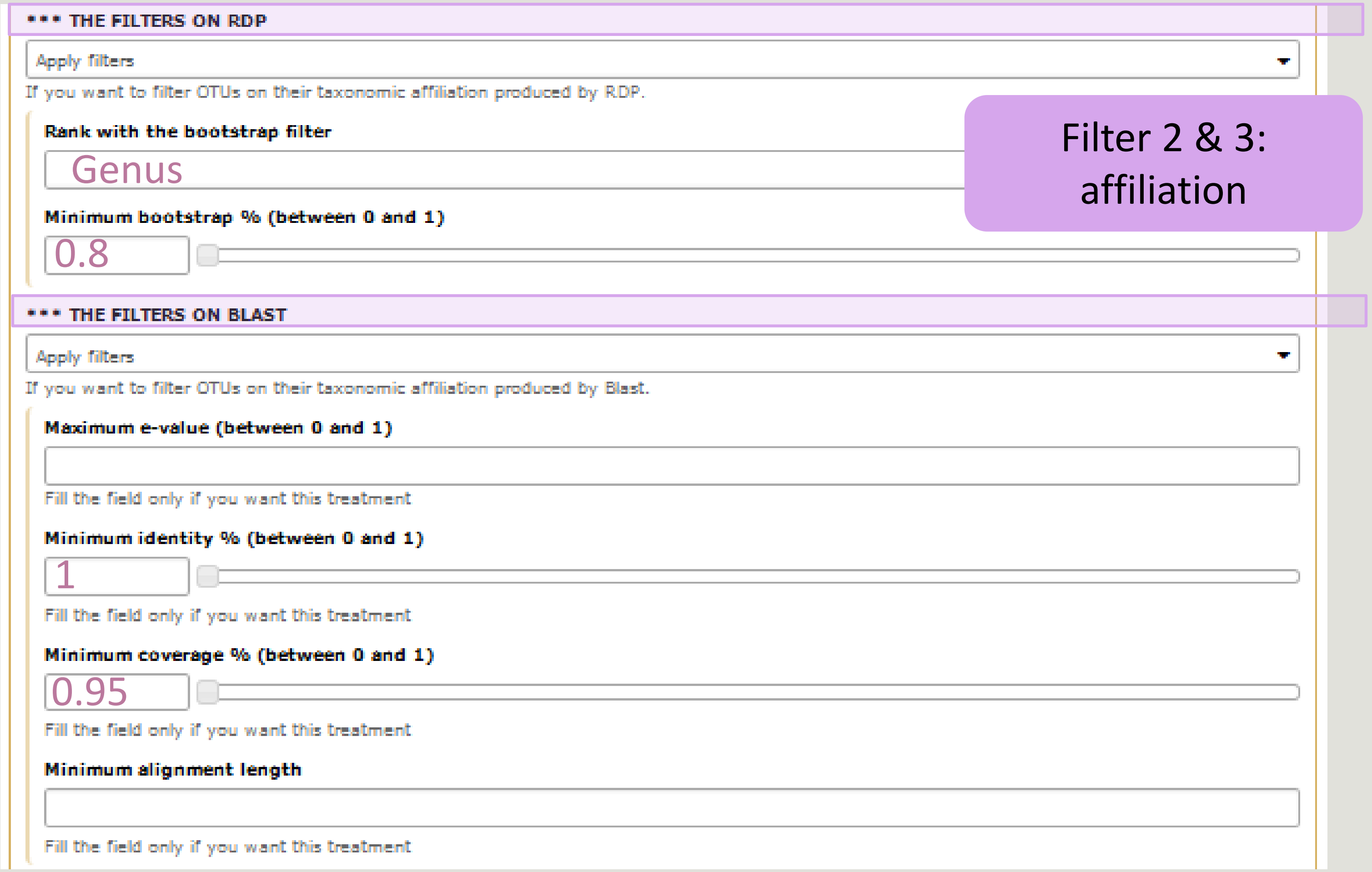
0.00005

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.



*** 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

Filter 2 & 3: affiliation

*** 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)

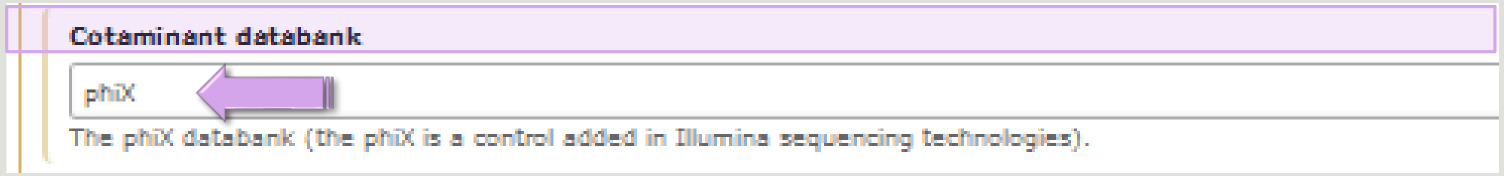
0.95

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! - 4

LAUNCH TOOL FILTERS

Exercise 4

Go to history « MiSeq contiged »

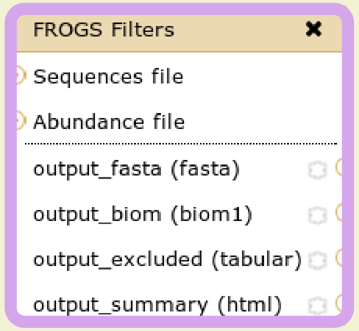
Launch « Filters » tool with non_chimera_abundanced1d3.biom, non_chimerad1d3.fasta

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.

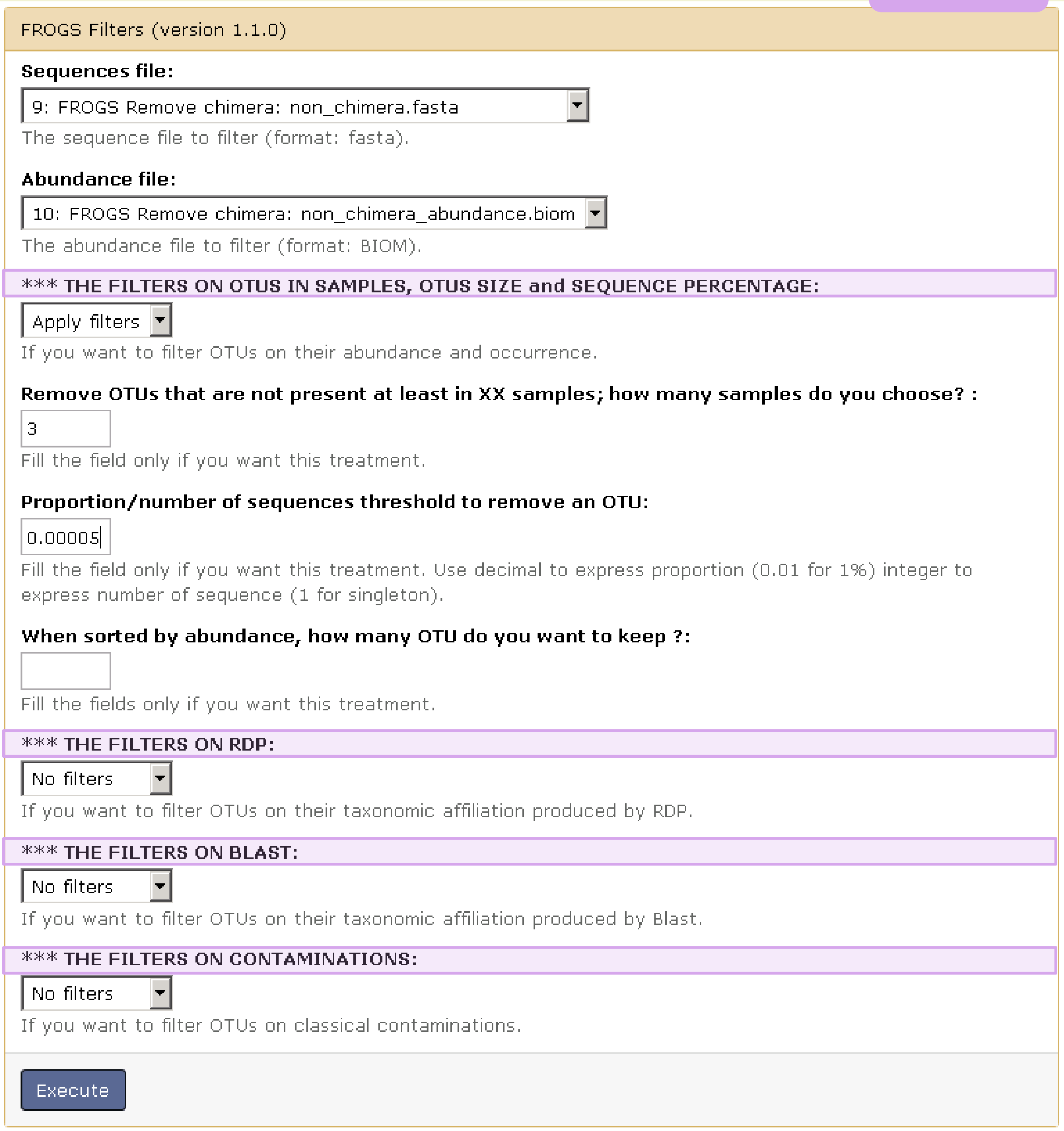


FROGS Filters ✖

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

Filters

Input



FROGS Filters (version 1.1.0)

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.

Remove OTUs that are not present at least in XX samples; how many samples do you choose? :
3
Fill the field only if you want this treatment.

Proportion/number of sequences threshold to remove an OTU:
0.00005
Fill the field only if you want this treatment. Use decimal to express proportion (0.01 for 1%) integer to express number of sequence (1 for singleton).

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

Fill the fields only if you want this treatment.

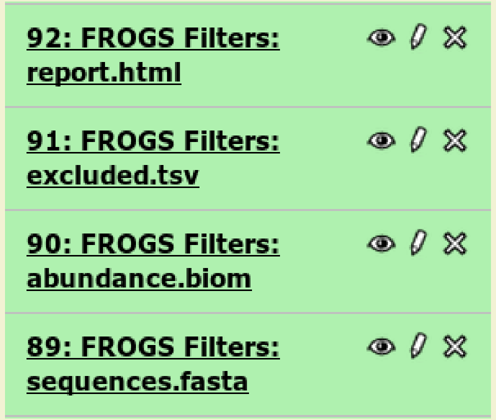
*** THE FILTERS ON RDP:
No filters
If you want to filter OTUs on their taxonomic affiliation produced by RDP.

*** THE FILTERS ON BLAST:
No filters
If you want to filter OTUs on their taxonomic affiliation produced by Blast.

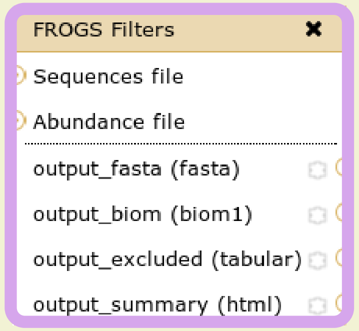
*** THE FILTERS ON CONTAMINATIONS:
No filters
If you want to filter OTUs on classical contaminations.

Execute

Output



- 92: FROGS Filters: report.html
- 91: FROGS Filters: excluded.tsv
- 90: FROGS Filters: abundance.biom
- 89: FROGS Filters: sequences.fasta



FROGS Filters ✖

Sequences file

Abundance file

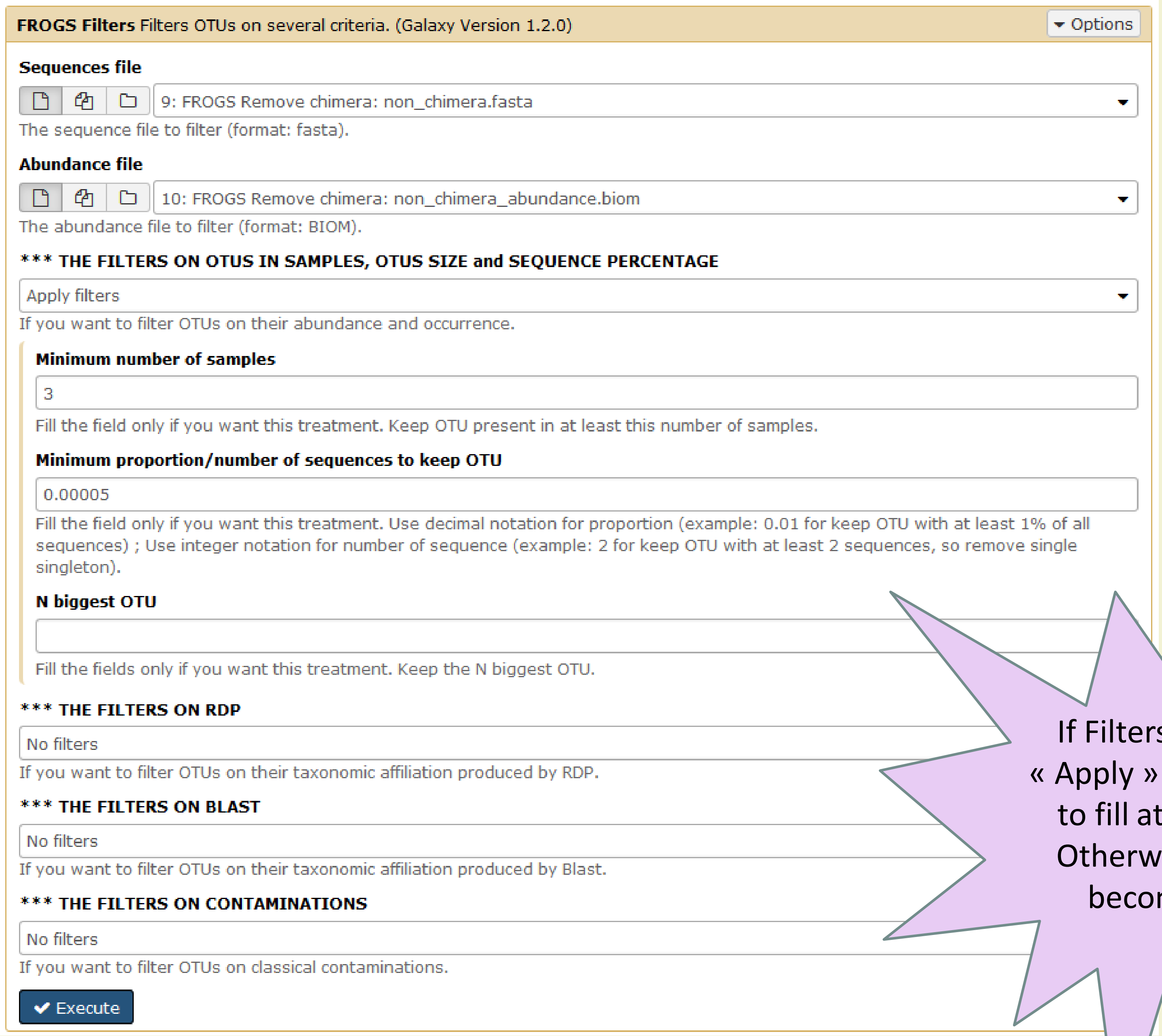
output_fasta (fasta)

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.

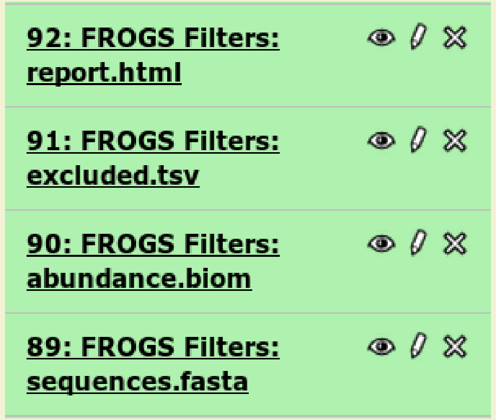
***** THE FILTERS ON CONTAMINATIONS**

No filters

If you want to filter OTUs on classical contaminations.

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

Output



92: FROGS Filters: report.html

91: FROGS Filters: excluded.tsv

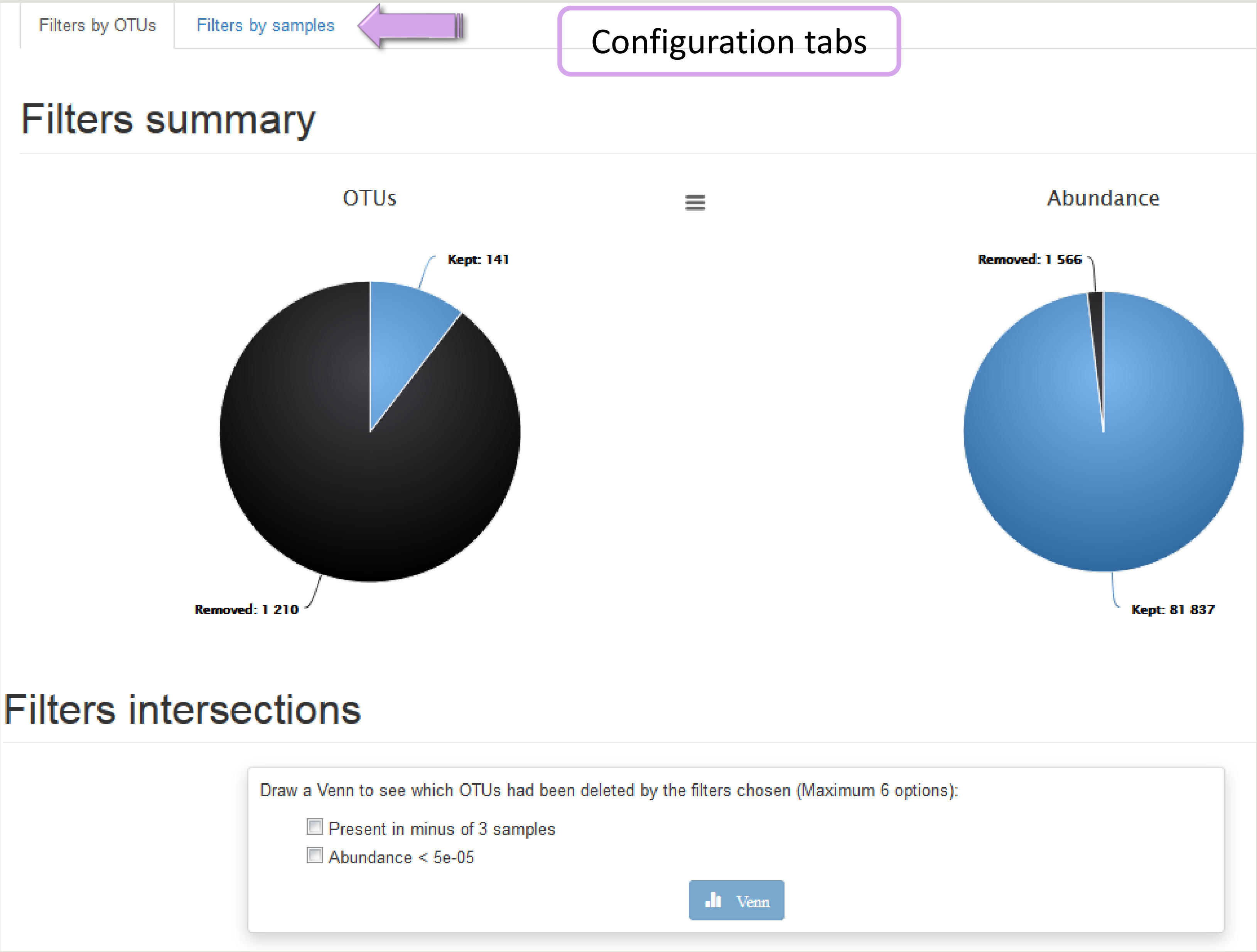
90: FROGS Filters: abundance.biom

89: FROGS Filters: sequences.fasta

MiSeq contiged

Exercise 6

1. What are the output files of “Filters” ?
2. Explore “FROGS Filter : report.html” file.
3. How many clusters have you removed ?
4. Build the Venn diagram on the two filters.
5. How many clusters 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.



Configuration tabs

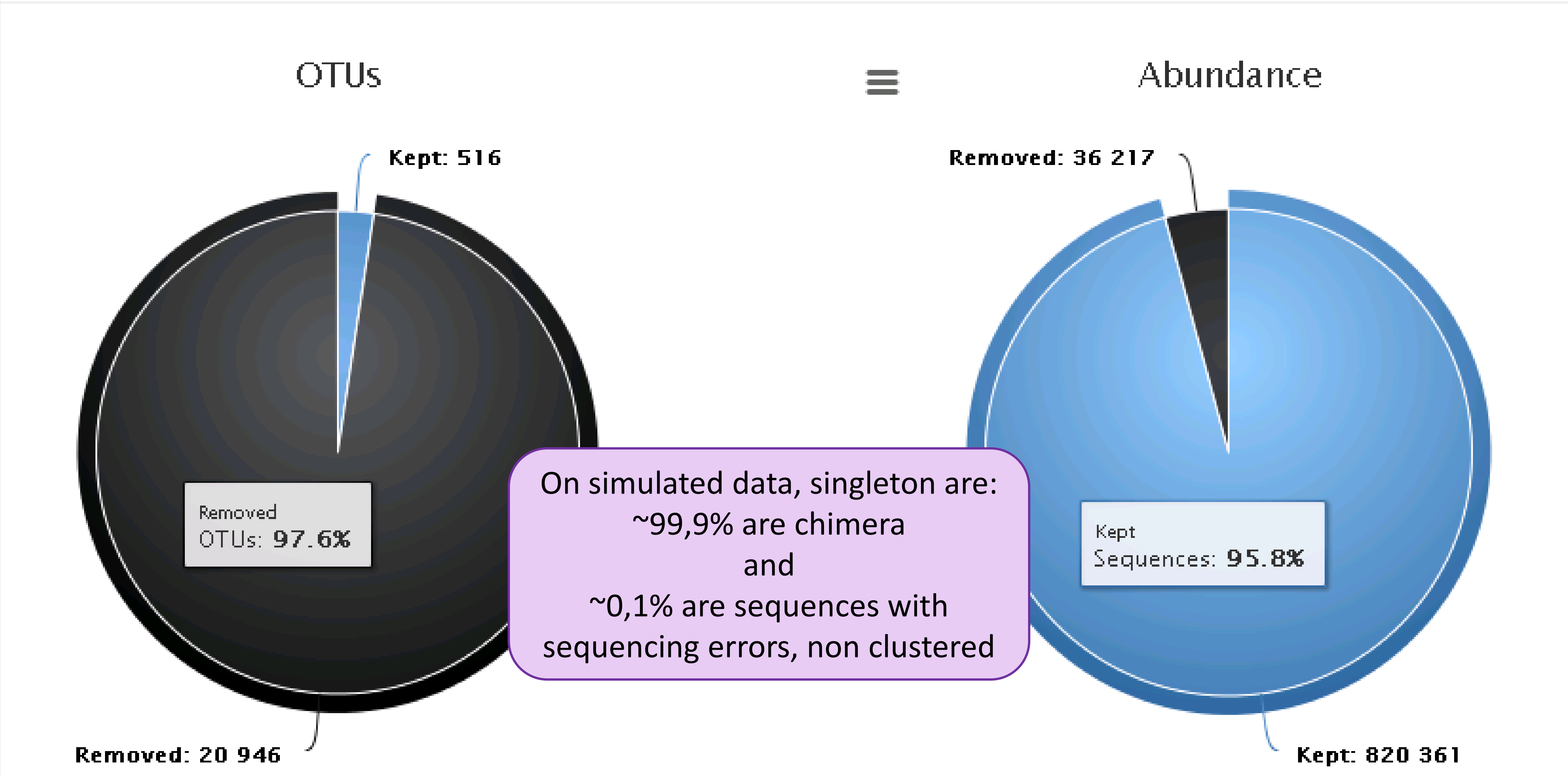
Filters summary

Filters intersections

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

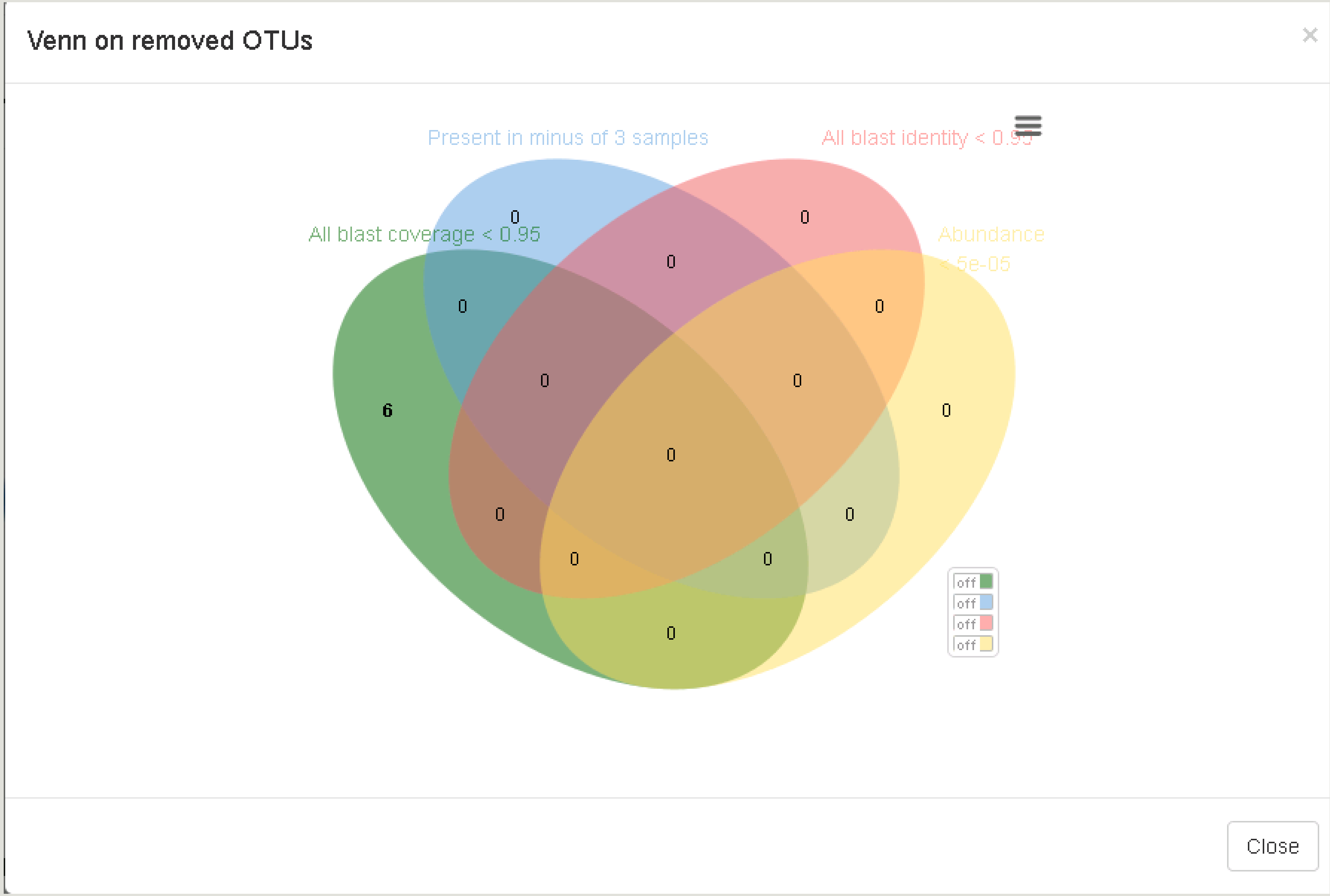
☐ Present in minus of 3 samples

☐ Abundance < 5e-05



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

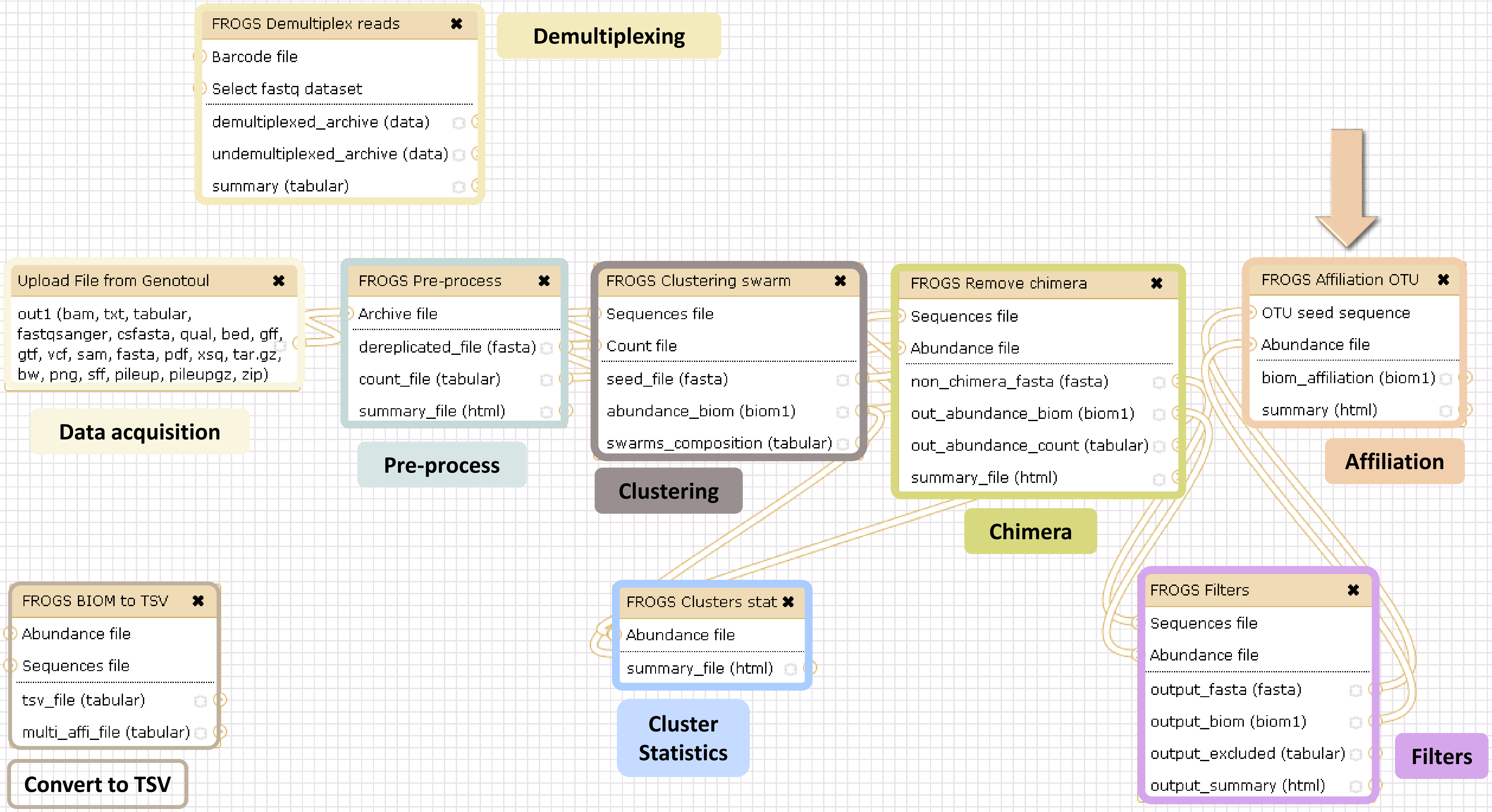
Removing little OTUs (conservation rate =0.005%) and non shared OTU (in less than 2 samples)

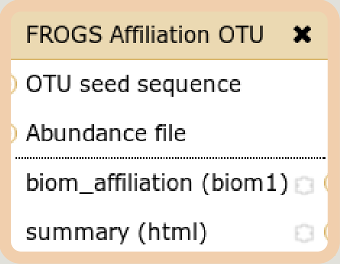


Venn on removed OTUs

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

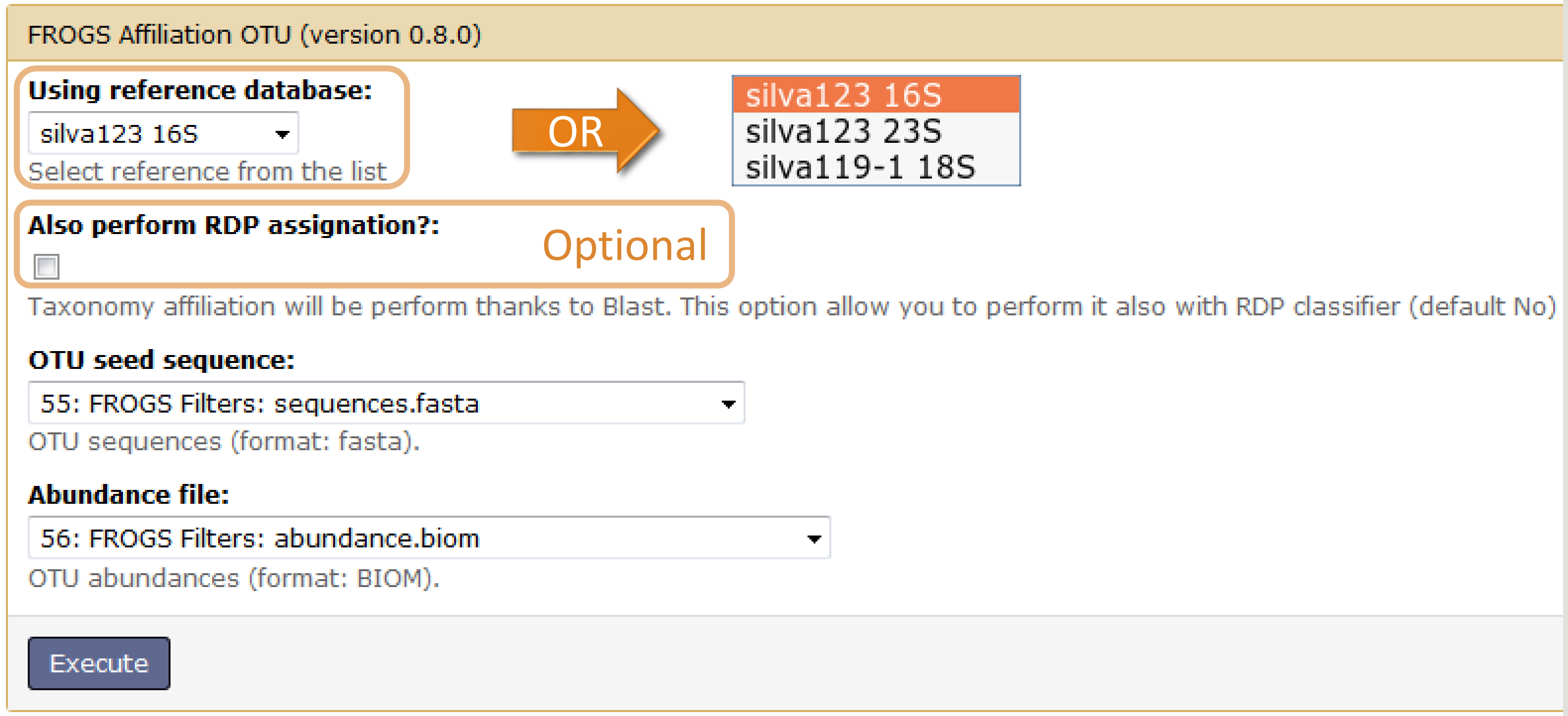
OTU seed sequence

Abundance file

biom_affiliation (biom1)

summary (html)

Affiliation



FROGS Affiliation OTU (version 0.8.0)

Using reference database:

silva123 16S

Select reference from the list

silva123 16S
silva123 23S
silva119-1 18S

Also perform RDP assignation?:

□

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

OTU seed sequence:

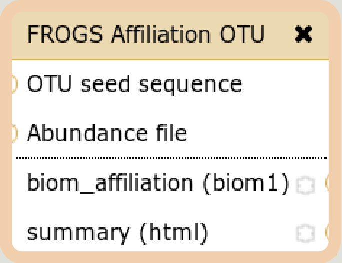
55: FROGS Filters: sequences.fasta

OTU sequences (format: fasta).

Abundance file:

56: FROGS Filters: abundance.biom

OTU abundances (format: BIOM).



FROGS Affiliation OTU ✖

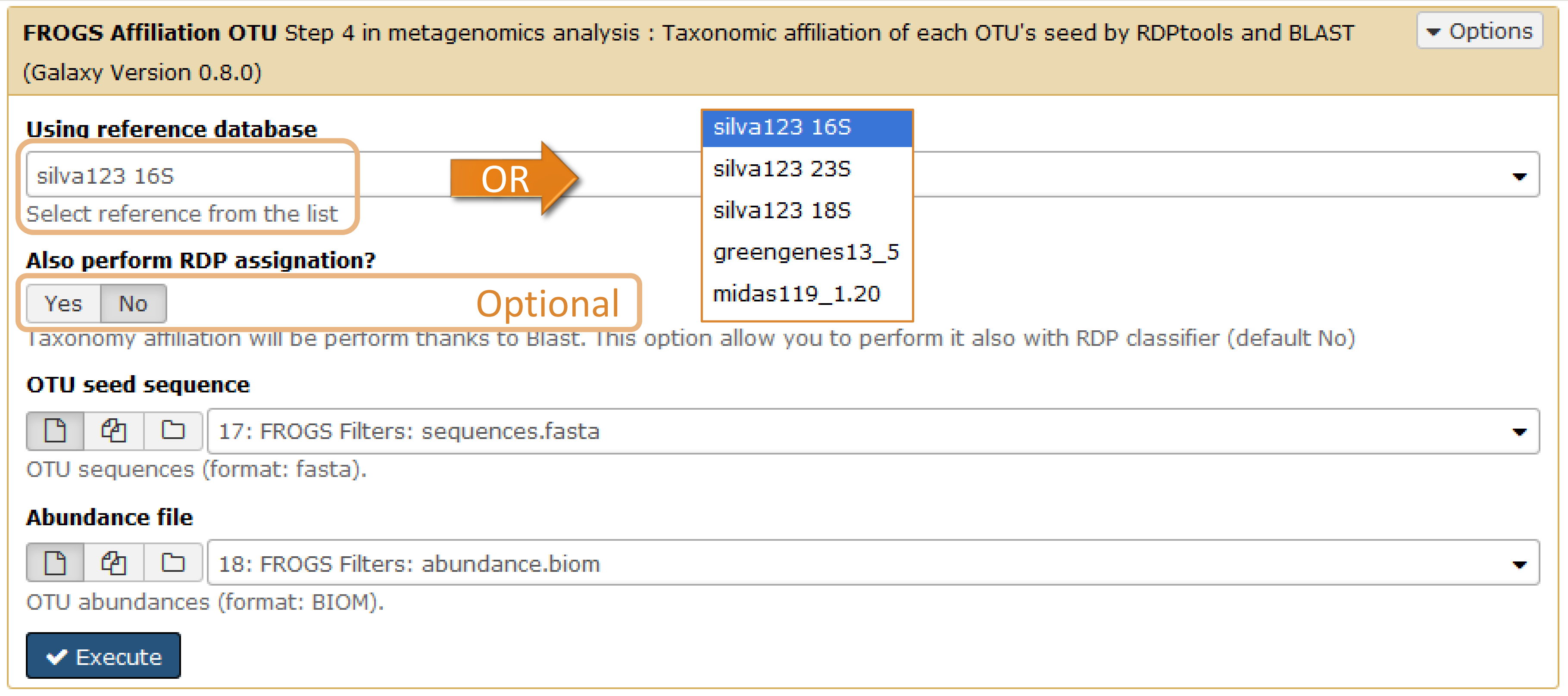
OTU seed sequence

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

OR

Also perform RDP assignation?

Yes [✓ No]

Optional

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).

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! – 5

LAUNCH THE « FROGS AFFILIATION » TOOL

Exercise 5.1

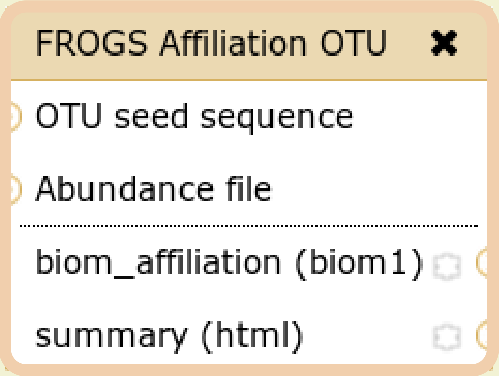
Go to « MiSeq contiged » history

Launch the « FROGS Affiliation » tool with

- SILVA 123 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 ✖

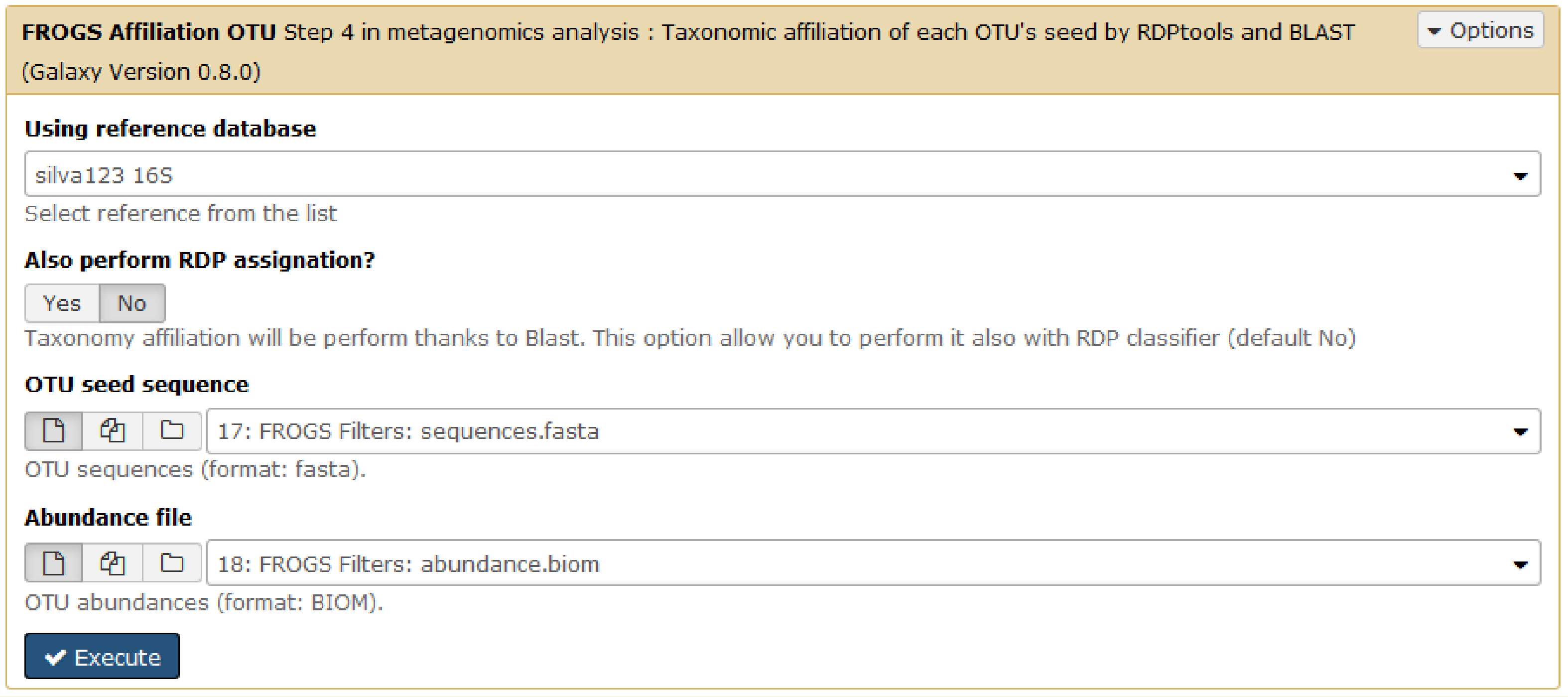
OTU seed sequence

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).

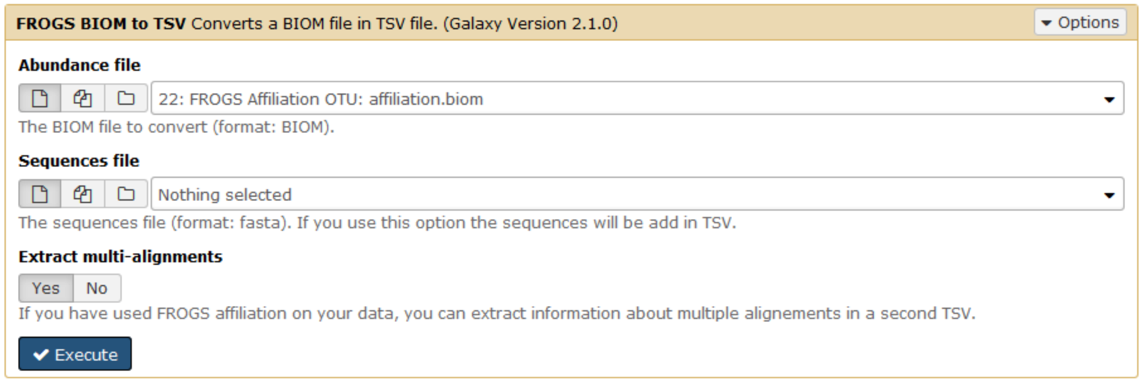
Abundance file

18: FROGS Filters: abundance.biom

OTU abundances (format: BIOM).

Exercise 5.1

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

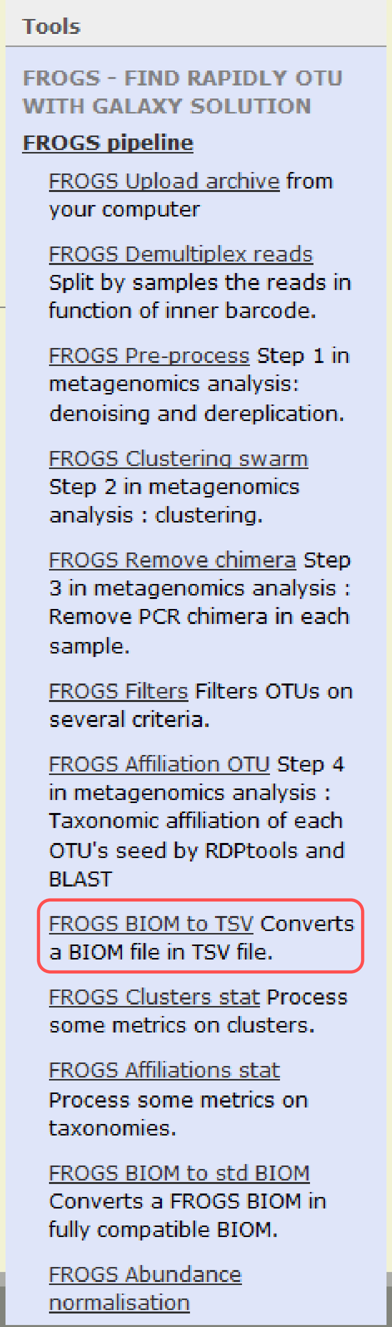
Nothing selected

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

Exercise 5.1

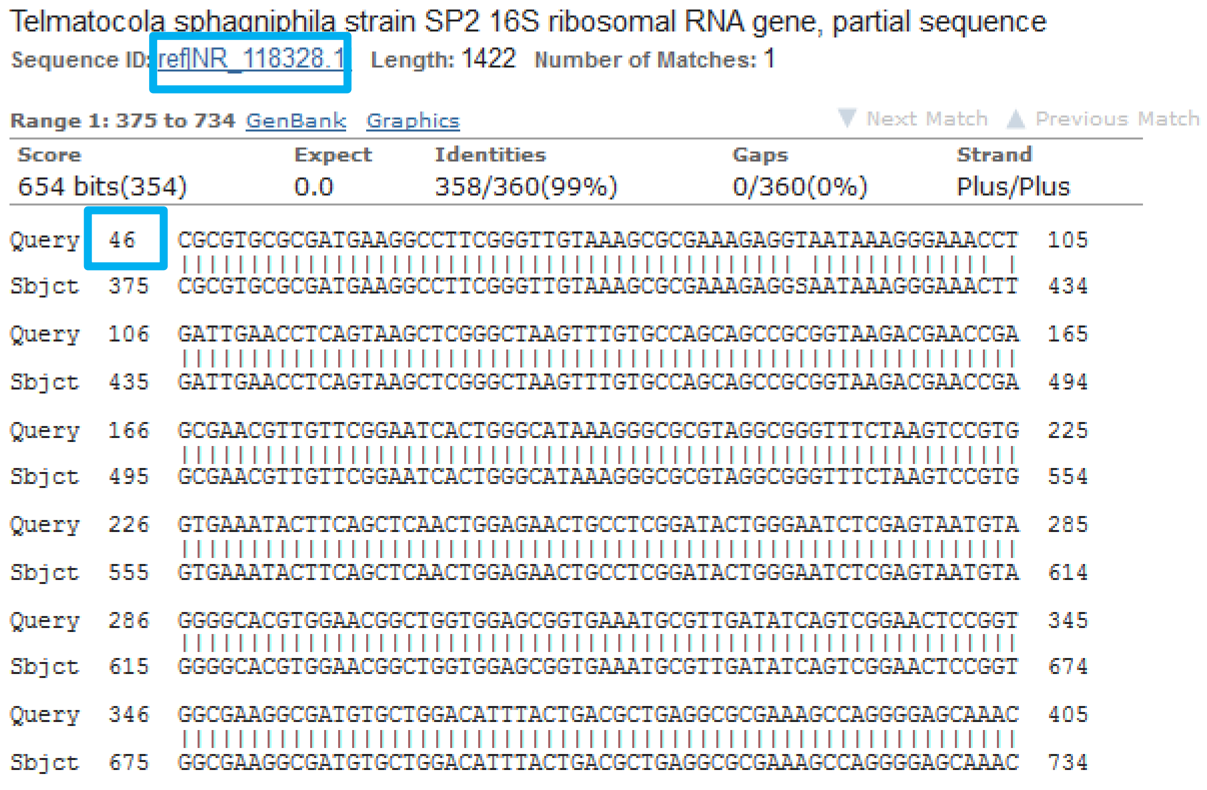
5. Understand Blast affiliations - Cluster_2388

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.



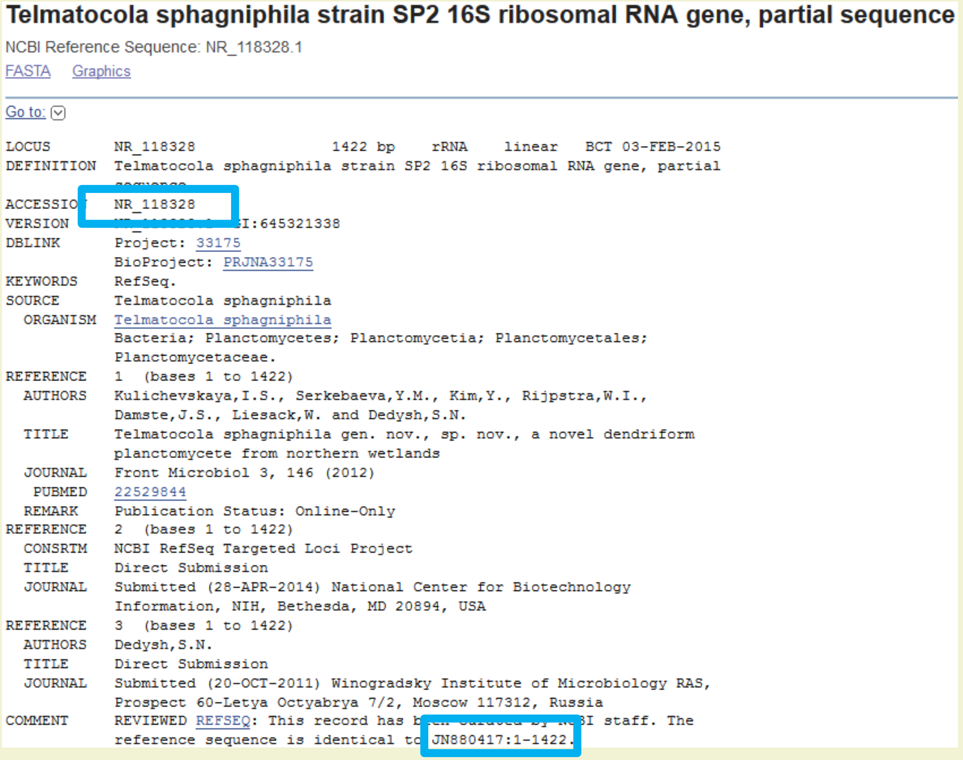
Telmatocola sphagniphila strain SP2 16S ribosomal RNA gene, partial sequence

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

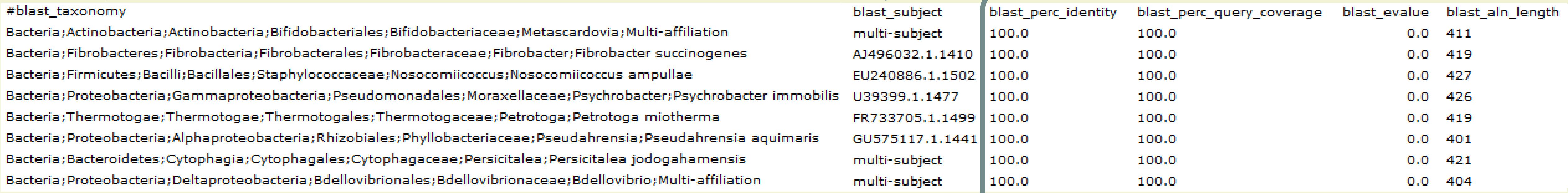
NCBI Reference Sequence: NR_118328.1

```
LOCUS       NR_118328                1422 bp    rRNA    linear   BCT 03-FEB-2015
DEFINITION  Telmatocola sphagniphila strain SP2 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   NR_118328
VERSION     NR_118328.1  GI: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 been curated by NCBI 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

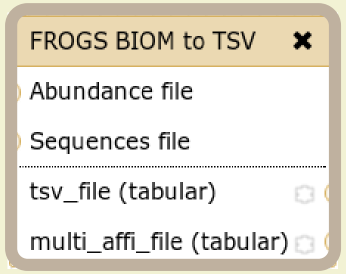
The reference sequence taxonomic affiliation is this one.



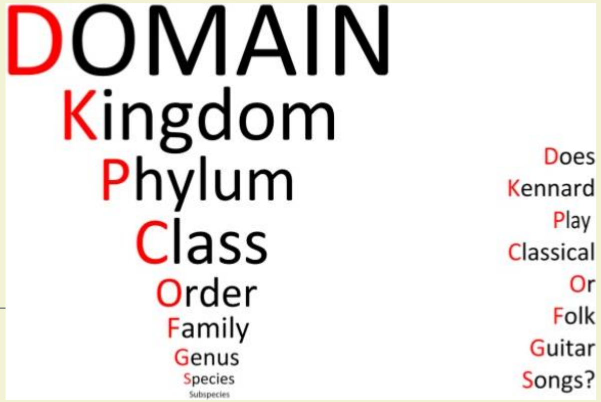
#blast_taxonomy	blast_subject	blast_perc_identity	blast_perc_query_coverage	blast_evalue	blast_aln_length
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Metascardovia;Multi-affiliation	multi-subject	100.0	100.0	0.0	411
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter;Fibrobacter 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

Evaluation variables of BLAST

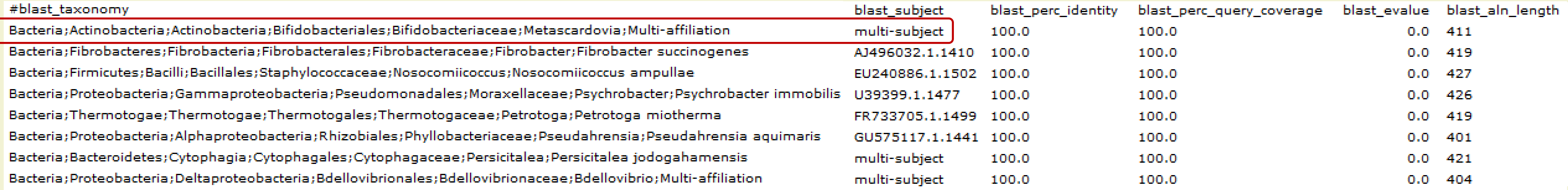
Convert to TSV



Blast columns



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

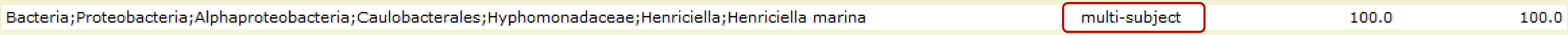


#blast_taxonomy	blast_subject	blast_perc_identity	blast_perc_query_coverage	blast_evalue	blast_aln_length
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Metascardovia;Multi-affiliation	multi-subject	100.0	100.0	0.0	411
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter;Fibrobacter 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

Blast columns

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

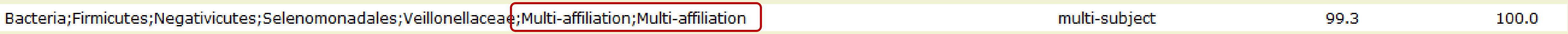


```
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Henriciella;Henriciella marina          multi-subject          100.0          100.0
```

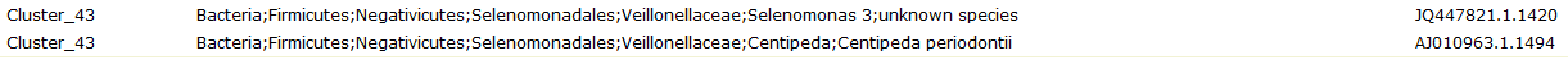
Cluster_11 has 2 identical blast hits, with identical species but with different strains (strains are not written in our data)

Blast columns

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



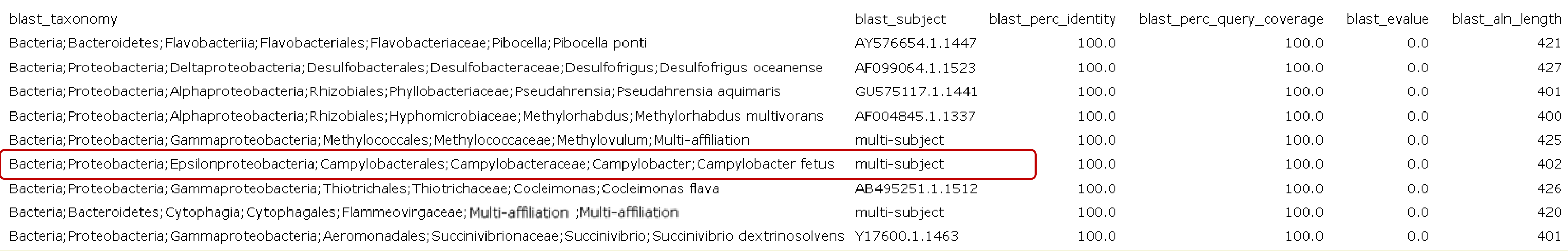
```
Bacteria;Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Multi-affiliation;Multi-affiliation          multi-subject          99.3          100.0
```



```
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

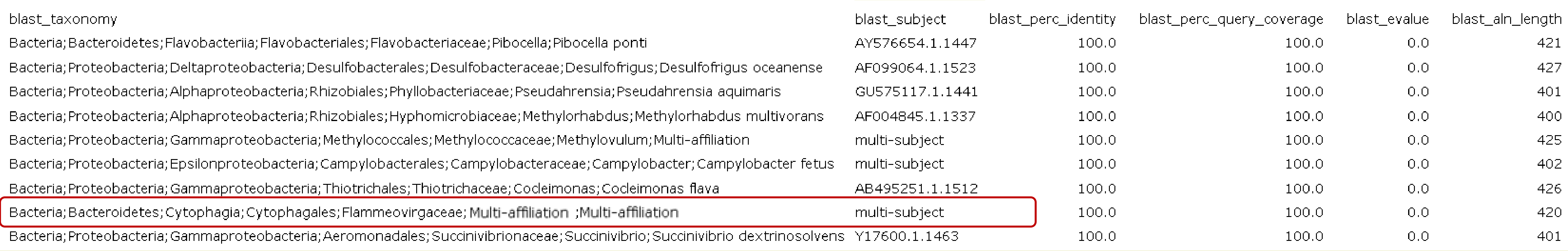
1st to 6th columns – Blast



blast_taxonomy	blast_subject	blast_perc_identity	blast_perc_query_coverage	blast_evalue	blast_aln_length
Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pibocella;Pibocella ponti	AY576654.1.1447	100.0	100.0	0.0	421
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfofrigus;Desulfofrigus oceanense	AF099064.1.1523	100.0	100.0	0.0	427
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia;Pseudahrensia aquimaris	GU575117.1.1441	100.0	100.0	0.0	401
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Methylorhabdus;Methylorhabdus multivorans	AF004845.1.1337	100.0	100.0	0.0	400
Bacteria;Proteobacteria;Gammaproteobacteria;Methylococcales;Methylococcaceae;Methylovulum;Multi-affiliation	multi-subject	100.0	100.0	0.0	425
Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Campylobacter;Campylobacter fetus	multi-subject	100.0	100.0	0.0	402
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas;Cocleimonas flava	AB495251.1.1512	100.0	100.0	0.0	426
Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Multi-affiliation ;Multi-affiliation	multi-subject	100.0	100.0	0.0	420
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Succinivibrionaceae;Succinivibrio;Succinivibrio dextrinosolvens	Y17600.1.1463	100.0	100.0	0.0	401

Cluster_6 has 38 identical blast hits, with different taxonomies as the species level

1st to 6th columns – Blast



blast_taxonomy	blast_subject	blast_perc_identity	blast_perc_query_coverage	blast_evalue	blast_aln_length
Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pibocella;Pibocella ponti	AY576654.1.1447	100.0	100.0	0.0	421
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfofrigus;Desulfofrigus oceanense	AF099064.1.1523	100.0	100.0	0.0	427
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia;Pseudahrensia aquimaris	GU575117.1.1441	100.0	100.0	0.0	401
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Methylorhabdus;Methylorhabdus multivorans	AF004845.1.1337	100.0	100.0	0.0	400
Bacteria;Proteobacteria;Gammaproteobacteria;Methylococcales;Methylococcaceae;Methylovulum;Multi-affiliation	multi-subject	100.0	100.0	0.0	425
Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Campylobacter;Campylobacter fetus	multi-subject	100.0	100.0	0.0	402
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas;Cocleimonas flava	AB495251.1.1512	100.0	100.0	0.0	426
Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Multi-affiliation ;Multi-affiliation	multi-subject	100.0	100.0	0.0	420
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Succinivibrionaceae;Succinivibrio;Succinivibrio dextrinosolvens	Y17600.1.1463	100.0	100.0	0.0	401

Cluster_8 has 2 identical blast hits, with different taxonomies as the genus level

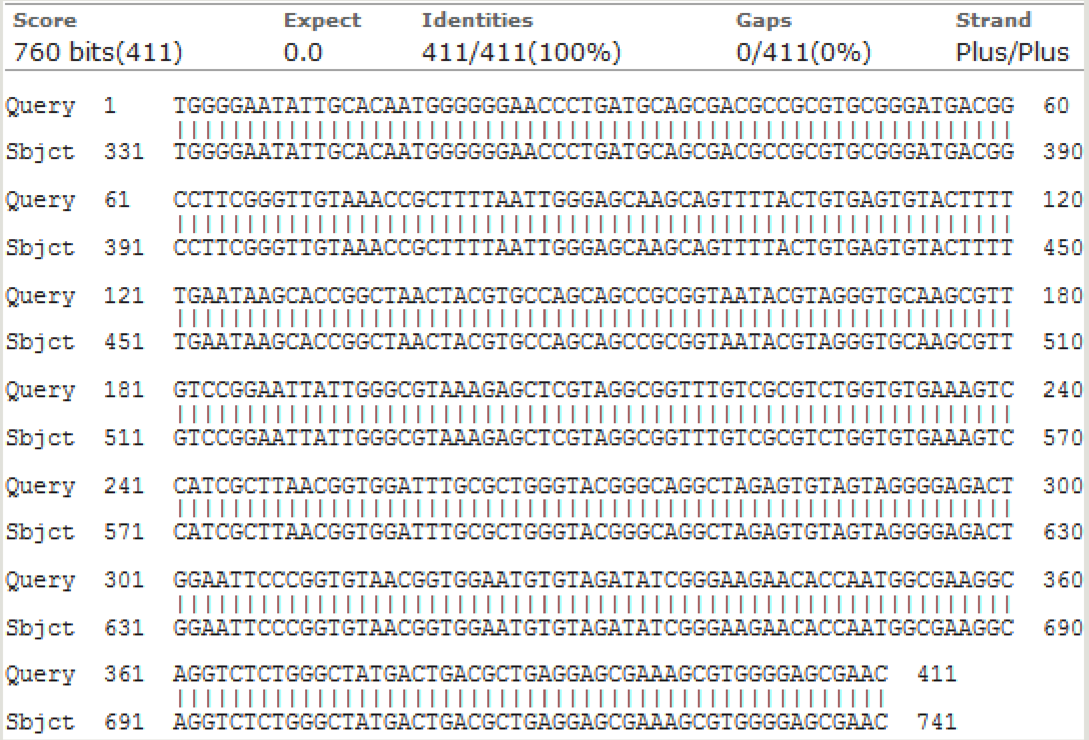
Blast variables : e-value

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)

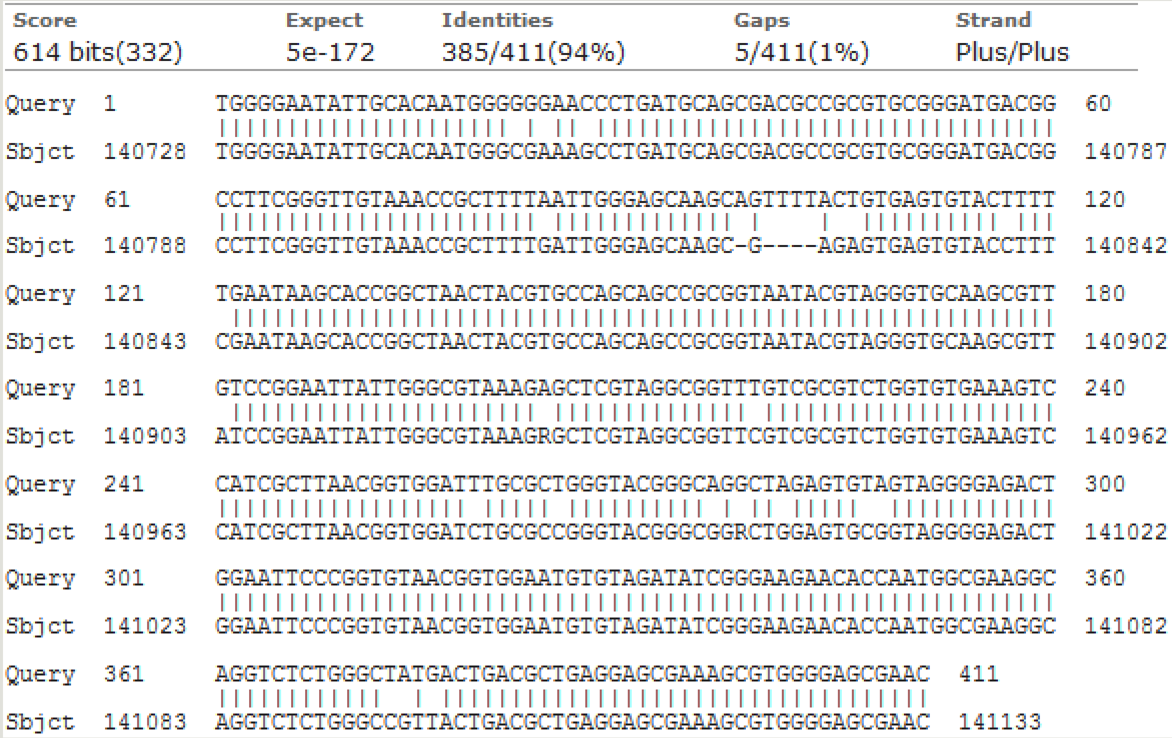


```
Score            Expect     Identities          Gaps          Strand
760 bits(411)    0.0        411/411(100%)       0/411(0%)     Plus/Plus

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)



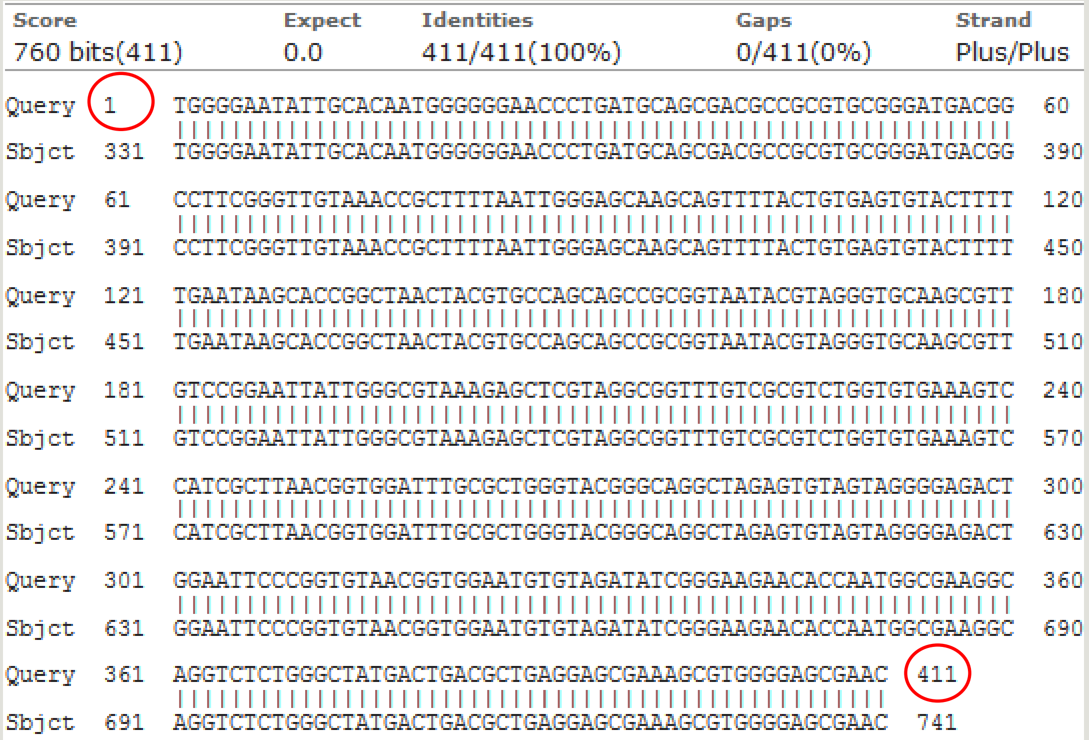
Score	Expect	Identities	Gaps	Strand
614 bits(332)	5e-172	385/411(94%)	5/411(1%)	Plus/Plus

```
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     AGGTCTCTGGGCTATGACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  411
               ||||||||||||  | |||||||||||||||||||||||||||||||||||
Sbjct  141083  AGGTCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC  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    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
```

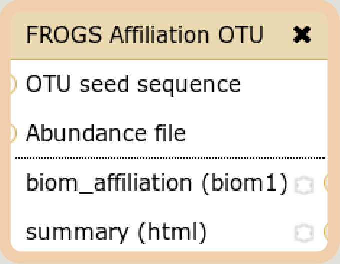
Query length = 411
100% coverage

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 ✖

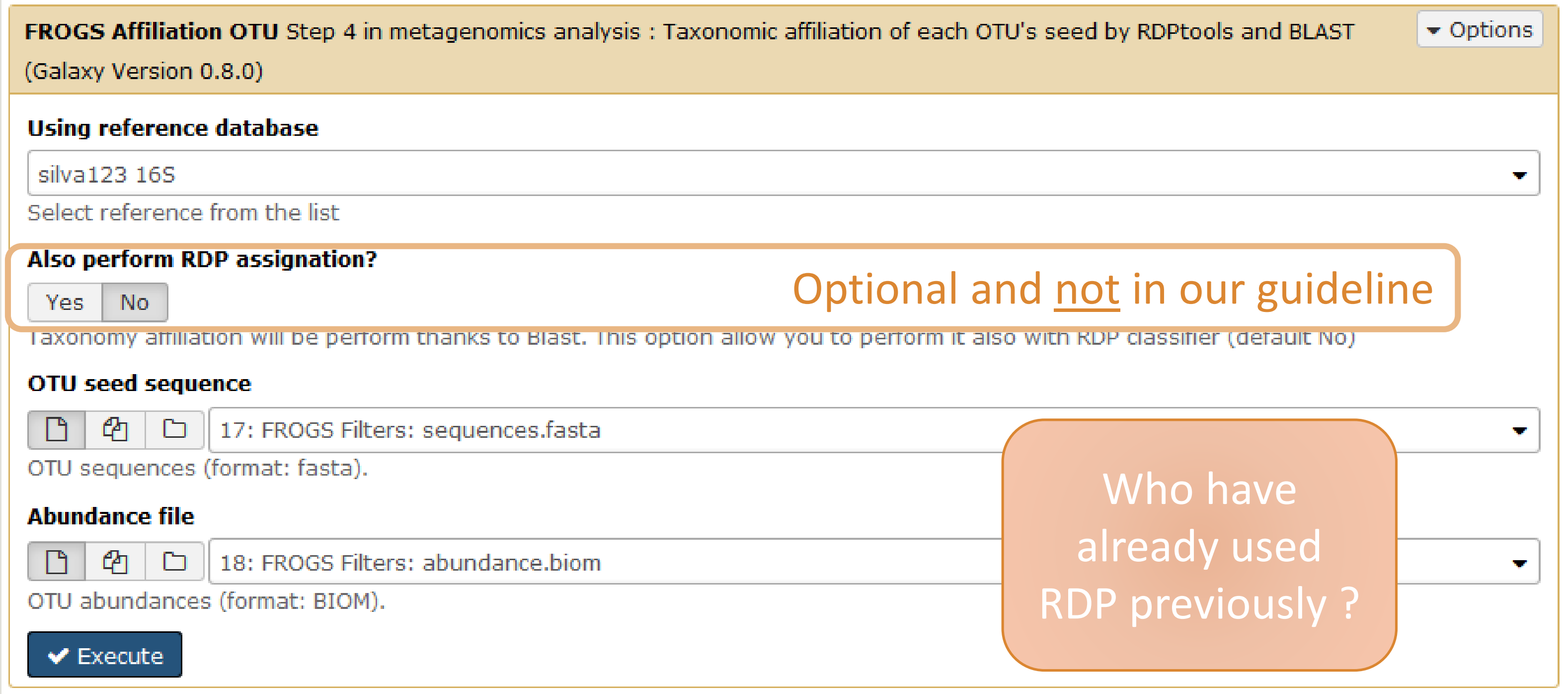
OTU seed sequence

Abundance file

biom_affiliation (biom1)

summary (html)

Affiliation



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)

Optional and <u>not</u> in our guideline

OTU seed sequence

17: FROGS Filters: sequences.fasta ▾

OTU sequences (format: fasta).

Abundance file

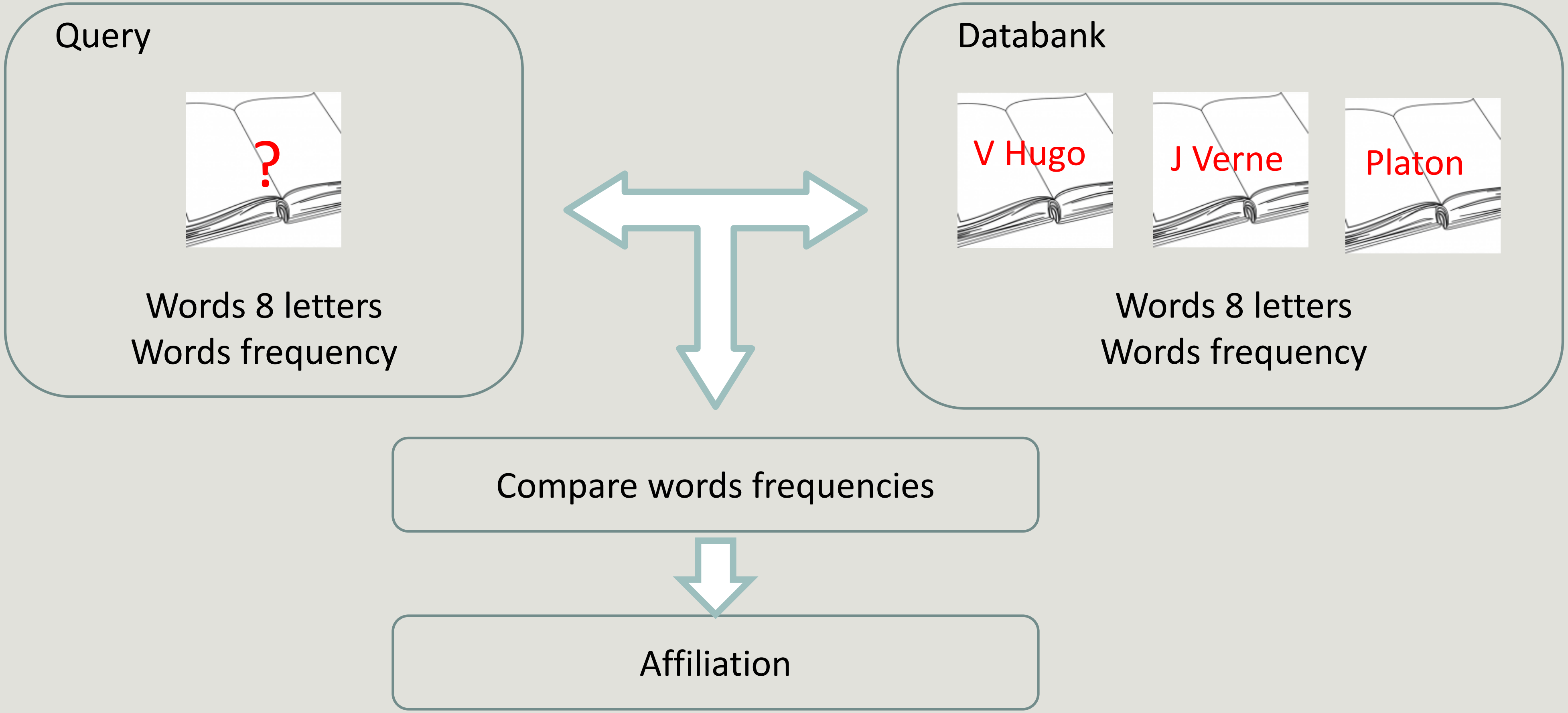
18: FROGS Filters: abundance.biom ▾

OTU abundances (format: BIOM).

Who have already used RDP previously ?

Escape RDP explanation

How works RDP ?



Query

?

Words 8 letters
Words frequency

Databank

V Hugo

J Verne

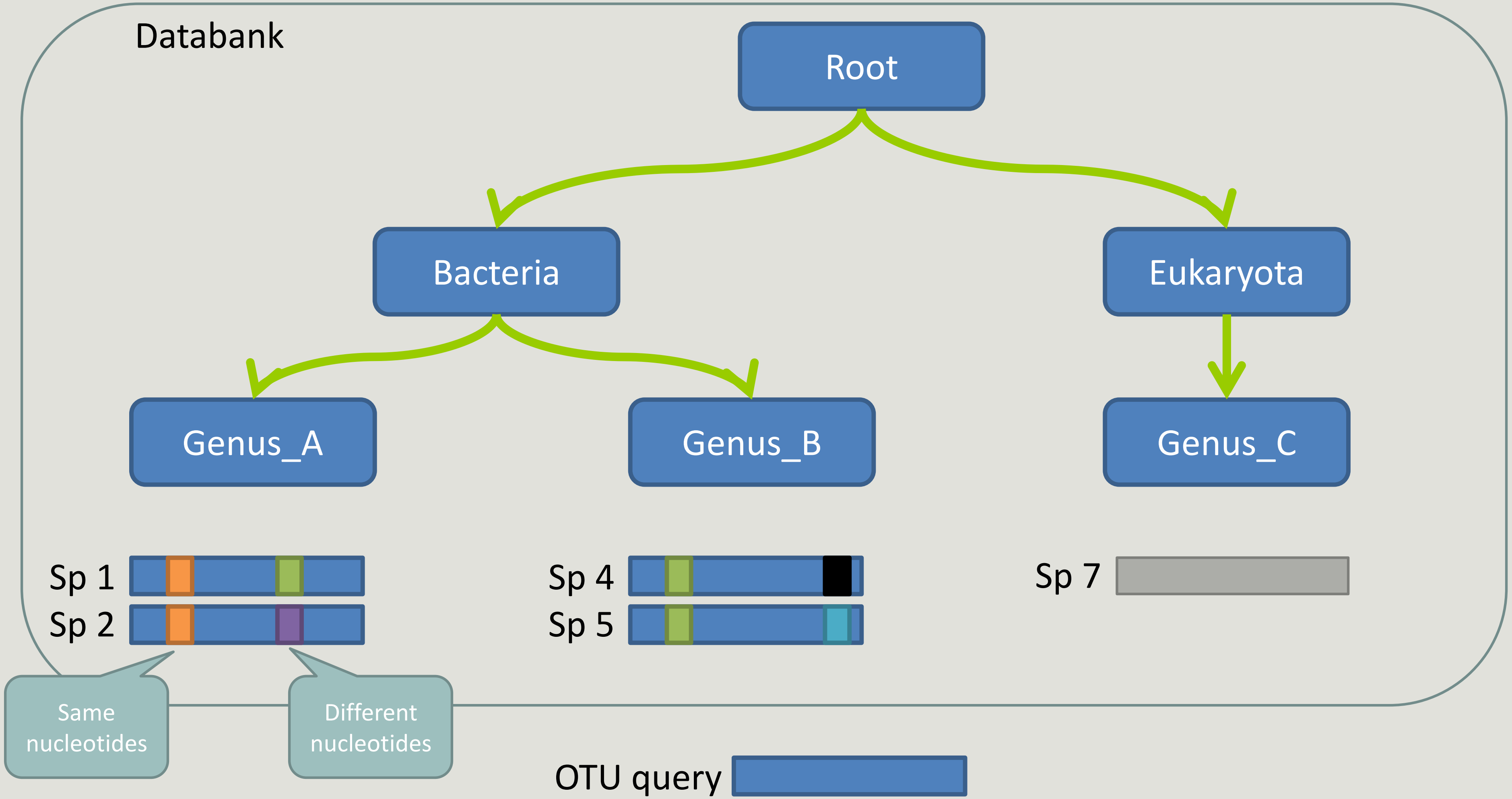
Platon

Words 8 letters
Words frequency

Compare words frequencies

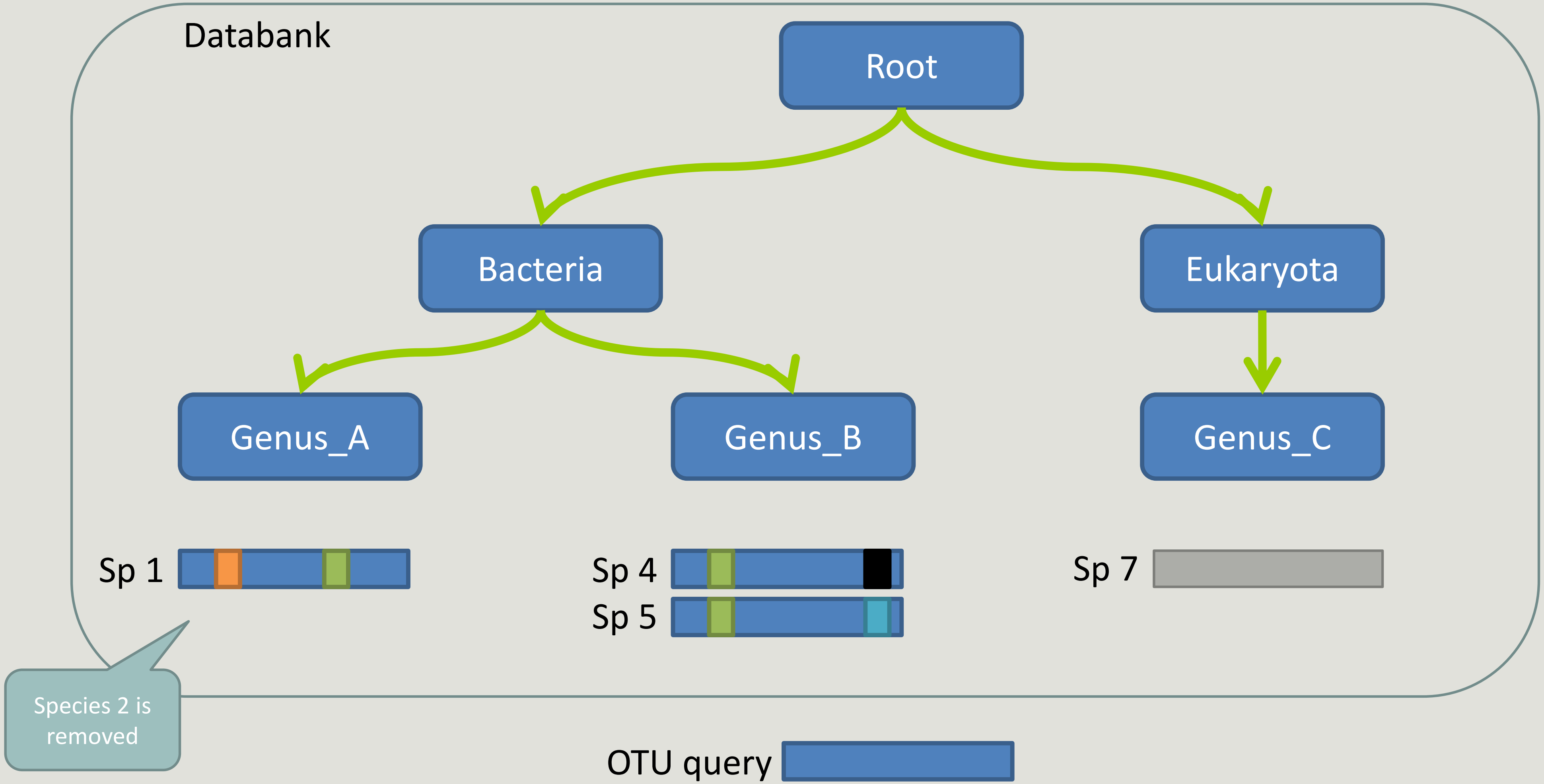
Affiliation

How works RDP ?

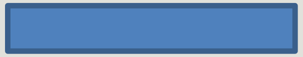


Result:
Bacteria(100) ; Genus_A(50) ; Sp1(25)

The dysfunctions of RDP ?



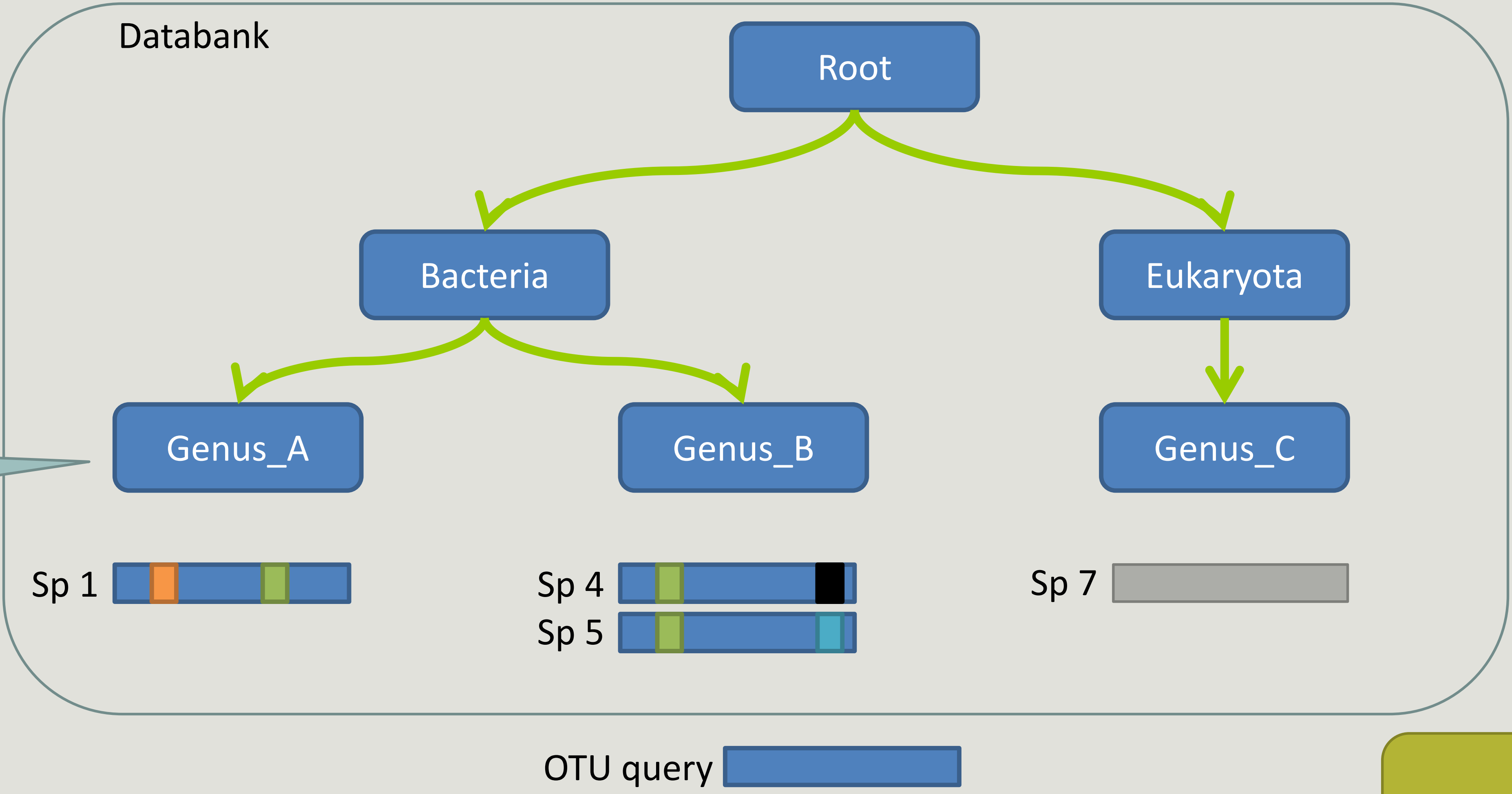
Species 2 is removed



Result:

?

The dysfunctions of RDP n°1 ?



Genus (50) was expected

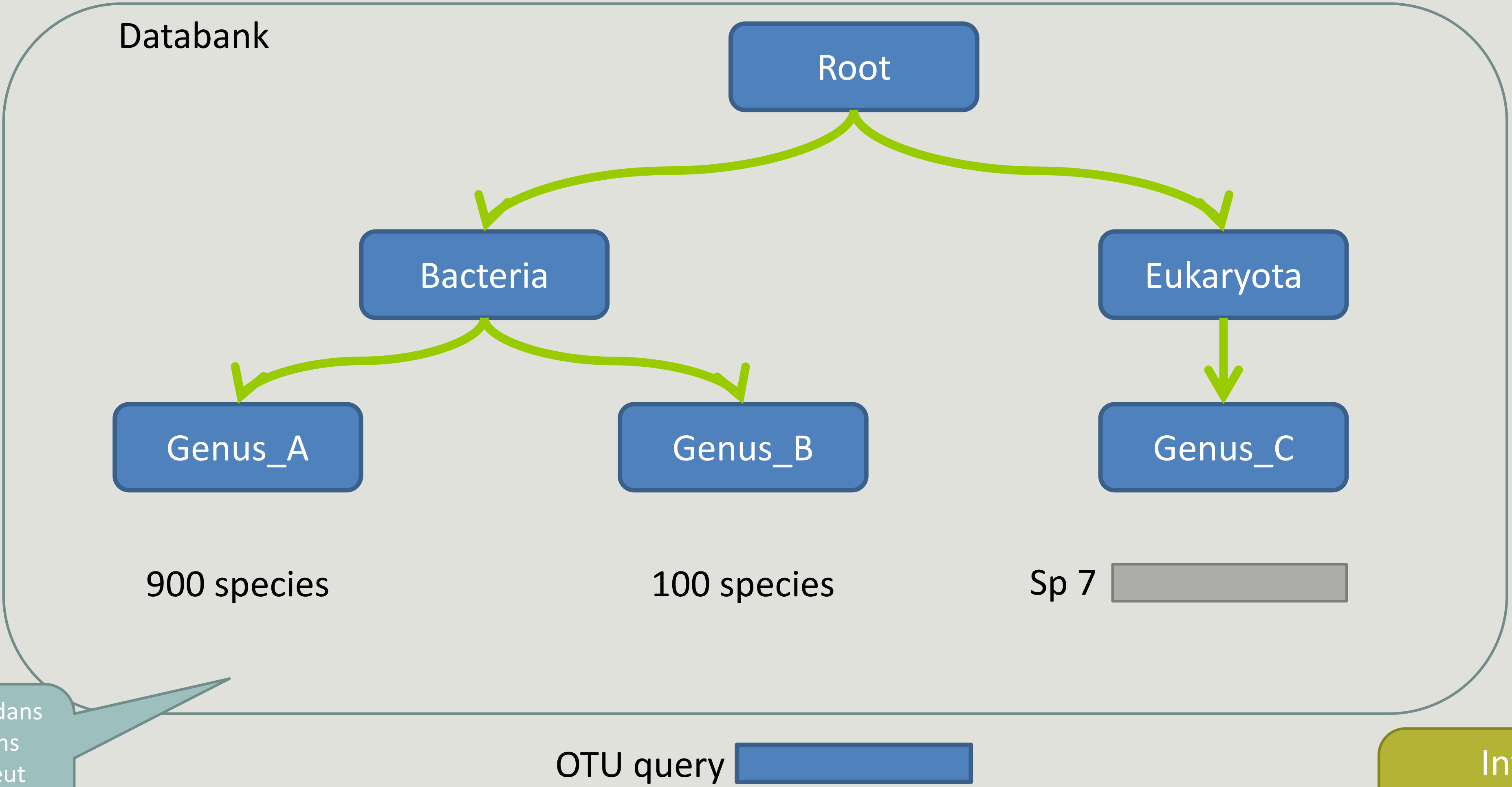
Genus (50) was expected
If seq order was inversed in banks, so we got the second chance

Order dependent

Result:

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

The dysfunctions of RDP n°2 ?



Databank

Root

Bacteria

Eukaryota

Genus_A

Genus_B

Genus_C

900 species

100 species

Sp 7

OTU query

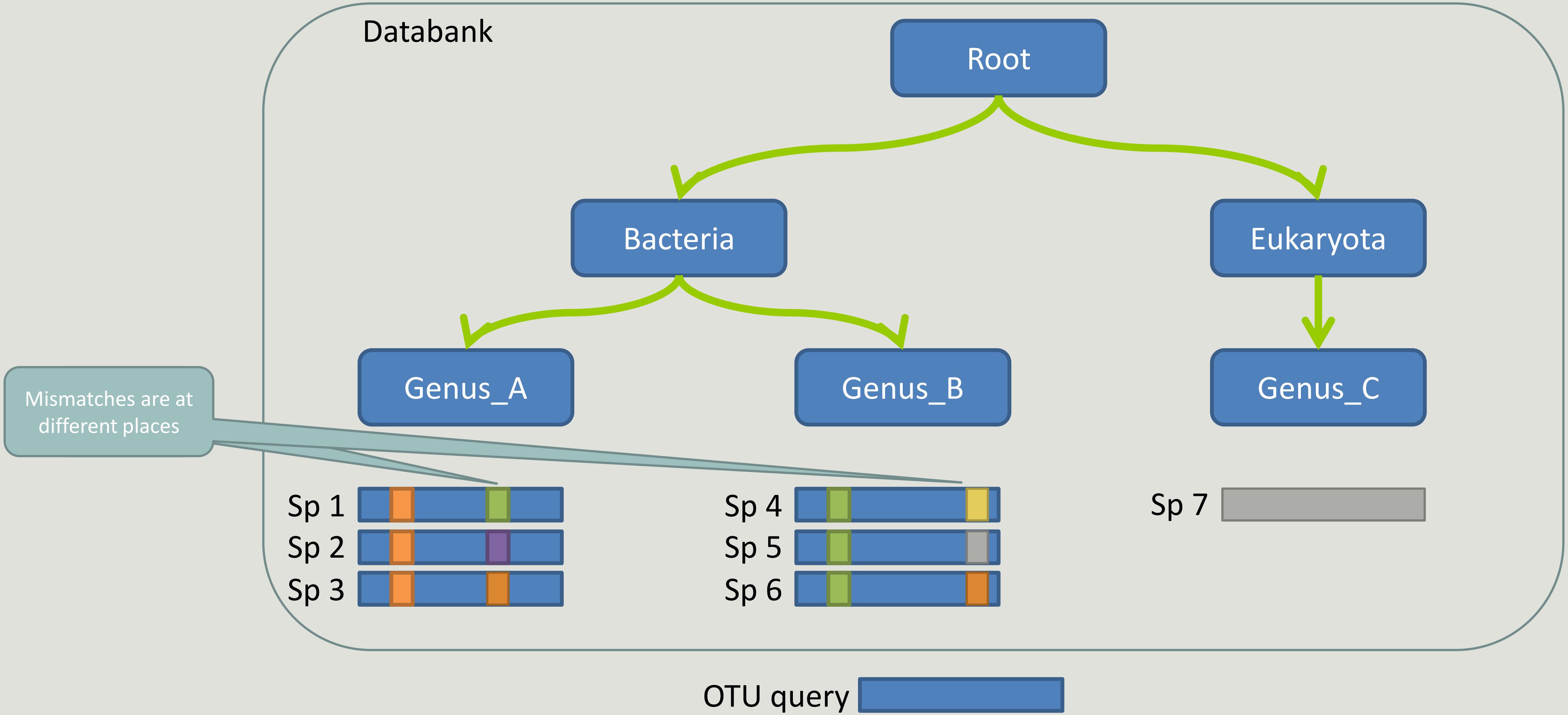
Beaucoup d’espèces dans un genre et peu dans l’autre, alors RDP peut donner des résultats très différents

Influenced by heterogeneity in last ranks

Result:

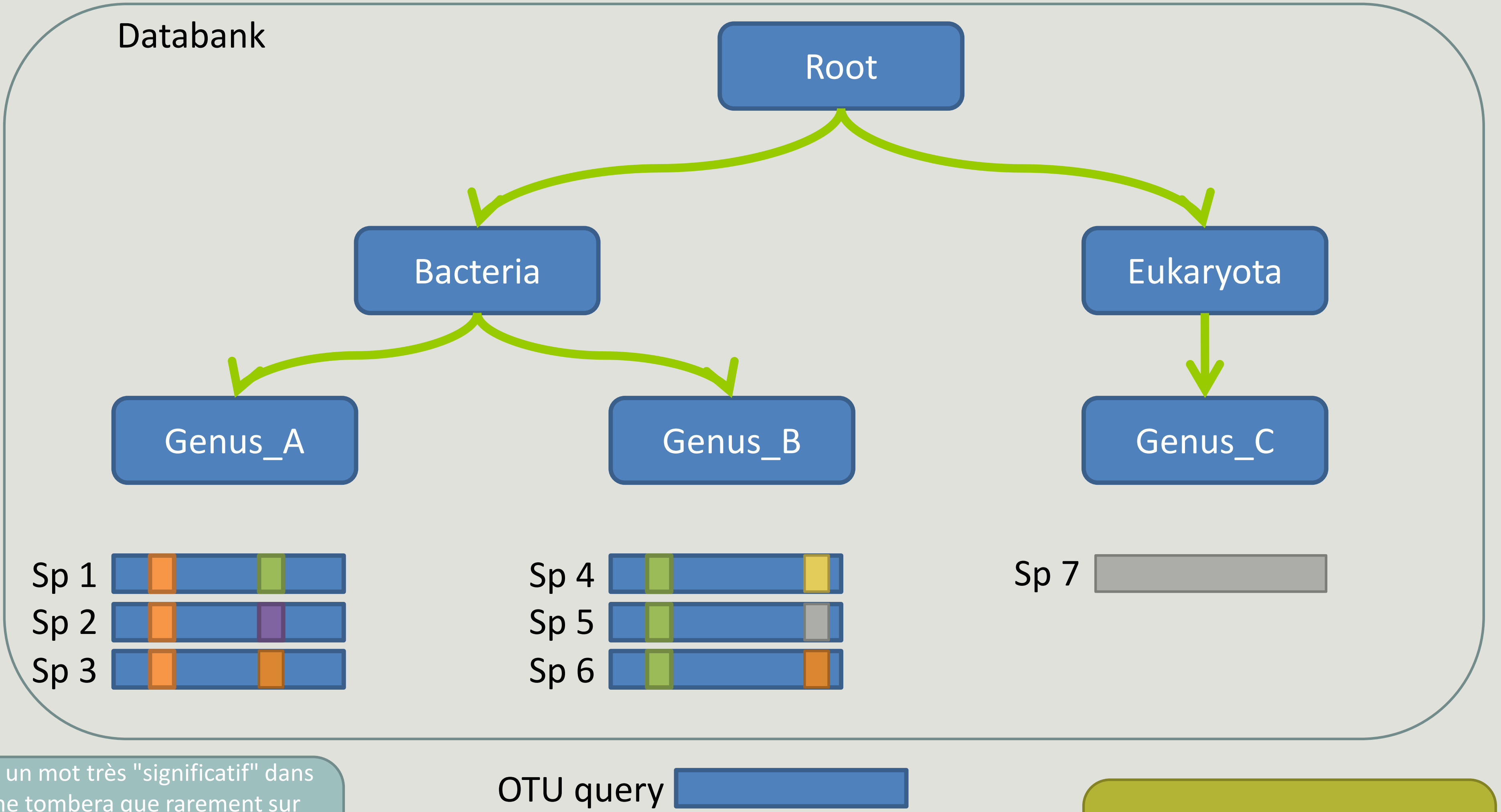
Bacteria(100); Genus_A(90); spX(0.1) OR Bacteria(100); Genus_B(10); spX(0.1)

The dysfunctions of RDP n°3 ?



Result:
?

The dysfunctions of RDP n°3 ?

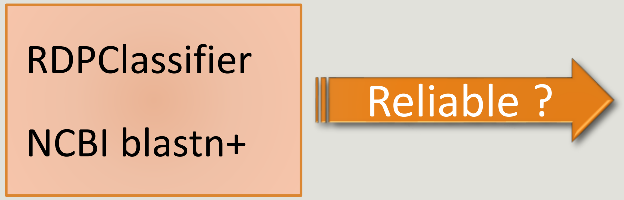


Si le mismatch se fait sur un mot très "significatif" dans le profil de k-mers, RDP ne tombera que rarement sur l'espèce lors du bootstrap. Avec une même distance d'édition (2 mismatchs) on peut donc avoir une grande différence de bootstrap pour peu que le mot affecté soit important dans le profil.

Result:
Bacteria(100); Genus_A(50); sp1(20)

Influenced by the divergences position

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

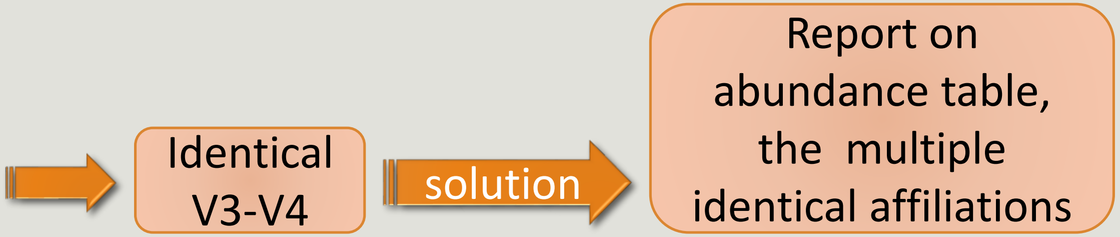


RDPClassifier

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

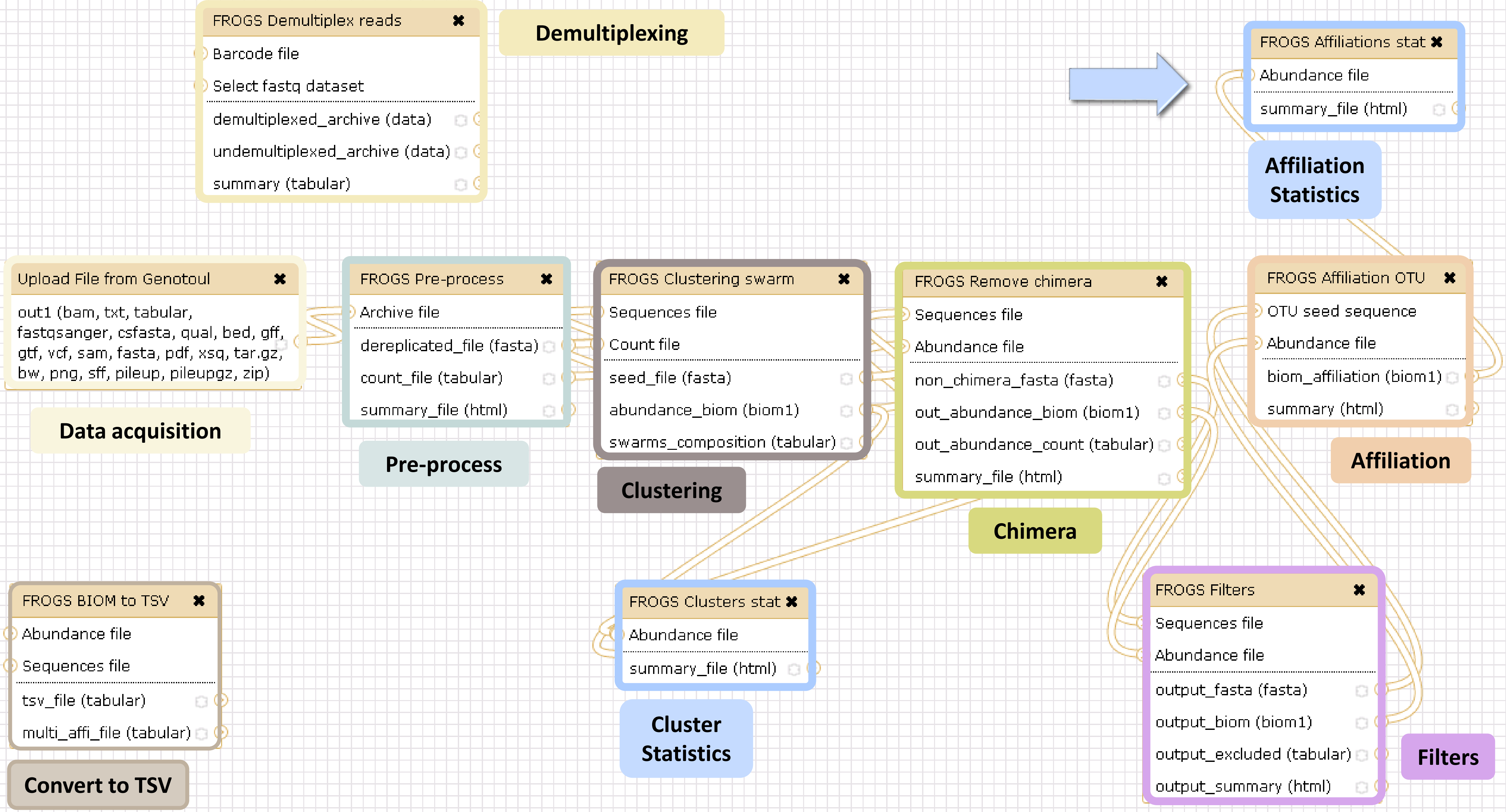
With the FROGS guideline

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)

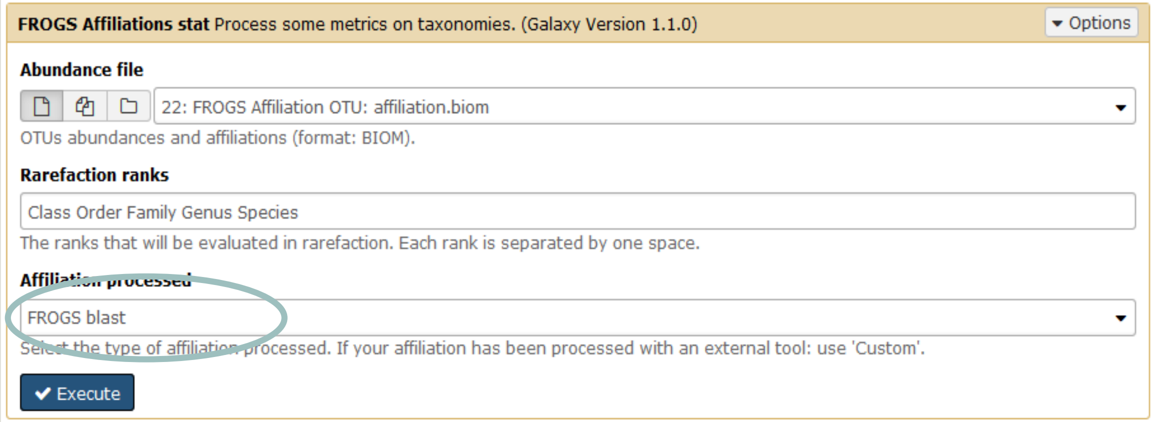
Convert to TSV

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

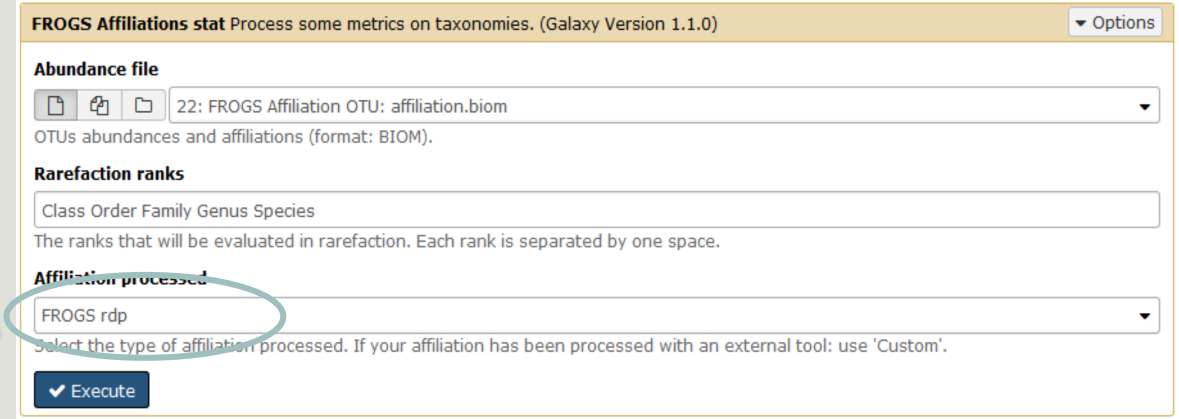
The ranks that will be evaluated in rarefaction. Each rank is separated by one space.

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

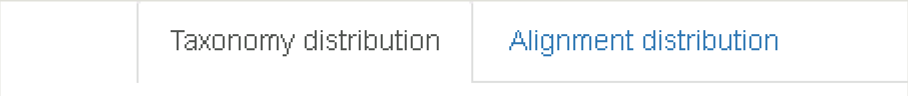
Class Order Family Genus Species

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

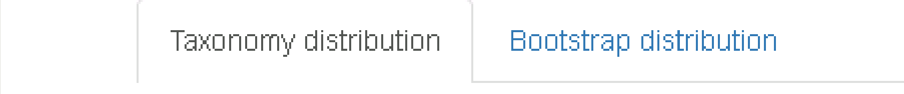
Affiliation processed

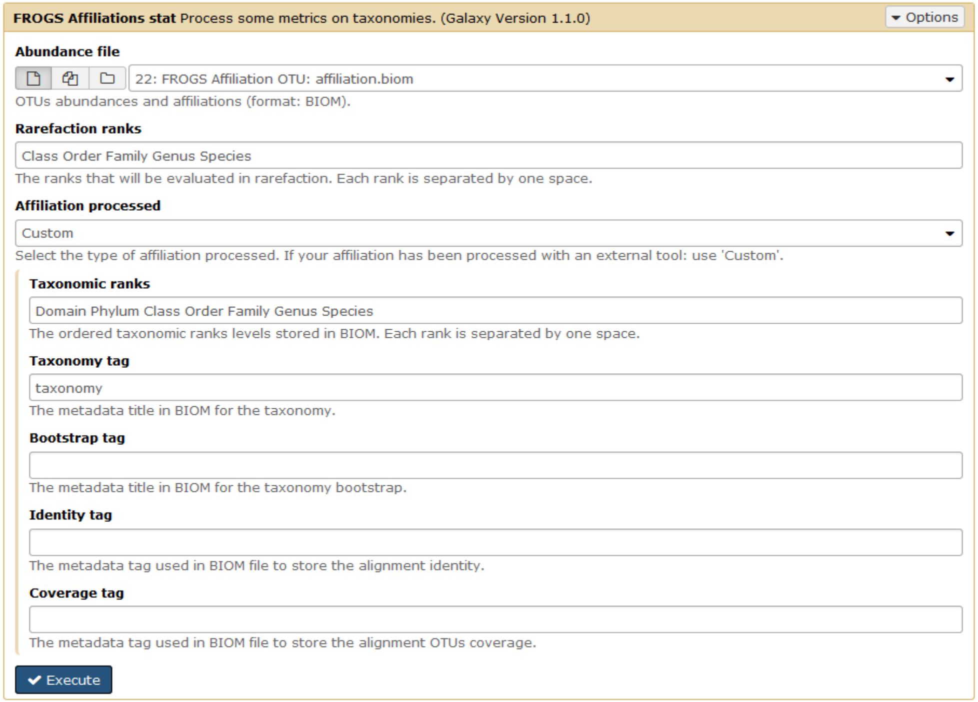
FROGS rdp

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.

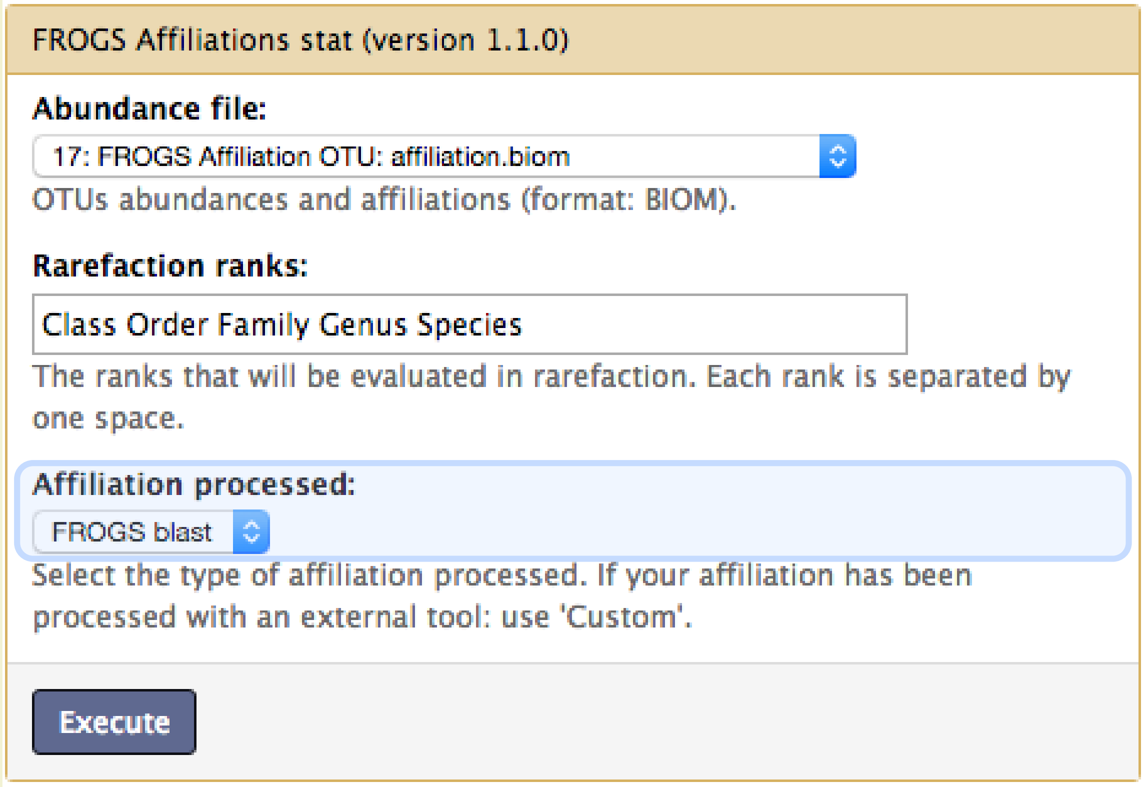
Identity tag

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 5.2



FROGS Affiliations stat (version 1.1.0)

Abundance file:

17: FROGS Affiliation OTU: affiliation.biom

OTUs abundances and affiliations (format: BIOM).

Rarefaction ranks:

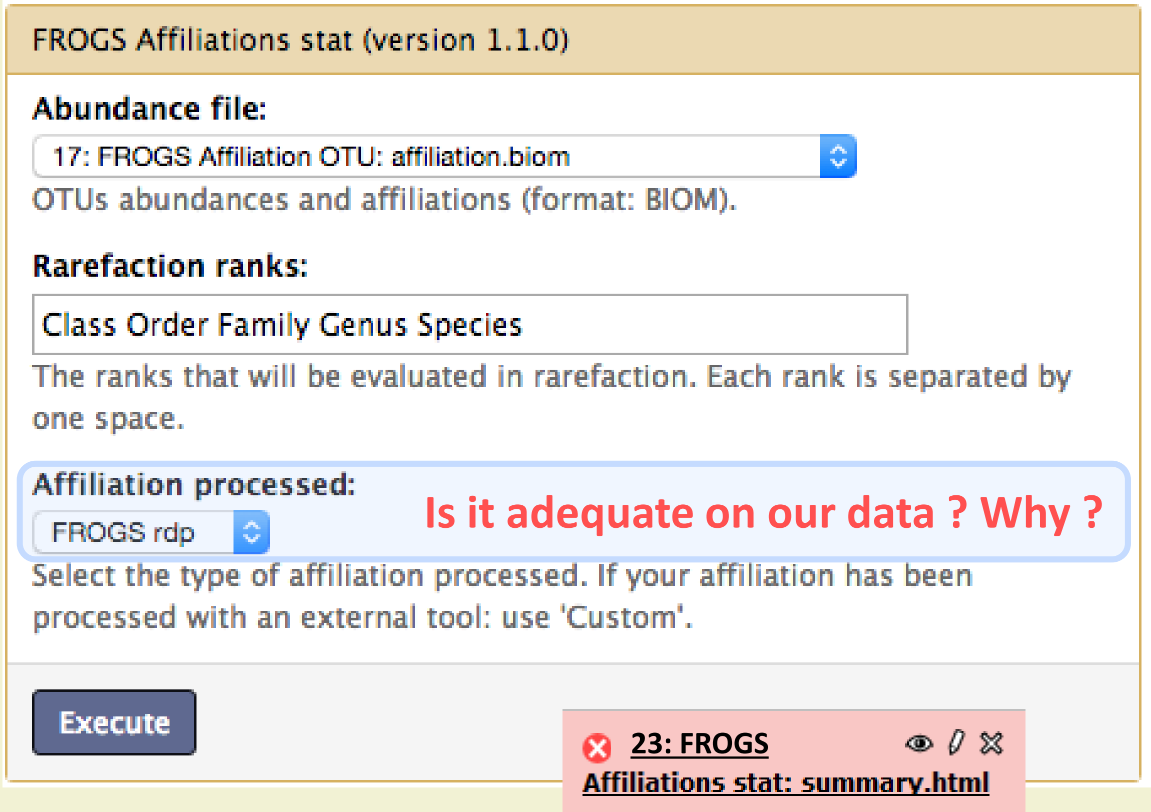
Class Order Family Genus Species

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

Is it adequate on our data ? Why ?

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

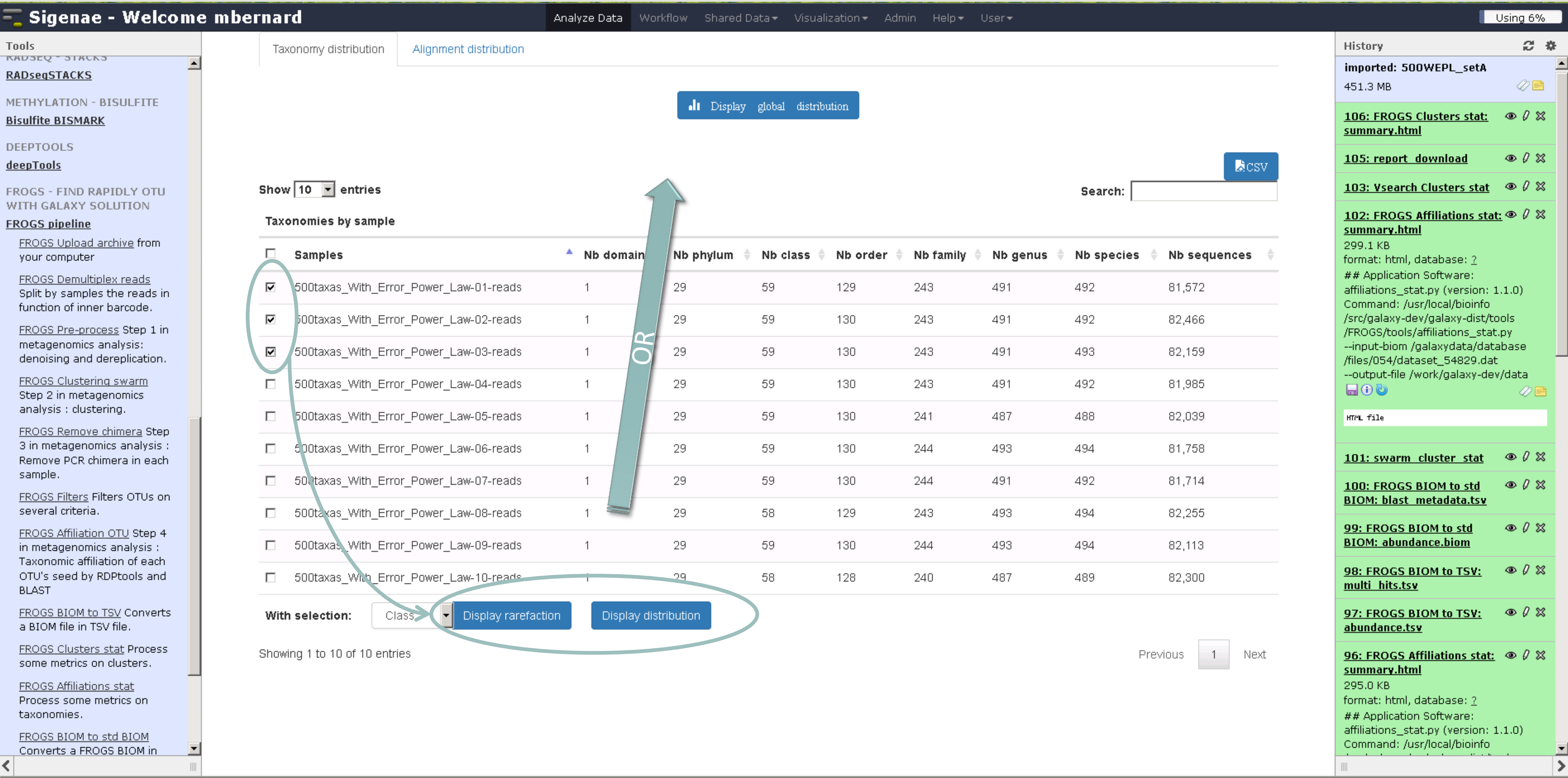
23: FROGS Affiliations stat: summary.html

Exercise 5.2

→ 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?



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

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

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

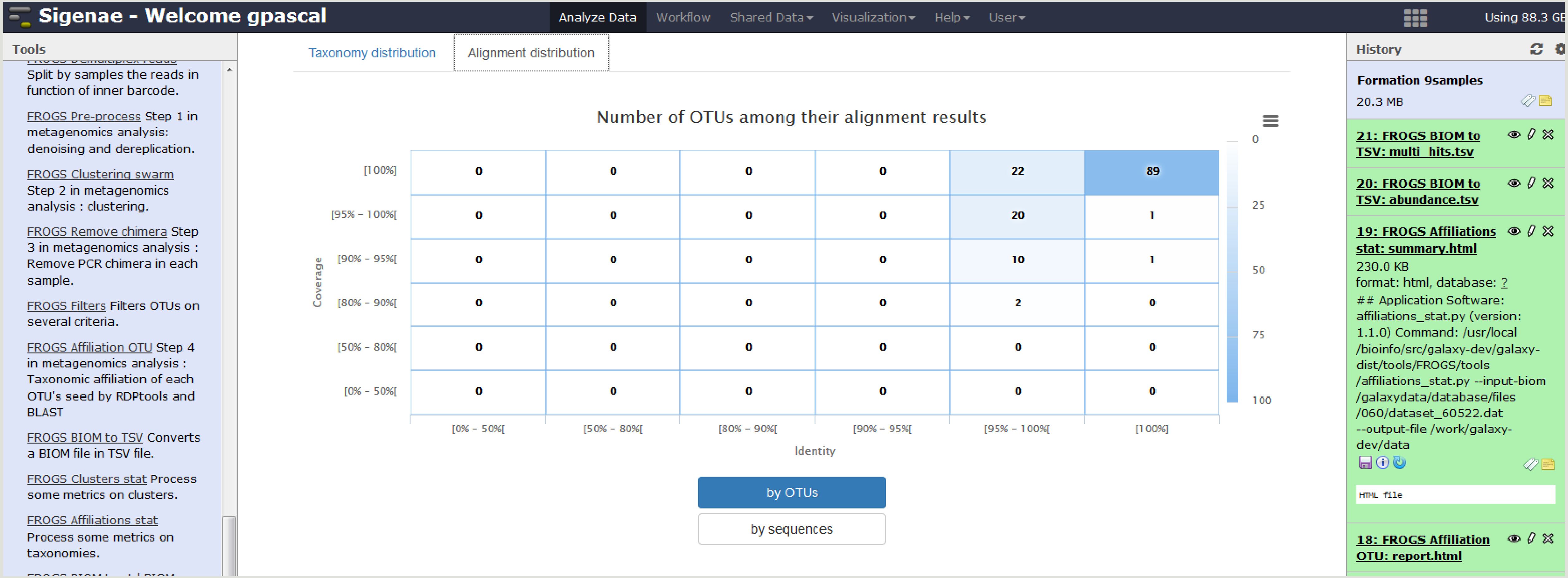
100: FROGS BIOM to std BIOM: blast_metadata.tsv

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



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

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: ?

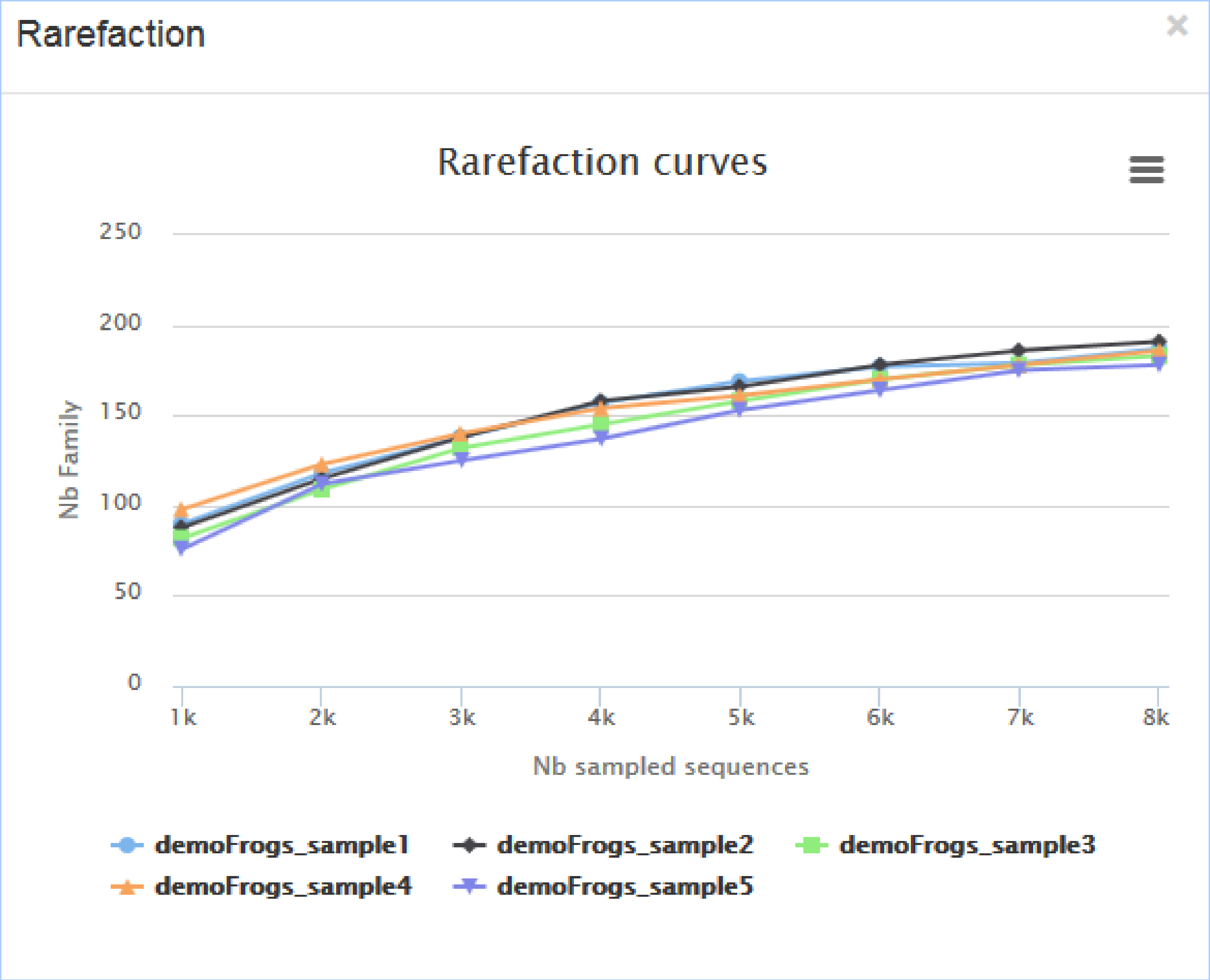
Application Software: affiliations_stat.py (version: 1.1.0) Command: /usr/local /bioinfo/src/galaxy-dev/galaxy-dist/tools/FROGS/tools /affiliations_stat.py --input-biom /galaxydata/database/files /060/dataset_60522.dat --output-file /work/galaxy-dev/data

HTML file

18: FROGS Affiliation OTU: report.html

Available only after AFFILIATION TOOL

Samples size ~8500 sequences



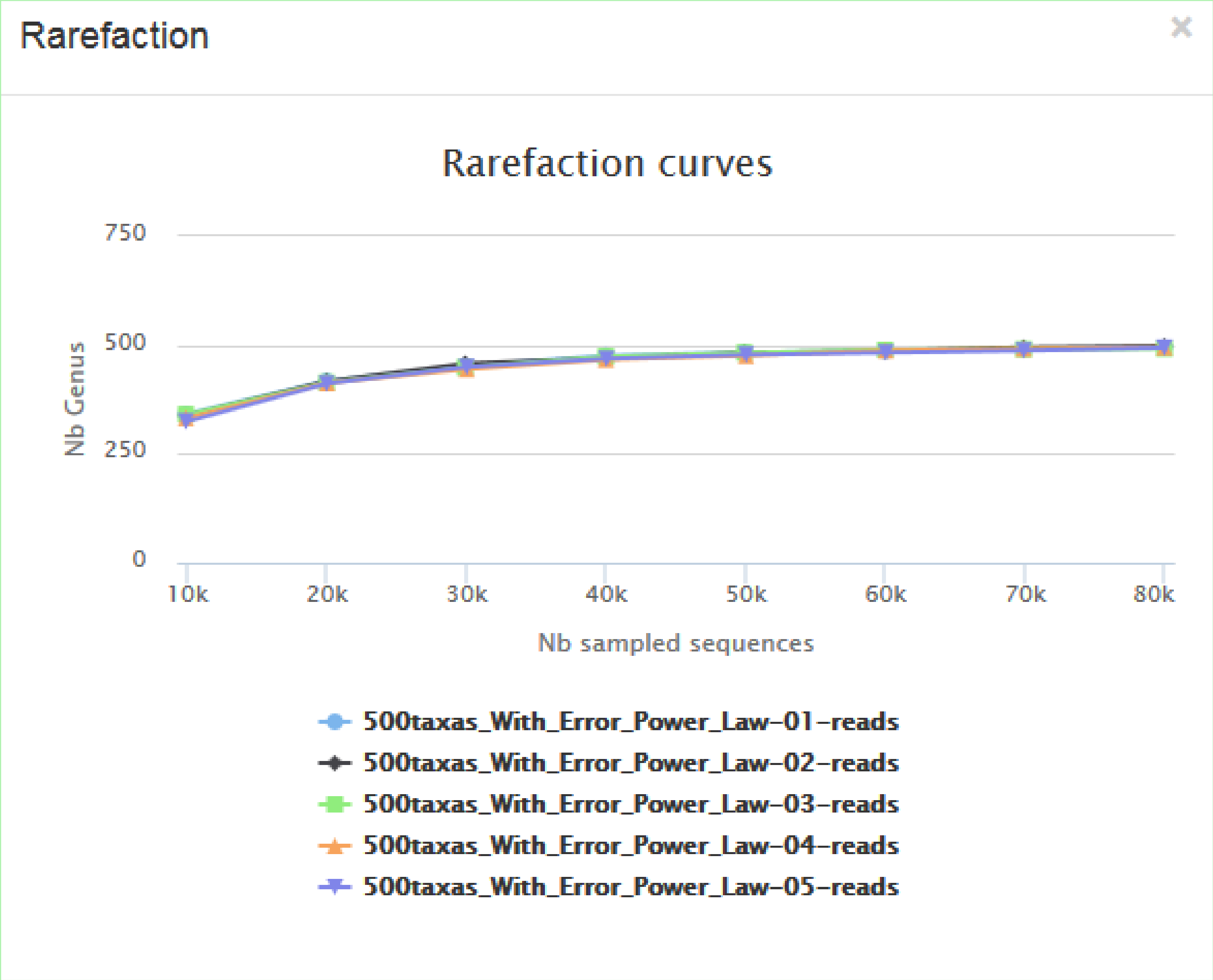
The curve continues to rise

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

Rarefaction tab

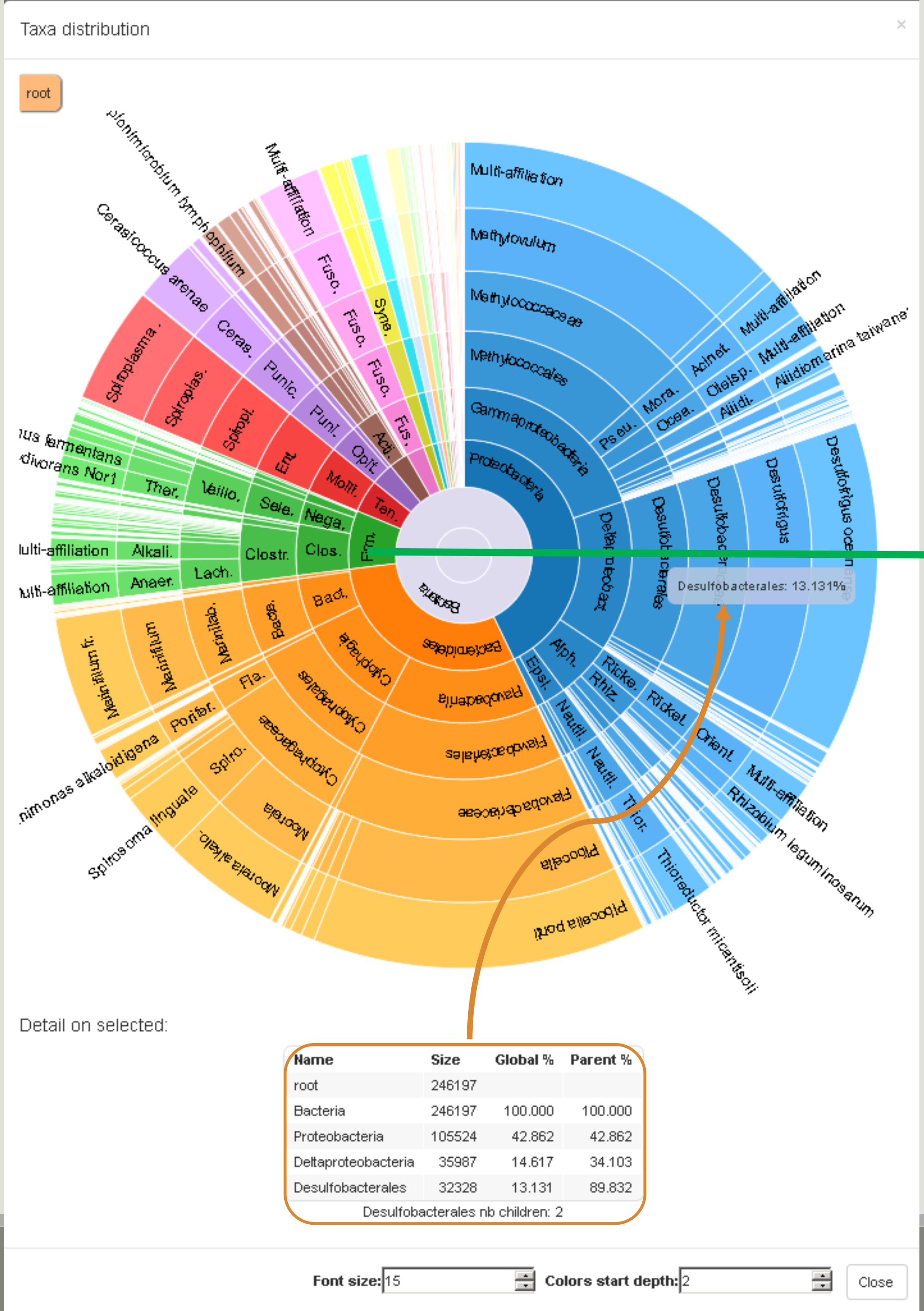
Available only after AFFILIATION TOOL

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



Desulfobacterales: 13.131%

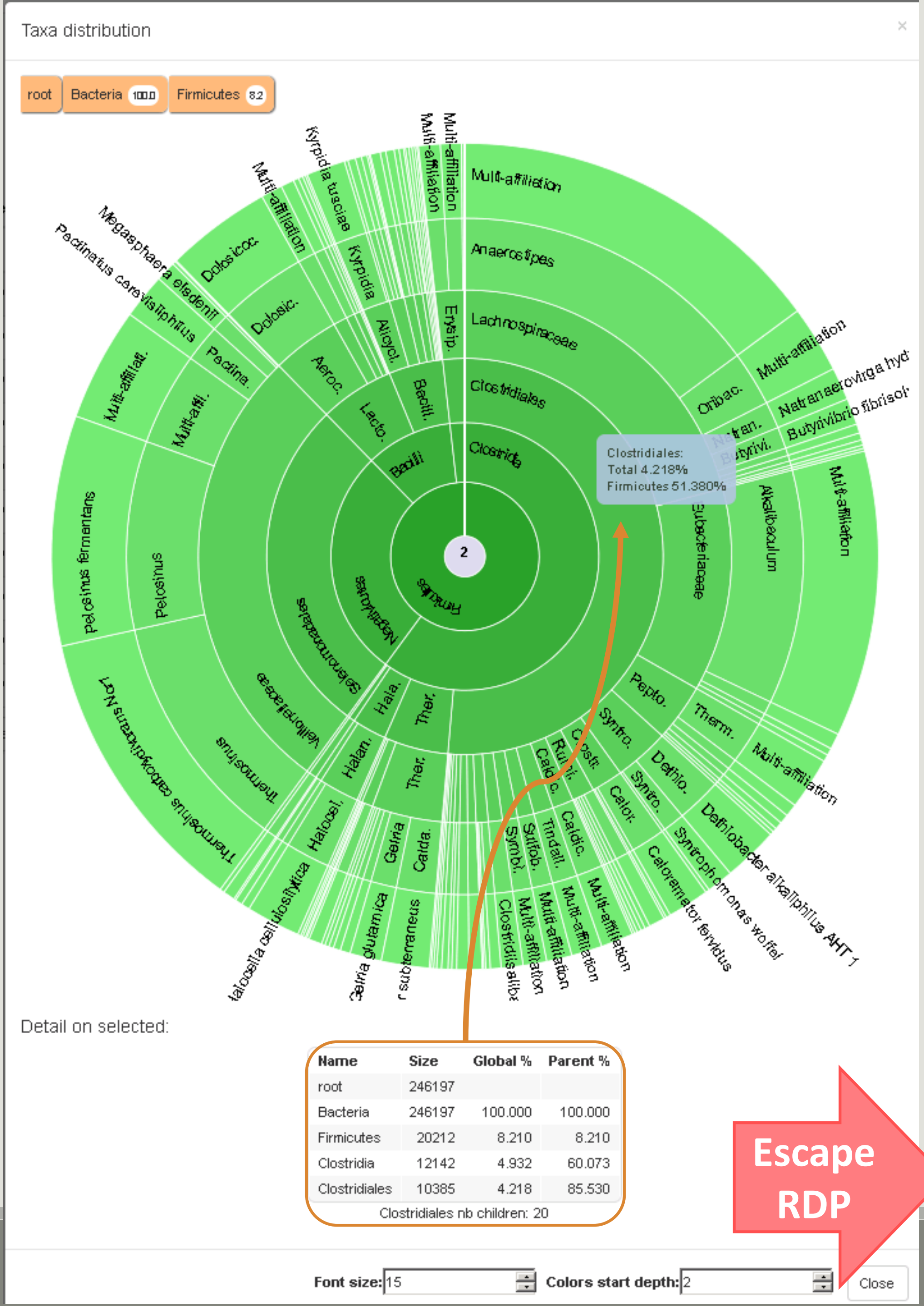
Detail on selected:

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



Clostridiales:
Total 4.218%
Firmicutes 51.380%

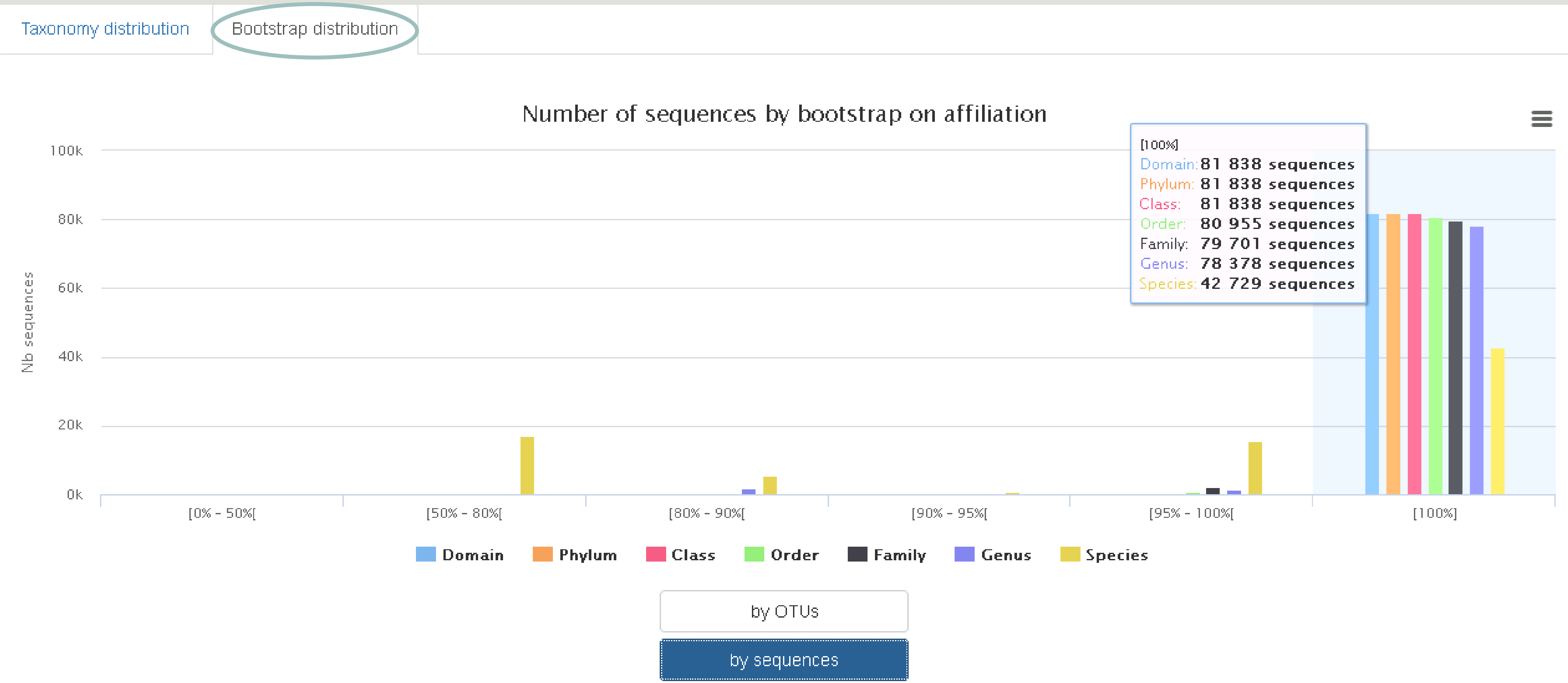
Detail on selected:

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

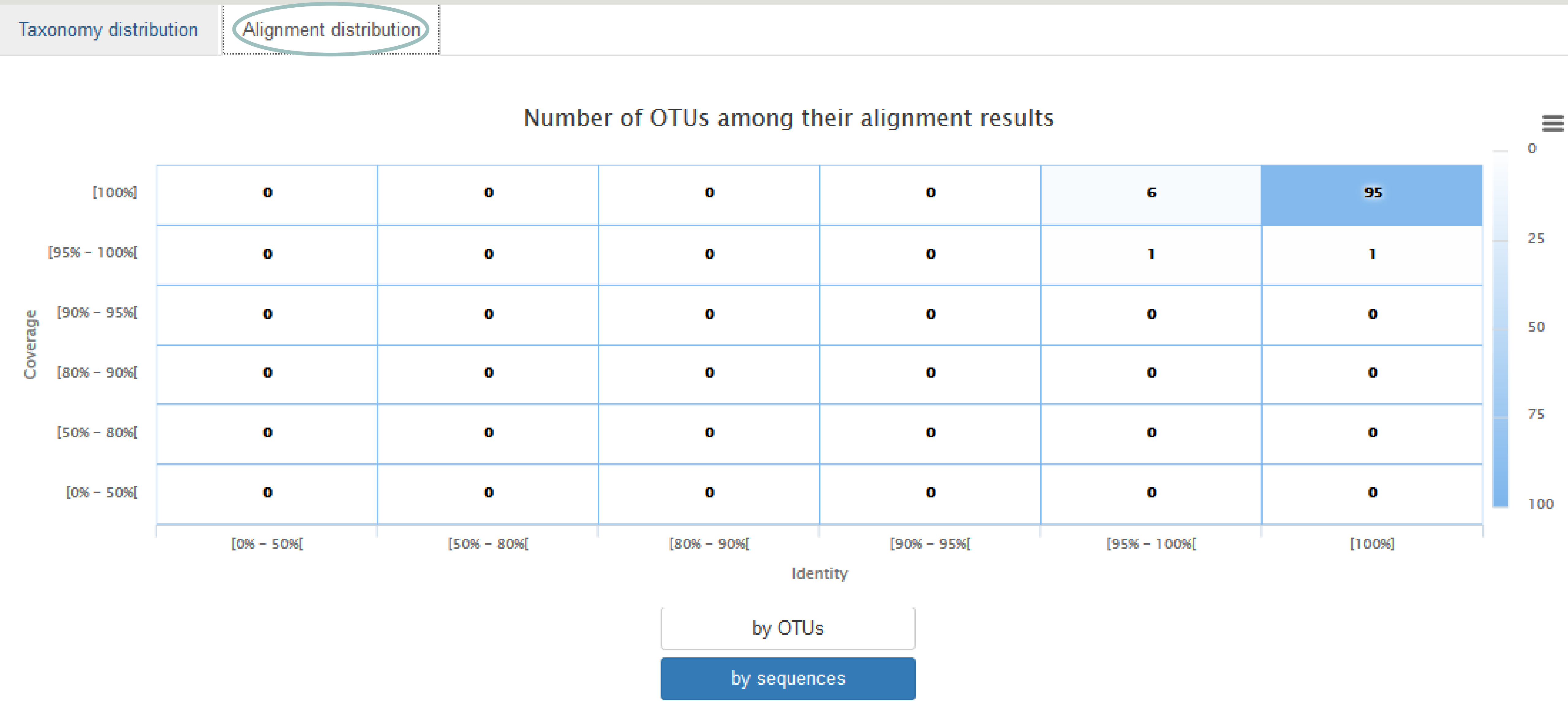
Clostridiales nb children: 20

Font size: 15 Colors start depth: 2 Close

Escape RDP

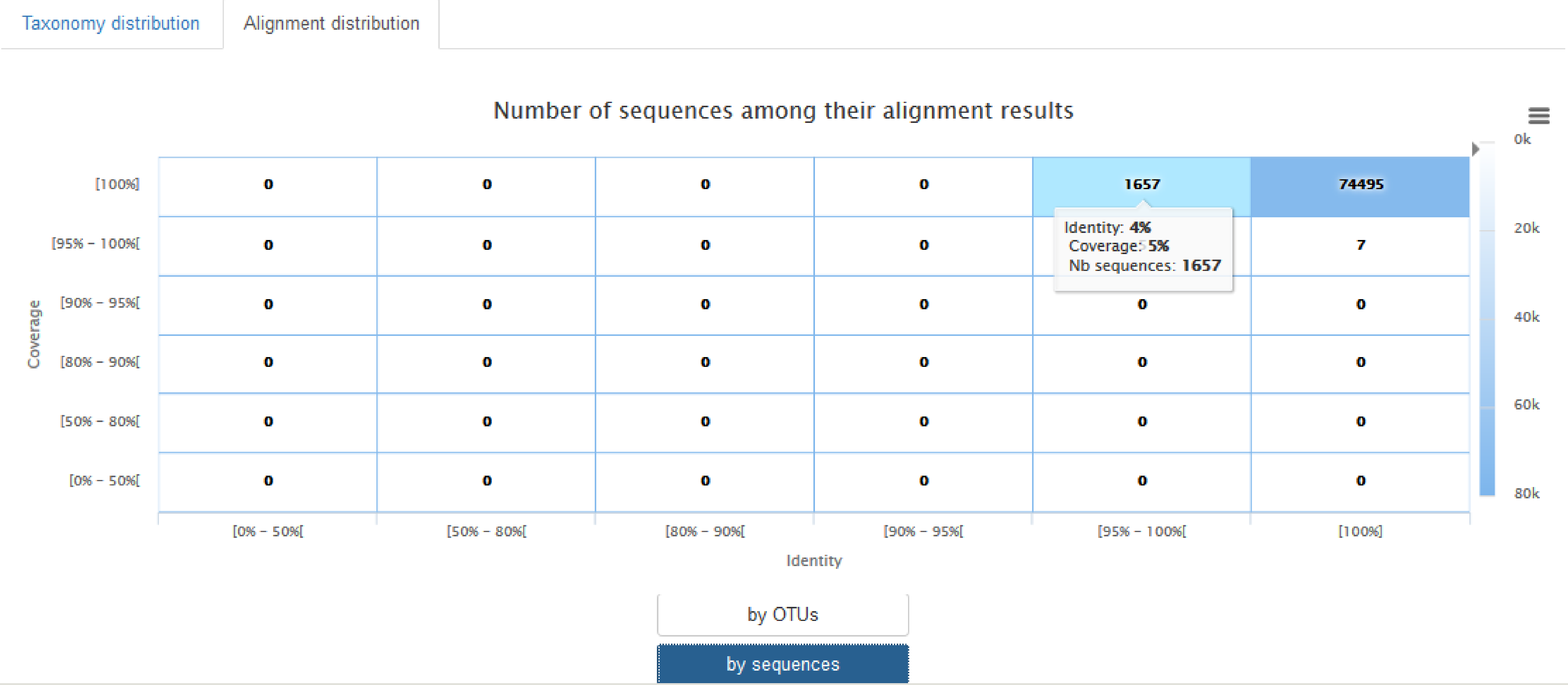


[100%]
Domain: 81 838 sequences
Phylum: 81 838 sequences
Class: 81 838 sequences
Order: 80 955 sequences
Family: 79 701 sequences
Genus: 78 378 sequences
Species: 42 729 sequences



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

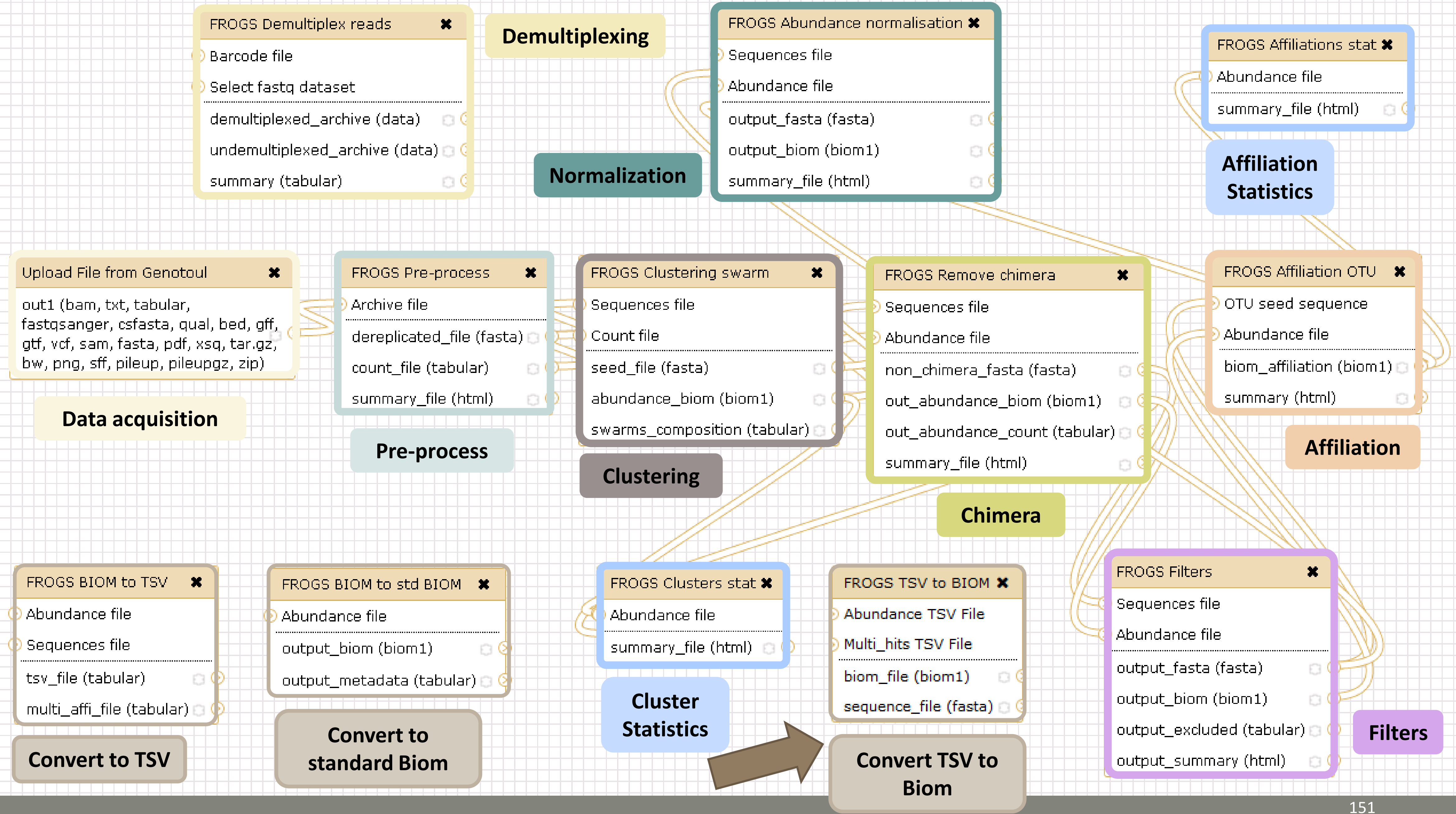


Number of sequences among their alignment results

Coverage \ Identity	[0% – 50%[	[50% – 80%[	[80% – 90%[	[90% – 95%[	[95% – 100%[	[100%]
[100%]	0	0	0	0	1657	74495
[95% – 100%[	0	0	0	0		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)

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

TSV to BIOM

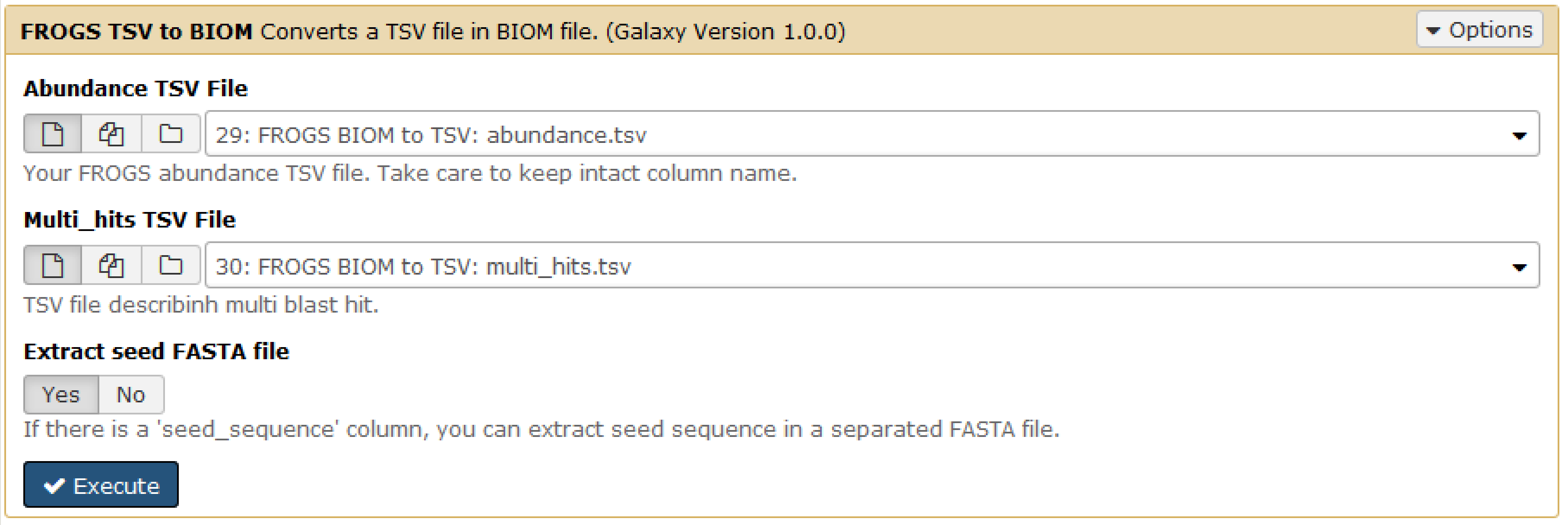
After modifying your abundance TSV file you can again:

- 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.

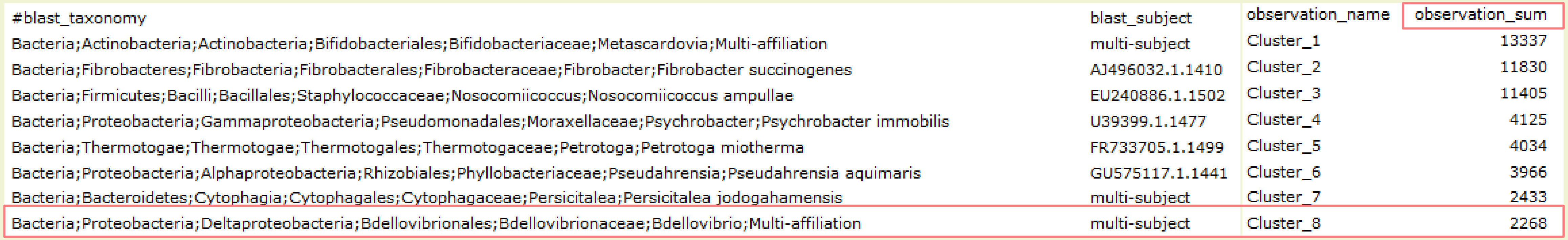
Your Turn! – 6

PLAY WITH TSV_TO_BIOM

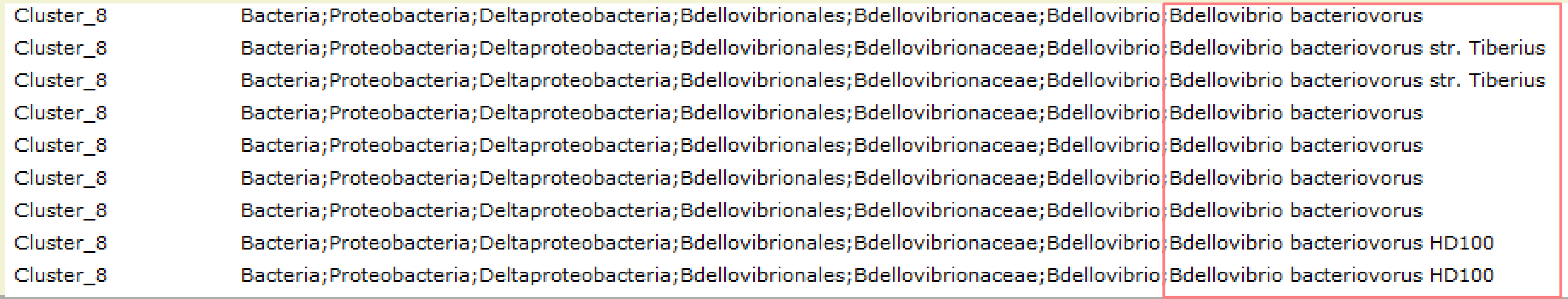
Exercise 6

→ objectives : Play with multi-affiliation and TSV_to_BIOM

1. Observe in Multi-hit.tsv and abundance.tsv cluster_8 annotation



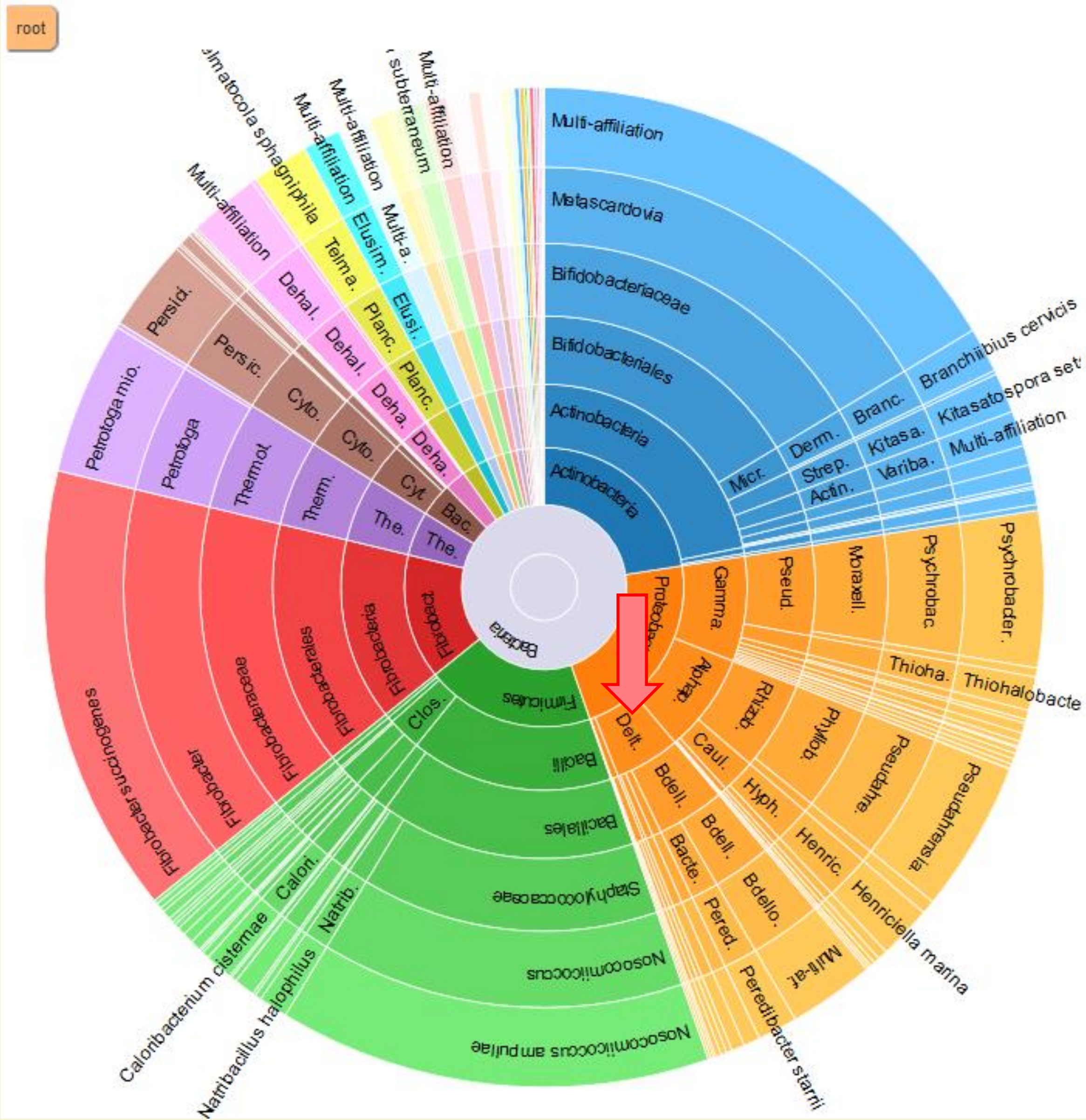
#blast_taxonomy	blast_subject	observation_name	observation_sum
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Metascardovia;Multi-affiliation	multi-subject	Cluster_1	13337
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter;Fibrobacter succinogenes	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

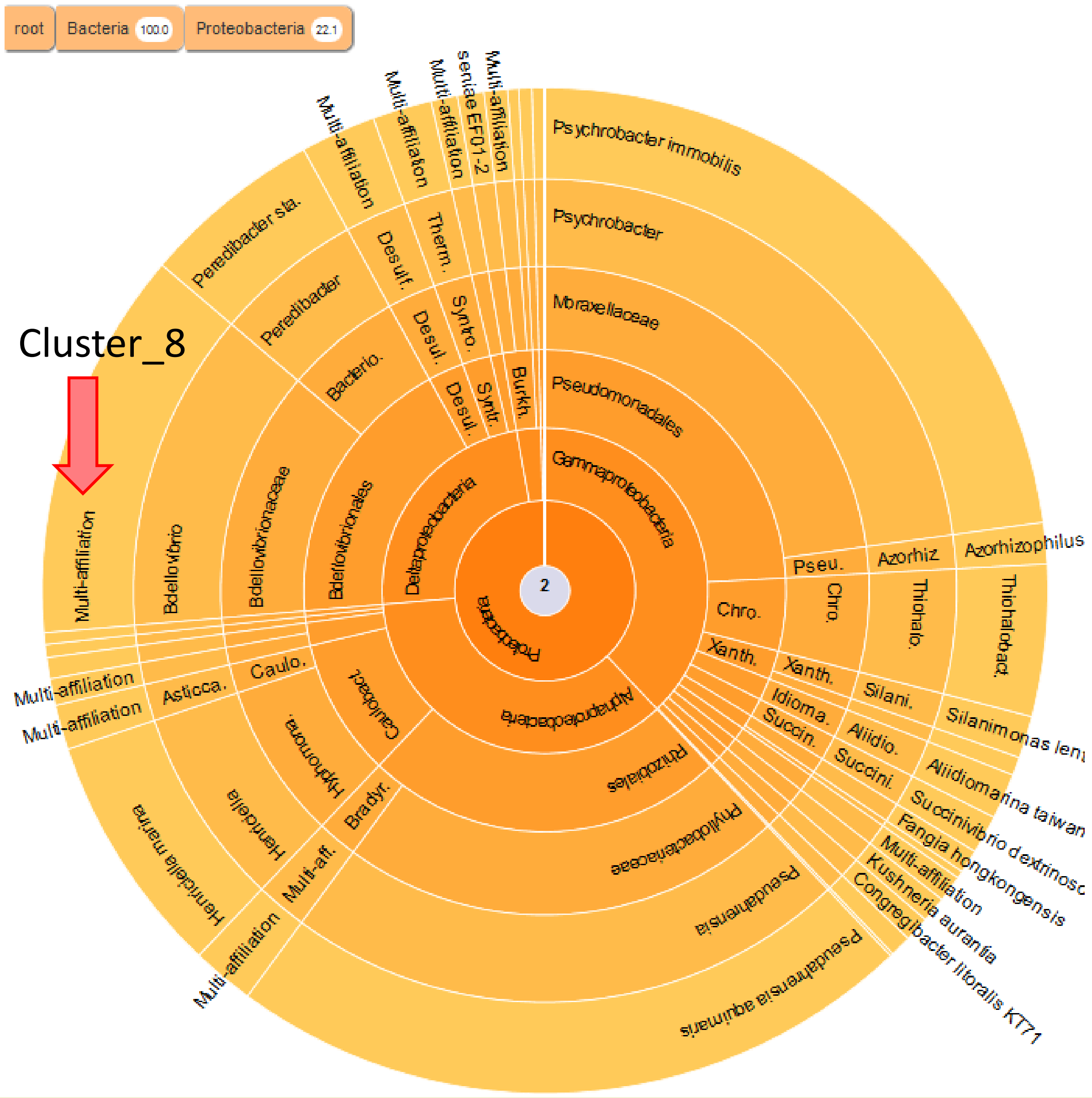


Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus	CP007656.1036900.1038415
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	Bdellovibrio bacteriovorus str. Tiberius	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 6

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

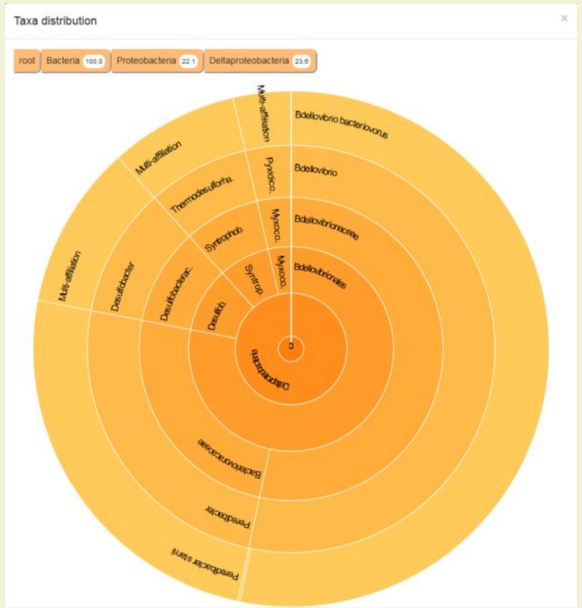
Exercise 6

4. Modify multihit.tsv and keep only :

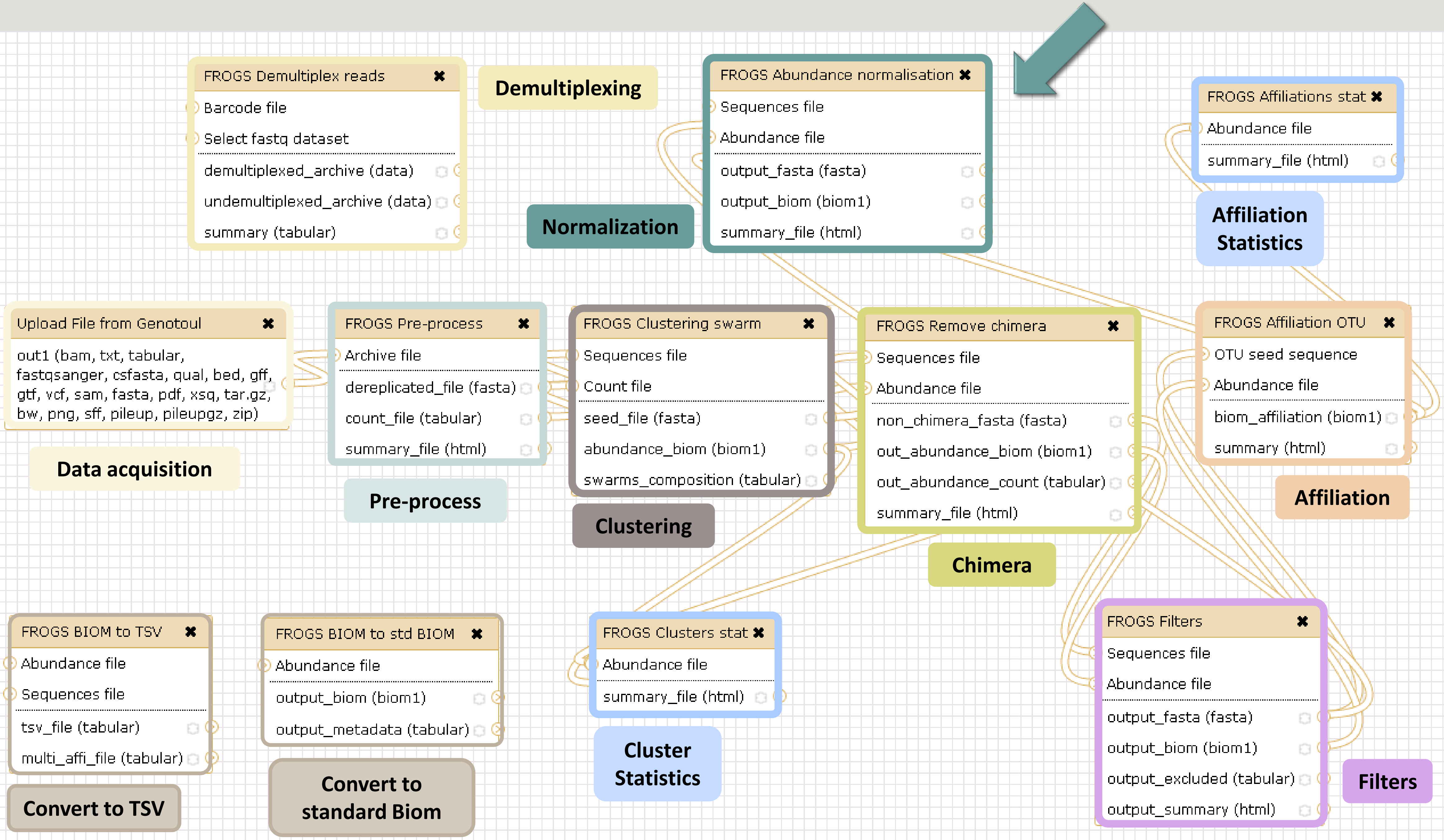
```
Cluster_8	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio;Bdellovibrio bacteriovorus	CP007656.1036900.1038415
```

Careful, no quotes 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



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 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

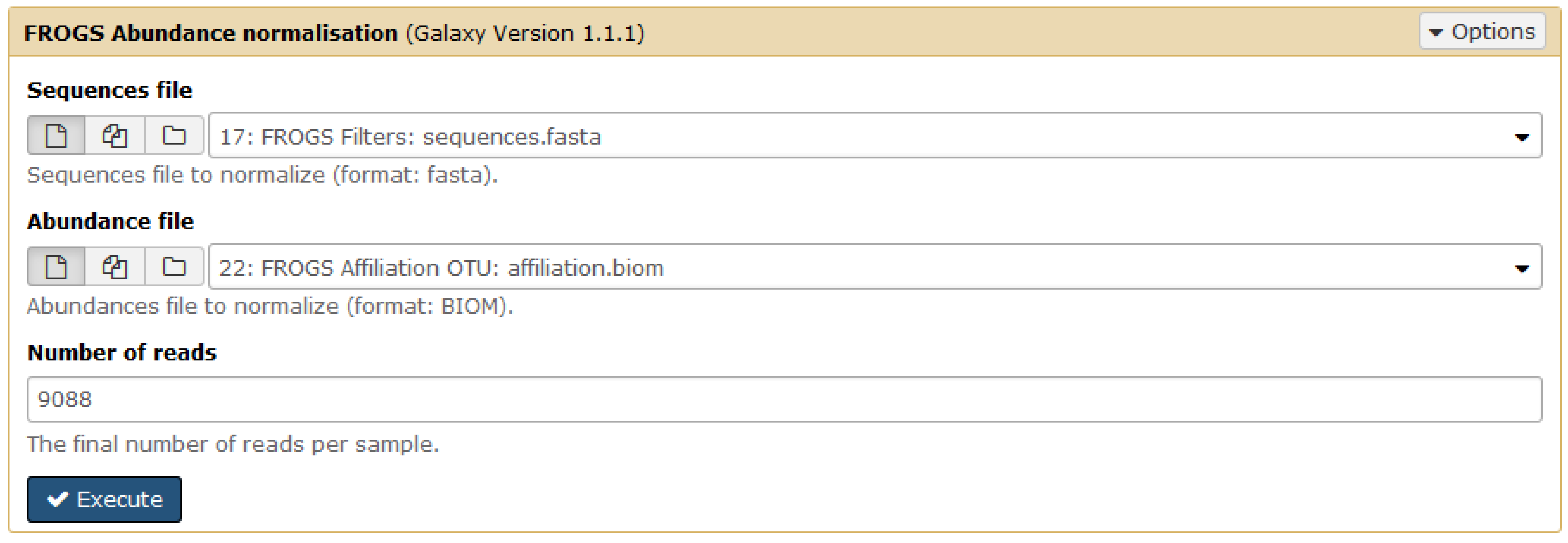
Your Turn! – 7

LAUNCH NORMALIZATION TOOL

Exercise 7

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

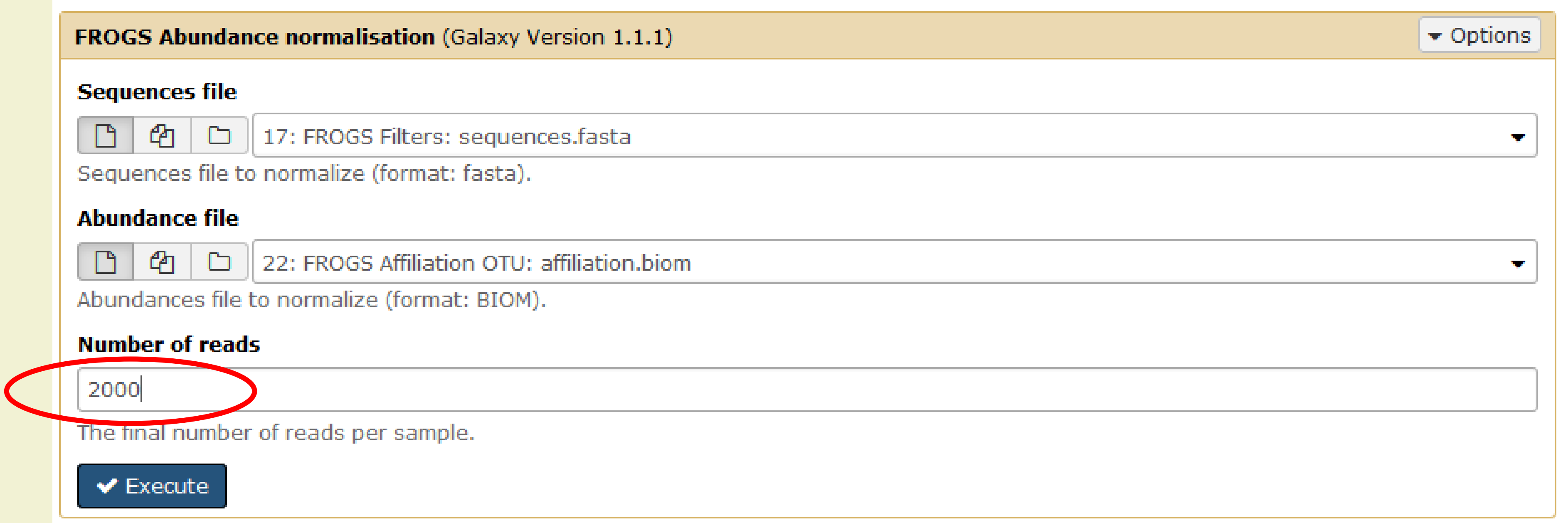
22: FROGS Affiliation OTU: affiliation.biom

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

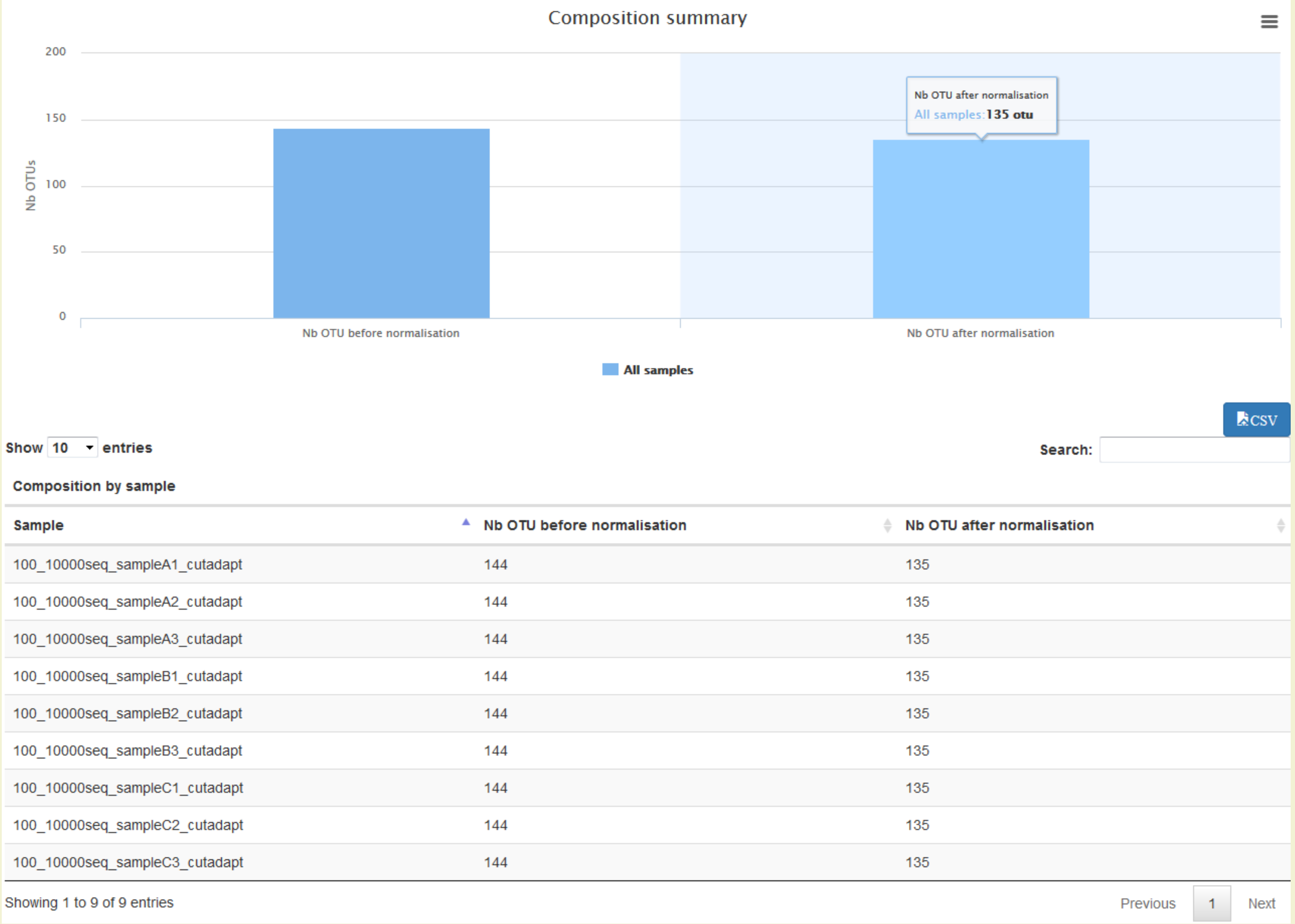
22: FROGS Affiliation OTU: affiliation.biom

Abundances file to normalize (format: BIOM).

Number of reads

2000

The final number of reads per sample.



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

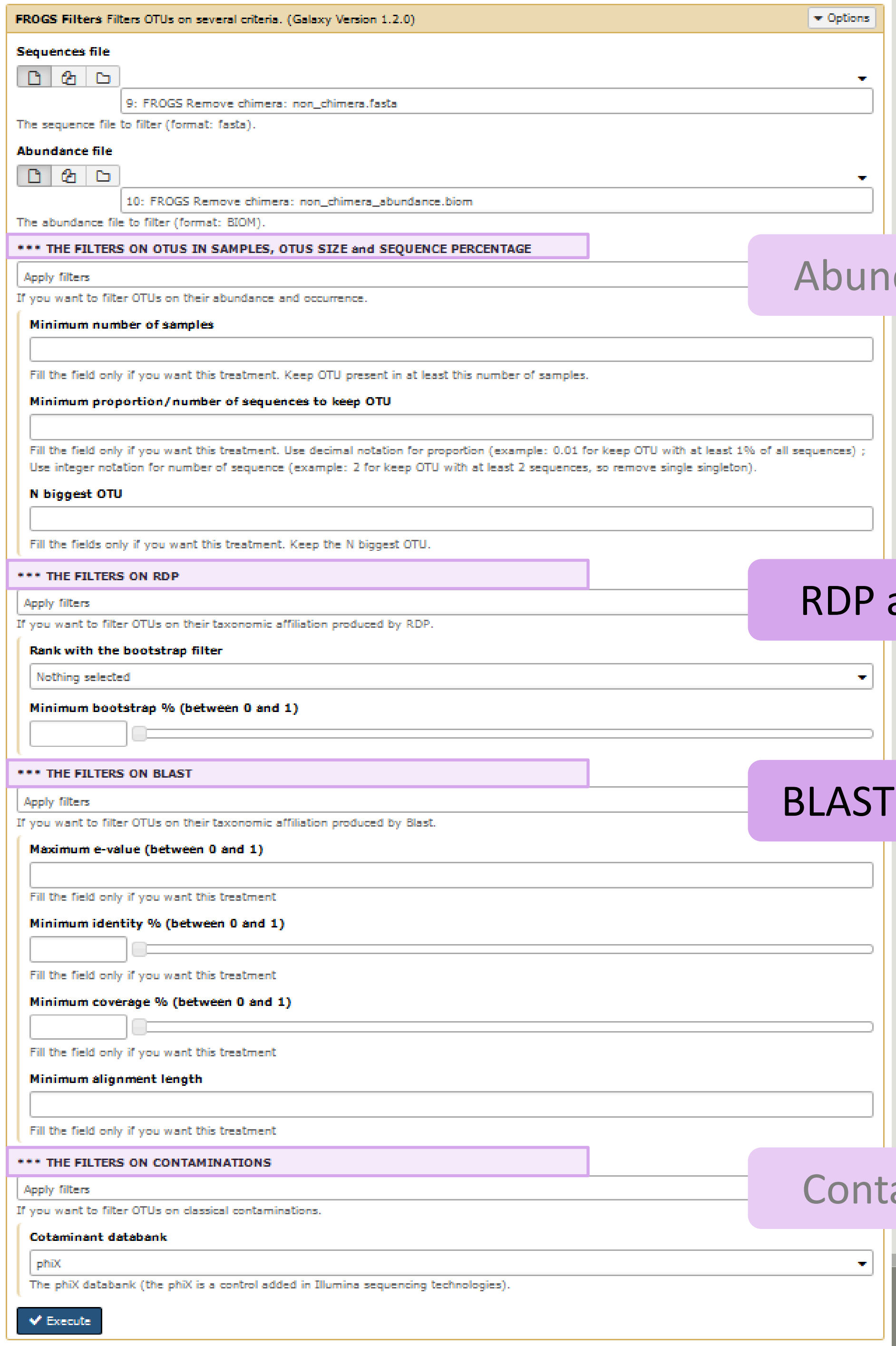
Sample	Nb OTU before normalisation	Nb OTU after normalisation
100_10000seq_sampleA1_cutadapt	144	135
100_10000seq_sampleA2_cutadapt	144	135
100_10000seq_sampleA3_cutadapt	144	135
100_10000seq_sampleB1_cutadapt	144	135
100_10000seq_sampleB2_cutadapt	144	135
100_10000seq_sampleB3_cutadapt	144	135
100_10000seq_sampleC1_cutadapt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

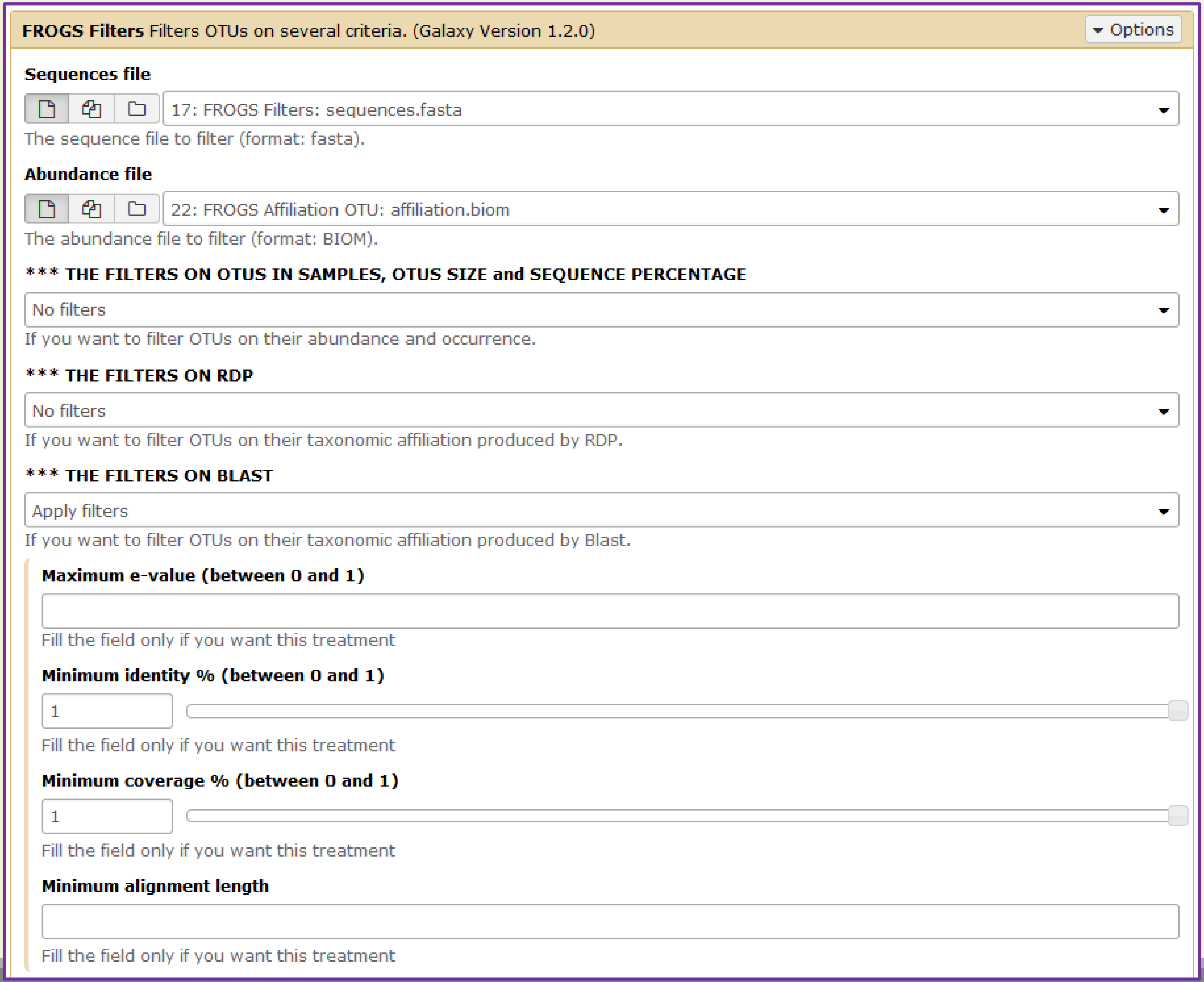
RDP affiliation filters

BLAST affiliation filters

Contamination filter

Exercise 8

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)

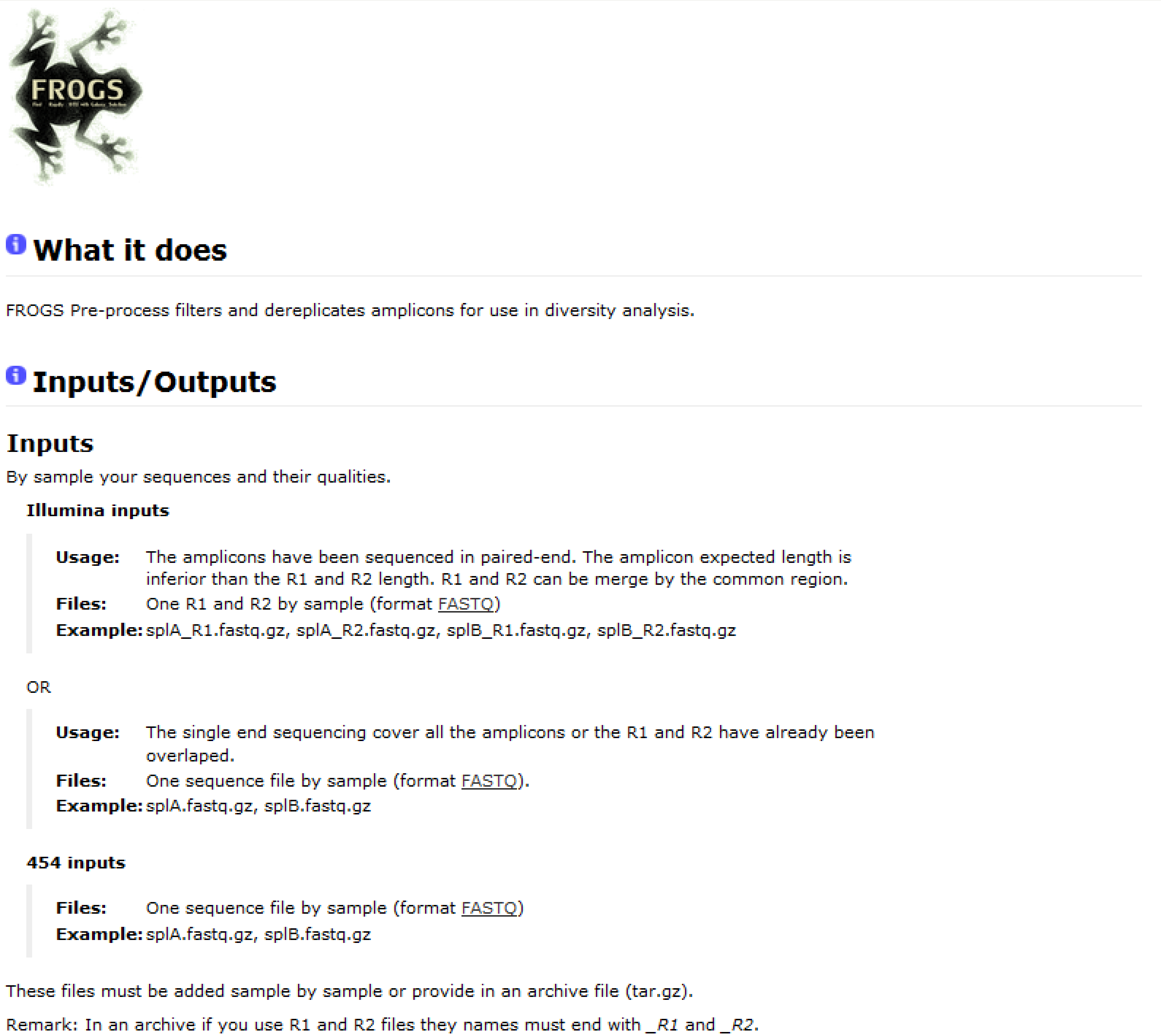
1

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

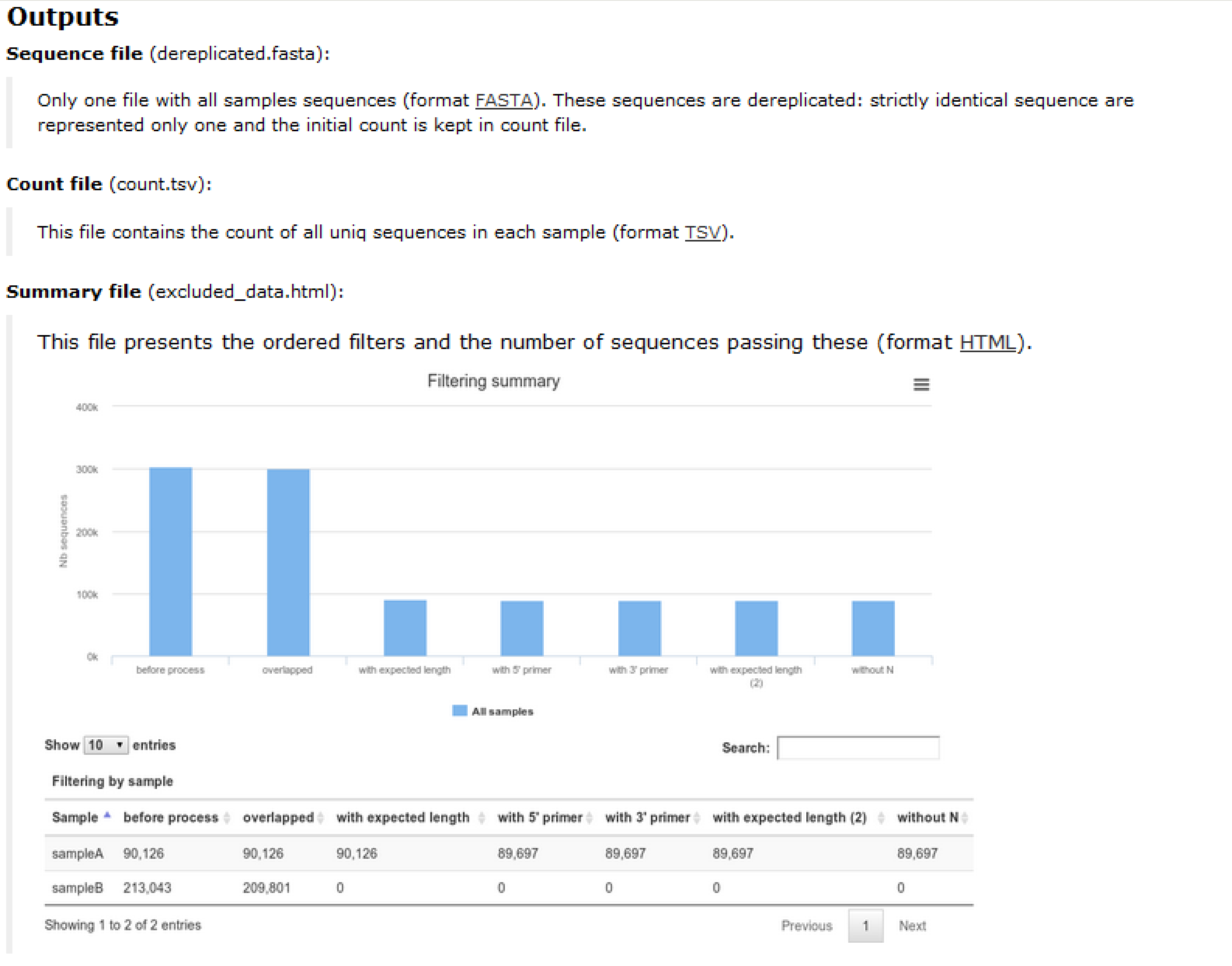
> **Usage:** The single end sequencing cover all the amplicons or the R1 and R2 have already been overlaped.
>
> **Files:** One sequence file by sample (format FASTQ).
>
> **Example:** splA.fastq.gz, splB.fastq.gz

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).

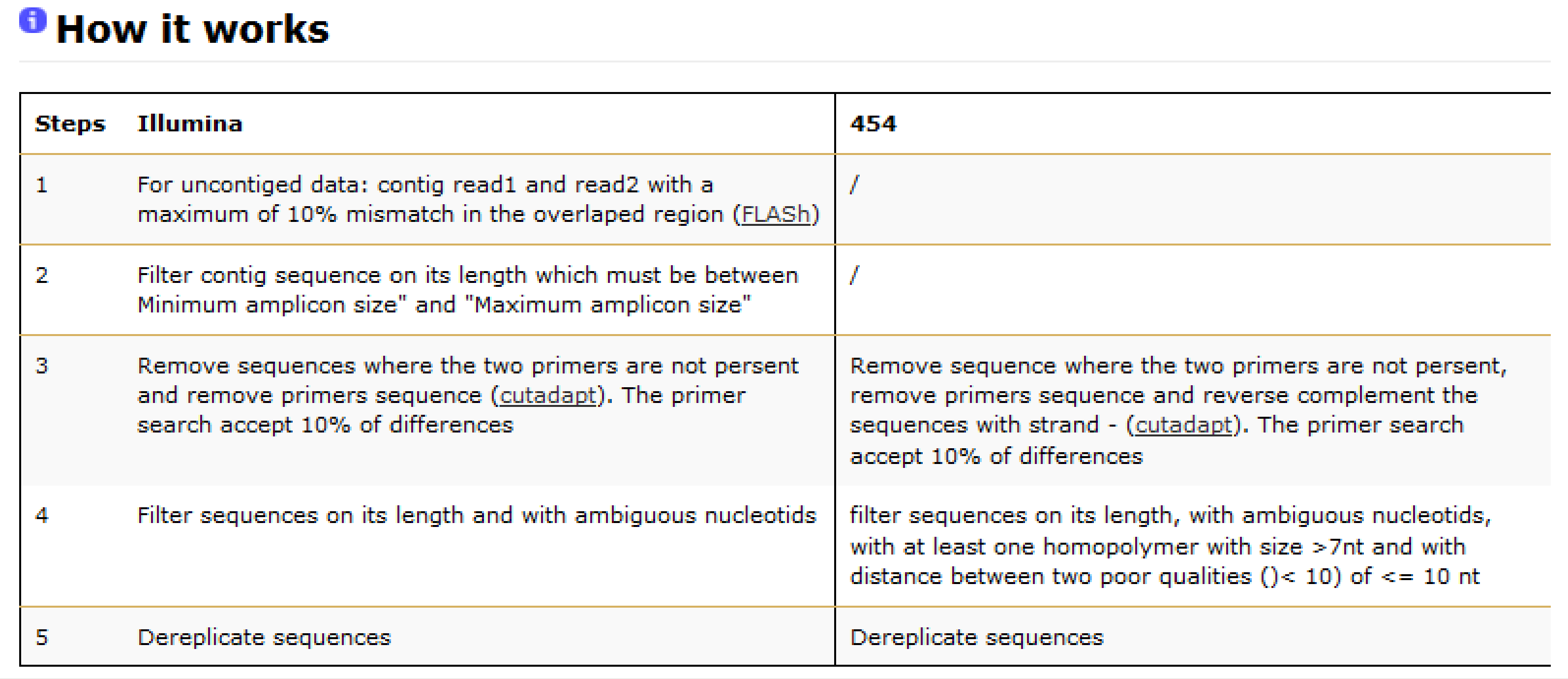
Show 10 ▾ entries

Search:

Filtering by sample

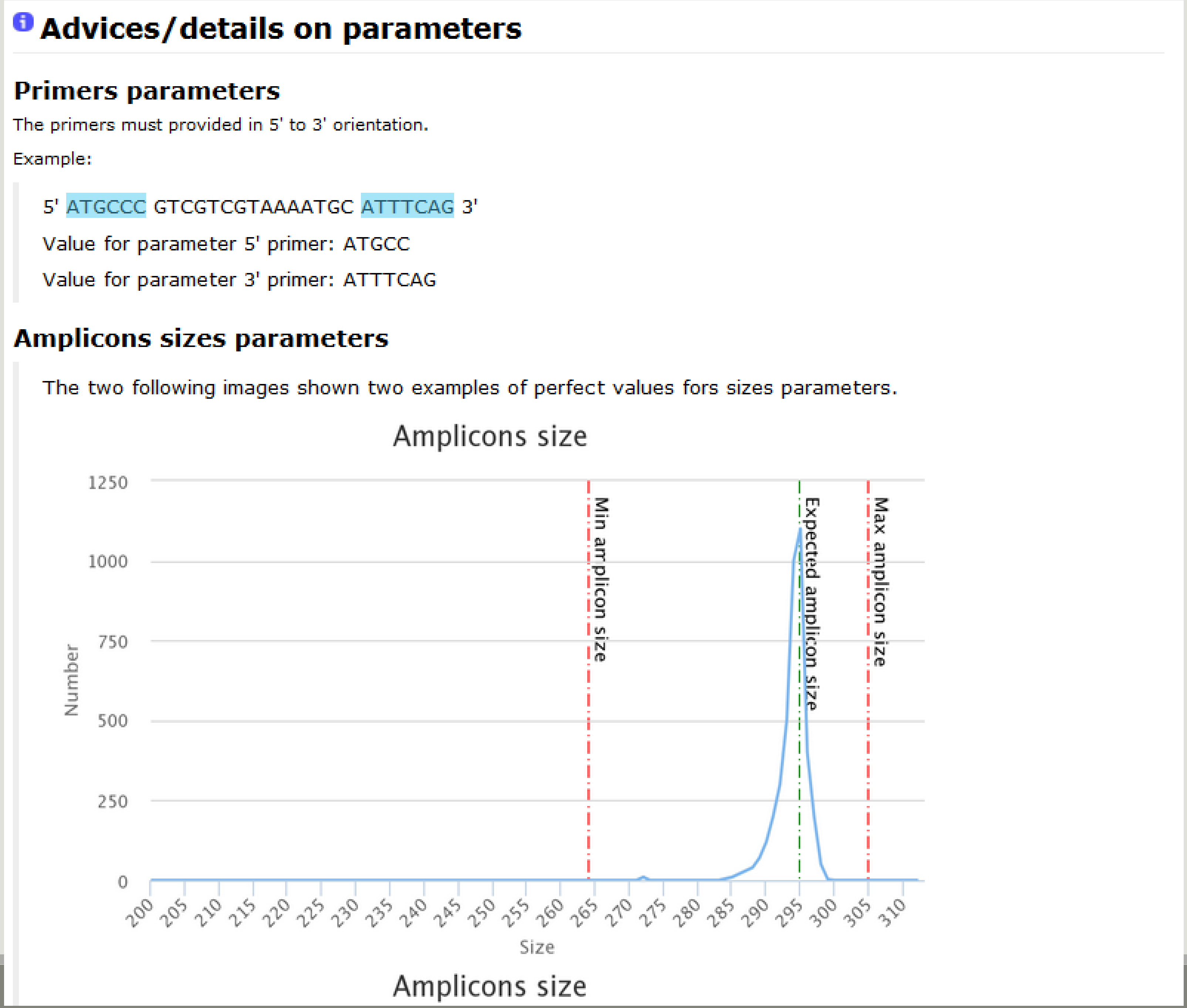
Sample ▲	before process	overlapped	with expected length	with 5' primer	with 3' primer	with expected length (2)	without N
sampleA	90,126	90,126	90,126	89,697	89,697	89,697	89,697
sampleB	213,043	209,801	0	0	0	0	0

Showing 1 to 2 of 2 entries



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.

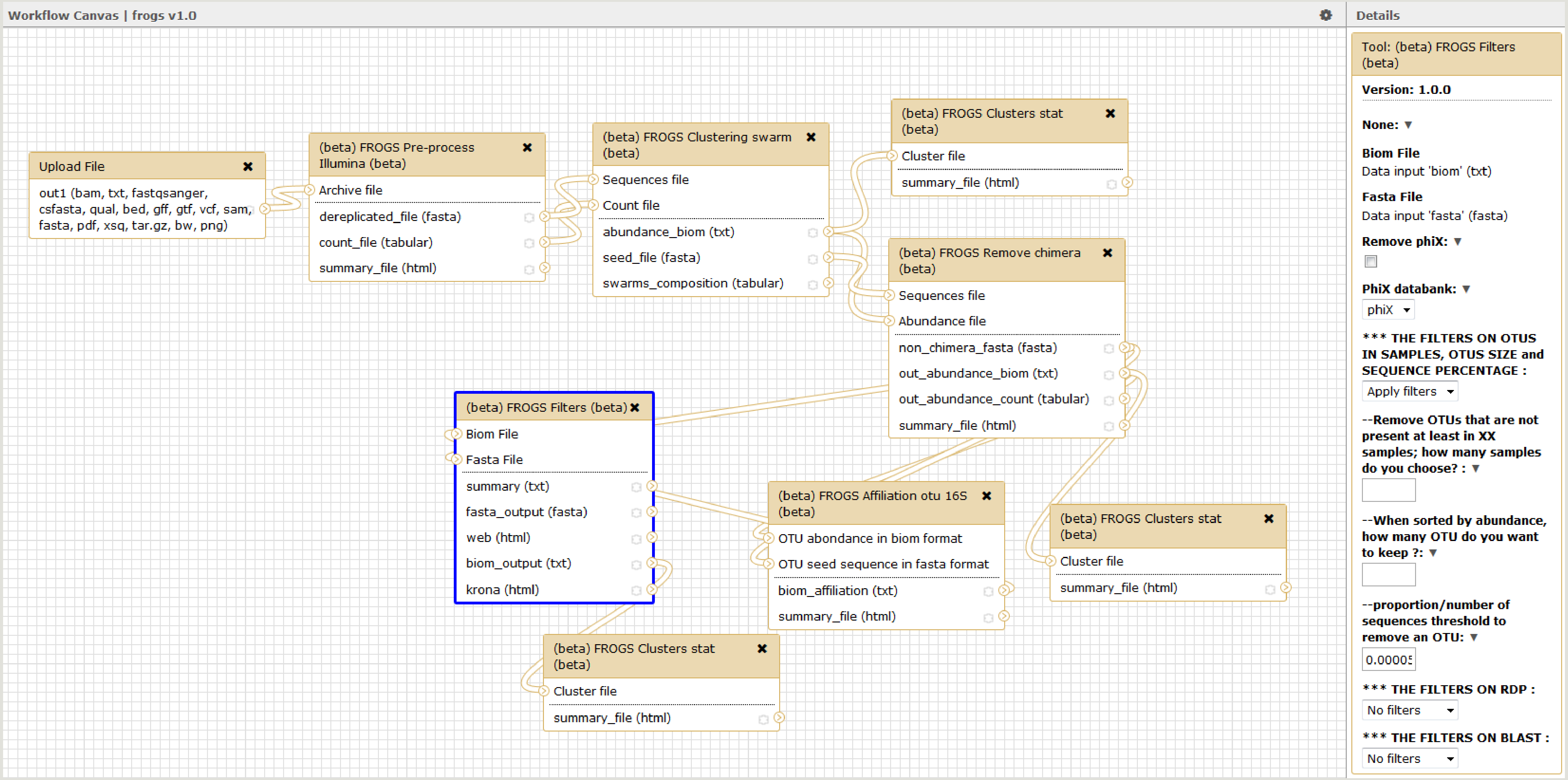
Example:

> 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.

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: ▼
0.00005

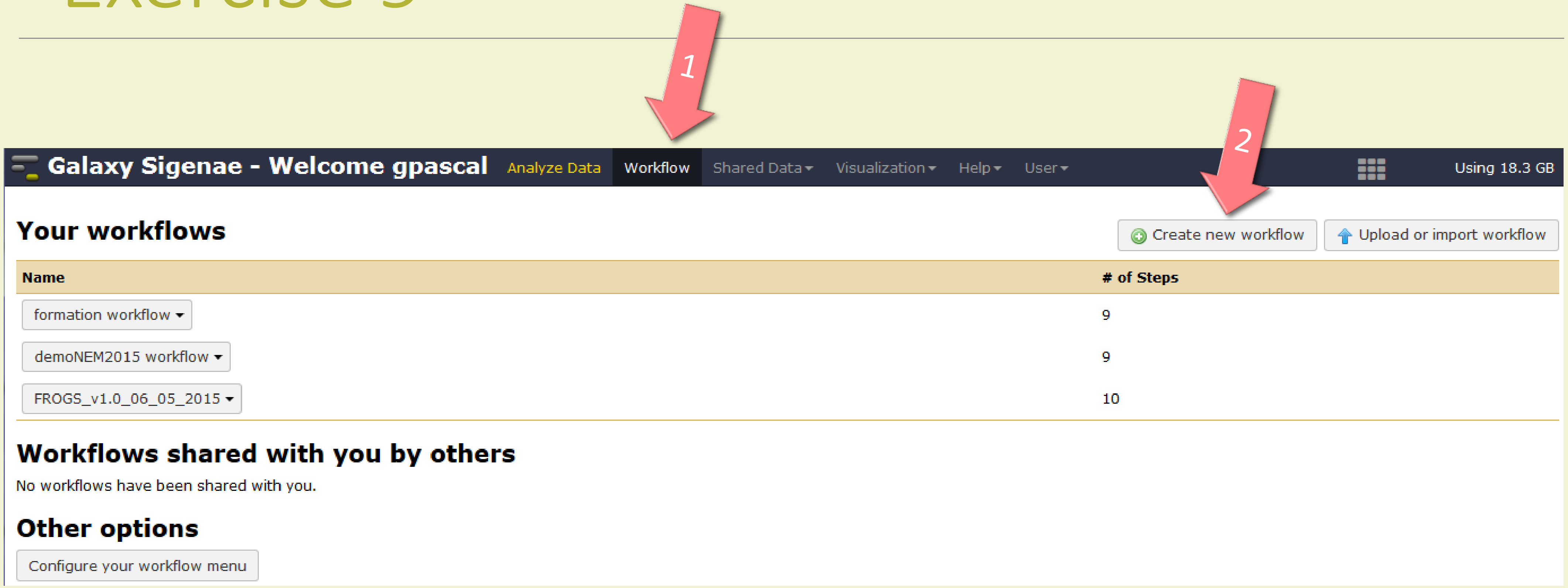
*** THE FILTERS ON RDP :
No filters

*** THE FILTERS ON BLAST :
No filters

Your Turn! – 9

CREATE YOUR OWN WORKFLOW !

Exercise 9



Your workflows

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

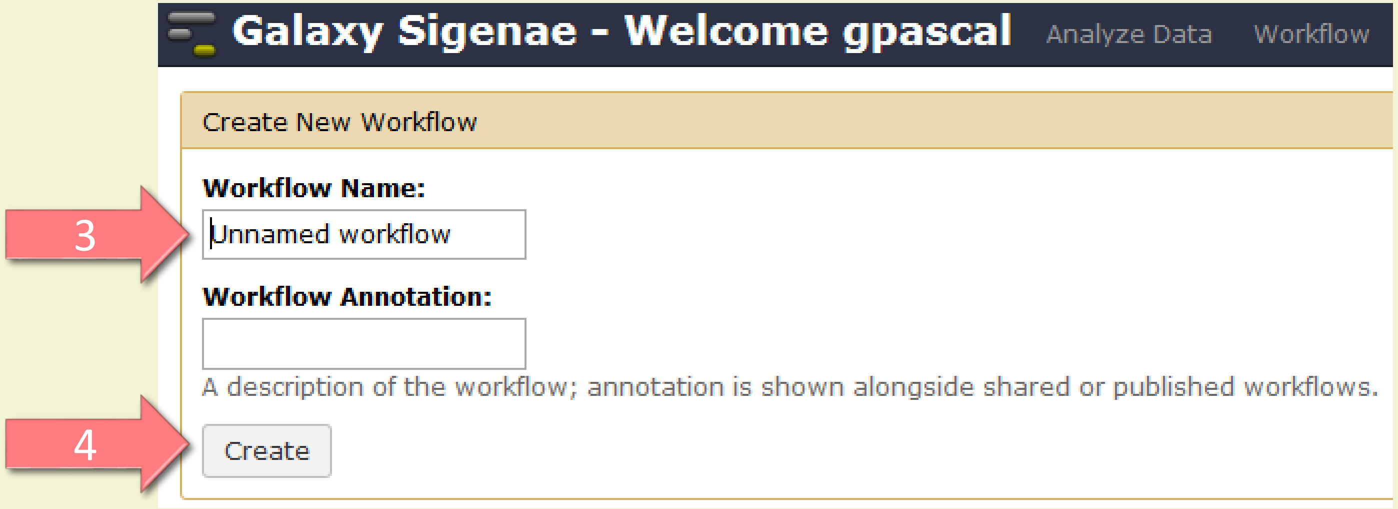
Workflows shared with you by others

No workflows have been shared with you.

Other options

Configure your workflow menu

Exercise 9



Galaxy Sigenae - Welcome gpascal

Create New Workflow

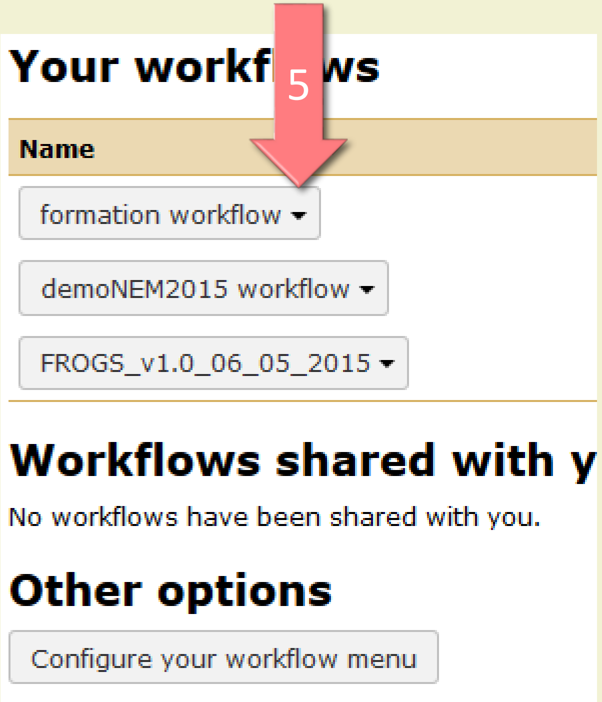
3 Workflow Name: Unnamed workflow

Workflow Annotation:

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

4

Exercise 9



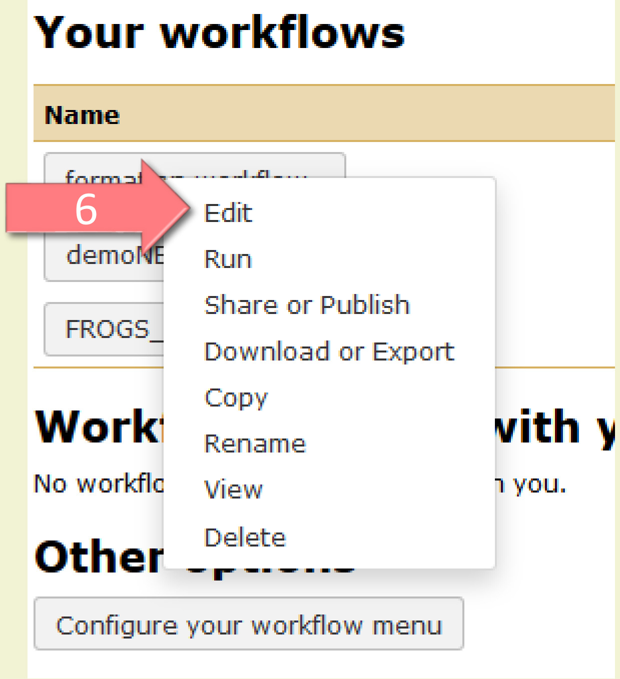
Your workflows

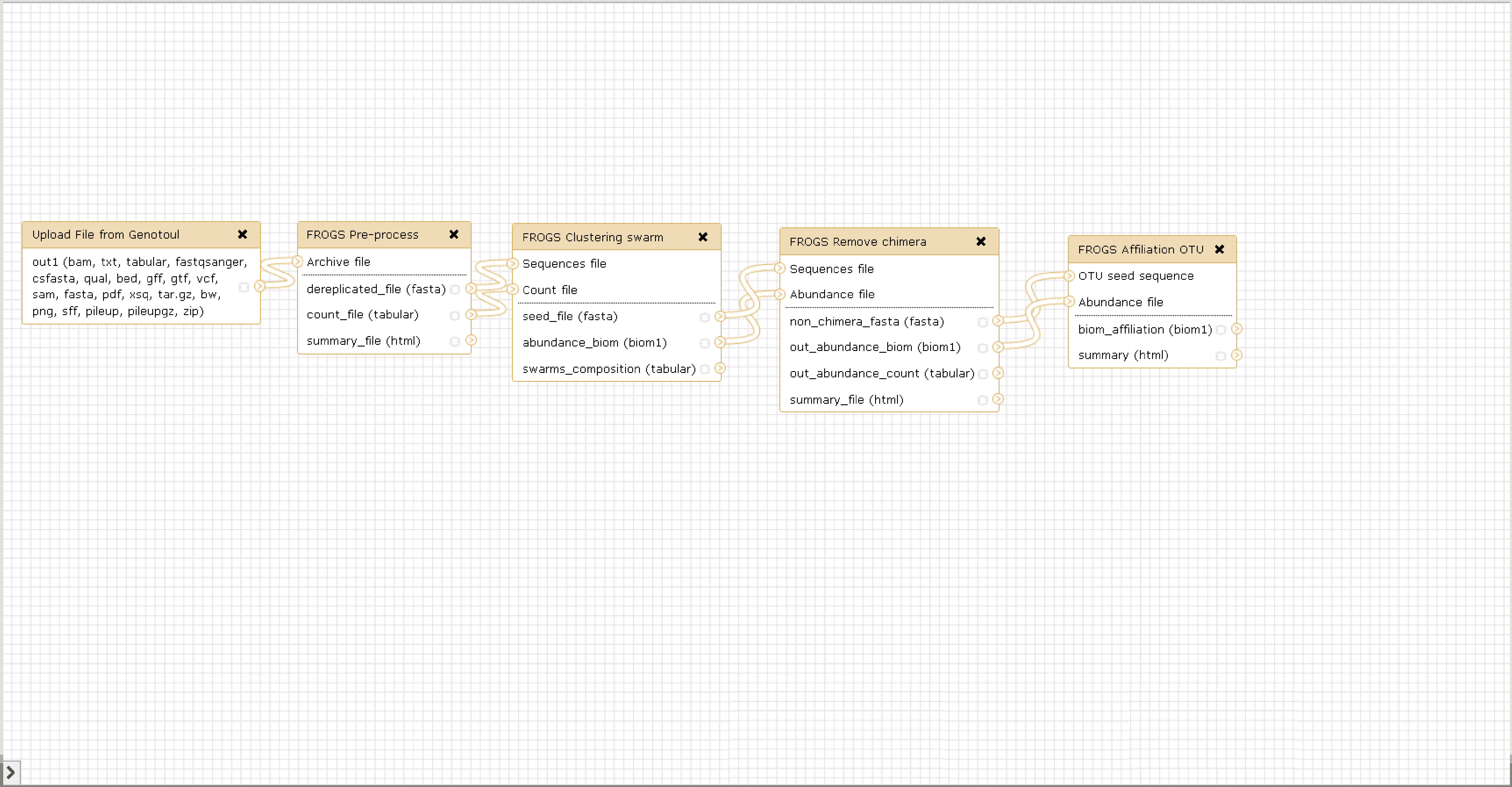
Name
formation workflow ▾
demoNEM2015 workflow ▾
FROGS_v1.0_06_05_2015 ▾

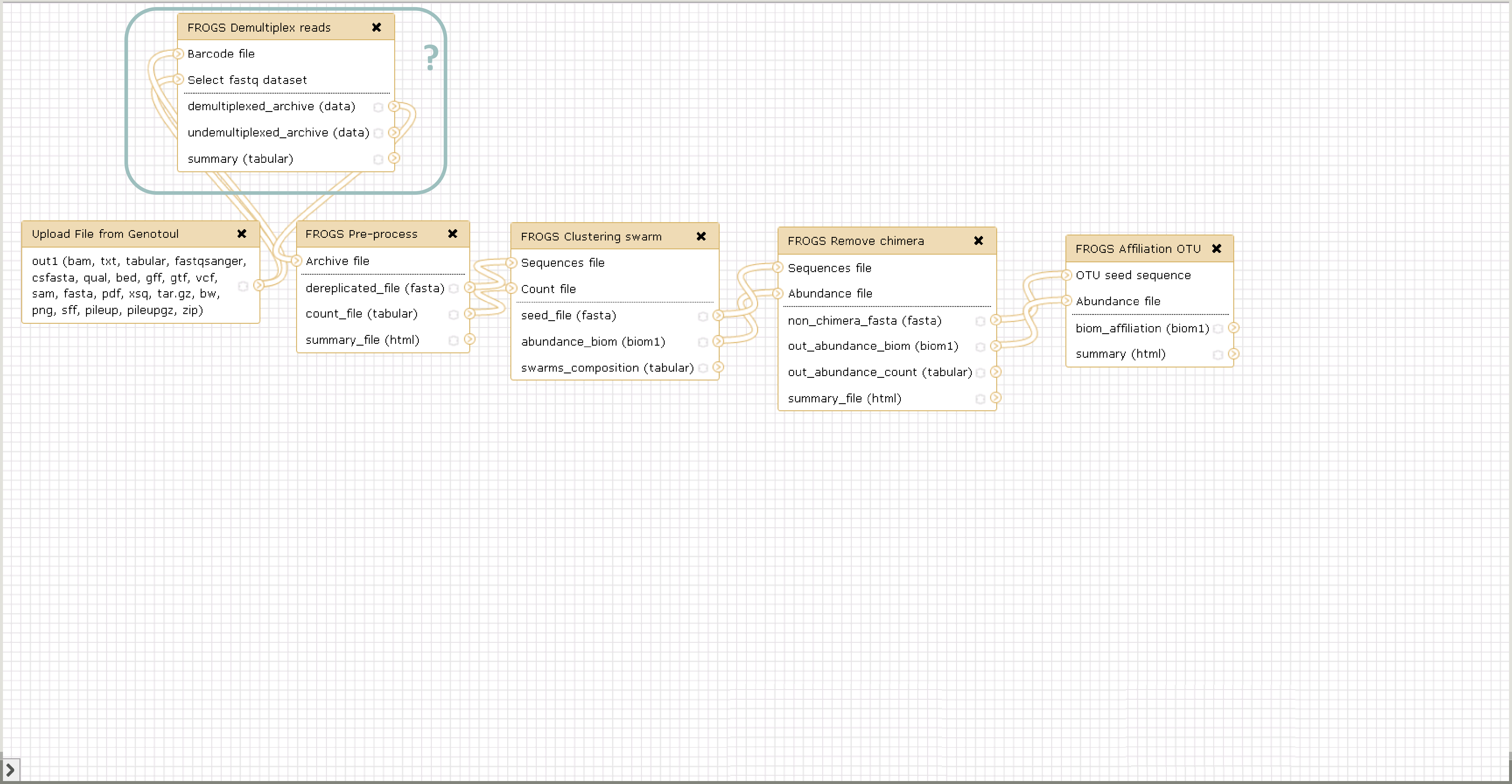
Workflows shared with y

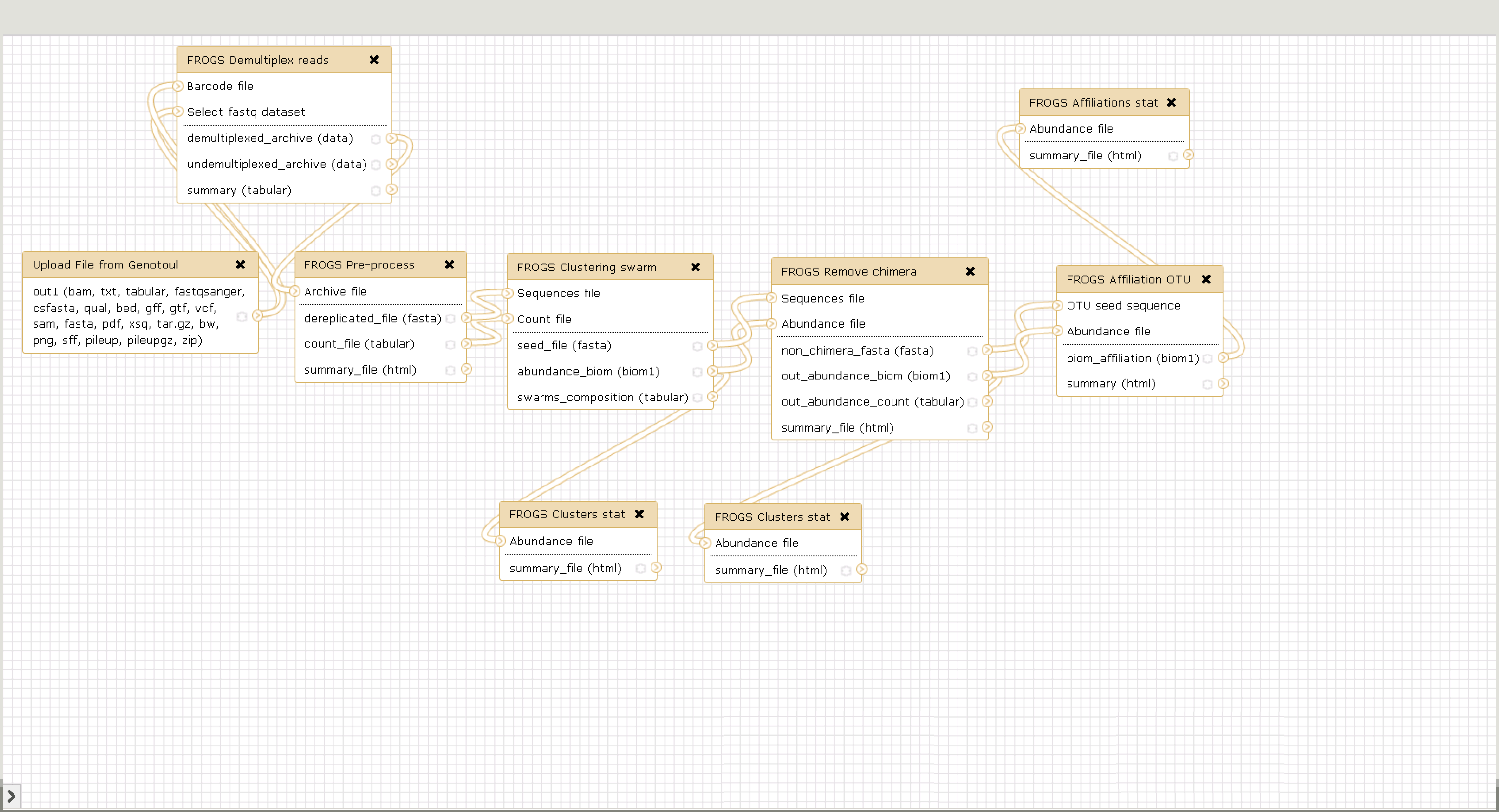
No workflows have been shared with you.

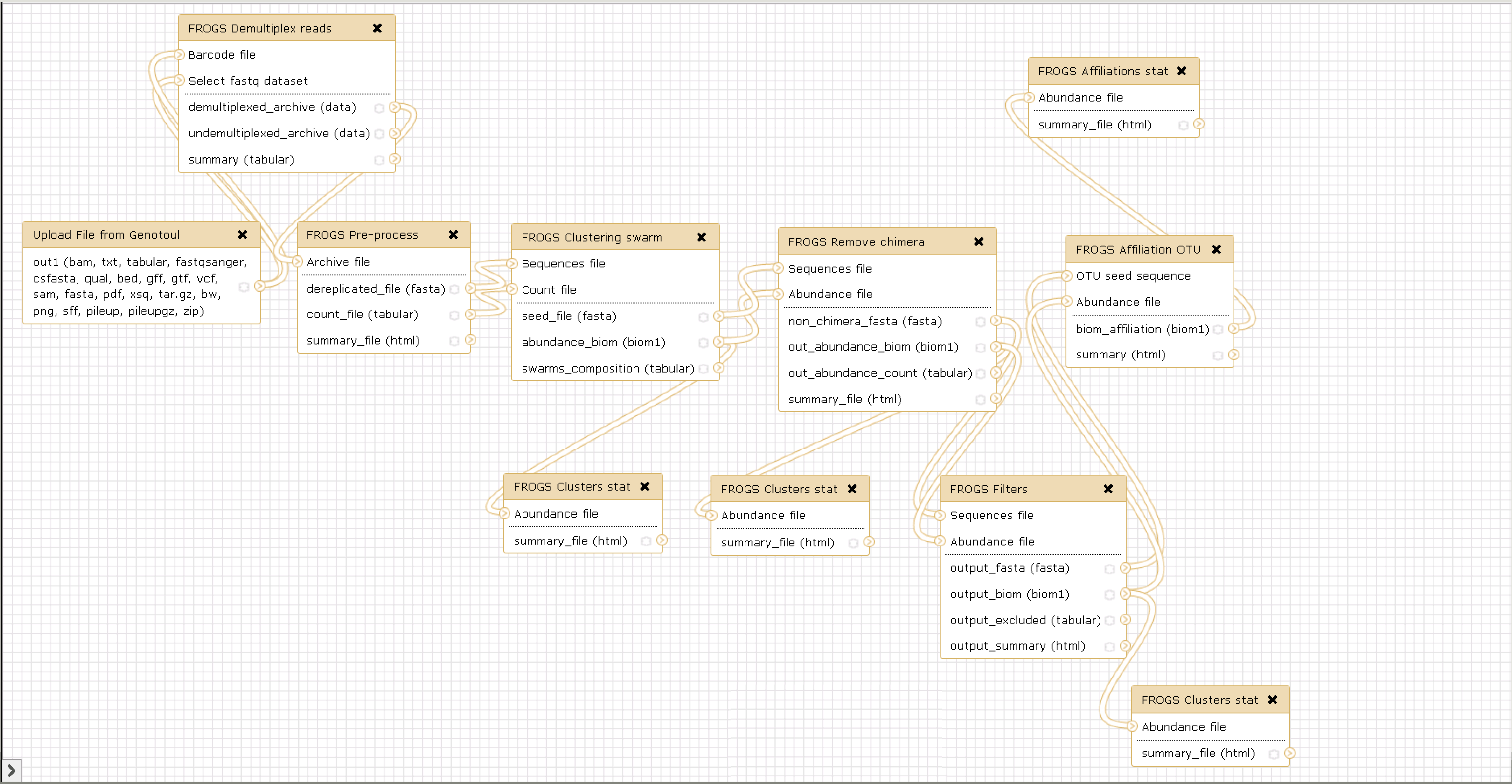
Other options

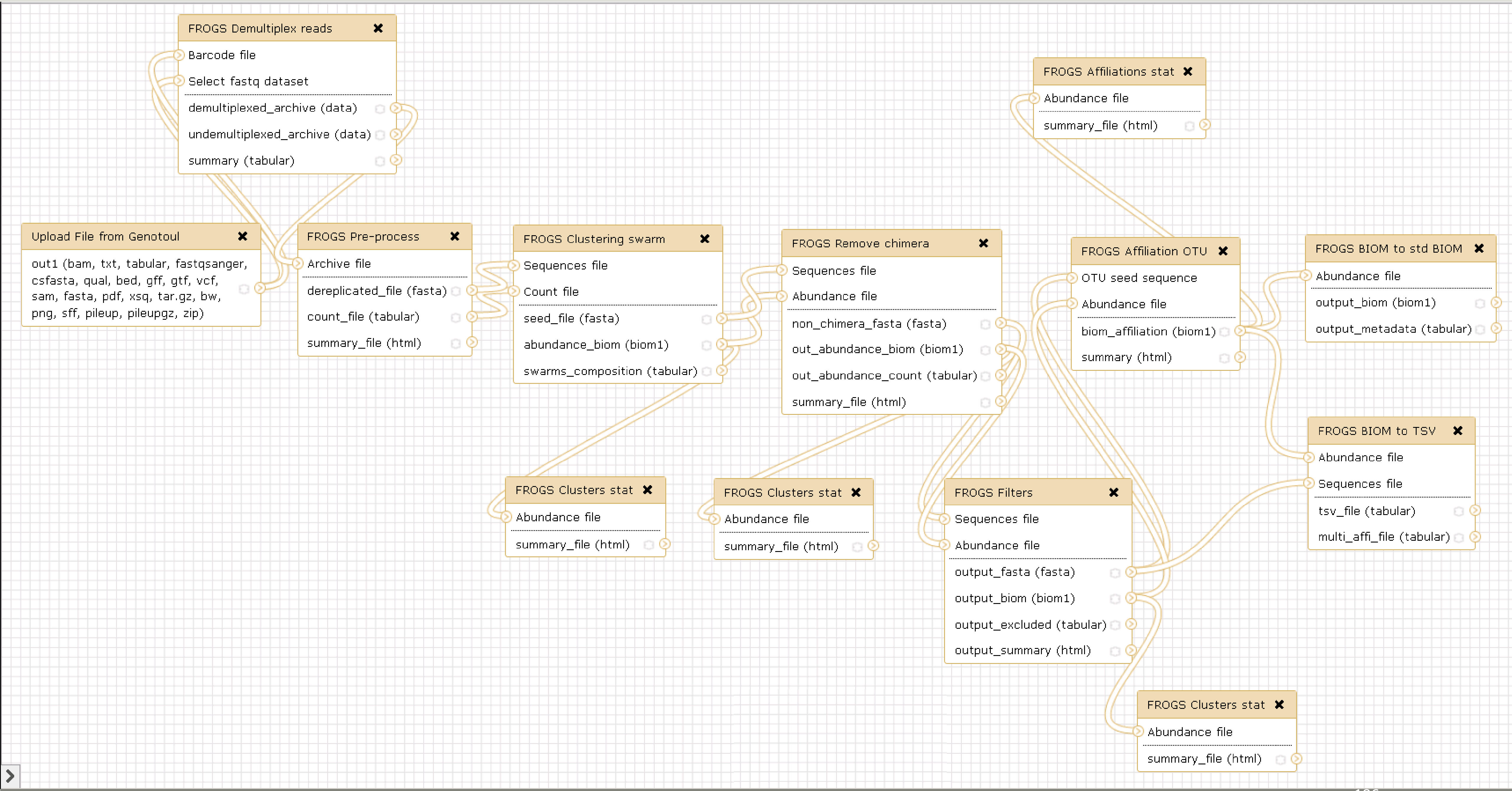


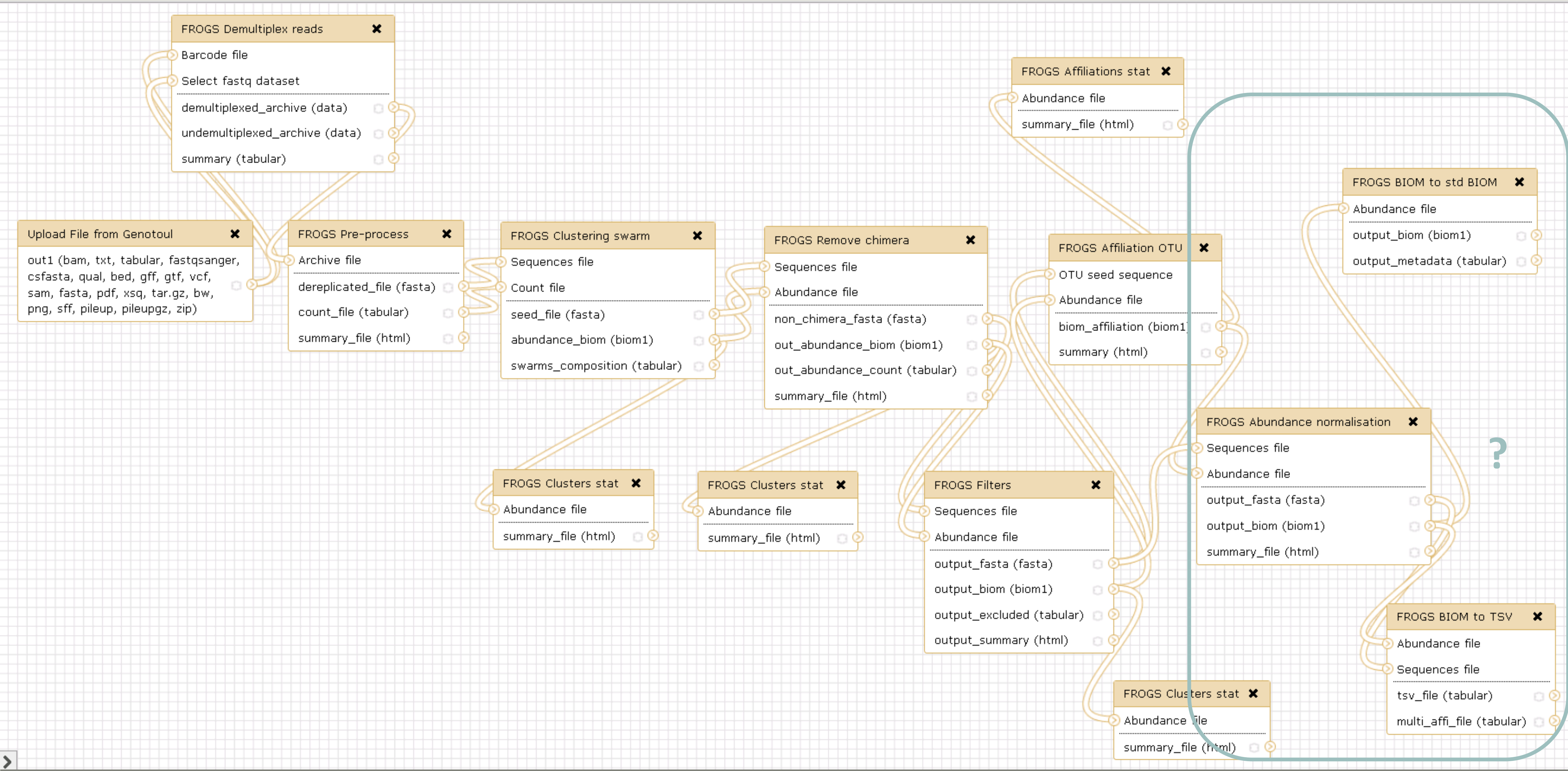

Upload File from Genotoul
out1 (bam, txt, tabular, fastqsanger, csfasta, qual, bed, gff, gtf, vcf, sam, fasta, pdf, xsq, tar.gz, bw, png, sff, pileup, pileupgz, zip)
FROGS Pre-process
Archive file
dereplicated_file (fasta)
count_file (tabular)
summary_file (html)
FROGS Clustering swarm
Sequences file
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)
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 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)
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 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 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)

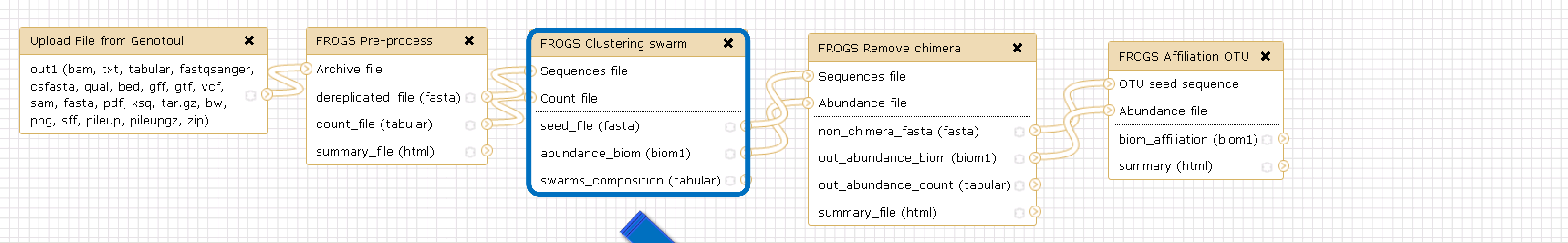
FROGS Clusters stat ✖
Abundance file
summary_file (html)

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)

?

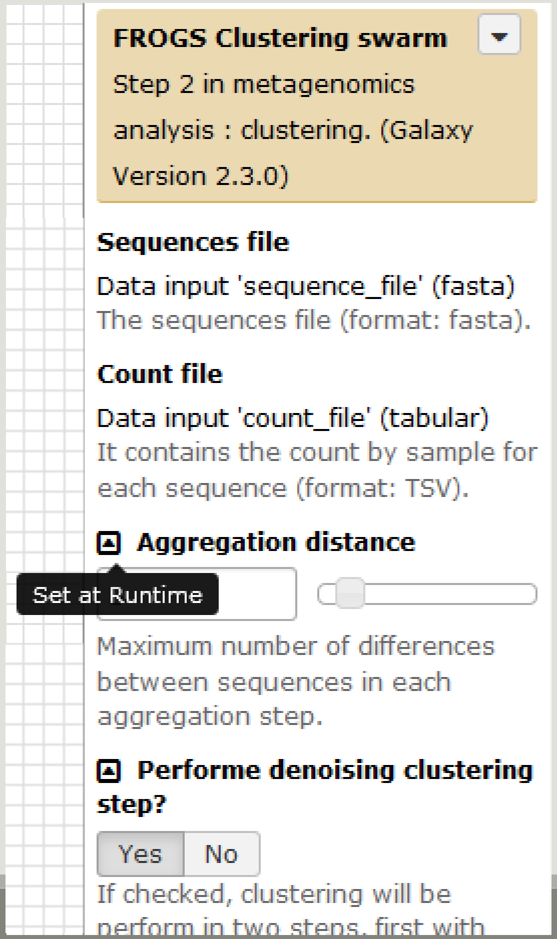
FROGS BIOM to TSV ✖
Abundance file
Sequences file
tsv_file (tabular)
multi_affi_file (tabular)



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

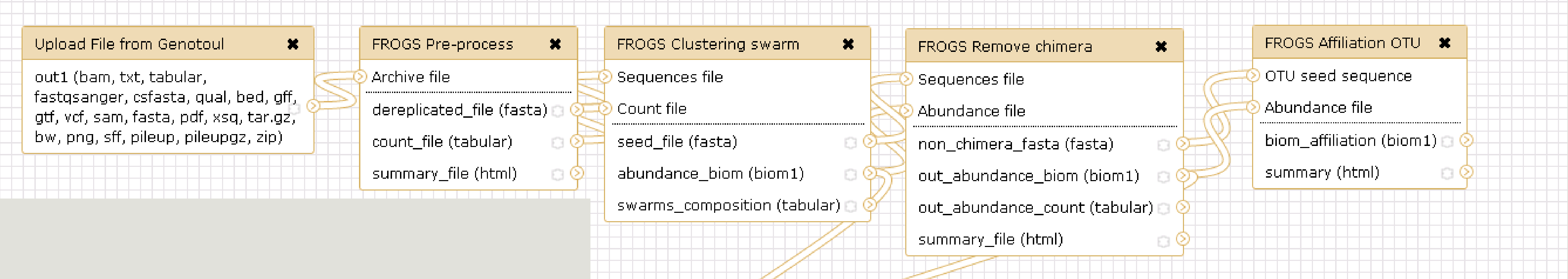
Set at Runtime

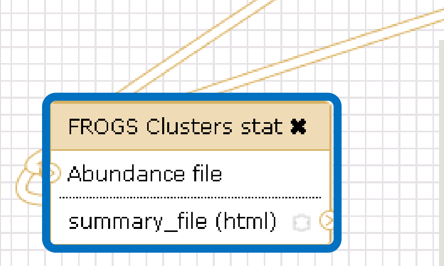
Maximum number of differences between sequences in each aggregation step.

Performe denoising clustering step?

Yes No

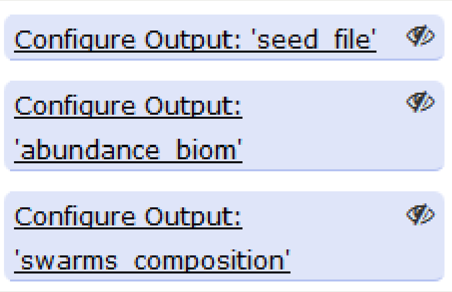
If checked, clustering will be perform in two steps, first with





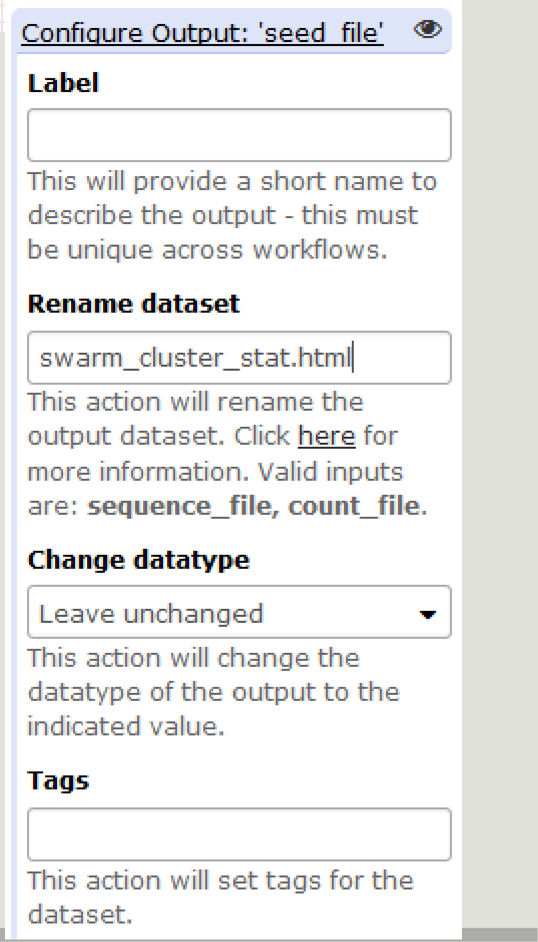
For each tool, think to:

- 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**.

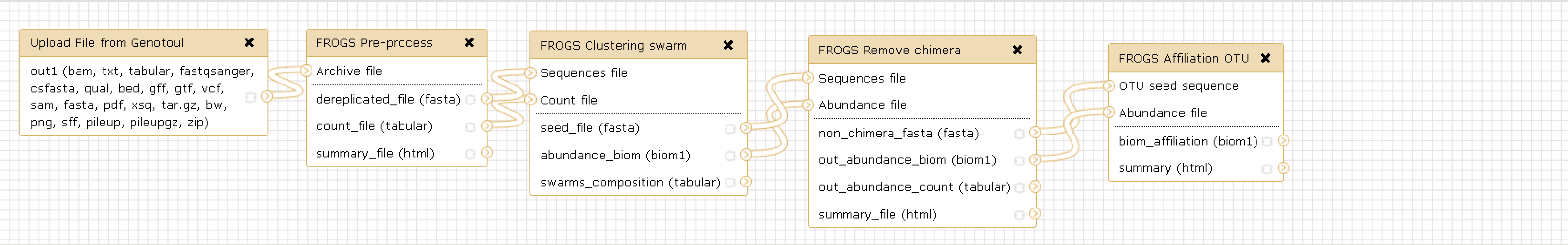
Change datatype

Leave unchanged

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

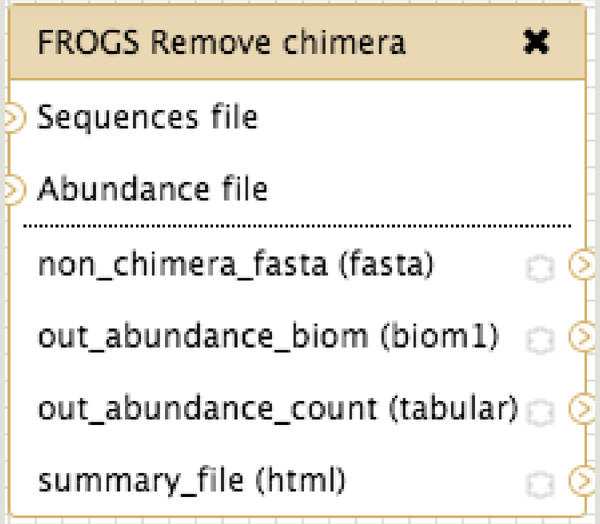
Tags

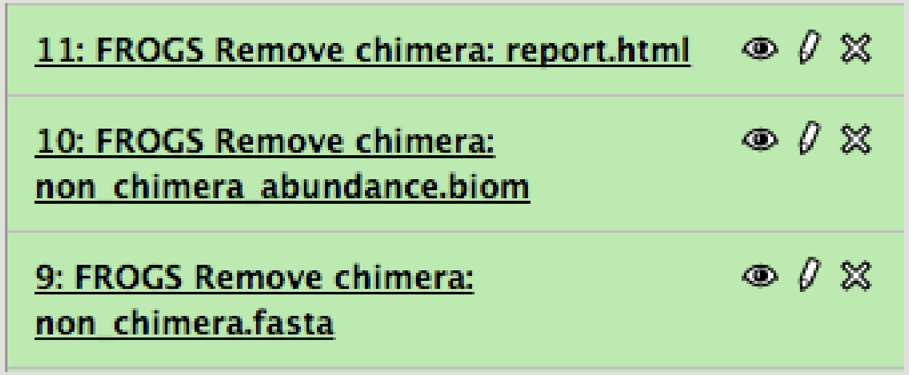
This action will set tags for the dataset.



For each tool, think to:

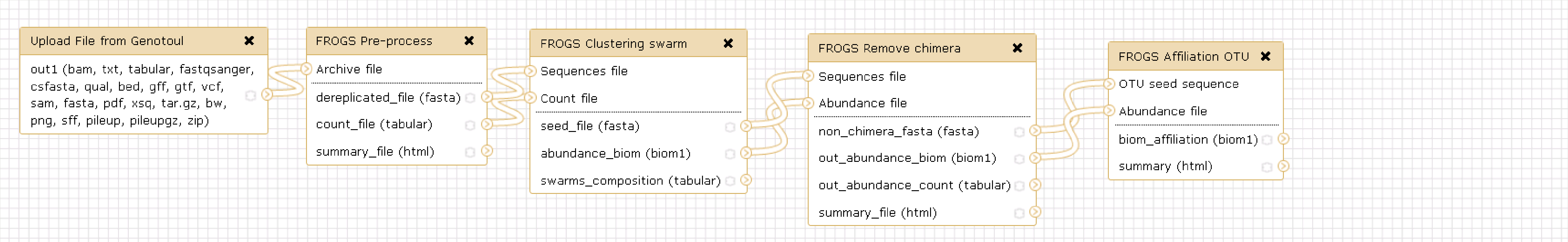
- Fixe parameter ?
- Automatically rename output files
- Hide intermediate files ?


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

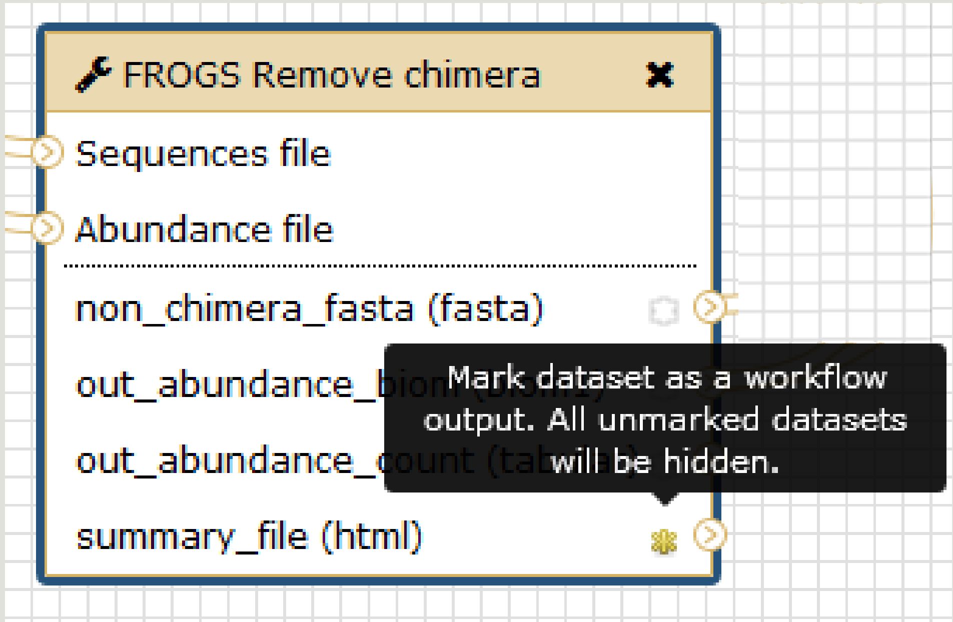
10: FROGS Remove chimera: non_chimera_abundance.biom

9: FROGS Remove chimera: non_chimera.fasta

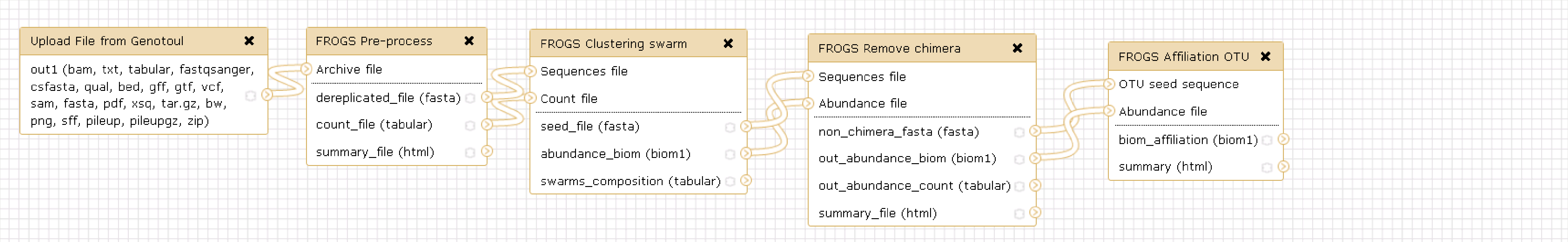


For each tool, think to:

- 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)

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

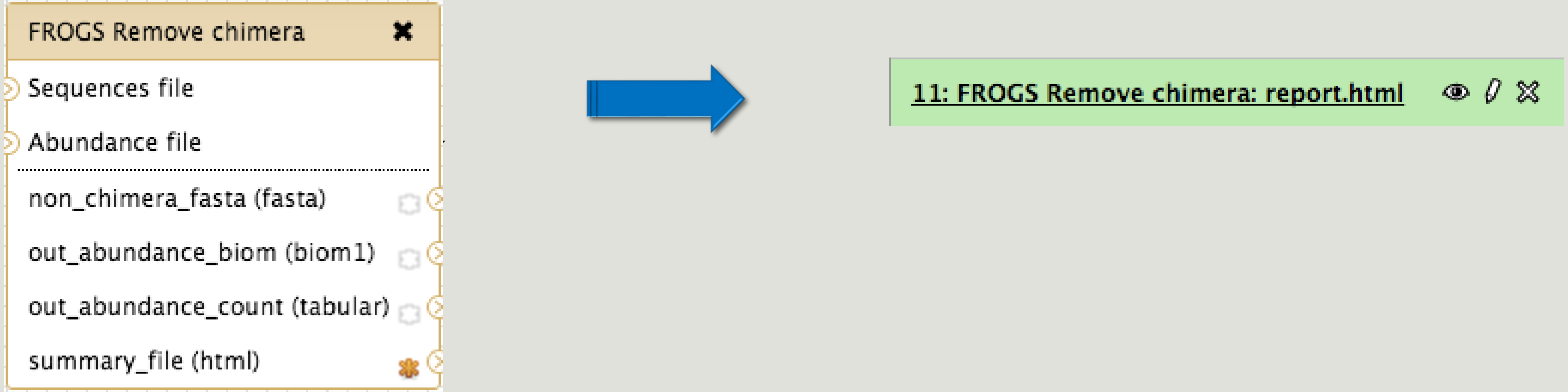
Abundance file

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)

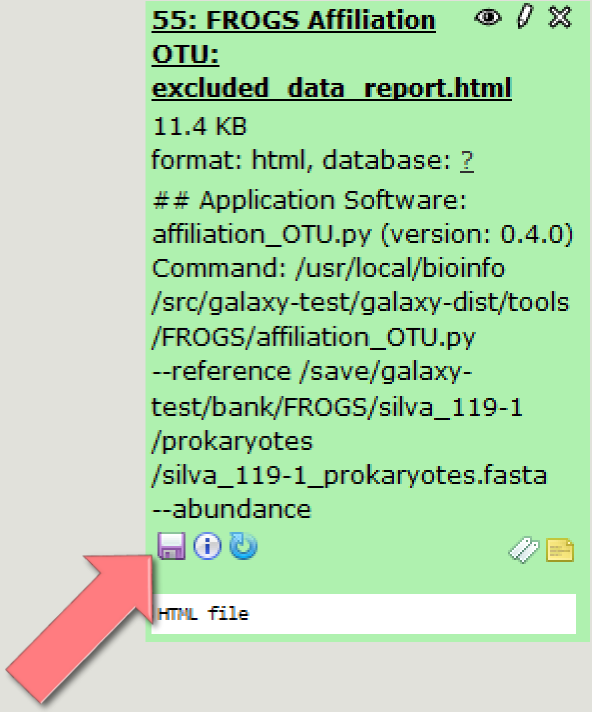
out_abundance_count (tabular)

summary_file (html)

11: FROGS Remove chimera: report.html

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: ?

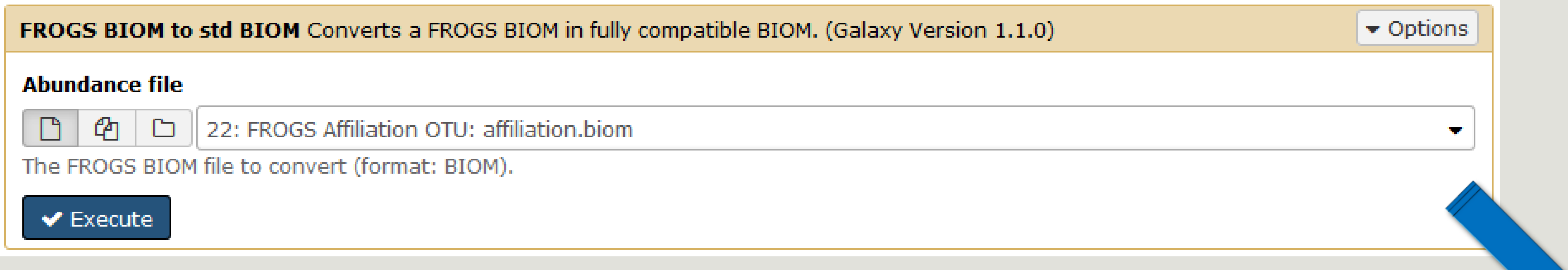
```
## Application Software:
affiliation_OTU.py (version: 0.4.0)
Command: /usr/local/bioinfo
/src/galaxy-test/galaxy-dist/tools
/FROGS/affiliation_OTU.py
--reference /save/galaxy-
test/bank/FROGS/silva_119-1
/prokaryotes
/silva_119-1_prokaryotes.fasta
--abundance
```

```
HTML file
```

FROGS BIOM to Standard BIOM

FROGS biom to standard Biom

This step is required to run R

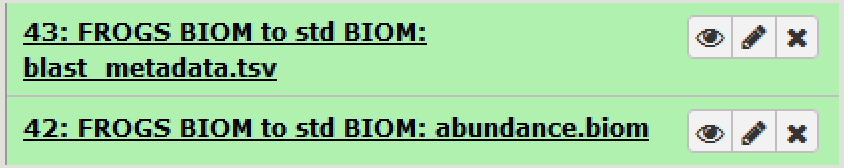


FROGS BIOM to std BIOM Converts a FROGS BIOM in fully compatible BIOM. (Galaxy Version 1.1.0)

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

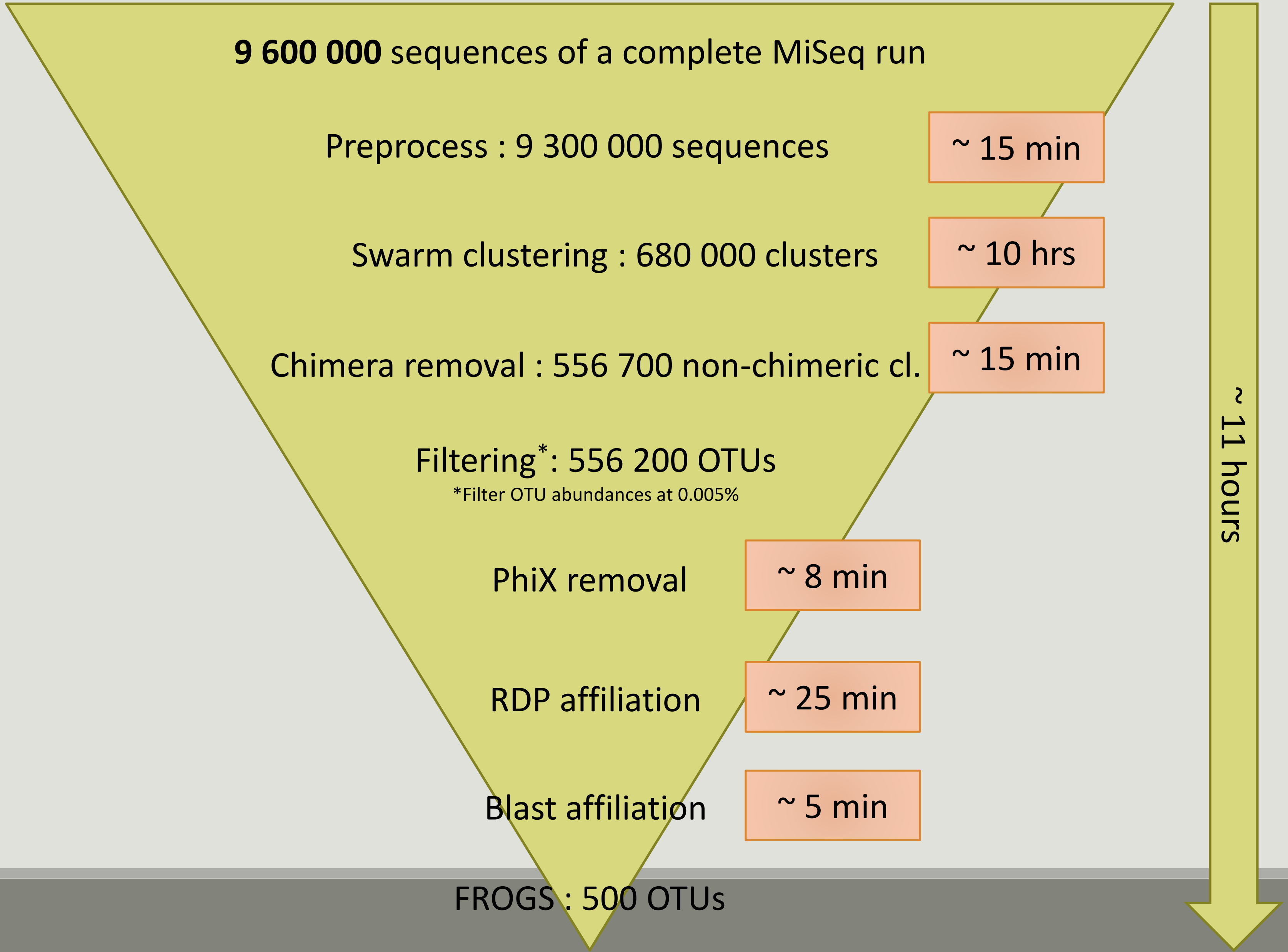
42: FROGS BIOM to std BIOM: abundance.biom

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

↓

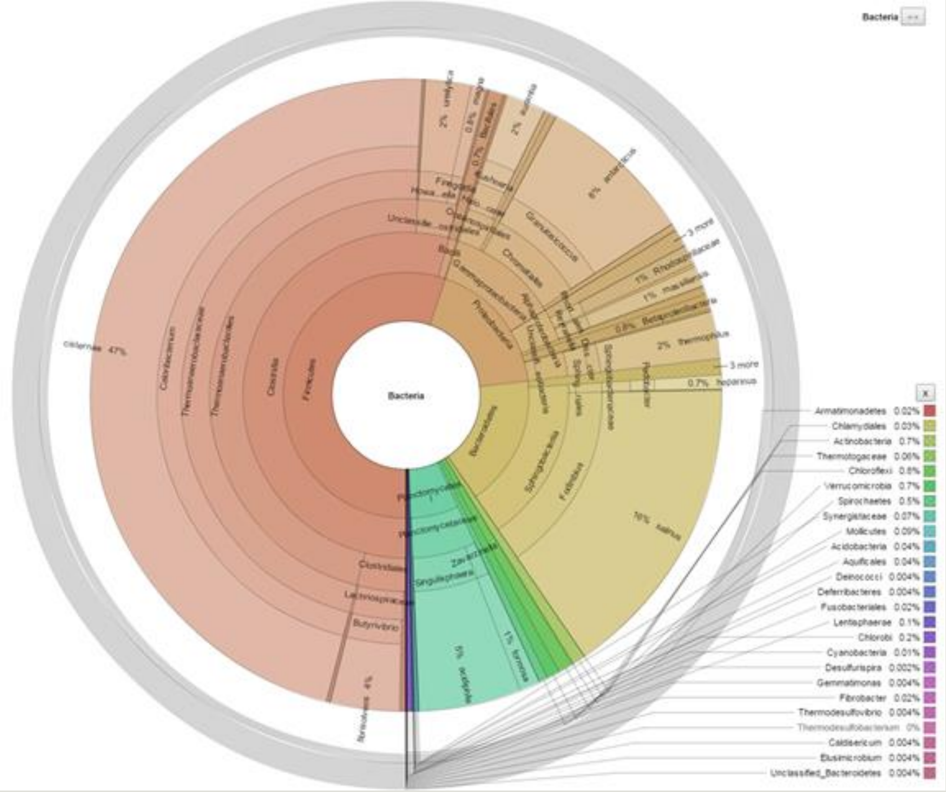
SWARM : 109 000 clusters

↓

VSEARCH: 21 000 clusters

↓

filters : 0.005% **505 OTUs**



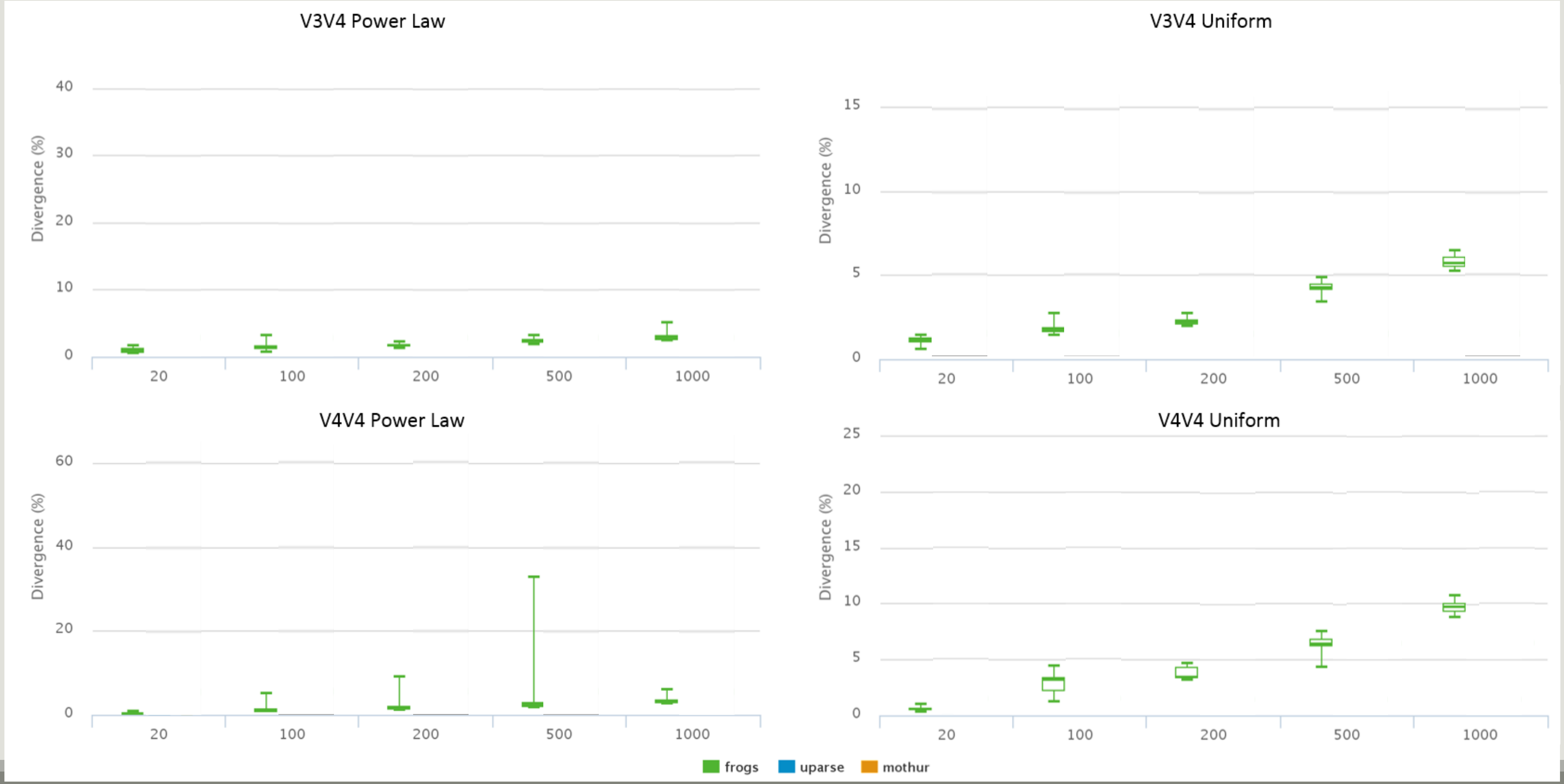
FROGS’ Accuracy

- 10 artificial samples of 100 000 sequences
- 25 sets of species
- 20, 100, 200, 500 or 1000 different species
- power law or a uniform distribution
- 5 to 20% of chimera
- 1.10^{+11} sequences were treated with FROGS, UPARSE and MOTHUR, with their guidelines, to compare their performances

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

FROGS' Accuracy

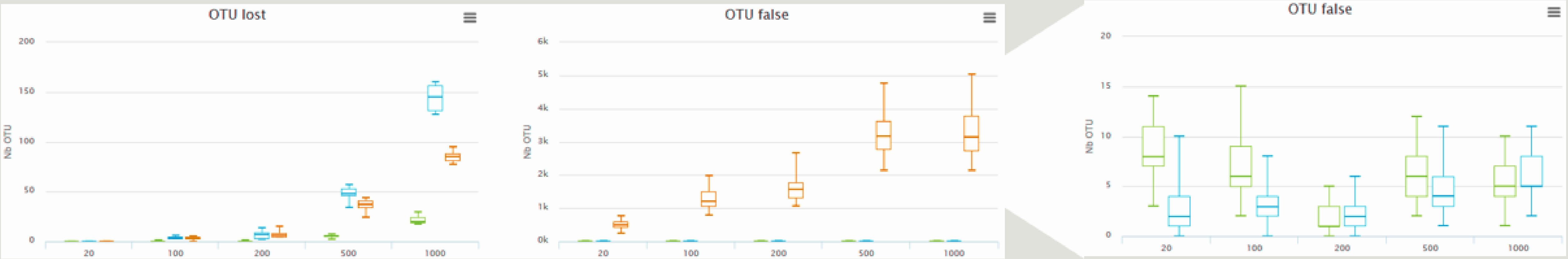
→ divergence at "genus" rank



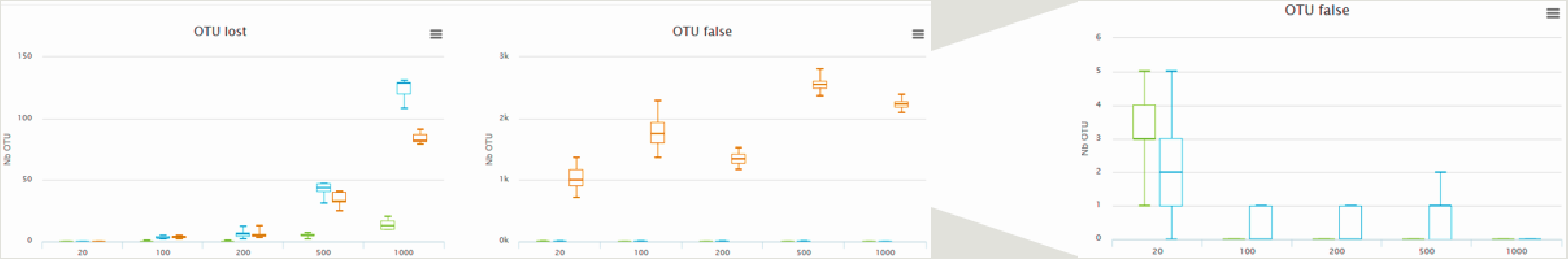
FROGS' Accuracy

→ Lost & False OTU

V3V4 Power Law



V3V4 Uniform

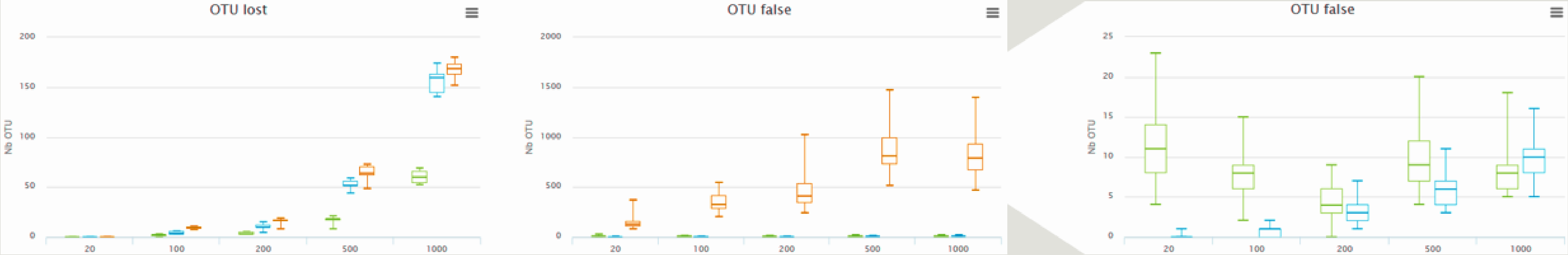


🟩 frogs 🟦 uparse 🟧 mothur

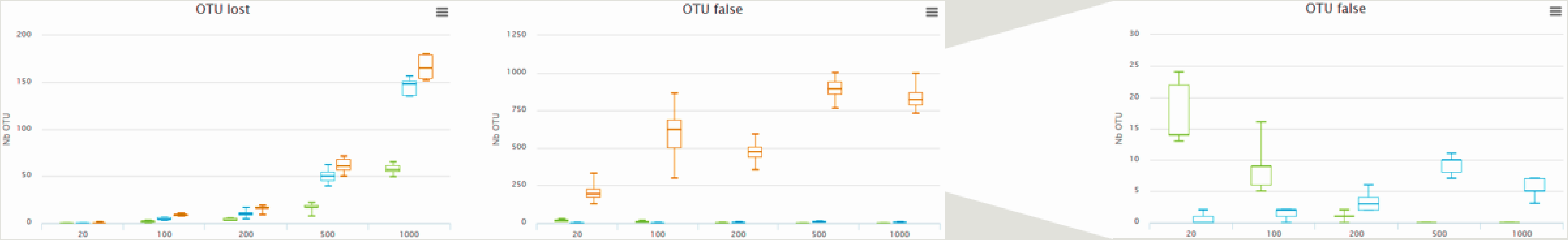
FROGS’ Accuracy

→ Lost & False OTU

V4V4 Power Law



V4V4 Uniform



🟩 frogs 🟦 uparse 🟧 mothur

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/fileadmin/user_upload/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

20th to 23th Februray 2017 4 days (is full !)

1 Galaxy day
2 FROGS days
1 Statistics phyloseq day (under R)

If we have time

- Change clustering option ad compare.
- Make a phylogenetic tree from sequences.fasta built with Filter Tool.

→ use the document about phylogeny.fr