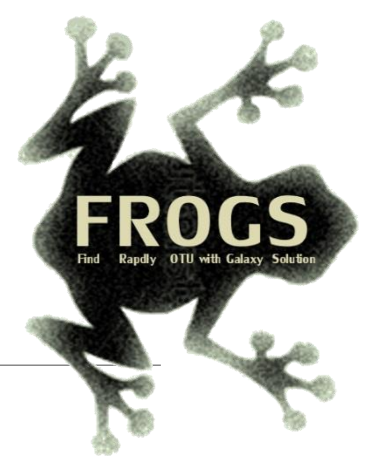


D- Training on Galaxy: Metabarcoding

May 2022 - Webinar

FROGS Practice on ITS data

LUCAS AUER, MARIA BERNARD, LAURENT CAUQUIL, MAHENDRA MARIADASSOU, GÉRALDINE PASCAL & OLIVIER RUÉ

GenPhySE

GABI

What is a ITS ?

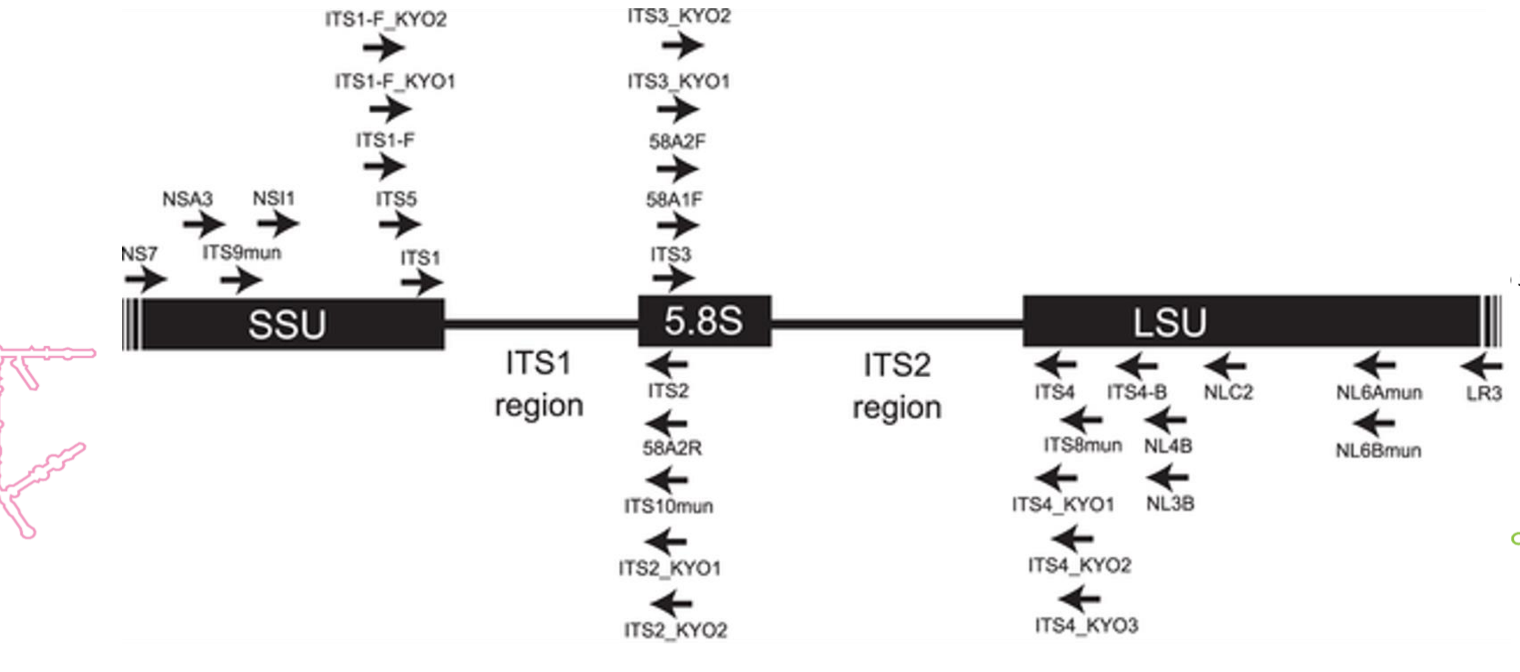
S. cerevisiae

Small SU
18S rRna

Large SU
25S rRna

Map of nuclear ribosomal RNA genes and their ITS regions.

S. cerevisiae SSU



S. cerevisiae LSU

18S

25S

5S

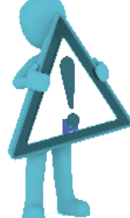
5.8S

Toju H, Tanabe AS, Yamamoto S, Sato H (2012) High-Coverage ITS Primers for the DNA-Based Identification of Ascomycetes and Basidiomycetes in Environmental Samples. PLOS ONE 7(7): e40863. https://doi.org/10.1371/journal.pone.0040863

Secondary Structures of rRNAs from All Three Domains of Life
Anton S. Petrov , Chad R. Bernier, Burak Gulen, Chris C. Waterbury, Eli Hershkovits, Chiaolong Hsiao, Stephen C. Harvey, Nicholas V. Hud, George E. Fox, Roger M. Wartell, Loren Dean Williams February 5, 2014 https://doi.org/10.1371/journal.pone.0088222

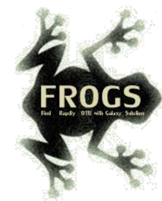
What is a ITS ?

- Size polymorphism of ITS (from 361 to 1475 bases in UNITE 7.1)
- Highly conserved regions of the neighboring of ITS1 and ITS2
- Lack of a generalist and abundant ITS databank (several small specialized databanks)
- Multiple copies* (14 to 1400 copies (mean at 113, median et 80))
- Do not target Glomeromycetes/Glomeromycota (→ alternative: 18S)



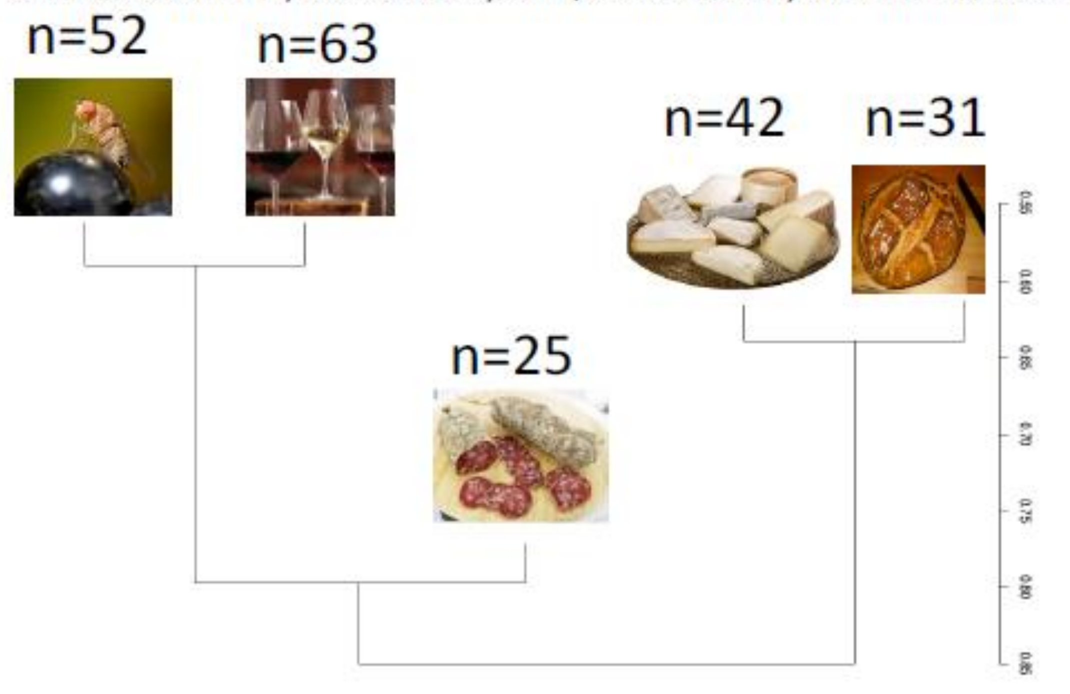
If your sequencing platform preprocesses your data, it has to keep short and long sequences

* https://doi.org/10.1111/mec.14995



ITS data form METABARFOOD project metaprogramme MEM

Yeast catalog in food ecosystem

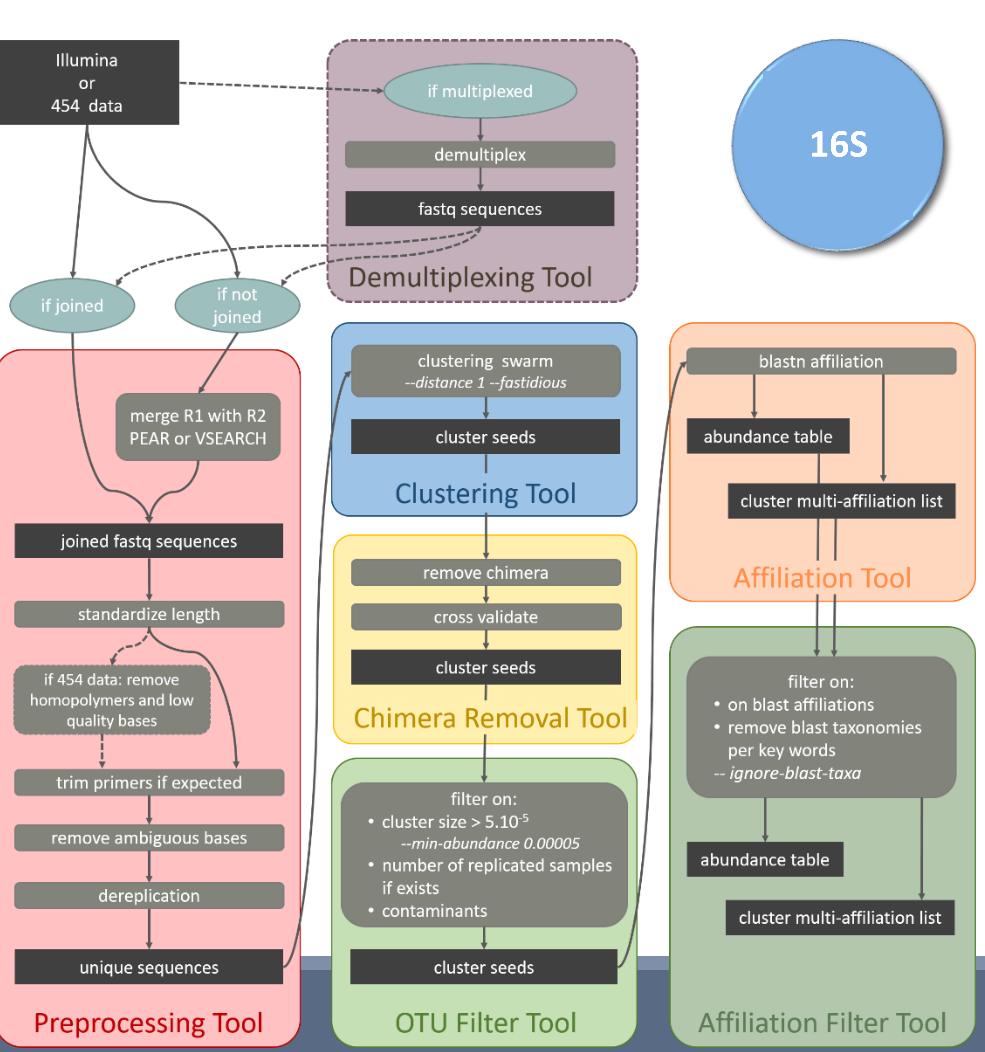


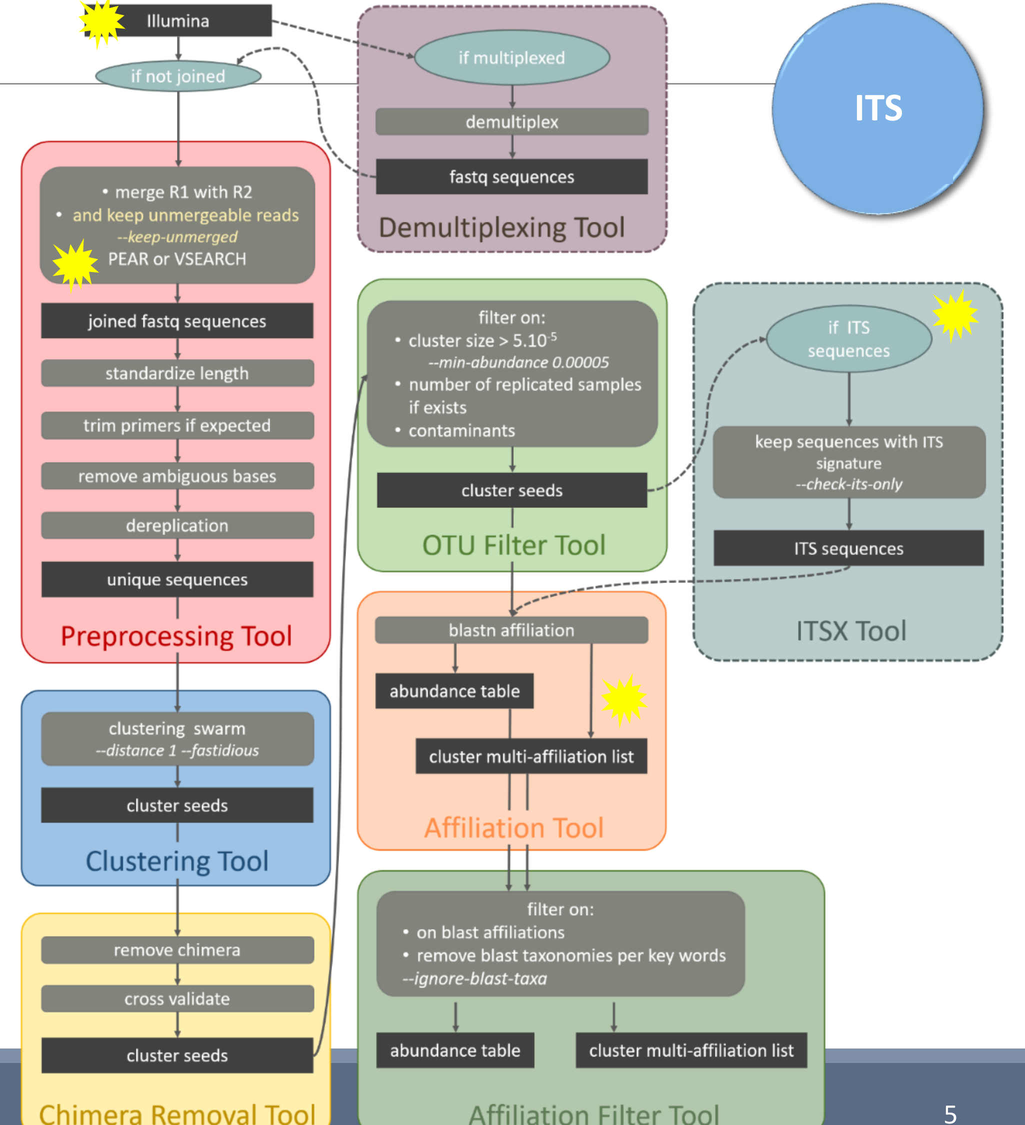
The universal fungal barcode, the Internal Transcribed Spacer (ITS) region, displays considerable size variation amongst yeasts and other micro-eukaryotes.

There are also several repeats leading to sequencing errors or termination.

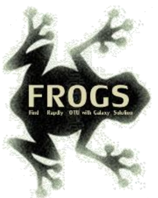
: Differences between 16S and ITS pipelines

Overview



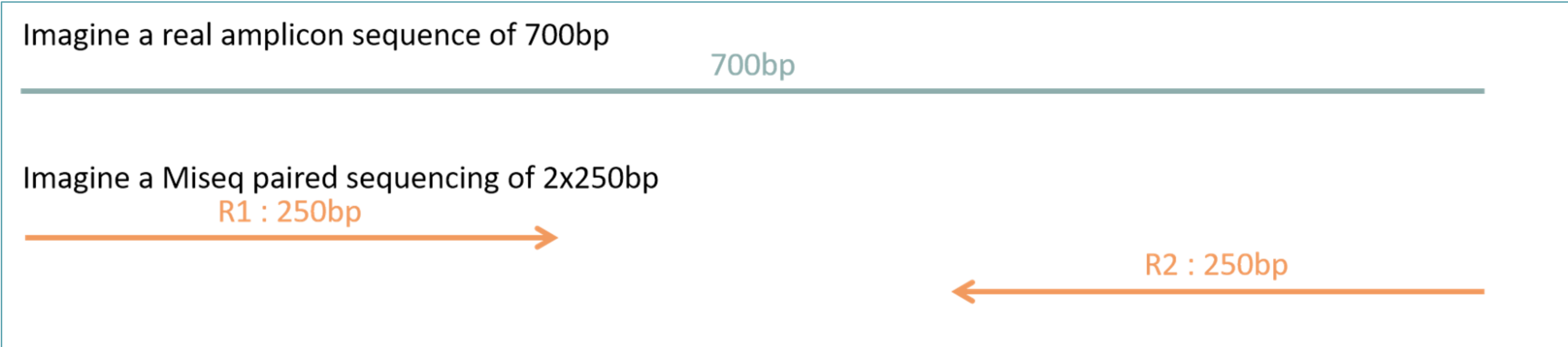


FROGS Pipeline

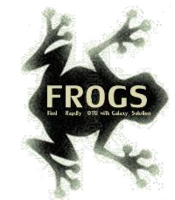


Minimal pipeline for ITS amplicon analyses

Problematic:
some ITS reads (Miseq sequencing) are non-overlapping sequences



Consequence: during bioinformatics process, these reads are lost and underlying organisms will be never represented in the abundance table.



Solution: in preprocess step – creation of “FROGS combined” sequences

Imagine a real amplicon sequence of 700bp

700bp

Imagine a Miseq paired sequencing of 2x250bp

R1 : 250bp

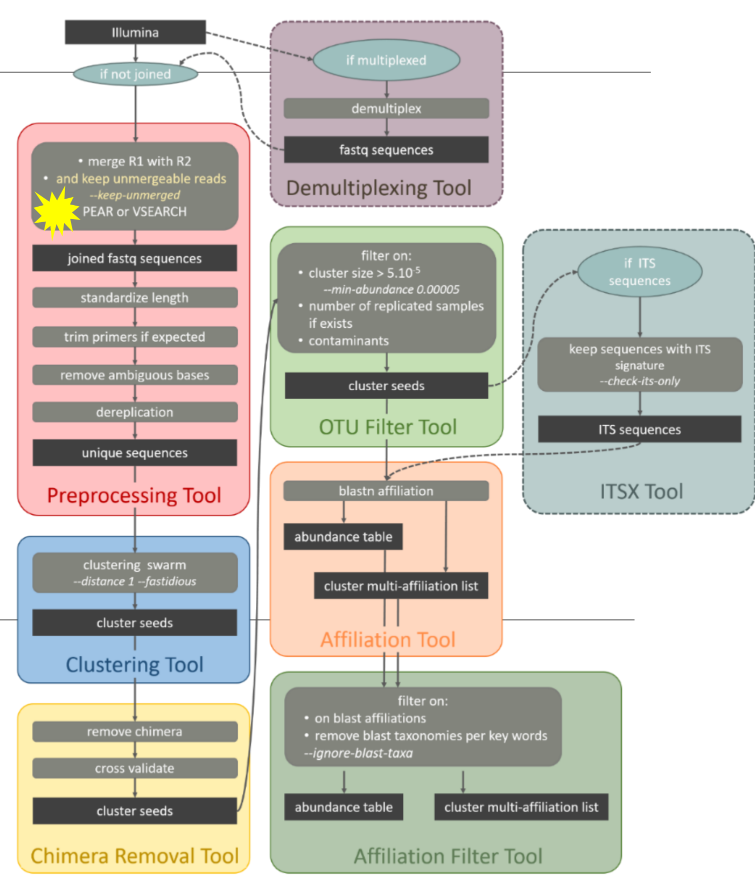
R2 : 250bp

Reconstructing amplicon sequence is not possible with overlap, an arbitrary sequence of 100Ns is added. It is named « FROGS combined »

NNNNNNNNNNNNNNNNNN

Combined sequence length : 600bp, with 100 Ns

Pre-process tool



ITS

FROGS Pre-process merging, denoising and dereplication. (Galaxy Version 3.2.3+galaxy2) Favorite Options

Sequencer

Illumina

Select the sequencing technology used to produce the sequences.

Input type

TAR Archive

Samples files can be provided in a single TAR archive or sample by sample (with one or two files each).

TAR archive file

1: ITS1.tar.gz

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

Are reads already merged ?

No

The archive contains 1 file by sample : R1 and R2 pair are already merged in one sequence.

Reads 1 size

250

The maximum read1 size.

Reads 2 size

250

The maximum read2 size.

Mismatch rate.

0.1

The maximum rate of mismatch in the overlap region

Merge software

Vsearch

Select the software to merge paired-end reads.

Would you like to keep unmerged reads?

Yes

No : Unmerged reads will be excluded; Yes : unmerged reads will be artificially combined with 100 N. (default No)

To keep FROGS combined sequences, choose YES

ITS

Minimum amplicon size

50

The minimum size for the amplicons (with primers).

Maximum amplicon size

490

The maximum size for the amplicons (with primers).

Sequencing protocol

Illumina standard

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

5' primer

CTTGGTCATTTAGAGGAAGTAA

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

3' primer

GCATCGATGAAGAACGCAGC

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

Email notification

No

Send an email notification when the job completes.

Execute

Primer 5': CTTGGTCATTTAGAGGAAGTAA
Primer 3': GCATCGATGAAGAACGCAGC

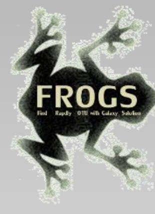
Exercise

Go to « ITS » history

Launch the pre-process tool on this data set

→ objective: understand preprocess report and « FROGS combined sequences »

ITS



Explore Preprocess report.html

Preprocess summary

Summary

1,000k
800k
600k
400k
200k
0

Nb sequences

input sequences : 990,529

990,529
882,089
877,708
877,703
877,656

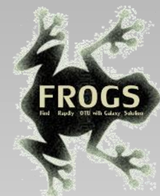
944,148
847,953
847,665
847,660
847,619

46,381
34,136
30,043
30,043
30,037

paired-end assembled
with 5' primer
with 3' primer
with expected length
without N

merged
artificial combined

ITS



2 tables:

Details on merged sequences

CSV

Show 10 entries   Search:

Samples	before process	% kept	paired-end assembled	with 5' primer	with 3' primer	with expected length	without N
complexe-ADN-1	56,284	85.79	52,757	48,305	48,292	48,292	48,288
echantillon1-1	32,883	81.01	31,137	26,653	26,640	26,640	26,639
echantillon1-2	56,166	91.15	53,736	51,225	51,200	51,200	51,195
echantillon1-3	84,102	71.56	80,060	60,224	60,188	60,187	60,181
echantillon2-1	53,405	86.65	51,004	46,282	46,276	46,275	46,275

Details on artificial combined sequences

CSV

Show 10 entries   Search:

Samples	before process	% kept	paired-end assembled	with 5' primer	with 3' primer	with expected length	without N
complexe-ADN-1	56,284	4.57	3,527	2,814	2,572	2,572	2,571
echantillon1-1	32,883	3.03	1,746	1,151	997	997	997
echantillon1-2	56,166	2.81	2,430	1,789	1,582	1,582	1,581
echantillon1-3	84,102	2.61	4,042	2,574	2,199	2,199	2,199
echantillon2-1	53,405	2.46	2,401	1,554	1,317	1,317	1,316

Own tag for combined sequences

```
>Cluster_20410 1:N:0:ATATAA
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
>Cluster_2881 1:N:0:ATATAA
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
>Cluster_10465 1:N:0:ATTACA
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
>Cluster_2714_FROGS_combined R1_desc:1:N:
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
>Cluster_6993_FROGS_combined R1_desc:1:N:
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
>Cluster_2580_FROGS_combined R1_desc:1:N:
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
```

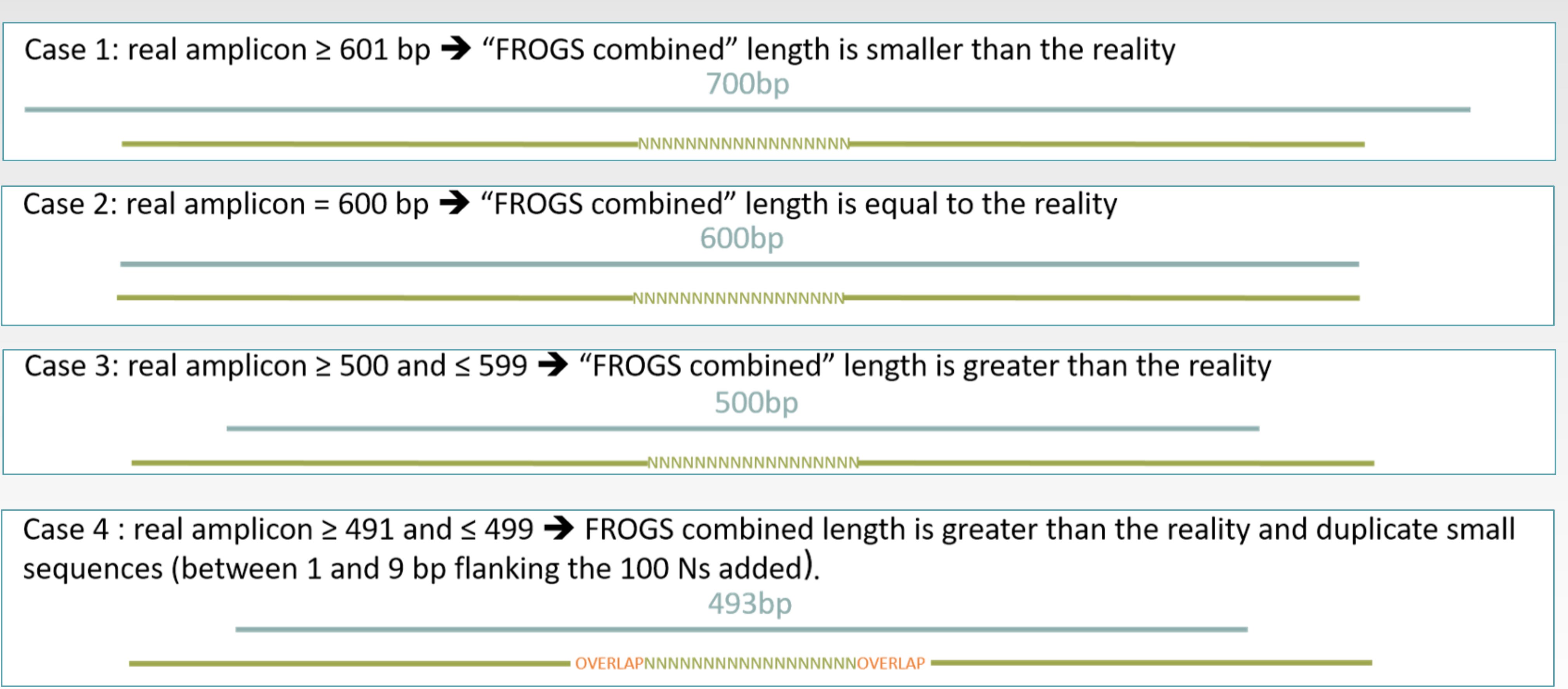
Filter only on minimum length for « combined ».

Minimum length = R1 + 100N + R2 – primers sizes

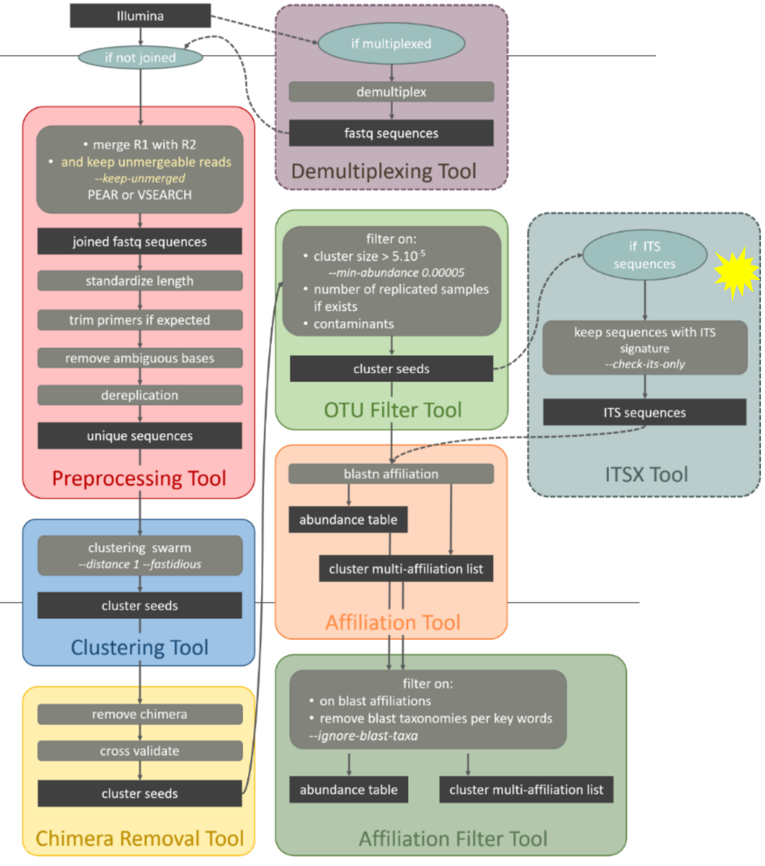
If the primers are very internal to the read, after trimming them, the combined sequence could be smaller than a read. FROGS rejects these cases.

FROGS "combined" sequences are **artificial** and present particular features especially on size.

Imagine a MiSeq sequencing of 2x250pb with reads impossible to overlap. So FROGS "combined" length = 600 bp.



ITSx tools

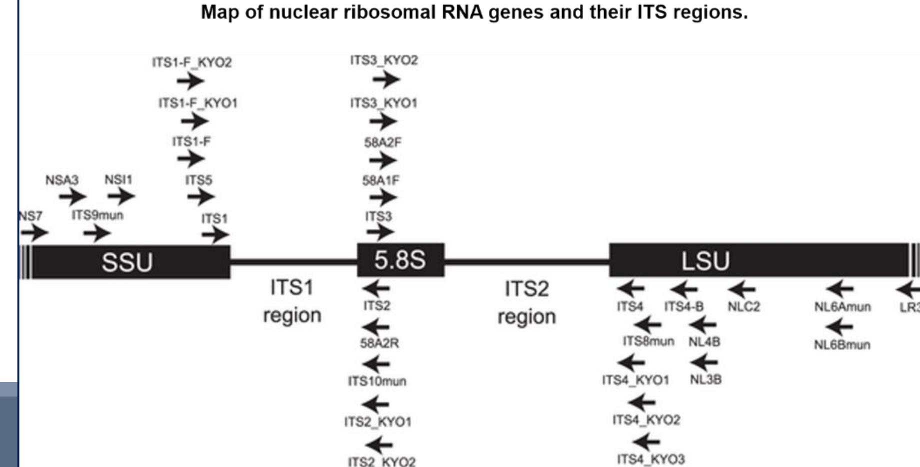


What is the purpose of the ITSx tool?

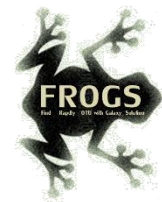
- ITSx is a tool to **filter** sequences.
- ITSx **identifies** and **trimms** ITS regions in sequences.
- It **excludes** the highly conserved neighboring sequences **SSU**, **5S** and **LSU** rRNA.
- If the ITS1 or ITS2 region is not detected, the sequence is discarded.
- You can choose to check only if the sequence is detected as an ITS.

  In this case, the sequence is not trimmed, only sequences not detected as ITS are rejected (*e.g.* contaminants).

Map of nuclear ribosomal RNA genes and their ITS regions.

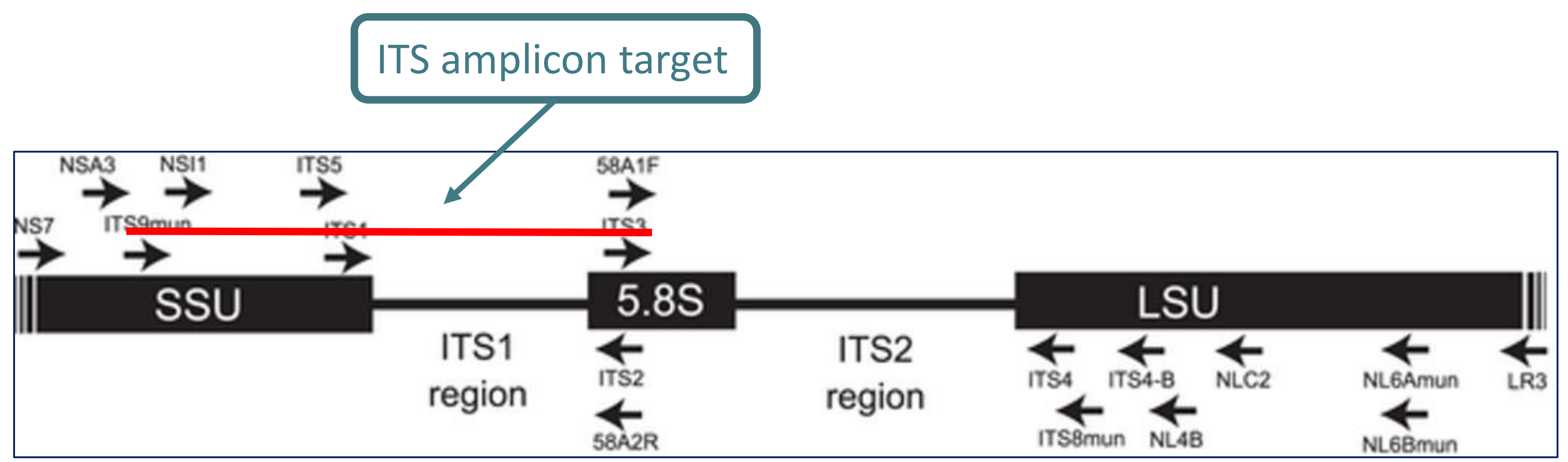


Bengtsson-Palme, J., et al. (2013), Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol Evol, 4: 914-919. https://doi.org/10.1111/2041-210X.12073



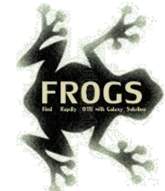
What is the purpose of the ITSx tool?

ITS amplicon target



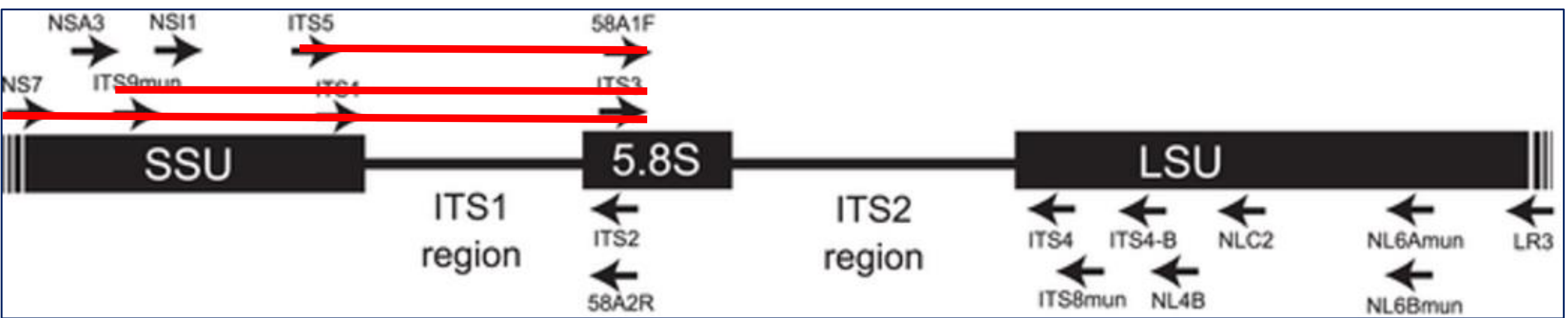
1st case: choose to trim
ITS1 is well detected
SSU part and 5.8S part are trimmed
Result:

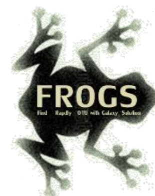
2nd case: choose to check only
ITS1 is well detected
SSU part and 5.8S part are not trimmed
Result:



Check only if sequence is detected as ITS? Yes or not?

- If not, only ITS1 or ITS2 part will be conserved
- This is interesting to keep only the ITS parts without the flanking sequences in case of :
  - comparison of sequenced amplicons with different primers targeting the same region to be amplified.
  - using a database with only ITS part





When should we use ITSx ?

After filtering !

FROGS Pre-process
- TAR archive file
- ☐ FROGS Pre-process: dereplicated.fasta (fasta)
- ☐ FROGS Pre-process: count.tsv (tabular)
- ☑ FROGS Pre-process: report.html (html)

FROGS Clustering swarm
- Sequences file
- Count file
- ☐ FROGS Clustering swarm: seed_sequences.fasta (fasta)
- ☐ FROGS Clustering swarm: clustering_abundance.biom (biom1)
- ☐ FROGS Clustering swarm: swarms_composition.tsv (tabular)

FROGS Remove chimera
- Sequences file
- Abundance file
- ☐ FROGS Remove chimera: non_chimera.fasta (fasta)
- ☐ FROGS Remove chimera: non_chimera_abundance.biom (biom1)
- ☑ FROGS Remove chimera: report.html (html)

FROGS OTU Filters
- Sequences file
- Abundance file
- ☐ FROGS OTU Filters: otuFilter_abundance.biom (biom1)
- ☐ FROGS OTU Filters: otuFilter_sequences.fasta (fasta)
- ☐ FROGS OTU Filters: excluded.tsv (tabular)
- ☑ FROGS OTU Filters: report.html (html)

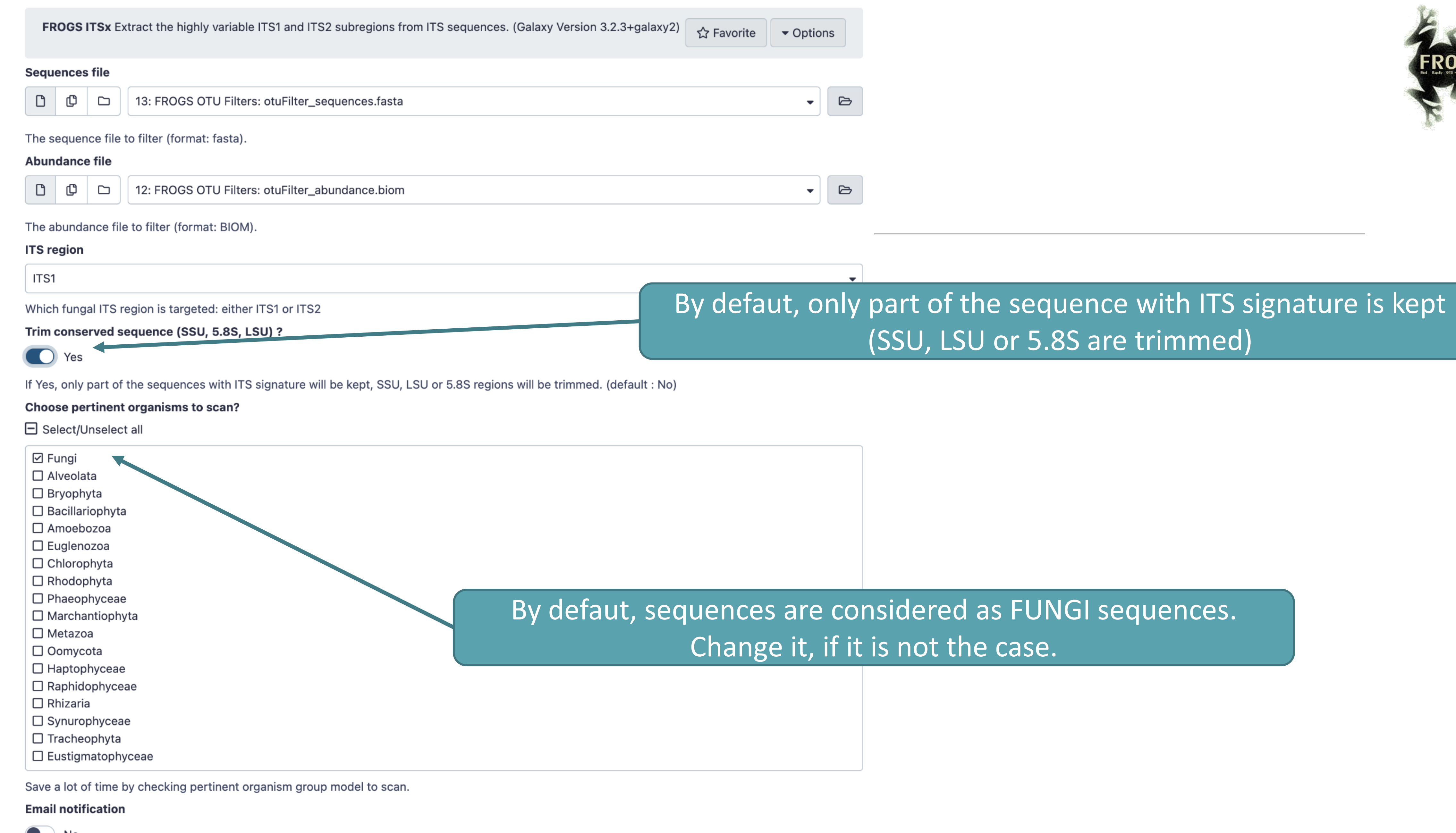
FROGS ITSx
- Sequences file
- Abundance file
- ☐ FROGS ITSx: nonITS_sequence.fasta (fasta)
- ☐ FROGS ITSx: ITS_sequence.fasta (fasta)
- ☐ FROGS ITSx: itsx_abundance.biom (biom1)
- ☑ FROGS ITSx: report.html (html)

ITSx is a fastidious step

FROGS Affiliation OTU
- OTU seed sequence
- Abundance file
- ☐ FROGS Affiliation OTU: affiliation_abundance.biom (biom1)
- ☑ FROGS Affiliation OTU: report.html (html)

FROGS Affiliation Filters
- Sequences file
- Abundance file
- ☑ FROGS Affiliation Filters: affiFilter_abundance.biom (biom1)
- ☑ FROGS Affiliation Filters: impacted_OTU.tsv (tabular)
- ☑ FROGS Affiliation Filters: impacted_OTU.multi-affiliations.tsv (tabular)
- ☑ FROGS Affiliation Filters: report.html (html)

FROGS BIOM to TSV
- Abundance file
- Sequences file (optional)
- ☑ FROGS BIOM to TSV: abundance.tsv (tabular)
- ☑ FROGS BIOM to TSV: multi-affiliations.tsv (tabular)

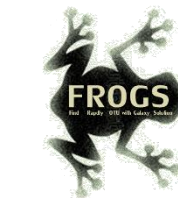

FROGS ITSx Extract the highly variable ITS1 and ITS2 subregions from ITS sequences. (Galaxy Version 3.2.3+galaxy2)
Favorite
Options
Sequences file
13: FROGS OTU Filters: otuFilter_sequences.fasta
The sequence file to filter (format: fasta).
Abundance file
12: FROGS OTU Filters: otuFilter_abundance.biom
The abundance file to filter (format: BIOM).
ITS region
ITS1
Which fungal ITS region is targeted: either ITS1 or ITS2
Trim conserved sequence (SSU, 5.8S, LSU) ?
Yes
If Yes, only part of the sequences with ITS signature will be kept, SSU, LSU or 5.8S regions will be trimmed. (default : No)
Choose pertinent organisms to scan?
Select/Unselect all
Fungi
Alveolata
Bryophyta
Bacillariophyta
Amoebozoa
Euglenozoa
Chlorophyta
Rhodophyta
Phaeophyceae
Marchantiophyta
Metazoa
Oomycota
Haptophyceae
Raphidophyceae
Rhizaria
Synurophyceae
Tracheophyta
Eustigmatophyceae
Save a lot of time by checking pertinent organism group model to scan.
Email notification
No
Send an email notification when the job completes.
Execute
By defaut, only part of the sequence with ITS signature is kept (SSU, LSU or 5.8S are trimmed)
By defaut, sequences are considered as FUNGI sequences. Change it, if it is not the case.
FROGS

Careful !

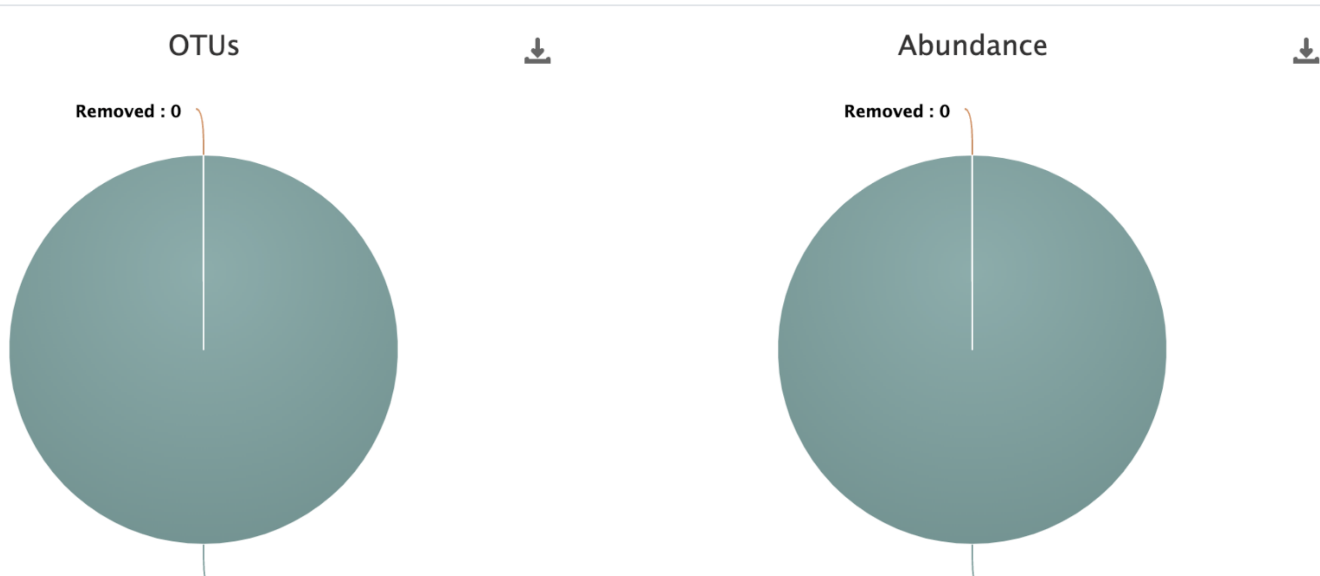
- The ITSx step is time consuming and has to be done on clusters. We advise our users to apply ITSx in 5th step:

1. Preprocess step,
2. Clustering step,
3. Chimera removing step,
4. Filter on OTUs abundances and replicats step,
5. ITSx if Fungi ITS amplicons.

Report.html, ITSX output



Filters (ITSx) summary



Filters (ITSx) by samples

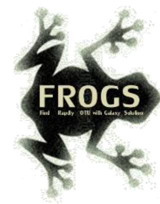
CSV

Show 10 entries

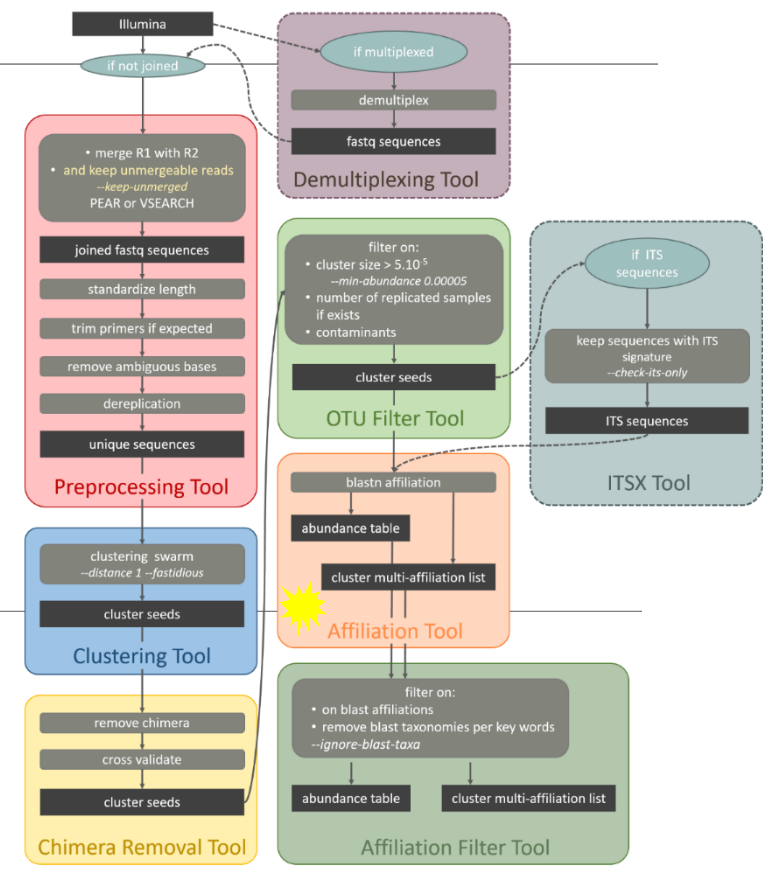
Search:

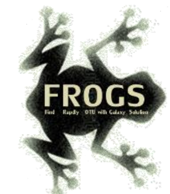
OTUs removed by sample

Sample name	Initial	Kept	Initial abundance	Kept abundance
complexe-ADN-1	92	92	46,438	46,438
echantillon1-1	71	71	26,497	26,497
echantillon1-2	71	71	50,940	50,940



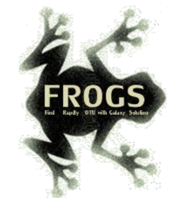
ITS Affiliation





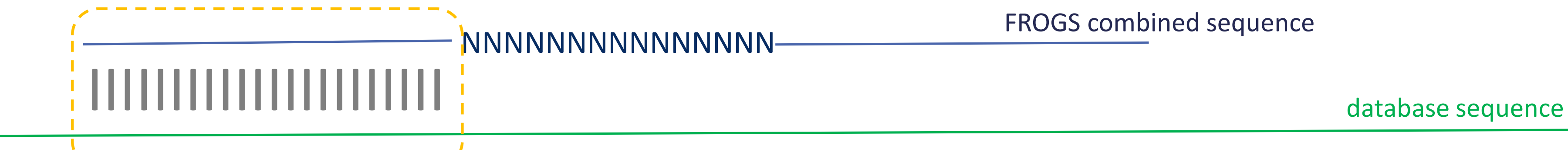
What is special about the affiliation of ITS/FROGS combined sequences?

- 2 alignment tools - blastn+ or needleall - are used to find alignments between each OTU and the database.
- Only the bests hits with the same score are reported.
- blastn+ is used for classical **merged read pair**, and blastn+ then needleall are used for **artificially combined sequence**.
- For each alignment, several metrics are computed: %identity, %coverage and alignment length.



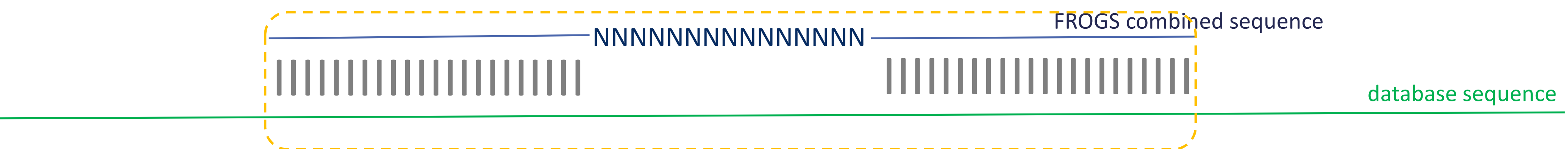
What is special about the affiliation of ITS/FROGS combined sequences?

- blastn+ *i.e.* a local aligner, is not usable for "**combined**" sequences



Between combined and the database sequence, alignment is perfect until N stretch with blastn+. Information about the 2nd part of sequence are not explored !

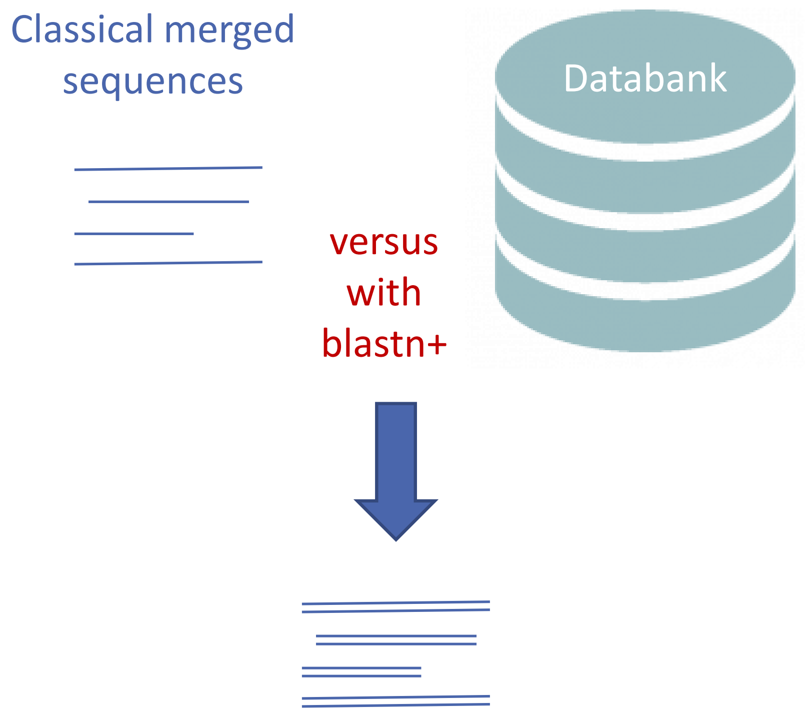
- It is necessary to use a global aligner *i.e.* Needleall (the sequence must be aligned in its entirety), but it is computationally too hard.



What is special about the affiliation of ITS/FROGS combined sequences?

Solution:

- 1st step treat classical merged sequences with blastn+



What is special about the affiliation of ITS/FROGS combined sequences?

- 2nd step for FROGS combined sequences: use blastn+ to create a small databank and align with needleall this small databank versus FROGS combined sequences

FROGS combined sequences

NNNNNNNNNNNNNNN
NNNNNNNNNNNNNNN
NNNNNNNNNNNNNNN
NNNNNNNNNNNNNNN

versus with blastn+

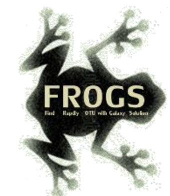
Databank

100 different best hits of **each R1** and **each R2**

a small databank

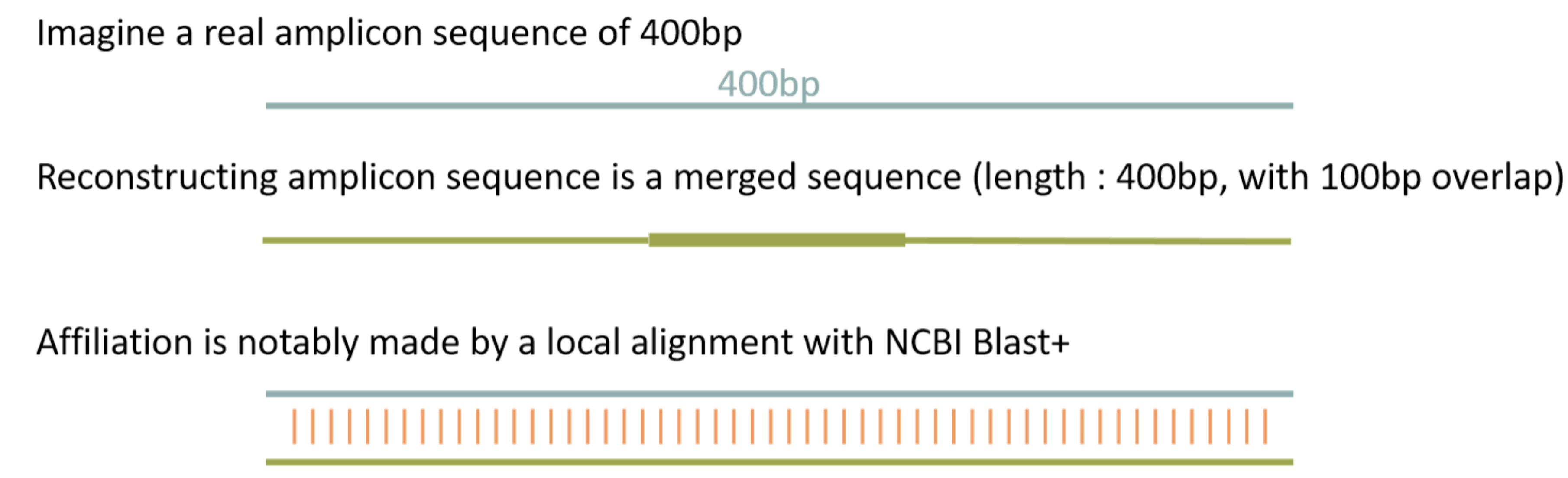
versus with needleall

Careful, with "combined" sequences, we introduced some modification on identity percentage



Case 1: a sequencing of overlapping sequences
i.e. 16S V3-V4 amplicon MiSeq sequencing

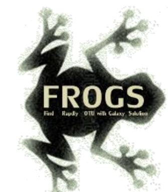
Imagine a real amplicon sequence of 400bp



Reconstructing amplicon sequence is a merged sequence (length : 400bp, with 100bp overlap)

Affiliation is notably made by a local alignment with NCBI Blast+

Imagine a perfect sequencing without error:
classical %id = number of matches / alignment length = 400 matches / 400 positions = 100% identity



Case2: a sequencing of non-overlapping sequences case of ITS1 amplicon MiSeq sequencing

Imagine a real amplicon sequence of 700bp

700bp

Reconstructing a FROGS combined sequence (length : 600bp, with 100Ns)

NNNNNNNNNNNNNNNNNN

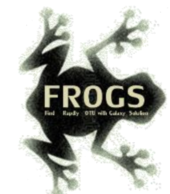
Affiliation could not be made by a local alignment but with a global alignment with Emboss needleall

****

NNNNNNNNNNNNNNNNNN

Imagine a perfect sequencing without error:

classical %id = number of matches / alignment length = (250+250 matches) / 700 positions = 71%



Case2: a sequencing of non-overlapping sequences case of ITS1 amplicon MiSeq sequencing

Filtering on %id will systematically removed “FROGS combined” OTUs.
So, we replaced the classical %id by a %id computed on the sequenced bases only.

% sequenced bases identity = number of matches / (seed length – artificial added N)

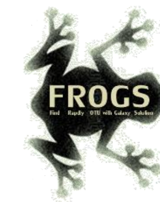
Case 1 : 16S V3V4 ➔ overlapped sequence

% sequenced bases identity = 400 matches / 400 bp = **100 %**

Case 2 : very large ITS1 ➔ “FROGS combined” shorter than the real sequence

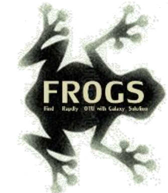
% sequenced bases identity = (250 + 250) / (600 - 100) = **100%**

This calculation allows the 100% identity score to be returned on FROGS "combined" shorter or longer than reality in case of perfect sequencing. And returns a lower percentage of identity in the case of repeated small overlaps kept in the FROGS "combined".



Affiliation Post-process

What is the purpose of the *Affiliation post-process* tool ?



This tool allows **grouping OTUs together** in accordance with the %id and %cov chosen by the user and according to the following criteria:

1. They must have the same affiliation

Or

2. If they have "multi-affiliation" tag in FROGS taxonomy, they must have in common in their list of possible affiliations at least one identical affiliation.

What is the purpose of the *Affiliation post-process* tool ?

In consequence:

The different affiliations involved in multi-affiliation are merged.

The abundances are added together.

It is the most abundant OTU seed that is kept.

FROGS Affiliation postprocess Optionnal step to resolve inclusive amplicon ambiguities and to aggregate OTUs based on alignment metrics (Galaxy Version 3.2.3+galaxy2)

Favorite | Options

FROGS

Sequences file

17: FROGS ITSx: ITS_sequence.fasta

The sequence file to filter (format: fasta).

Abundance file

20: FROGS Affiliation OTU: affiliation_abundance.biom

The abundance file to filter (format: BIOM).

Yes, we have combined sequences

Is this an amplicon hyper variable in length?

Yes

Multi-affiliation tag may be resolved by selecting the shortest amplicon reference. For this you need the reference fasta file of your targetted amplicon.

Using reference database

ITS UNITE Fungi 8.3

same database used for affiliation

Select reference from the list

minimum identity for aggregation

100

OTUs will be aggregated if they share the same taxonomy with at least X% identity.

minimum coverage for aggregation

100

OTUs will be aggregated if they share the same taxonomy with at least X% alignment coverage.

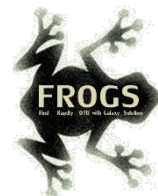
Email notification

No

Send an email notification when the job completes.

Execute

Here, we wanted to aggregate OTUs only if they are identical



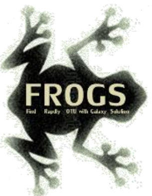
Exemple

After Preprocessing + Clustering + OTU Filter:

```
>Cluster_3
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATATCCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGT
ATTTTTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCTCTC

>Cluster_54
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATATCCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGT
ATTTTTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGTTCCG

>Cluster_414_FROGS_combined
AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATATCCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGT
ATTTTTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCAGATCGGAANNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTCCGATCTCTTGGT
CATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACTATATCCACTTACACCTGTGAACTGTTCTACTAC
TTGACGCAAGTCGAGTATTTTTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCTCTC
```



Exemple

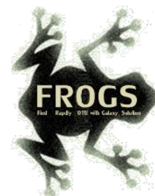
After Preprocessing + Clustering + OTU Filter + **ITSX** :

>Cluster_3
GTGATTGCCTTTATAGGCTTATAACTATATCCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGTATTTTTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATA

>Cluster_54
GTGATTGCCTTTATAGGCTTATAACTATATCCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGTATTTTTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATA

>Cluster_414_FROGS_combined
GTGATTGCCTTTATAGGCTTATAACTATATCCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGTATTTTTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATA

These 3 sequences have become **strictly identical** !



Exemple

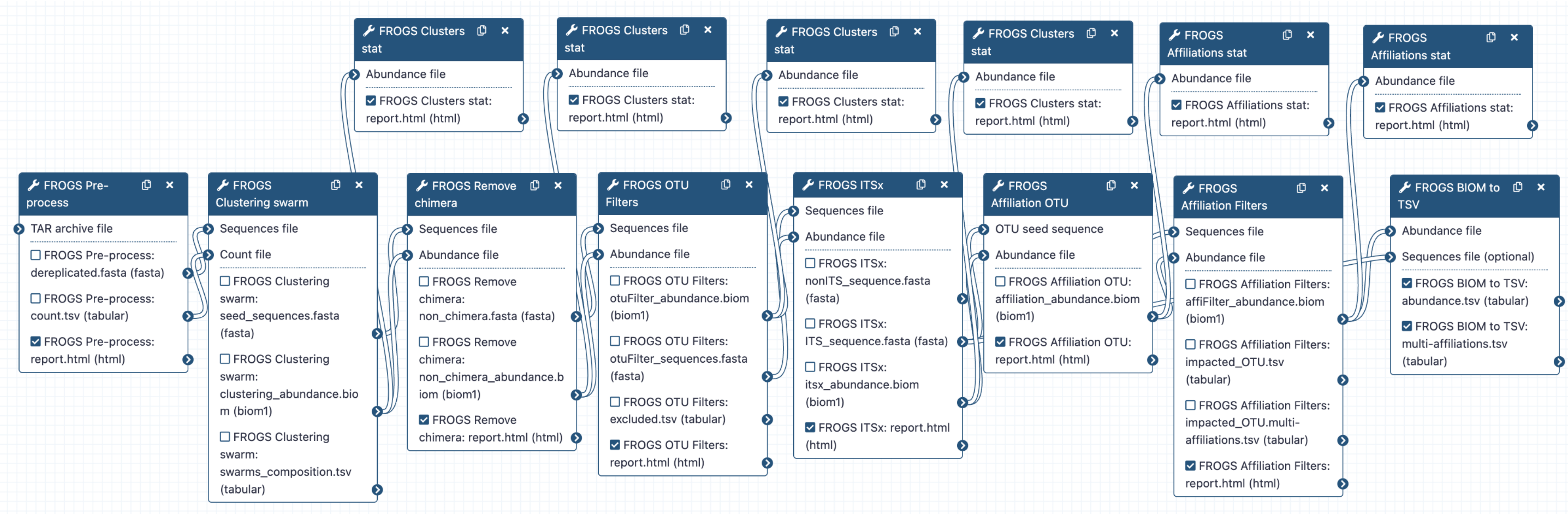
After Preprocessing + Clustering + OTU Filter + ITSX + **Affiliation Post-process** :

Cluster_3, Cluster_54 and Cluster_414_FROGS_combined are **aggregated** in a same OTU

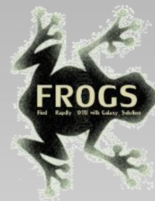
FROGS Affiliation postprocess: aggregation_composition.txt

Cluster_1 Cluster_244 Cluster_448_FROGS_combined Cluster_471_FROGS_combined
Cluster_2 Cluster_320 Cluster_357 Cluster_435 Cluster_468 Cluster_312 Cluster_364 Cluster_477 Cluster_466 Cluster_480
Cluster_3 Cluster_54 Cluster_414_FROGS_combined
Cluster_4 Cluster_15 Cluster_27 Cluster_42 Cluster_67 Cluster_77 Cluster_137 Cluster_209 Cluster_422
Cluster_5 Cluster_5171
Cluster_6 Cluster_53
Cluster_9 Cluster_71
Cluster_7

Workflow creation



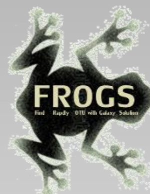
A workflow links FROGS steps together and when it is launched, all the steps run automatically.



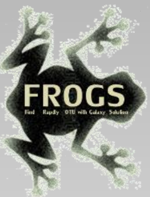
Practice

CREATE YOUR OWN WORKFLOW !

Exercise



Exercise



Create Workflow

Name

ITS_formation

3

Annotation

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

✖ Cancel ✔ Create

4

Exercise

Search Workflows | + Create | Import

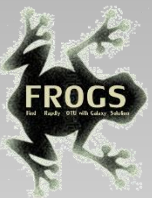
Name	Tags	Updated	Sharing	Bookmarked	
ITS_formation		a few seconds ago		☐	▶
16S		3 minutes ago		☐	▶
ITS		5 minutes ago		☐	▶

5

6

Name	Tags
ITS_formation	

- Edit
- Copy
- Download
- Rename
- Share
- View
- Delete



Solution of exercise:

FROGS Clusters stat
- Abundance file
- ☑ FROGS Clusters stat: report.html (html)

FROGS Clusters stat
- Abundance file
- ☑ FROGS Clusters stat: report.html (html)

FROGS Clusters stat
- Abundance file
- ☑ FROGS Clusters stat: report.html (html)

FROGS Clusters stat
- Abundance file
- ☑ FROGS Clusters stat: report.html (html)

FROGS Affiliations stat
- Abundance file
- ☑ FROGS Affiliations stat: report.html (html)

FROGS Affiliations stat
- Abundance file
- ☑ FROGS Affiliations stat: report.html (html)

FROGS Pre-process
- TAR archive file
- ☐ FROGS Pre-process: dereplicated.fasta (fasta)
- ☐ FROGS Pre-process: count.tsv (tabular)
- ☑ FROGS Pre-process: report.html (html)

FROGS Clustering swarm
- Sequences file
- Count file
- ☐ FROGS Clustering swarm: seed_sequences.fasta (fasta)
- ☐ FROGS Clustering swarm: clustering_abundance.biom (biom1)
- ☐ FROGS Clustering swarm: swarms_composition.tsv (tabular)

FROGS Remove chimera
- Sequences file
- Abundance file
- ☐ FROGS Remove chimera: non_chimera.fasta (fasta)
- ☐ FROGS Remove chimera: non_chimera_abundance.biom (biom1)
- ☑ FROGS Remove chimera: report.html (html)

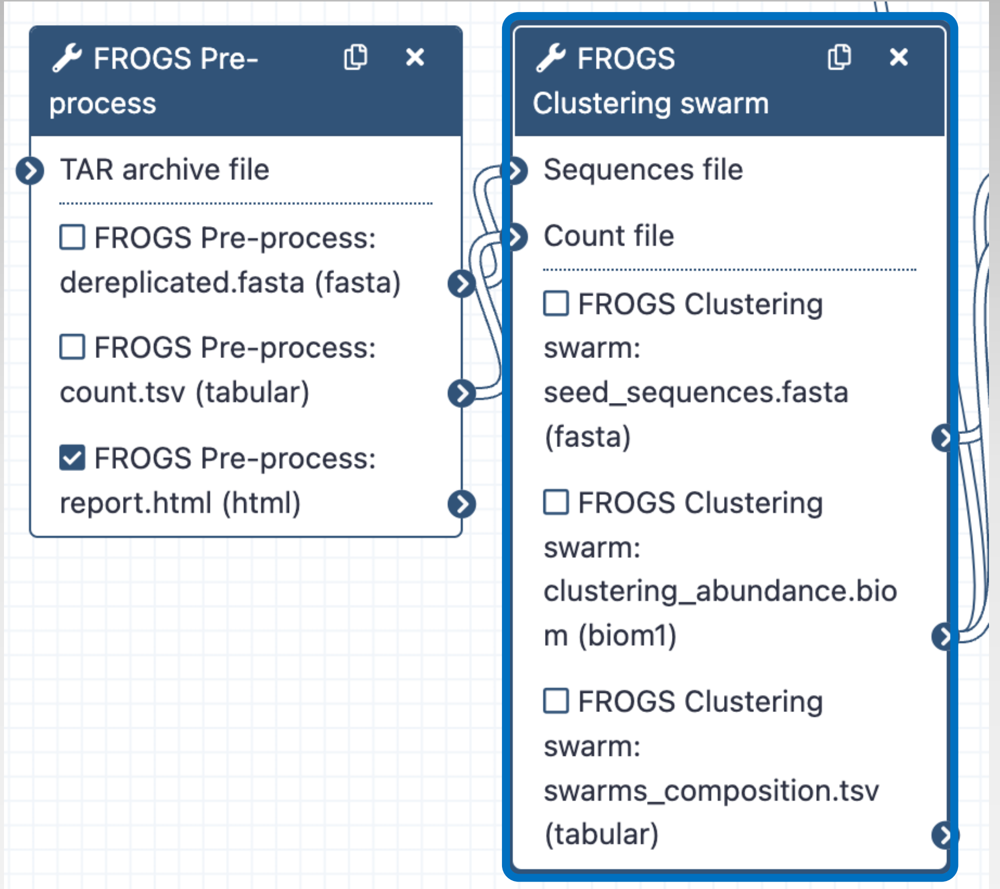
FROGS OTU Filters
- Sequences file
- Abundance file
- ☐ FROGS OTU Filters: otuFilter_abundance.biom (biom1)
- ☐ FROGS OTU Filters: otuFilter_sequences.fasta (fasta)
- ☐ FROGS OTU Filters: excluded.tsv (tabular)
- ☑ FROGS OTU Filters: report.html (html)

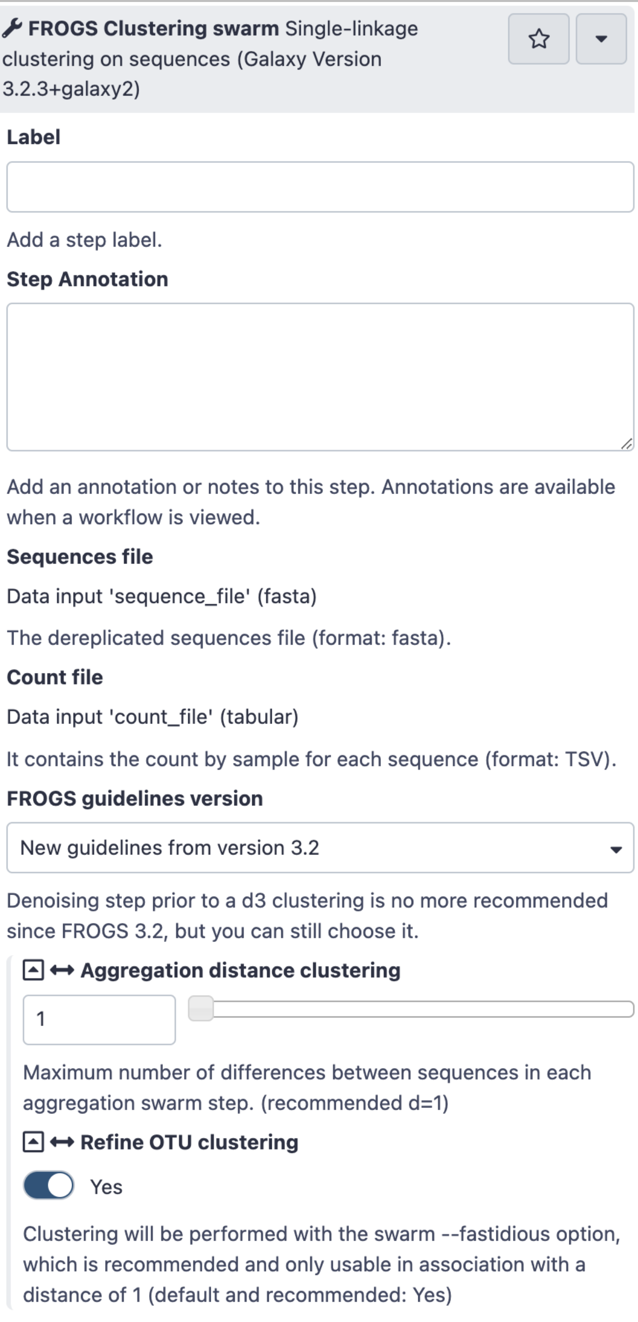
FROGS ITSx
- Sequences file
- Abundance file
- ☐ FROGS ITSx: nonITS_sequence.fasta (fasta)
- ☐ FROGS ITSx: ITS_sequence.fasta (fasta)
- ☐ FROGS ITSx: itsx_abundance.biom (biom1)
- ☑ FROGS ITSx: report.html (html)

FROGS Affiliation OTU
- OTU seed sequence
- Abundance file
- ☐ FROGS Affiliation OTU: affiliation_abundance.biom (biom1)
- ☑ FROGS Affiliation OTU: report.html (html)

FROGS Affiliation Filters
- Sequences file
- Abundance file
- ☐ FROGS Affiliation Filters: affiFilter_abundance.biom (biom1)
- ☐ FROGS Affiliation Filters: impacted_OTU.tsv (tabular)
- ☐ FROGS Affiliation Filters: impacted_OTU.multi-affiliations.tsv (tabular)
- ☑ FROGS Affiliation Filters: report.html (html)

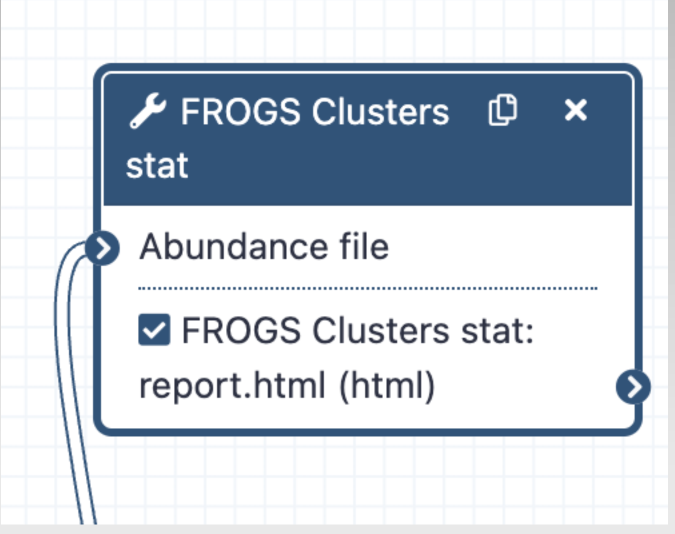
FROGS BIOM to TSV
- Abundance file
- Sequences file (optional)
- ☑ FROGS BIOM to TSV: abundance.tsv (tabular)
- ☑ FROGS BIOM to TSV: multi-affiliations.tsv (tabular)



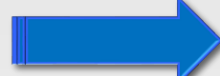


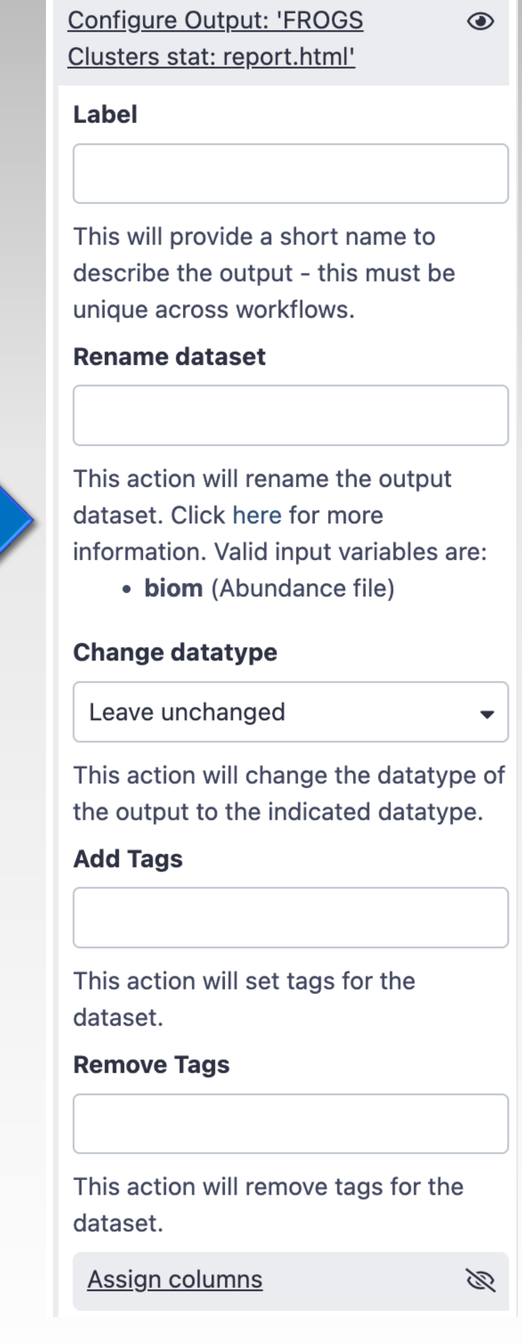
For each tool, think to:

1. Set parameters



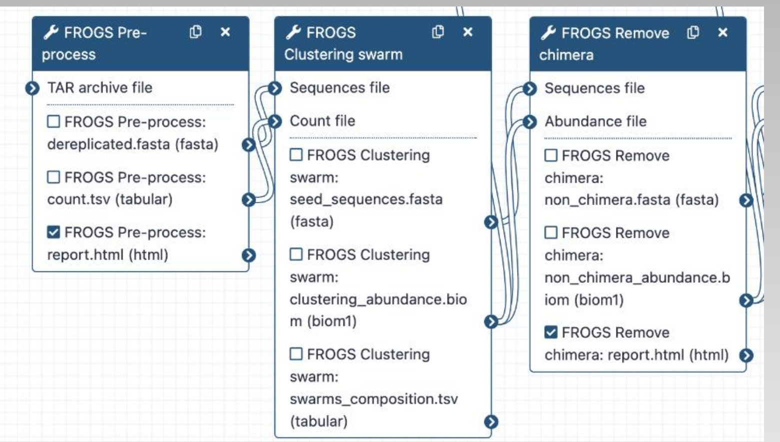
Configure Output: 'FROGS Clusters stat: report.html'





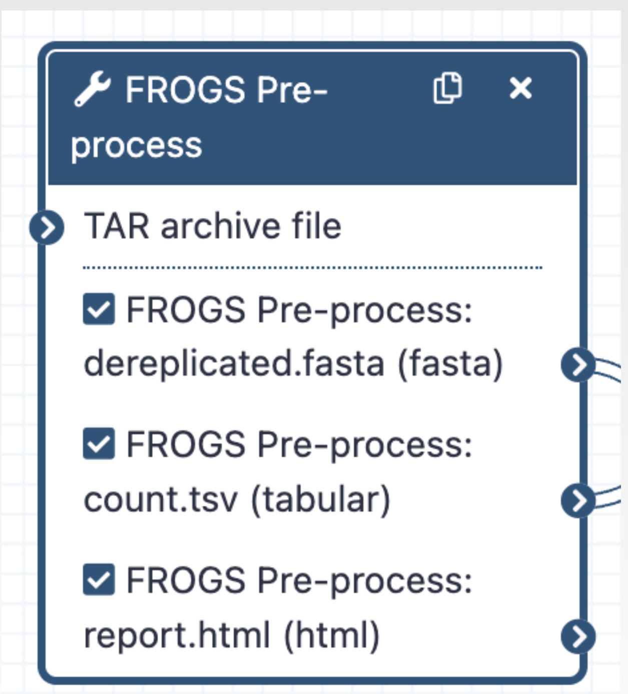
For each tool, think to:

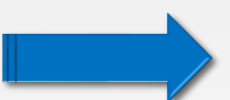
1. Set parameters
2. Rename output files



For each tool, think to:

1. Set parameters
2. Rename output files
3. Hide intermediate files

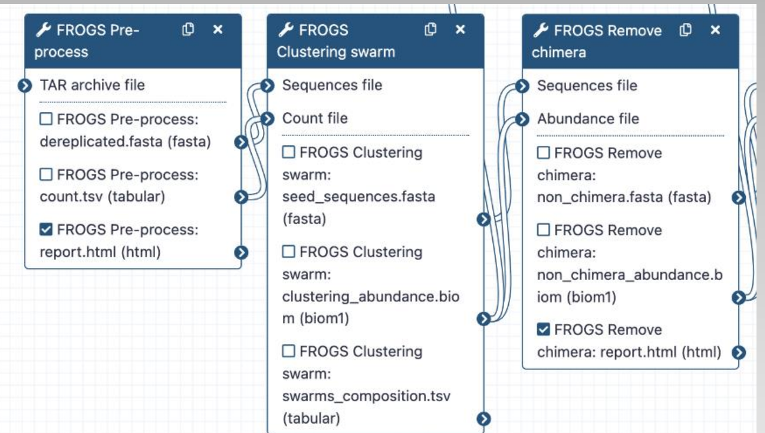




11: FROGS Remove chimera: report.html

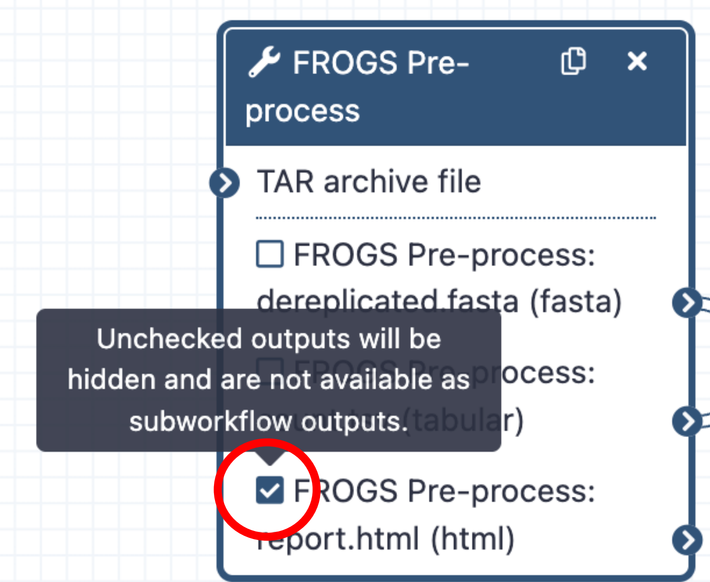
10: FROGS Remove chimera: non_chimera_abundance.biom

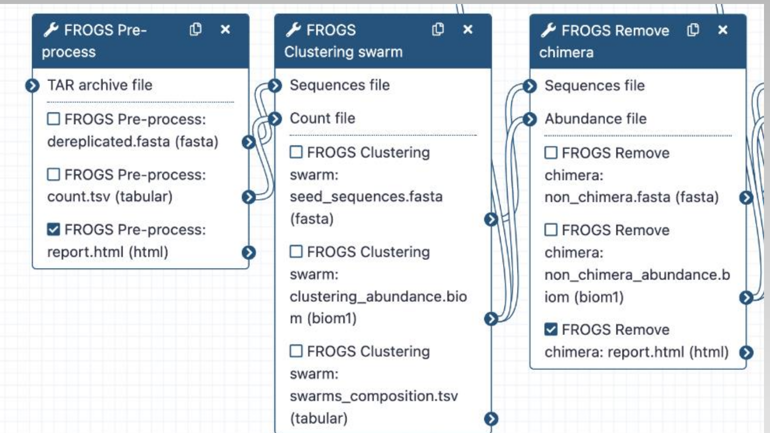
9: FROGS Remove chimera: non_chimera.fasta



For each tool, think to:

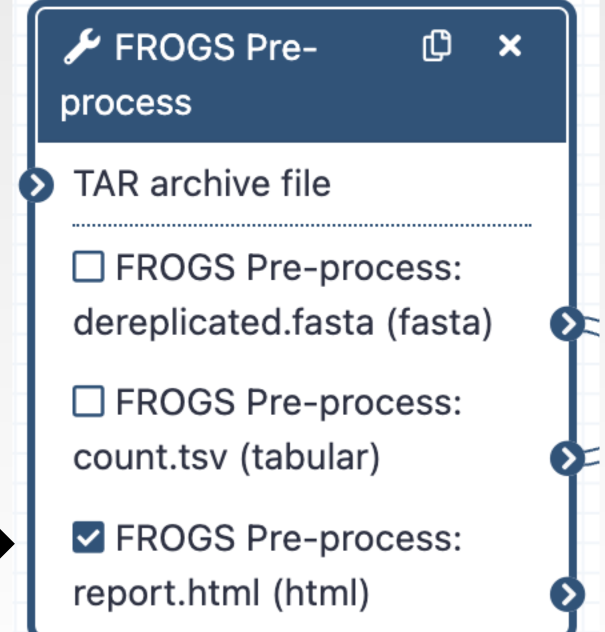
1. Set parameters
2. Rename output files
3. Hide intermediate files

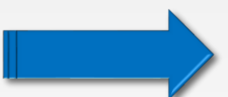




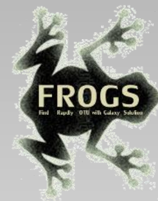
For each tool, think to:

1. Set parameters
2. Rename output files
3. Hide intermediate files





11: FROGS Remove chimera: report.html



Could you integrate « upload file » in the workflow ?

Get Data

Upload File from your computer

Server upload file from path

Server dir upload files from folder path

UCSC Main table browser

UCSC Archaea table browser

SRA server

EBI SRA ENA SRA

modENCODE fly server

?

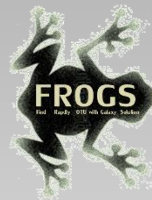
FROGS Pre-process

TAR archive file

FROGS Pre-process: dereplicated.fasta (fasta)

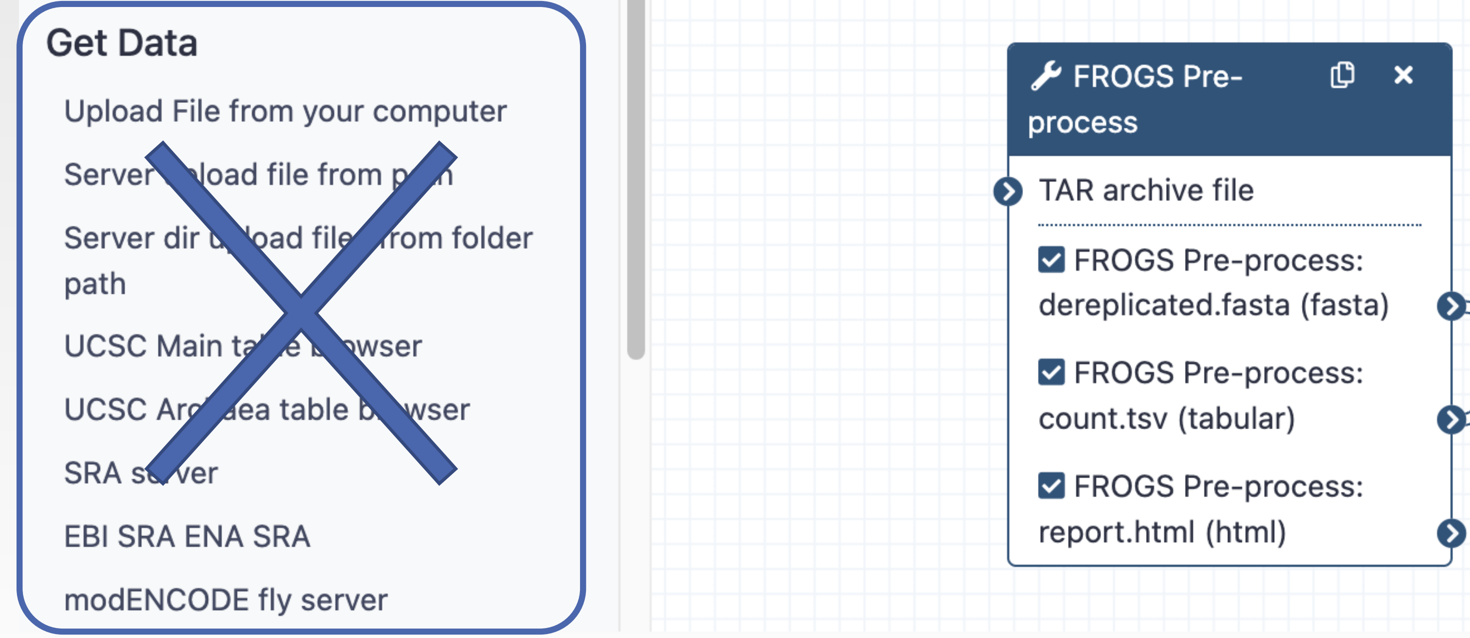
FROGS Pre-process: count.tsv (tabular)

FROGS Pre-process: report.html (html)

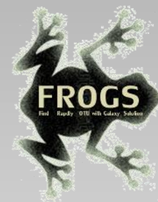


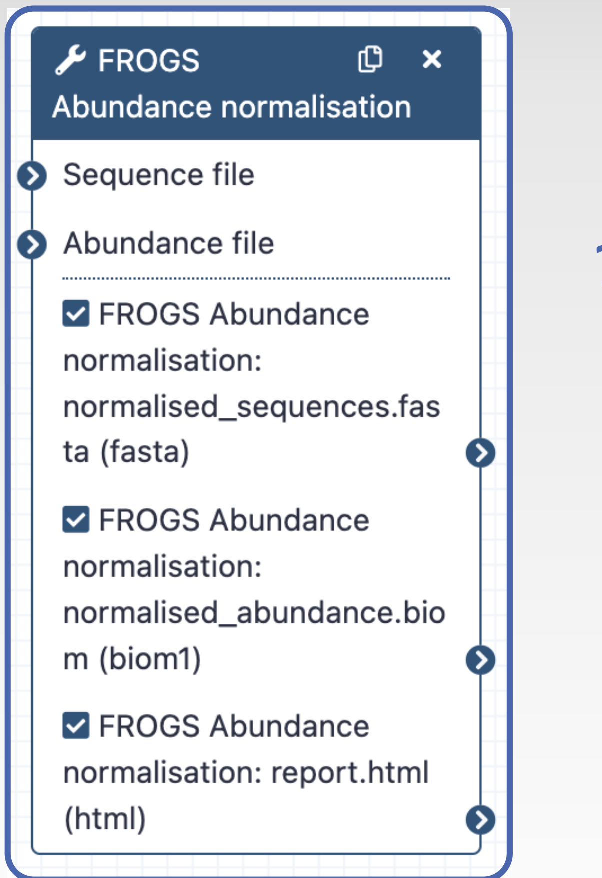
Could you integrate « upload file » in the workflow ?

Upload file cannot be automitized because the workflow, at each run, will be processed with different input data

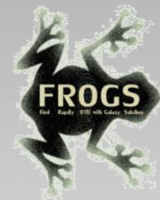


Could you integrate « Normalisation tool » in the workflow ?





?



Could you integrate « Normalisation tool » in the workflow ?

Yes but only if you select
« sampling by the number of
sequence of the smallest sample »

FROGS Abundance normalisation Normalise OTU abundance.
(Galaxy Version 4.0.0+galaxy1)

Label

Add a step label.

Step Annotation

Add an annotation or notes to this step. Annotations are available when a workflow is viewed.

Sequence file

Data input 'input_fasta' (fasta)

Sequence file to normalise (format: fasta).

Abundance file

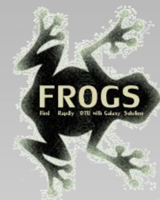
Data input 'input_biom' (biom1)

Abundance file to normalise (format: BIOM).

Sampling method

- [x] Sampling by the number of sequences of the smallest sample
- [ ] Select a number of sequences

Sampling by the number of sequences of the smallest sample, or select a number manually



Exercise

When your workflow is built

1. Run your own workflow with ITS data with :

http://genoweb.toulouse.inra.fr/~formation/15_FROGS/Webinar_data/ITS1.tar.gz

2. Import metadata for statistics analyses

http://genoweb.toulouse.inra.fr/~formation/15_FROGS/Webinar_data/metadata_ITS.tsv

3. Run FROGS_stat tools