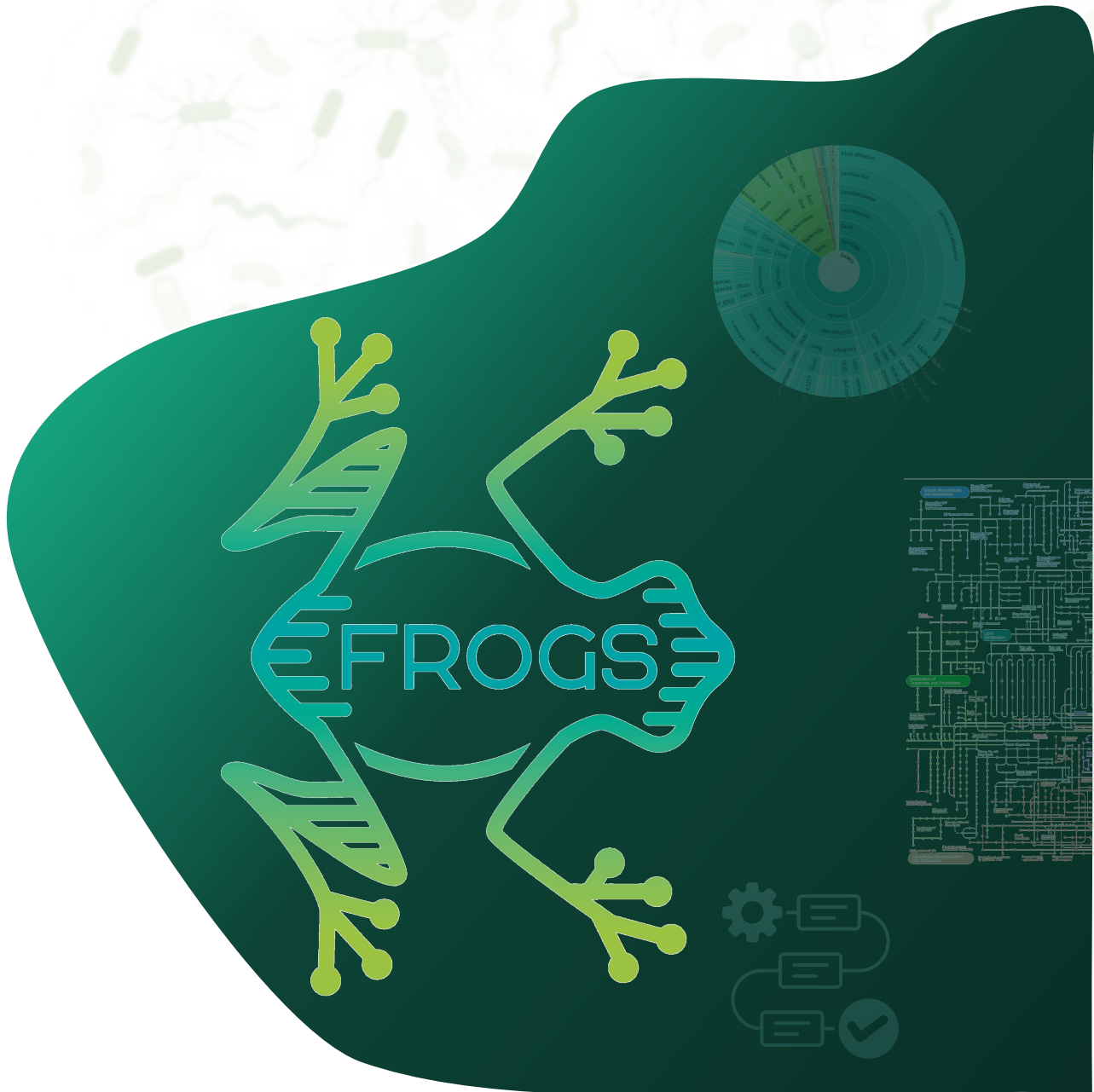
Before starting the training

There are some things to consider.

Lucas AUER, Gabryelle Agoutin, Maria BERNARD, Géraldine PASCAL, Maëlle POMIÈS & Olivier RUÉ



9 am (first day 8.45 am) to 5.30 pm

2 short coffee breaks morning and afternoon

Lunch 12.30 am to 1.30 pm

J0

Get Started with Galaxy

To be done autonomously on galaxy platform

J1

Metabarcoding overview: the key concepts

- Barcode
- Technical sources of noise and errors
- Short reads
- Long reads
- Bioinformatics analysis

FROGS Core

- Presentation of the datasets
- 16S short-read processing
- Data analysis
- ASV (Swarm/DADA2)

J2

FROGS Core

- Remove chimera
- ASV filters
- ITSX

FROGS Core

- Taxonomic affiliation
- Phylogenetic tree building
- Companion tools

between two croaks

Practical work

application of acquired knowledge to a small dataset

Autonomously

J3

FROGS Stat

- Import data
- Alpha diversity
- Beta diversity

A stand-alone application on a set of long read data

- presentation of the datasets
- read processing

J4

FROGS Stat

DesSeq analysis

FROGS Func

Predicting functional abundances based only on marker gene sequences

Practical work

- Apply all FROGS steps to the long-read dataset
- Find the parameters
- Analyze the outputs
- Understand the processes

FROGS Teams

Developers

Maria BERNARD

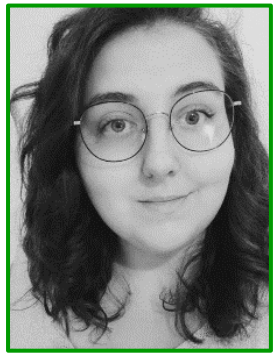
OLIVIER RUÉ

Biology and Statistical experts

Lucas AUER

Galaxy support

Patrice DÉHAIS



Gabryelle AGOUTIN

Databank manager

Maelle POMIÈS

Web master

Géraldine PASCAL

Coordinator

FROGS papers

Frédéric Escudié, Lucas Auer, Maria Bernard, Mahendra Mariadassou, Laurent Cauquil, Katia Vidal, Sarah Maman, Guillermina Hernandez-Raquet, Sylvie Combes, Géraldine Pascal.

"FROGS: Find, Rapidly, OTUs with Galaxy Solution." *Bioinformatics,* Volume 34, Issue 8, 15 April 2018, Pages 1287–1294

Maria Bernard, Olivier Rué, Mahendra Mariadassou and Géraldine Pascal; FROGS: a powerful tool to analyse the diversity of fungi with special management of internal transcribed spacers, *Briefings in Bioinformatics* 2021, 10.1093/bib/bbab318

Bioinformatics, 2017, 1–8
doi: 10.1093/bioinformatics/btx791
Advance Access Publication Date: 7 December 2017
Original Paper

OXFORD

Sequence analysis

FROGS: Find, Rapidly, OTUs with Galaxy Solution

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†The authors wish it to be known that, in their opinion, the first two authors should be regarded as Joint First Authors.
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Abstract

Motivation: Metagenomics leads to major advances in microbial ecology and biologists need user friendly tools to analyze their data on their own.
Results: This Galaxy-supported pipeline, called FROGS, is designed to analyze large sets of ampli-con sequences and produce abundance tables of Operational Taxonomic Units (OTUs) and their taxonomic affiliation. The clustering uses Swarm. The chimera removal uses VSEARCH, combined with original cross-sample validation. The affiliation output to highlight databases conf ous graphical illustrations are produced alon for the detection and quantification of OTUs robust and highly sensitive. It compares fa QIIME.
Availability and implementation: Source cod geraldinepascal/FROGS.git. A companion wel
Contact: geraldine.pascal@inra.fr
Supplementary information: Supplementary

1 Introduction

The expansion of high-throughput sequencing of rRN has opened new horizons for the study of microbial By making it possible to study all micro-organisms f environment without the need to cultivate them, meta led to major advances in many fields of microbial ecol study of the impact of microbiota on human and anima

Briefings in Bioinformatics, 22(6), 2021, 1–6

https://doi.org/10.1093/bib/bbab318
Problem Solving Protocol

OXFORD

FROGS: a powerful tool to analyse the diversity of fungi with special management of internal transcribed spacers

Maria Bernard[†], Olivier Rué[†], Mahendra Mariadassou and Géraldine Pascal

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[†]Maria Bernard and Olivier Rué are joint first authors.

Abstract

Fungi are present in all environments. They fulfil important ecological functions and play a crucial role in the food industry. Their accurate characterization is thus indispensable, particularly through metabarcoding. The most frequently used markers to monitor fungi are ITSs. These markers are the best documented in public databases but have one main weakness: polymerase chain reaction amplification may produce non-overlapping reads in a significant fraction of the fungi. When these reads are filtered out, traditional metabarcoding pipelines lose part of the information and consequently produce biased pictures of the composition and structure of the environment under study. We developed a solution that enables processing of the entire set of reads including both overlapping and non-overlapping, thus providing a more accurate picture of fungal communities. Our comparative tests using simulated and real data demonstrated the effectiveness of our solution, which can be used by both experts and non-specialists on a command line or through the Galaxy-based web interface.

Key words: fungi; ITS; metabarcoding; workflow; amplicon; metagenomics

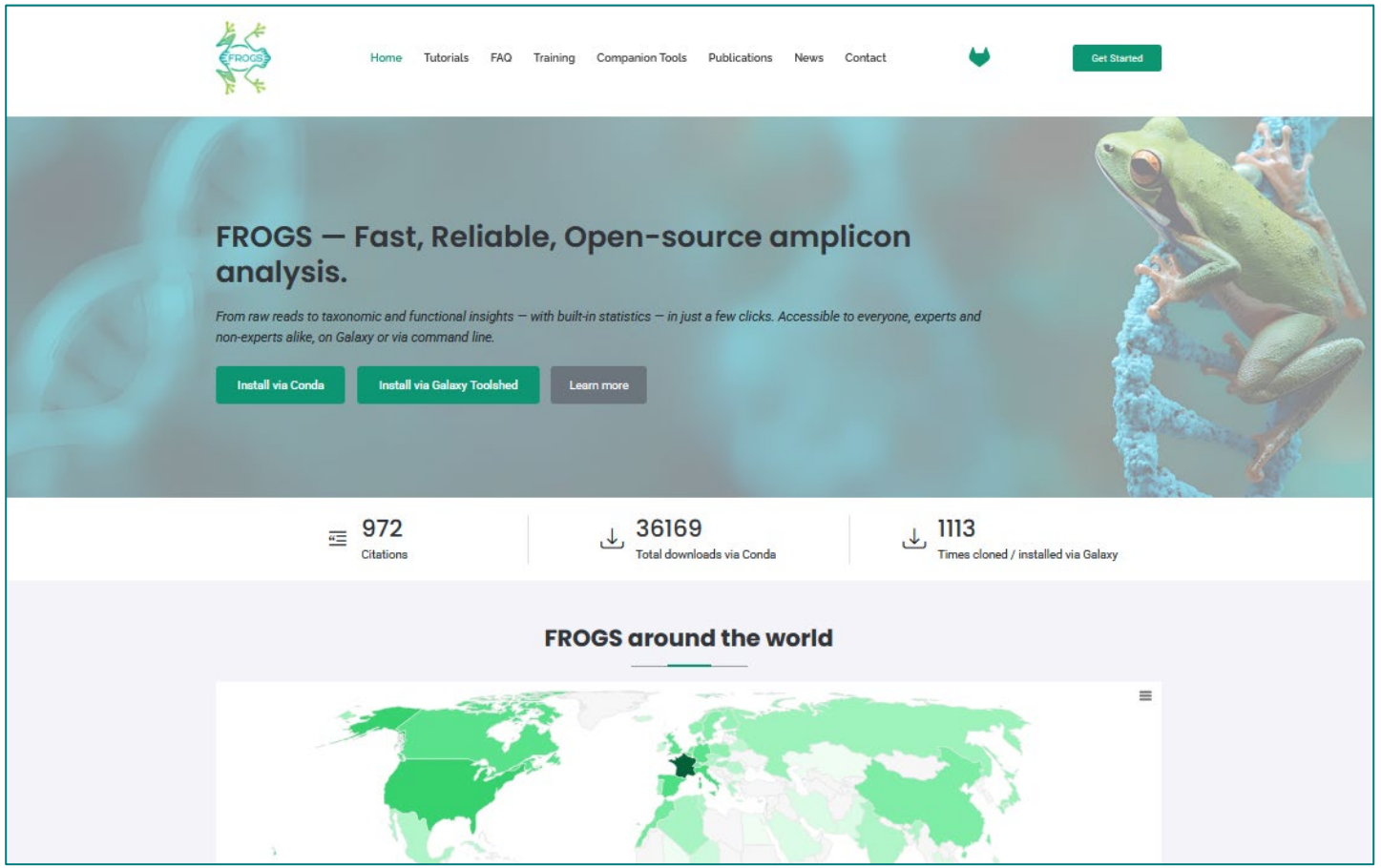
Introduction

Using amplicon sequencing to describe the microbial composition of an environment is a time saving and cost-effective strategy and can be used even for very large-scale surveys [1]. Most studies currently focus on the bacterial fraction of microbial communities but the fungal fraction is equally important, as fungi are ubiquitous and provide several ecosystem services [2]. Unfortunately, studying the fungal fraction using metabarcoding has its own challenges. Indeed, in fungi, there is no equivalent of the 16S rRNA gene, which is widely used and highly suitable for bacteria. The best candidates are internal transcribed spacers (ITS), but these are more difficult to manipulate. The main problem with ITS is size polymorphism, with a size range of 361–1475 bases in UNITE 7.1 [3] (unlike 16S where 95% of the sequences have a length between 1205 and 1556 bases). Most studies describing ITS data analyses process either (i) paired-end reads but filter out non-overlapping, non-mergeable reads, thus systematically discarding taxa with longer ITS, or (ii) single-end reads, thus limiting taxonomic resolution and losing the benefit of information contained in longer sequences [4, 5].

Maria Bernard is a bioinformatics engineer. She is a member of a platform team conducting NGS sequence analysis and designing software. She specializes in workflow development in particular for metabarcoding analysis.
Olivier Rué is a bioinformatics engineer. He is in charge of data analysis at the Migale bioinformatics facility. He specializes in the analysis of metabarcoding and metagenomics data.
Mahendra Mariadassou has a PhD in statistics. He is involved in the development of new statistical methods and tools for metabarcoding analysis.
Géraldine Pascal has a PhD in bioinformatics and coordinates the FROGS project. She is currently involved in designing solutions for long read problems, workflow development and metagenomics analysis.
Submitted: 19 April 2021; **Received (in revised form):** 19 July 2021

https://frogs.inrae.fr

FROGS website



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