IntegronFinder Documentation

Release 2.0rc6

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IntegronFinder is a program that detects integrons in DNA sequences. The program is available on a webserver *Galaxy Pasteur*, or by command line (IntegronFinder on github).

- You already read the *paper* and want to install it ? Click *here*
- You did not read the paper (yet) but you would like to have rapid introduction to integrons and the program? click *here*

CHAPTER

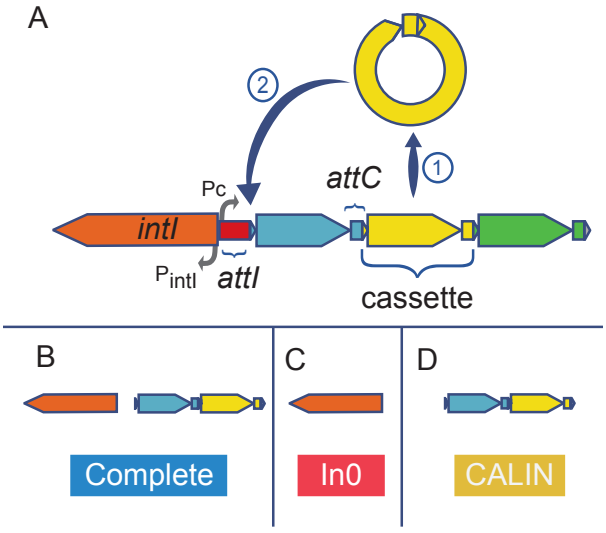
ONE

USER GUIDE

1.1 User Guide

1.1.1 Introduction

Integrons are major genetic element, notorious for their major implication in the spread of antibiotic resistance genes. More generally, integrons are gene-capturing platform, whose broader evolutionary role remains poorly understood. IntegronFinder is able to detect with high accuracy integron in DNA sequences. It is accurate because it combines the use of HMM profiles for the detection of the essential protein, the site-specific integron integrase, and the use of Covariance Models for the detection of the recombination site, the *attC* site.



How does it work ?

For each sequence in the input file:

- First, IntegronFinder annotates the DNA sequence's CDS with Prodigal.
- Second, IntegronFinder detects independently integron integrase and *attC* recombination sites. The Integron integrase is detected by using the intersection of two HMM profiles:

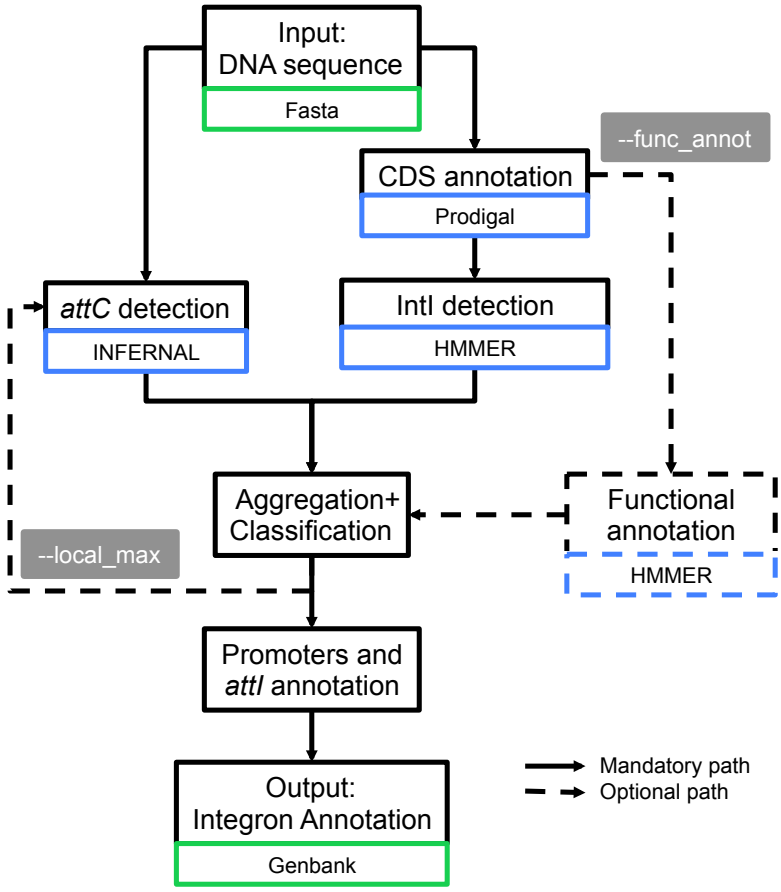
  - one specific of tyrosine-recombinase (PF00589)
  - one specific of the integron integrase, near the patch III domain of tyrosine recombinases.

The *attC* recombination site is detected with a covariance model (CM), which models the secondary structure in addition to the few conserved sequence positions.

- Third, the results are integrated, and IntegronFinder distinguishes 3 types of elements:
  - **complete integron (panel B above)** Integron with integron integrase nearby *attC* site(s)
  - **In0 element (panel C above)** Integron integrase only, without any *attC* site nearby
  - **CALIN element (panel D above)** Cluster of *attC* sites Lacking INtegrase nearby. A rule of thumb to avoid false positive is to filter out singleton of *attC* site.

IntegronFinder can also annotate gene cassettes (CDS nearby *attC* sites) using Resfams, a database of HMM profiles aiming at annotating antibiotic resistance genes. This database is provided but the user can add any other HMM profiles database of its own interest.

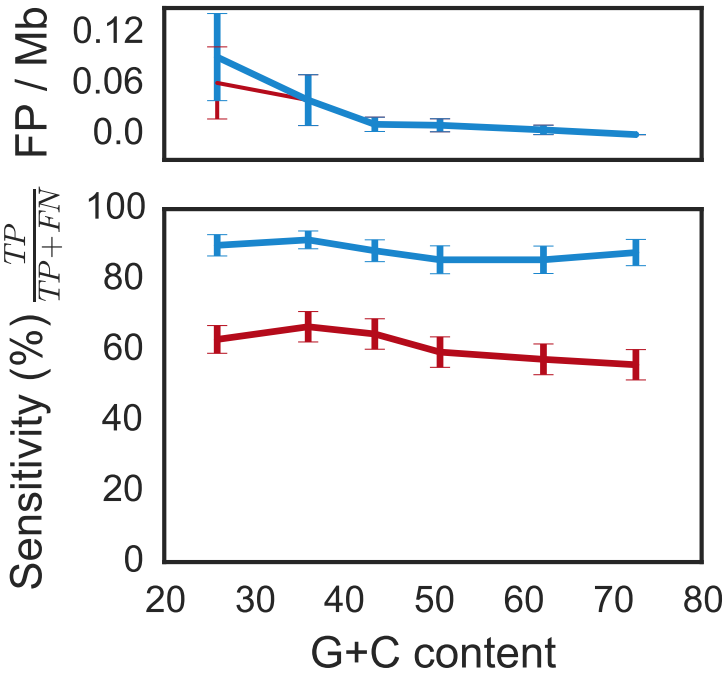
When available, IntegronFinder annotates the promoters and attI sites by pattern matching.



Does it work ?

Yes! The estimated sensitivity is 61% on average with the default option and goes up to 88% with the `--local_max` option. The missing *attC* sites are usually at the end of the array. The False positive rate with the `--local_max` option is estimated between 0.03 False Positive per Megabases (FP/Mb) to 0.72 FP/Mb. This leads to a probability of finding 2 consecutive false *attC* sites within 4kb between 4.10^-6 and 7.10^-9. Overall, the probability of finding an integron in a chromosome (including finding a part of it) is more than 95%. Finally, these parameters do not depend on the G+C percent of the given replicon. See the paper for more information (freely accessible).

	Default	local_max
Sensitivity	61.20%	88.03%
FP rate	0.02 FP/Mb	0.03 FP/Mb
Mean time	2.59 s	86.59 s



The time in the table correspond to the average time per run with a pseudogenome having attC sites on a Mac Pro, 2 x 2.4 GHz 6-Core Intel Xeon, 16 Gb RAM, with options –cpu 20 and –no-proteins.

Note: The time does not vary depending of the mode (default or local_max), and is about a couple of second, if the replicon does not contain any *attC* site.

1.1.2 Installation

IntegronFinder dependencies

IntegronFinder is built with Python >= 3.4, and a few libraries are needed:

- Python >=3.4
- Pandas (>=0.22)
- Numpy (>=1.14.2)
- Biopython (>=1.70)
- Matplotlib (>=2.2.2)
- colorlog

From version 1.5.1, integron_finder will check and install theses libraries for you.

In addition, IntegronFinder has external dependencies, which have to be installed prior the use of the program (click to access the corresponding website).

- HMMER 3.1b2
- INFERNAL 1.1
- Prodigal V2.6.2
- Nextflow (for parallelization)

After installation of these programs, they should be in your `$PATH` (*i.e.* you can type in a terminal `hmmsearch`, `cmsearch`, or `prodigal` and a `command not found` shall not be displayed). If you have them installed somewhere else, please refer to integron_finder's parameters to give complete path to IntegronFinder.

Installation procedure

Warning: When installing a new version (up to 2.0 included) of IntegronFinder, do not forget to *uninstall* the previous version installed !

Warning: If You upgrading from version prior to 2.0 to 2.0 be careful the python used changed for 3.x. The python 2.7 is not supported anymore. So if you installed `integron_finder` within a virtualenv you need to create a new one based on python3.

From Version 2.0

System wide installation

1. Open a terminal and hit:

```
sudo pip install integron_finder
```

Warning: On recent Debian/Ubuntu the –user option is forced. So use of –root option give an unexpected behavior and you cannot use –prefix option at all unless you add option –system for instance

```
sudo pip install --system integron_finder
```

or

```
pip install --prefix=/tmp/test_if --system integron_finder
```

2. To get an updated version (no need to uninstall):

```
sudo pip install -U integron_finder
```

User wide installation

1. Open a terminal and hit:

```
pip install --user integron_finder
```

Installation in a virtualenv

The virtual environment (virtualenv) is a system to isolate a python program from the system and avoid libraries conflict. So you can install a different python or libraries version than your system in each virtualenv. So if you update the system it will not change anything for your program and *vice versa*. If you want to remove the program just remove the virtual environment.

Create a virtual environment:

```
python3 -m venv Integron_Finder
```

or on some systems:

```
virtualenv -p python3 Integron_Finder
```

activate you virtualenv:

```
source Integron_Finder/bin/activate
```

The name of the virtualenv appear in parenthesis at the beginning of the prompt. Then install integron_finder:

```
pip install integron_finder
```

To run integron finder, you have to activate (once per session) the virtual environment:

```
source  Integron_Finder/bin/activate
```

When you do not need to use integron_finder just deactivate the virtual environment. In the active terminal just type:

```
deactivate
```

The integron_finder command will disappear from the path. The name of the virtualenv disappear from the prompt.

Conda Package

From 2.0 version, Integron_Finder is available as conda package. Integron_finder is in bioconda From 2.0 version, Integron_Finder is available as [conda](https://conda.io/docs/index.html) package. Integron_finder is in [bioconda](https://bioconda.github.io/) channel. (The advantage with this solution is that it will install prodigal, hmmer, and infernal too.)

1. install conda
2. Set up channels

   ```
   conda config --add channels defaults
   conda config --add channels conda-forge
   conda config --add channels bioconda
   ```

3. install integron_finder

   ```
   conda install integron_finder
   ```

   (The advantage with this solution is that it will install prodigal, hmmer, and infernal too.)

From Version 1.5.1 and after

1. Open a terminal and hit:

```
(sudo) pip install integron_finder
```

2. To get an updated version (no need to uninstall):

```
(sudo) pip install -U integron_finder
```

For Version 1.5 and before

1. Download the latest release that can be installed like this (v1.5)
2. Uncompress it
3. In a shell (*e.g.* a terminal), go to the directory and run:

```
(sudo) python setup.py install
```

Note: Super-user privileges (*i.e.*, `sudo`) are necessary if you want to install the program in the general file architecture.

Note: If you do not have the privileges, or if you do not want to install IntegronFinder in the Python libraries of your system, you can install IntegronFinder in a virtual environment. See virtualenv or if you're using Canopy, see Canopy CLI

Warning: The installer does not work with pure setuptools procedure, it does not work in egg. Unless you disable egg by using the `--root` option. `python setup.py install --root /prefix/where/to/install/integron_finder`

Uninstallation procedure

From Version 1.5.1 and after

To uninstall IntegronFinder, run in the following command:

```
(sudo) pip uninstall integron_finder
```

It will uninstall integron_finder executable

From Version 1.0 to Version 1.5

Go to the directory from where you installed IntegronFinder (e.g. Integron_Finder-1.5), and run:

```
(sudo) python setup.py uninstall
```

How to install Python

The purpose of this section is to provide some help about installing python dependencies for IntegronFinder if you never installed any python package.

As IntegronFinder has not been tested on Windows, we assume Unix-based operating system. For Windows users, the best would be to install a unix virtual machine on your computer.

Usually a python distribution is already installed on your machine. However, if you don't know how to install libraries, we recommend to re-install it from a distribution which contains pre-compiled libraries. There are two main distributions (click to access website):

- Enthought Canopy
- Anaconda

Download version 3.x which correspond to your machine, then make sure that python from these distributions is the default one (you can possibly choose that in the preference and/or during installation). Make sure Biopython is installed, otherwise, you will have to install Biopython. `pip` or `conda` are recommended as a python packages installer.

It works as follow:

```
(sudo) pip install Biopython==1.71
```

To install version 1.71 of Biopython (recommended for IntegronFinder).

Note: If you don't manage to install all the packages, try googling the error, or don't hesitate to ask a question on stackoverflow.

1.1.3 What's new ?

In Version 2.0

Here are the major changes between versions 1.x and 2.0. Essentially, it has be designed such as it becomes easier to find integrons with high confidence in huge datasets (but it works also for small datasets).

- IntegronFinder now accepts multifasta files as input.

- Only three files are created by default (see output section for details about the other possible output files):
  - A file with all integrons and their elements detected in all sequences in the input file.
  - A summary file with the number and type of integrons per sequence.
  - A file with standard output
- IntegronFinder can be run in parallel easily with a provided Nextflow script that is (almost) ready to use.
- We diversify the installation methods, so it can be easily deployed on a variety of machine. Notably, we built a singularity container which will allow a smooth installation on clusters.
- CALINs are now reported when they have at least 2 *attC* sites (instead of 1 before). This value can be changed by the user with *–calin-threshold x*
- Promoters and attI sites are not detected by default to increase speed
- It is now easy to obtain multiple alignements of detected attC sites
- Improve the documentation, especially on the developer part so anyone can contribute.
- Add unit (or non regression) tests.

1.1.4 Quick start

We assume here that the program is *installed*.

You can see all available options with:

```
integron_finder -h
```

For impatient

Go to the directory containing your input file(s), or specify the path to that file and call:

```
integron_finder mysequences.fst
```

or:

```
integron_finder path/to/mysequences.fst
```

It will perform a search, and outputs the results in a directory called `Results_Integron_Finder_mysequences`.

Input and Outputs

Inputs

`integron_finder` can take as an input:

- a fasta file
- a multi-fasta file
- many (multi-)fasta files

Outputs

By default, `integron_finder` will output 3 files under `Results_Integron_Finder_mysequences`:

- `mysequences.integrons` : A file with all integrons and their elements detected in all sequences in the input file.
- `mysequences.summary` : A summary file with the number and type of integrons per sequence.
- `integron_finder.out` : A copy standard output. The stdout can be silenced with the argument `--mute`

The amount of log in the standard output can be controlled with `--verbose` for more or `--quiet` for less, and both are cumulative arguments, eg. `-vv` or `-qq`.

Other files can be created on demand:

- `--gbk`: Creates a Genbank files with all the annotations found (present in the `.integrons` file)
- `--pdf`: Creates a simple pdf graphic with complete integrons
- `--split-results`: Creates a `.integrons` a `.summary` file per replicon if the input is a multifasta file.
- `--keep-tmp`: Keep temporary files. See *Keep intermediate files* for more.

For everyone

Note: The different options will be shown separately, but they can be used altogether unless otherwise stated.

Thorough local detection

This option allows a much more sensitive search of *attC* sites. It will be slower if integrons are found, but will be as fast if nothing is detected.

```
integron_finder mysequences.fst --local-max
```

CALIN detection

By default CALIN are reported if they are composed of at least 2 *attC* sites, in order to avoid false positives. This value was chosen as CALIN with 2 attC sites were unlikely to be false positive. The probability of a false CALIN with at least 2 attC sites within 4kb was estimated between 4.10^-6 and 7.10^-9. However, one can modify this value with the option *–calin-threshold* and use a lower or higher value depending on the risk one is willing to take:

```
integron_finder mysequences.fst --calin-threshold 1
```

Note: If `--local-max` is called, it will run around CALINs with single attC sites, even if `--calin-threshold` is 2. The filtering step is done after the search with local max in that case.

Functional annotation

This option allows to annotate cassettes given HMM profiles. As Resfams database is distributed, to annotate antibiotic resistance genes, just use:

```
integron_finder mysequences.fst --func-annot
```

IntegronFinder will look in the directory `Integron_Finder-x.x/data/Functional_annotation` and use all `.hmm` files available to annotate. By default, there is only `Resfams.hmm`, but one can add any other HMM file here. Alternatively, if one wants to use a database which is present elsewhere on the user's computer without copying it into that directory, one can specify the following option

```
integron_finder mysequences.fst --path_func_annot bank_hmm
```

where `bank_hmm` is a file containing one absolute path to a hmm file per line, and you can comment out a line

```
~/Downloads/Integron_Finder-x.x/data/Functional_annotation/Resfams.hmm
~/Documents/Data/Pfam-A.hmm
# ~/Documents/Data/Pfam-B.hmm
```

Here, annotation will be made using Pfam-A et Resfams, but not Pfam-B. If a protein is hit by 2 different profiles, the one with the best e-value will be kept.

Search for promoter and *attI* sites

By default `integron_finder` look for *attC* sites and site-specific integron integrase,, If you want to search for known promoters (integrase, Pc-int1 and Pc-int3) and AttI sites in integrons elements you need to add the `--promoter-attI` option on the command line.

Keep intermediate results

Integrons finder needs some intermediate results to run completely. It includes notably the protein file in fasta (mysequences.prt), but also the outputs from hmmer and infernal. A folder containing these outputs is generated for each replicon and have name `tmp_<replicon_id>` This directory is removed at the end. You can keep this directory to analyse further each `integron_finder` steps with the option `--keep-tmp`. Using this argument allows you to rerun `integron_finder` on the same sequences without redetecting proteins and attC sites. It is useful if one wants to change clustering parameters, evalues of attC sites, or size of them. Note that it won't search for new attC sites so it is better to start with relaxed parameters and then rerun `integron_finder` with more strict parameters. See the section *for integron diggers* for more informations

For each tmp file, there are:

- `<replicon_id>.fst`: a single fasta file with the replicon_name
- `<replicon_id>.prt`: a multifasta file with the sequences of the detected proteins.
- `<replicon_id>_intI_table.res`: hmm result for the intI hmm profile in tabular format
- `<replicon_id>_intI.res`: hmm result for the intI hmm profile
- `<replicon_id>_phage_int_table.res`: hmm result for the tyrosine recombinase hmm profile in tabular format
- `<replicon_id>_phage_int.res`: hmm result for the tyrosine recombinase hmm profile in tabular format
- `<replicon_id>_attc_table.res`: cmsearch result for the attC sites covariance model in tabular format

- `<replicon_id>_attc.res`: significant (according to `evalue-attc`) attC sites aligned in stockholm format
- `integron_max.pickle`: pickle file so `integron_finder` reuse this instead of re-running the local_max part

Topology

By default, IntegronFinder assumes that

- your replicon is considered as **circular** if there is **only one replicon** in the input file.
- your replicons are considered as **linear** if there are **several replicons** in the input file.

However, you can change this default behavior and specify the default topology with options `--circ` or `--lin`:

```
integron_finder --lin mylinearsequence.fst
integron_finder --circ mycircularsequence.fst
```

If you have multiple replicon in the input file with different topologies you can specify a topology for each replicon by providing a topology file. The syntax for the topology file is simple:

- one topology by line
- one line start by the seqid followed by 'circ' or 'lin' for circular or linear topologies.

example:

```
seq_id_1 circ
seq_id_2 lin
```

You can also mix the options `--circ` or `--lin` with option `--topology-file`:

```
integron_finder --circ --topology-file path/to/topofile mysequencess.
↪fst
```

In the example above the default topology is set to *circular*. The replicons specified in topofile supersede the default topology.

Warning: However, if the replicon is smaller than `4 x dt` (where `dt` is the distance threshold, so 4kb by default), the replicon is considered linear to avoid clustering problem. The topology used to searching integron is report in the **.integrons file*

For big data people

Parallelization

The time limiting part are HMMER (search integrase) and INFERNAL (search *attC* sites). So if you have to analyze one or few replicons the user can set the number of CPU used by HMMER and INFERNAL:

```
integron_finder mysequences.fst --cpu 4
```

Default is 1.

If you want to deal with a fasta file with a lot of replicons (from 10 to more than thousand) we provide a workflow to parallelize the execution of the data. This mean that we cut the data input into chunks (by default of one replicon) then execute IntegronFinder in parallel on each replicon (the number of parallel tasks can be limited) then aggregate the results in one global summary. The workflow use the nextflow framework and can be run on a single machine or a cluster.

First, you have to install nextflow first, and *integron_finder*. Then we provide 2 files (you need to download them from the IntegronFinder github repo.)

- *parallel_integron_finder.nf* which is the workflow itself in nextflow syntax
- *nextflow.config* which is a configuration file to execute the workflow.

The workflow file should not be modified. Whereas the profile must be adapted to the local architecture.

The file *nextflow.config* provide for profiles:

- a standard profile for local use
- a cluster profile
- a standard profile using singularity container
- a cluster profile using singularity container

Warning: On Ubuntu Bionic Beaver (18.04) The default java is not suitable to run nextflow So you have to install another jvm

> sudo add-apt-repository ppa:webupd8team/java sudo apt-get update sudo apt-get install oracle-java8-installer

for more details see: https://medium.com/coderscorner/installing-oracle-java-8-in-ubuntu-16-10-845507b13343

so now install nextflow. If you have capsule error like

```
CAPSULE EXCEPTION: Error resolving dependencies. while processing␣
↪attribute Allow-Snapshots: false (for stack trace, run with -
↪Dcapsule.log=verbose)
Unable to initialize nextflow environment
```

install nextflow (>=0.29.0) as follow (change the nextflow version with the last release)

```
wget -O nextflow http://www.nextflow.io/releases/v0.30.2/nextflow-0.
↪30.2-all
chmod 777 nextflow
```

for more details see: https://github.com/nextflow-io/nextflow/issues/770#issuecomment-400384617

How to get parallel_integron_finder

The release contains the workflow *parallel_integron_finder.nf* and the *nextflow.config* at the top level of the archive But If you use pip to install Integron_Finder you have not easily access to them. But they can be downloaded or executed directly by using nextflow.

to download it

```
nextflow pull gem-pasteur/Integron_Finder
```

to get the latest version or use *-r* option to specify a version

```
nextflow pull -r release_2.0 gem-pasteur/Integron_Finder
```

to see what you download

```
nextflow see Integron_Finder
```

to execute it directly

```
nextflow run gem-pasteur/Integron_Finder -profile standard --replicons␣
↪all_coli.fst --circ
```

or:

```
nextflow run -r release_2.0 gem-pasteur/Integron_Finder -profile␣
↪standard --replicons all_coli.fst --circ
```

standard profile

This profile is used if you want to parallelize IntegronFinder on your machine. You can specify the number of tasks in parallel by setting the queueSize value

```
standard {
        executor {
            name = 'local'
            queueSize = 7
        }
        process{
            executor = 'local'
            $integron_finder{
                errorStrategy = 'ignore'
                cpu=params.cpu
            }
        }
 }
```

If you installed IntegronFinder with singularity, just uncomment the container line in the script, and set the proper path to the container.

All options available in non parallel version are also available for the parallel one. except the `--outdir` which is not available and `--replicons` option which is specific to the parallelized version. `--replicons` allows to specify the path of a file containing the replicons.

A typical command line will be:

```
./parallel_integron_finder.nf -profile standard --replicons all_coli.
↪fst --circ
```

Note: Joker as * or ? can be used in path to specify several files as input.

But **do not forget** to protect the wild card from the shell for instance by enclosing your glob pattern with simple quote.

```
nextflow run -profile standard parallel_integron_finder.nf --replicons
↪'replicons_dir/*.fst'
```

Two asterisks, i.e. **, works like * but crosses directory boundaries. Curly brackets specify a collection of sub-patterns.

```
nextflow run -profile standard parallel_integron_finder.nf --replicons
↪'data/**.fa'
nextflow run -profile standard parallel_integron_finder.nf --replicons
↪'data/**/*.fa'
nextflow run -profile standard parallel_integron_finder.nf --replicons
↪'data/file_{1,2}.fa'
```

The first line will match files ending with the suffix *.fa* in the *data* folder and recursively in all its sub-folders. While the second one only match the files which have the same suffix in any sub-folder in the data path. Finally the last example capture two files: *data/file_1.fa*, *data/file_2.fa*

More than one path or glob pattern can be specified in one time using comma. **Do not** insert spaces surrounding the comma

```
nextflow run -profile standard parallel_integron_finder --replicons
↪'some/path/*.fa,other/path/*.fst'
```

The command above will analyze all files ending by *.fa* in */some/path* with *.fst* extension in *other/path*

For further details see: https://www.nextflow.io/docs/latest/channel.html#frompath

---

Note: The option *–outdir* is not allowed. Because you can specify several replicon files as input, So in this circumstances specify only one name for the output is a none sense.

Note: The options starting with one dash are for nextflow workflow engine, whereas the options starting by two dashes are for integron_finder workflow.

Note: Replicons will be considered linear by default (see above), here we use *–circ* to consider replicons circular.

Note: If you specify several input files, the split and merge steps will be parallelized.

If you execute this line, 2 kinds of directories will be created.

- One named *work* containing lot of subdirectories this for all jobs launch by nextflow.
- Directories named *Results_Integron_Finder_XXX* where XXX is the name of the replicon file. So, one directory per replicon file will be created. These directories contain the final results as in non parallel version.

cluster profile

The cluster profile is intended to work on a cluster managed by SLURM. If You cluster is managed by an other drm change executor name by the right value (see nextflow supported cluster)

You can also managed

- The number of task in parallel with the *executor.queueSize* parameter (here 500). If you remove this line, the system will send in parallel as many jobs as there are replicons in your data set.

- The queue with *process.queue* parameter (here common,dedicated)
- and some options specific to your cluster management systems with *process.clusterOptions* parameter

```
cluster {
    executor {
        name = 'slurm'
        queueSize = 500
    }

    process{
        executor = 'slurm'
        queue= 'common,dedicated'
        clusterOptions = '--qos=fast'
        $integron_finder{
            cpu=params.cpu
        }
    }
}
```

To run the parallel version on cluster, for instance on a cluster managed by slurm, I can launch the main nextflow process in one slot. The parallelization and the submission on the other slots is made by nextflow itself. Below a command line to run parallel_integron_finder and use 2 cpus per integron_finder task, each integron_finder task can be executed on different machines, each integron_finder task claim 2 cpus to speed up the attC sites or integrase search:

```
sbatch --qos fast -p common nextflow run  parallel_integron_finder.nf -
↪profile cluster --replicons all_coli.fst --cpu 2 --local-max --gbk --
↪circ
```

The results will be the same as describe in local execution.

singualrity profiles

If you use the singularity integron_finder image, use the profile *standard_singularity*. With the command line below nextflow will download parallel_integron_finder from github and download the integron_finder image from the singularity-hub so you haven't to install anything except nextflow and singularity.

```
nextflow run gem-pasteur/Integron_Finder -profile standard_singularity␣
↪--replicons all_coli.fst --circ
```

You can also use the integron_finder singularity image on a cluster, for this use the profile *cluster_singularity*.

```
sbatch --qos fast -p common nextflow run  gem-pasteur/Integron_
↪Finder:2.0 -profile cluster_singualrity --replicons all_coli.fst --
↪cpu 2 --local-max --gbk --circ
```

In the case of your cluster cannot reach the world wide web. you have to download the singularity image

```
singularity pull --name Integron_Finder shub://gem-pasteur/integron_
↪finder:2.0
```

the move the image on your cluster modify the nextflow.config to point on the location of the image, and adapt the cluster options (executor, queue, . . .) to your architecture

```
 cluster_singularity {
        executor {
            name = 'slurm'
            queueSize = 500
        }

        process {
            container = /path/to/integron_finder
            queue = 'common,dedicated'
            clusterOptions = '--qos=fast'
            withName: integron_finder {
                cpus = params.cpu
            }
        }
        singularity {
            enabled = true
            runOptions = '-B /pasteur'
            autoMounts = false
       }
    }
}
```

then run it

```
sbatch --qos fast -p common nextflow run  ./parallel_integron_finder.
↪nf -profile cluster_singualrity --replicons all_coli.fst --cpu 2 --
↪local-max --gbk --circ
```

If you want to have more details about the jobs execution you can add some options to generate report:

Execution report

To enable the creation of this report add the `-with-report` command line option when launching the pipeline execution. For example:

```
nextflow run  ./parallel_integron_finder.nf -profile standard -with-
↪report [file name] --replicons
```

It creates an HTML execution report: a single document which includes many useful metrics about a workflow execution. For further details see https://www.nextflow.io/docs/latest/tracing.html#execution-report

Trace report

In order to create the execution trace file add the `-with-trace` command line option when launching the pipeline execution. For example:

```
nextflow run  ./parallel_integron_finder.nf -profile standard -with-
↪trace --replicons
```

It creates an HTML timeline for all processes executed in your pipeline. For further details see https://www.nextflow.io/docs/latest/tracing.html#timeline-report

Timeline report

To enable the creation of the timeline report add the `-with-timeline` command line option when launching the pipeline execution. For example:

```
nextflow run  ./parallel_integron_finder.nf -profile standard -with-
↪timeline [file name] --replicons ...
```

It creates an execution tracing file that contains some useful information about each process executed in your pipeline script, including: submission time, start time, completion time, cpu and memory used. For further details see https://www.nextflow.io/docs/latest/tracing.html#trace-report

For integron diggers

Many options are set to prevent false positives. However, one may want higher sensitivity at the expense of having potentially false positives. Ultimately, only experimental experiments will tell whether a given *attC* sites or integrase is functional.

Also, note that because of how local_max works (ie. around already detected elements), true *attC* sites may be found thanks to false *attC* sites, because false *attC* sites may trigger local_max around

them. Hence, one may want to use very relaxed parameters first with the `--keep-tmp` flag to rerun the analysis on the same data while restrincting the parameters.

Clustering of elements

attC sites are clustered together if they are on the same strand and if they are less than 4 kb apart (`-dt 4000` by default). To cluster an array of *attC* sites and an integron integrase, they also must be less than 4 kb apart. This value has been empirically estimated and is consistent with previous observations showing that biggest gene cassettes are about 2 kb long. This value of 4 kb can be modified though:

```
integron_finder mysequences.fst --distance-thresh 10000
```

or, equivalently:

```
integron_finder mysequences.fst -dt 10000
```

This sets the threshold for clustering to 10 kb.

Note: The option `--outdir` allows you to chose the location of the Results folder (`Results_Integron_Finder_mysequences`). If this folder already exists, IntegronFinder will not re-run analyses already done, except functional annotation. It allows you to re-run rapidly IntegronFinder with a different `--distance-thresh` value. Functional annotation needs to re-run each time because depending on the aggregation parameters, the proteins associated with an integron might change.

Integrase

We use two HMM profiles for the detection of the integron integrase. One for tyrosine recombinase and one for a specific part of the integron integrase. To be specific we use the intersection of both hits, but one might want to use the union of both hits (and sees whether it exists cluster of attC sites nearby non integron-integrase...). To do so, use:

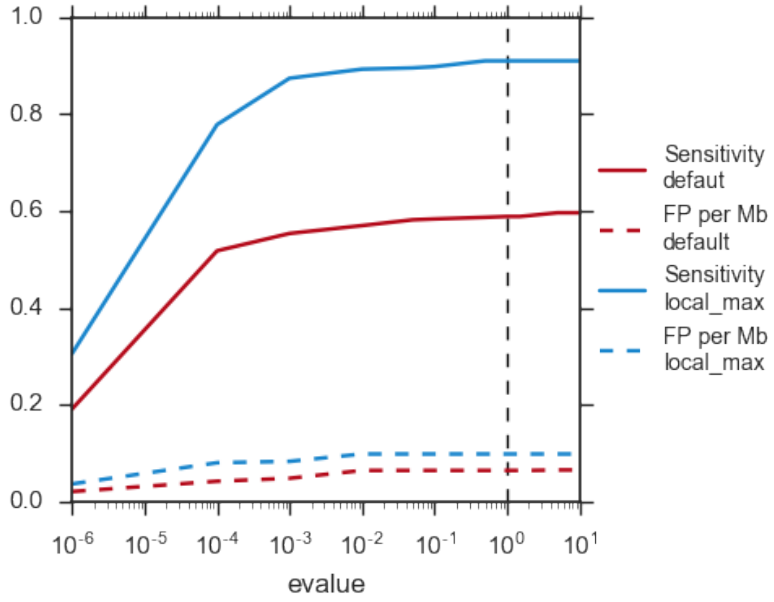
```
integron_finder mysequences.fst --union-integrases
```

attC evalue

The default evalue is 1. Sometimes, degenerated *attC* sites can have a evalue above 1 and one may want to increase this value to have a better sensitivity.

```
integron_finder mysequences.fst --evalue-attc 5
```

Here is a plot of how the sensitivity and false positive rate evolve as a function of the evalue:



Note: If one wants to have maximum sensitivity, use a high evalue (max is 10), and then integron_finder can be run again on the same data with a lower evalue. It won't work the other way around (starting with low evalue), as attC sites are not searched again.

attC size

By default, *attC* sites' size ranges from 40 to 200bp. This can be changed with the `--min-attc-size` or `--max-attc-size` parameters:

```
integron_finder mysequences.fst --min-attc-size 50 --max-attc-size 100
```

Palindromes

attC sites are more or less palindromic sequences, and sometimes, a single *attC* site can be detected on the 2 strands. By default, the one with the highest evalue is discarded, but you can choose to keep them with the following option:

```
integron_finder mysequences.fst --keep-palindromes
```

attC alignements

One can get the alignements of *attC* sites in the temporary files (use `--keep-tmp`) to have them. Under `Results_Integron_Finder_mysequences/tmp_repliconA/repliconA_attc.res` one can find alignements of *attC* sites from repliconA, in Stokholm format, where R and L core regions are aligned with each others:

```
# STOCKHOLM 1.0
#=GF AU Infernal 1.1.2

ACBA.0917.00019.0001/315102-315161          GUCUAACAAUUC---
 ↪GUUCAAGCcgacgccgcu.....................................................
 ↪ucgcggcgcgGCUUAACUCAAGC----GUUAGAU
#=GR ACBA.0917.00019.0001/315102-315161 PP ************...
 ↪******************.....................................................
 ↪************************....*******
ACBA.0917.00019.0001/313260-313368          ACCUAACAAUUC---
 ↪GUUCAAGCcgagaucgcuucgcggccgcggaguuguucggaaaaauugucacaacgccgcggccgcaaagcgcuccgGCUU
 ↪---GUUGGGC
#=GR ACBA.0917.00019.0001/313260-313368 PP ************...
 ↪************************************************************************************
 ↪...*******
ACBA.0917.00019.0001/313837-313906          GCCCAACAUGGC---
 ↪GCUCAAGCcgaccggccagcccu.......................................
 ↪gcgggcuguccgucgGCUUAGCUAGGGC----GUUAGAG
#=GR ACBA.0917.00019.0001/313837-313906 PP ************...
 ↪**********************.....................................
 ↪****************************....*******
#=GC SS_cons                                <<<<<<<--------<<<-<<<<.....
 ↪.....................................................................>>>>>
 ↪>>----------->>>>>>>
#=GC RF                                     [Rsec=]========[=Lsec=].....
 ↪.....................................................................
 ↪[Lprim]==========[Rprim]
//
```

Which you can manipulate easily with `esl-alimanip` tools provided by infernal (the following examples should work if your `cmsearch` is in your `PATH`). You can convert the same alignement in dna alphabet (cmsearch use RNA alphabet):

```
$ esl-alimanip --dna Results_Integron_Finder_mysequences/tmp_ACBA.0917.
 ↪00019.0001/ACBA.0917.00019.0001_attc.res
# STOCKHOLM 1.0
#=GF AU Infernal 1.1.2

ACBA.0917.00019.0001/315102-315161          GTCTAACAATTC---
 ↪GTTCAAGCCGACGCCGCT-----------------------------------------------------
 ↪TCGCGGCGCGGCTTAACTCAAGC----GTTAGAT
```

(continues on next page)

(continued from previous page)

```
#=GR ACBA.0917.00019.0001/315102-315161 PP ************...
 ↪******************.....................................................
 ↪************************....********
ACBA.0917.00019.0001/313260-313368         ACCTAACAATTC---
 ↪GTTCAAGCCGAGATCGCTTCGCGGCCGCGGAGTTGTTCGGAAAAATTGTCACAACGCCGCGGCCGCAAAGCGCTCCGGCTT
 ↪---GTTGGGC
#=GR ACBA.0917.00019.0001/313260-313368 PP ************...
 ↪*********************************************************************************
 ↪...********
ACBA.0917.00019.0001/313837-313906         GCCCAACATGGC---
 ↪GCTCAAGCCGACCGGCCAGCCCT---------------------------------------
 ↪GCGGGCTGTCCGTCGGCTTAGCTAGGGC----GTTAGAG
#=GR ACBA.0917.00019.0001/313837-313906 PP ************...
 ↪************************.....................................
 ↪*****************************....********
#=GC SS_cons                                <<<<<<<--------<<<-<<<<.....
 ↪.....................................................................>>>>>
 ↪>>----------->>>>>>>
#=GC RF                                     [Rsec=]========[=Lsec=].....
 ↪.....................................................................
 ↪[Lprim]==========[Rprim]
//
```

You can also convert it to fasta format:

```
$ esl-alimanip --dna --outformat afa Results_Integron_Finder_
 ↪mysequences/tmp_ACBA.0917.00019.0001/ACBA.0917.00019.0001_attc.res
>ACBA.0917.00019.0001/315102-315161
GTCTAACAATTC---GTTCAAGCCGACGCCGCT--------------------------
----------------------TCGCGGCGCGGCTTAACTCAAGC----GTTAGAT
>ACBA.0917.00019.0001/313260-313368
ACCTAACAATTC---GTTCAAGCCGAGATCGCTTCGCGGCCGCGGAGTTGTTCGGAAAAA
TTGTCACAACGCCGCGGCCGCAAAGCGCTCCGGCTTAACTCAGGC----GTTGGGC
>ACBA.0917.00019.0001/313837-313906
GCCCAACATGGC---GCTCAAGCCGACCGGCCAGCCCT----------------------
-----------------GCGGGCTGTCCGTCGGCTTAGCTAGGGC----GTTAGAG
```

The possible outformat are:

- stockholm
- pfam
- a2m
- psiblast
- afa

1.1.5 web server

Galaxy

You can access IntegronFinder online, on the Galaxy server of the Pasteur institute

How to use it

Registration on the Galaxy server of the Pasteur institute is not required to use the tool. Yet, if you wish to keep your history, we recommend you to register.

1. Upload your sequence with **Get Data - Upload File** in the menu on the left
2. Select your file in the **Replicon file** list of Integron Finder
3. Select the options you want
4. Click on **Execute**

If you want more options:

3. Select **Show** on advanced parameters
4. Select the options you want
5. Click on **Execute**

You can see the role of the different functions in the *tutorial* page.

Results

Once the job is finished, you get your results on right panel. All files contain the log of the run which tells you how many integrons have been found for each types along with the number of *attC* sites per type. There are 4 different outputs created:

- **Raw results archive:** An archive containing all raw results.
- **Integrons annotations:** A tabular file listing all the elements and their caracteristics.
- **GenBank:** The GenBank file of the input sequence with the annotation corresponding to the elements found (integrase, *attC*, promoter, attI, etc. . .).
- **Graphics:** Simple representation of one or more complete integrons found. The representation is very basic and a better representation can be obtained from the GenBank file and a software (eg Geneious) to represent it.

For each of the aforementioned files, you can save them by clicking on the download button.

1.1.6 References

If you use this software, please cite:

- Identification and analysis of integrons and cassette arrays in bacterial genomes Jean Cury; Thomas Jove; Marie Touchon; Bertrand Neron; Eduardo PC Rocha. **Nucleic Acids Research**, 2016; doi: 10.1093/nar/gkw319

Please cite also the following articles:

- Nawrocki, E.P. and Eddy, S.R. (2013) Infernal 1.1: 100-fold faster RNA homology searches. **Bioinformatics**, 29, 2933-2935.
- Eddy, S.R. (2011) Accelerated Profile HMM Searches. **PLoS Comput Biol**, 7, e1002195.
- Hyatt, D., Chen, G.L., Locascio, P.F., Land, M.L., Larimer, F.W. and Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. **BMC Bioinformatics**, 11, 119.

and if you use ResFams, cite the corresponding articles:

- Gibson, M.K., Forsberg, K.J. and Dantas, G. (2015) Improved annotation of antibiotic resistance determinants reveals microbial resistomes cluster by ecology. **ISME J**, 9, 207-216.

CHAPTER

TWO

DEVELOPER GUIDE

2.1 Developer Guide

This part is for developers, who want to work on IntegronFinder scripts.

2.1.1 Developer installation

If you are not part of the project, start by forking IntegronFinder repository. For that, sign in to your account on github, and go to https://github.com/gem-pasteur/Integron_Finder. Then, click on 'Fork' (under your account icon). This will create a copy of the repository, but with your username instead of 'gem-pasteur'.

create a virtual environment:

```
virtualenv -p python3 Integron_Finder
```

activate you virtualenv:

```
source Integron_Finder/bin/activate
```

then install integron_finder in developer mode:

```
pip install -e "git+https://github.com/gem-pasteur/Integron_Finder
↪#egg=integron_finder[dev]"
```

or clone your repository manually, then install it

```
mkdir src
cd src
git clone https://github.com/gem-pasteur/Integron_Finder
cd Integron_Finder
pip install -e ".[dev]"
```

It installs the requirements and create a directory in the virtualenv src/integron_finder and create links in the virtualenv. So `integron_finder` is runnable and you can modify the sources and run it again without to reinstall the project.

Note: *[dev]* allow to install extra dependencies to generate documentation, compute test coverage ...

Warning: Debian/Ubuntu distribution *–user* is the default. So the *–prefix* option does not work and the *–root* opton has unexpected behavior. Therefore the best solution is to use *–user* or a virtualenv.

2.1.2 Send changes to upstream repository

If you want to integrate your code in the upstream (main) repository, you need to create a pull request.

1. Read the Contibuting guide
2. Create a new branch with `<your branch name>` a descriptive name (e.g. 'adding-xx-feature', 'fixing-typos', etc.), so that others understand what your are working on.
3. Work on it
4. Test that your work does not break the tests. add tests corresponding to your code
5. Push your local branch on your integron_finder clone on github

```
git push --set-upstream origin <your branch name>
```

6. ask for pull request
   - Go to your forked repository on github *https://github.com/<your_login>/Integron_Finder/pulls*
   - Click on 'New pull request'
   - Choose your repository and the branch on which you did your changes in 'head fork' (right-hand side), and choose 'gem-pasteur/Integron_Finder' with the branch on which you want to merge (probably master) in 'base fork' (left-hand side).
   - A green 'Able to merge' text should appear if git is able to automatically merge the 2 branches. In that case, click on 'Create pull request', write your comments on the changes you made, why etc, and save. We will receive the pull request.

2.1.3 Tests

IntegronFinder is provided with unit tests. You can find them in `tests` directory. You can use them to check that your changes did not break the previous features, and you can update them, and add your own tests for the new features.

Tests are done using unittest.

Running tests

To run the tests -v option is to increase the verbosity of the output:

```
python setup.py test
```

or:

```
python tests/run_tests.py -vv
```

or:

```
python tests/run_tests.py -vv tests/test_utils.py
```

to run specific tests.

If you also want to get code coverage (you need to install coverage):

```
coverage run  --source integron_finder tests/run_tests.py
```

Add `-vv` to get more details on each test passed/failed. If you want to see the coverage in html output, run (after executing the command above):

```
coverage html
```

The html coverage report will be generated in `coverage_html/index.html`.

Adding tests

If you want to create a new test file, adding a file in tests directory, must start with `test_`. Then, write your TestCase by inherits from IntegronTest and your tests using unittest framework (see examples in existing files), and *run them*.

2.1.4 Documentation

Documentation is done using `sphinx`. Source files are located in `doc/sources`. To generate the documentation you just have to run the makefile located in *doc* directory.

```
make html
```

To generate the documentation in *html* format or

```
make latexpdf
```

to generate the documentation in pdf format (for this option you need to have latex installed on your compute)

You can complete them.

2.1.5 Architecture Overview

Project files and directories

Files

COPYING The integron_finder licensing.

COPYRIGHT The integron finder copy rights holders.

MANIFEST.in What must be or should not included in the distribution.

README.md The file to red in first.

requirements.txt The requirements need to use integron_finder.

requirements_dev.txt The extra requirements to develop on integron_finder.

setup.cfg The setup.py configuration file.

setup.py The file to define how to build/install/release/test/... integron finder.

Directories

integron_finder The core of the projects contains integron_finder library The **scripts/finder** contain the main entry point.

tests Contains all needed for tests, the tests themselves, are a the top level and the name must start by `test_`. The data directory contains all data needed to perform the tests. (see *Tests* for further details)

doc Contains the documentation write in sphinx. The **source** directory contains the .rst files, whereas the **build** directory contains the generated documentation. To know how to contribute or generate documentation see *Documentation*

Singularity Contains the definition file for singularity container.

data TODO

dist This directory is generated when a distribution is created (`python setup.py sdist`).

Technical overview

The main entry point is in integron_finder/scripts/finder.py there are 3 functions

`intgeron_finder.scripts.main()` which is the real main entry point

`main` call `scripts/finder.parse_args()` which parse the commandline and generate a `config.Config` object. and do a loop over replicon and run `intgeron_finder.scripts/find_integron_in_one_replicon()`

all results are store in a directory named `Results_Integron_Finder_<replicon_file_name>` this directory is created by `intgeron_finder.scripts/find_integron_in_one_replicon()` store results in this directory or in a subdirectory call tmp_<replicon_id> these subdirectories will be keep only if `--keep-tmp` option is set, otherwise they are removed at the end of the `intgeron_finder.scripts/find_integron_in_one_replicon()`

when all replicons are computed the `main` function call `integron_finder.utils.merge_results()` to gather all results files `<replicons_id>.integtrons` and generate a unique file with these information.

to have details on `find_integron_in_one_replicon` works see *Introduction*

2.1.6 Integron_finder API Reference

annotation

`integron_finder.annotation.`**`add_feature`**(*replicon*, *integron_desc*, *prot_db*, *dist_threshold*)

Add integron annotation to the replicon.

Parameters

- **`replicon`** (a `Bio.Seq.SeqRecord` object.) – The Replicon to annotate
- **`integron_desc`** (a `pandas.DataFrame`) – integron description
- **`prot_db`** (a *`integron_finder.prot_db.ProteinDB`* object.) – the path to the fasta file containing the translation of the replicon.
- **`dist_threshold`** (*`int`*) – Two elements are aggregated if they are distant of dist_threshold or less.

integron_finder.annotation.**func_annot**(*integrons*, *replicon*, *prot_db*, *hmm_files*, *cfg*, *out_dir='.'*, *evalue=10*, *coverage=0.5*)

Call hmmmer to annotate CDS associated with the integron.
Use Resfams per default (Gibson et al, ISME J., 2014)

Parameters

- **integrons** (list of *integron_finder.integron.Integron* objects.) – integrons list to annotate
- **replicon** (Bio.Seq.SeqRecord object) – replicon where the integrons were found (genomic fasta file)
- **prot_db** (integron.prot_db.ProteinDB object.) – the protein database corresponding to the replicon translation
- **hmm_files** (*List[str]*) – List of path of hmm profiles to use to scan the prot_file
- **cfg** (*integron_finder.config.Config*) – the configuration for this analyse
- **out_dir** (*str*) – the path of the directory where to store the results
- **evalue** (*float*) –
- **coverage** (*float*) –

Returns

None.

But several files per hmm file are produced.

- subseqprot.tmp: fasta file containing a subset of protfile (the proteins belonging to the integron)
- <hmm>_fa.res: an output of the hmm search.
- <hmm>_fa_table.res: an output of the hmm search in tabulated format.

attc

integron_finder.attc.**find_attc_max**(*integrons*, *replicon*, *distance_threshold*, *model_attc_path*, *max_attc_size*, *min_attc_size*, *evalue_attc=1.0*, *circular=True*, *out_dir='.'*, *cpu=1*)

Look for attC site with cmsearch –max option which remove all heuristic filters. As this

option make the algorithm way slower, we only run it in the region around a hit. We call it local_max or eagle_eyes.

Default hit

```
                  attC
_____________________-->____-->_________-->_______________
_______<---------_____________________________________________
          intI
               ^-------------------------------------^
              Search-space with --local_max
```

Updated hit

```
                  attC           ***          ***
_____________________-->____-->___-->___-->___-->________
_______<---------_____________________________________________
          intI
```

Parameters

- **integrons** (list of `Integron` objects.) – the integrons may contain or not attC or intI.
- **replicon** (`Bio.Seq.SeqRecord` object.) – replicon where the integrons were found (genomic fasta file).
- **distance_threshold** (*int*) – the maximal distance between 2 elements to aggregate them.
- **evalue_attc** (*float*) – evalue threshold to filter out hits above it.
- **model_attc_path** (*str*) – path to the attc model (Covariance Matrix).
- **max_attc_size** (*int*) – maximum value for the attC size.
- **circular** (*bool*) – True if replicon is circular, False otherwise.
- **out_dir** (*str*) – The directory where to write results used indirectly by some called functions as `infernal.local_max()` or *infernal.expand*.
- **cpu** (*int*) – call local_max with the right number of cpu

Returns

Return type `pd.DataFrame` object

integron_finder.attc.**search_attc**(*attc_df*, *keep_palindromes*, *dist_threshold*, *replicon_size*)

Parse the attc data set (sorted along start site) for the given replicon and return list of arrays. One array is composed of attC sites on the same strand and separated by a distance less than dist_threshold.

Parameters

- **attc_df** (pandas.DataFrame) –
- **keep_palindromes** (*bool*) – True if the palindromes must be kept in attc result, False otherwise
- **dist_threshold** (*int*) – the maximal distance between 2 elements to aggregate them
- **replicon_size** (*int*) – the replicon number of base pair

Returns a list attC sites found on replicon

Return type list of pandas.DataFrame objects

config

class integron_finder.config.**Config**(*args*)

Config object hold values issue from command lines

__init__(*args*)

Initialize self. See help(type(self)) for accurate signature.

__weakref__

list of weak references to the object (if defined)

default_topology

The default topology available values are: 'circ' for circular or 'lin' for linear.

func_annot_path

The absolute path to the directory containing file needed for the functional annotation

input_dir

The absolute path to the directory where is located the replicon

log_level

Returns the level to apply to loggers. 0 <= level <=50

Return type int

model_attc_name

The name of the attc model

model_attc_path

The absolute path to the attC model file

model_dir
The absolute path to the directory containing the models

model_integrase
The absolute path to the integrase model file

model_len

Returns The length of the attc model (corresponding to CLEN field).

Raises IOError if model_attc_path does match an existing file RuntimeError if the file doe not content CLEN field.

outdir
The absolute path where to write the results directory

replicon_path
The absolute path to the replicon

result_dir
The absolute path to results directory

tmp_dir(*replicon_id*)
The absolute path of the tmp results dir.

hmm

integron_finder.hmm.**read_hmm**(*replicon_id*, *prot_db*, *infile*, *cfg*, *evalue=1.0*, *coverage=0.5*)
Function that parse hmmer –out output and returns a pandas DataFrame filter output by evalue and coverage. (Being % of the profile aligned)

Parameters

- **replicon_id** (*str*) – the id of the replicon
- **prot_db** (*integron_finder.prot_db.ProteinDB* object.) – The protein database corresponding to the replicon translation
- **infile** (*str*) – the hmm output (in tabulated format) to parse
- **cfg** (*integron_finder.config.Config* object.) – the config
- **evalue** (*float*) – filter out hits with evalue greater tha evalue.
- **coverage** (*float*) – filter out hits with coverage under coverage (% of the profile aligned)

Returns

data Frame with columns:

"Accession_number", "query_name", "ID_query", "ID_prot", "strand", "pos_beg", "pos_end", "evalue"
each row correspond to a hit.

Return type a `pandas.DataFrame`

`integron_finder.hmm.`**`scan_hmm_bank`**(*path*)

Parameters **`path`** (*str*) –

- if the path is a dir: return all files ending with .hmm in the dir
- if the path is a file: parse the file, each line must be an expression (glob) pointing to hmm files

Returns lists of hmm files to consider for annotation

Return type list of str

Raises **`IOError`** – if the path does not exists

infernal

`integron_finder.infernal.`**`expand`**(*replicon*, *window_beg*, *window_end*, *max_elt*, *df_max*, *circular*, *dist_threshold*, *model_attc_path*, *max_attc_size=200*, *min_attc_size=40*, *evalue_attc=1.0*, *search_left=False*, *search_right=False*, *out_dir='.'*, *cpu=1*)

for a given element, we can search on the left hand side (if integrase is on the right for instance) or right hand side (opposite situation) or both side (only integrase or only attC sites)

Parameters

- **`replicon`** (a `Bio.Seq.SeqRecord` object.) – The Replicon to annotate
- **`window_beg`** (*int*) – start of window to search for attc (position of protein)
- **`window_end`** (*int*) – end of window to search for attc (position of protein)
- **`max_elt`** (`pandas.DataFrame` object) – DataFrame with columns:

```
Accession_number cm_attC  cm_debut  cm_fin   pos_
↪beg   pos_end sens   evalue
```

and each row is an occurrence of attc site

- **df_max** (pandas.DataFrame object) – DataFrame with columns

```
Accession_number cm_attC  cm_debut  cm_fin   pos_
↪beg   pos_end sens   evalue
```

and each row is an occurrence of attc site

- **circular** (*bool*) – True if replicon topology is circular otherwise False.
- **dist_threshold** (*int*) – Two elements are aggregated if they are distant of dist_threshold [4kb] or less
- **max_attc_size** (*int*) – The maximum value for the attC size
- **min_attc_size** (*int*) – The minimum value for the attC size
- **model_attc_path** (*str*) – the path to the attc model file
- **evalue_attc** (*float*) – evalue threshold to filter out hits above it
- **search_left** (*bool*) – trigger the local_max search on the left of the already detected element
- **search_right** (*bool*) – trigger the local_max search on the right of the already detected element
- **out_dir** (*str*) – The path to directory where to write results
- **cpu** (*int*) – the number of cpu use by expand

Returns a copy of max_elt with attC hits

Return type pandas.DataFrame object

integron_finder.infernal.**find_attc**(*replicon_path*, *replicon_id*, *cmsearch_path*, *out_dir*, *model_attc*, *incE=1.0*, *cpu=1*)

Call cmsearch to find attC sites in a single replicon.

Parameters

- **replicon_path** (*str*) – the path of the fasta file representing the replicon to analyse.
- **replicon_id** (*str*) – the id of the replicon to analyse.
- **cmsearch_path** (*str*) – the path to the cmsearch executable.
- **out_dir** (*str*) – the path to the directory where cmsearch outputs will be stored.
- **model_attc** (*str*) – path to the attc model (Covariance Matrix).

- **incE** (*float*) – consider sequences <= this E-value threshold as significant (to get the alignment with -A)
- **cpu** (*int*) – the number of cpu used by cmsearch.

Returns None, the results are written on the disk.

Raises **RuntimeError** – when cmsearch run failed.

integron_finder.infernal.**local_max**(*replicon*, *window_beg*, *window_end*, *model_attc_path*, *strand_search='both'*, *evalue_attc=1.0*, *max_attc_size=200*, *min_attc_size=40*, *cmsearch_bin='cmsearch'*, *out_dir='.'*, *cpu_nb=1*)

Parameters

- **replicon** (Bio.Seq.SeqRecord object.) – The name of replicon (without suffix)
- **window_beg** (*int*) – Start of window to search for attc (position of protein).
- **window_end** (*int*) – End of window to search for attc (position of protein).
- **model_attc_path** (*str*) – The path to the covariance model for attc (eg: attc_4.cm) used by cmsearch to find attC sites
- **strand_search** (*str*) – The strand on which to looking for attc. Available values:
  - 'top': Only search the top (Watson) strand of target sequences.
  - 'bottom': Only search the bottom (Crick) strand of target sequences
  - 'both': search on both strands
- **evalue_attc** (*float*) – evalue threshold to filter out hits above it
- **max_attc_size** (*int*) – The maximum value fot the attC size
- **min_attc_size** (*int*) – The minimum value fot the attC size
- **cmsearch_bin** (*str*) – The path to cmsearch
- **out_dir** (*str*) – The path to directory where to write results
- **cpu_nb** (*int*) – The number of cpu used by cmsearch

Returns DataFrame with same structure as the DataFrame returns by *read_infernal()* where position are converted on position on replicon and attc are filtered by evalue, min_attc_size, max_attc_size also write

a file with intermediate results <replicon_id>_subseq_attc_table_end.res this file store the local_max results before filtering by max_attc_size and min_attc_size

Return type `pandas.DataFrame` object

`integron_finder.infernal.`**`read_infernal`**(*infile*, *replicon_id*, *len_model_attc*, *evalue=1*, *size_max_attc=200*, *size_min_attc=40*)

Function that parse cmsearch –tblout output and returns a pandas DataFrame

Parameters

- **`infile`** (*`str`*) – the path to the output of cmsearch in tabulated format (–tblout)
- **`replicon_id`** (*`str`*) – the id of the replicon are the integrons were found.
- **`len_model_attc`** (*`int`*) – the length of the attc model
- **`evalue`** (*`float`*) – evalue threshold to filter out hits above it
- **`size_max_attc`** (*`int`*) – The maximum value fot the attC size
- **`size_min_attc`** (*`int`*) – The minimum value fot the attC size

Returns

table with columns:

”Accession_number”, “cm_attC”, “cm_debut”, “cm_fin”, “pos_beg”, “pos_end”, “sens”, “evalue”

and each row is a hit that match the attc covariance model.

Return type `pandas.DataFrame` object

integrase

`integron_finder.integrase.`**`find_integrase`**(*replicon_id*, *prot_file*, *out_dir*, *cfg*)

Call Prodigal for Gene annotation and hmmer to find integrase, either with phage_int HMM profile or with intI profile.

Parameters

- **`replicon_id`** (*`str`*) – The Replicon identifier to search integrase into

- **prot_file** (*str*) – the path to the fasta file containing the translation of the replicon.
- **out_dir** (*str*) – the relative path to the directory where prodigal outputs will be stored
- **cfg** (a *integron_finder.config.Config* object) – the configuration

Returns None, the results are written on the disk

integron

class integron_finder.integron.**Integron**(*replicon*, *cfg*)

Integron object represents an object composed of an integrase, attC sites and gene cassettes. Each element is characterized by their coordinates in the replicon, the strand (+ or -), the ID of the gene (except attC). The object Integron is also characterized by the ID of the replicon.

__init__(*replicon*, *cfg*)

Parameters

- **replicon** (a Bio.Seq.SeqRecord object) – The replicon where integrons has been found
- **cfg** (a *integron_finder.config.Config* object) – the configuration

__weakref__

list of weak references to the object (if defined)

add_attC(*pos_beg_attC*, *pos_end_attC*, *strand*, *evalue*, *model*)

Adds attC site to the Integron object.

Parameters

- **pos_beg_attC** (*int*) – the position on the replicon of the beginning attc site
- **pos_end_attC** (*int*) – the position on replicon of the end of the attc site
- **strand** (*int*) – the strand where is found the attc 1 for forward, -1 for reverse
- **evalue** (*float*) – the evalue associated to this attc site
- **model** (*str*) – the name of attc model (for instance attc4)

add_attI()

Looking for Att1 sites and add them to this integron.

add_integrase(*pos_beg_int*, *pos_end_int*, *id_int*, *strand_int*, *evalue*, *model*)
Adds integrases to the integron. Should be called once.

Parameters

- **pos_beg_int** (*int*) – the position on the replicon of the beginning integrase site
- **pos_end_int** (*int*) – the position on replicon of the end of the integrase site
- **id_int** (*str*) – The protein id corresponding to the integrase
- **strand_int** (*int*) – the strand where is found the attc 1 for forward, -1 for reverse
- **evalue** (*float*) – the evalue associated to this attc site
- **model** (*str*) – the name of integrase model (for instance intersection_tyr_intI)

add_promoter()
Looks for known promoters if they exists within your integrons element. It takes 1s for about 13kb.

add_proteins(*prot_db*)

Parameters **prot_db** (integron.prot_db.ProteinDB object.) – The protein db corresponding to the translation of the replicon

describe()

Returns

DataFrame describing the integron object The columns are:

"pos_beg", "pos_end", "strand", "evalue", "type_elt", "model", "distance_2attC", "annotation", "considered_topology"

draw_integron(*file=None*)
Represent the different element of the integrons if file is provide save the drawing on the file otherwise display it on screen.

Parameters **file** (*str*) – the path to save the integron schema (in pdf format)

has_attC()

Returns True if integron has attc sites False otherwise.

has_integrase()

Returns True if integron has integrase False otherwise.

type()

Returns

The type of the integrons:

- 'complete' : Have one integrase and at least one attC
- 'CALIN' : Have at least one attC
- 'In0' : Just an integrase intI

Return type str

integron_finder.integron.**find_integron**(*replicon*, *prot_db*, *attc_file*, *intI_file*, *phageI_file*, *cfg*)

Function that looks for integrons given rules :

- presence of intI
- presence of attC
- d(intI-attC) <= 4 kb
- d(attC-attC) <= 4 kb

It returns the list of all integrons, be they complete or not. found in attC files + integrases file which are formatted as follow : intI_file : Accession_number ID_prot strand pos_beg pos_end evalue attc_file : Accession_number attC cm_debut cm_fin pos_beg pos_end sens evalue

Parameters

- **replicon** (Bio.Seq.SeqRecord object) – the name of the replicon
- **prot_db** (a *integron_finder.prot_db.ProteinDB* object.) – the protein database corresponding to the replicon translation
- **attc_file** (path to cmsearch output or pd.Dataframe) – the output of cmsearch or the result of parsing of this file by read_infernal
- **intI_file** (*str*) – the output of hmmsearch with the integrase model
- **phageI_file** (*str*) – the output of hmmsearch with the phage model
- **cfg** (a *integron_finder.config.Config* object) – configuration

Returns list of all integrons, be they complete or not

Retype list of *Integron* object

prot_db

The prot_db module contains classes to handle protein file and protein description which can be either generate by Prodigal or Provide by Gembase. It also provide an interface to abstract the way

to get protein sequences and descriptions

class integron_finder.prot_db.**ProteinDB**(*replicon*, *cfg*, *prot_file=None*)

AbstractClass defining the interface for ProteinDB. ProteinDB provide an abstraction and a way to access to proteins corresponding to the replicon/contig CDS.

__getitem__(*seq_id*)

Parameters **prot_seq_id** (*str*) – the id of a protein sequence

Returns The Sequence corresponding to the prot_seq_id.

Return type Bio.SeqRecord object

Raises **KeyError** – when seq_id does not match any sequence in DB

__init__(*replicon*, *cfg*, *prot_file=None*)

Initialize self. See help(type(self)) for accurate signature.

__iter__()

Returns a generator which iterate on the protein seq_id which constitute the contig.

Return type generator

__weakref__

list of weak references to the object (if defined)

_make_db()

Returns an index of the sequence contains in protfile corresponding to the replicon

_make_protfile()

Create fasta file with protein corresponding to the nucleic sequence (replicon)

Returns the path of the created protein file

Return type str

get_description(*gene_id*)

Parameters **gene_id** (*str*) – a protein/gene identifier

Returns The description of the protein corresponding to the gene_id

Return type *SeqDesc* namedtuple object

Raises

- **IntegronError** – when gene_id is not a valid Gembase gene identifier
- **KeyError** – if gene_id is not found in GembaseDB instance

protfile

Returns The absolute path to the protein file corresponding to contig id

Return type str

class integron_finder.prot_db.**ProdigalDB**(*replicon*, *cfg*, *prot_file=None*)

Creates proteins from Replicon/contig using prodigal and provide facilities to access them.

__getitem__(*prot_seq_id*)

Parameters **prot_seq_id** (*str*) – the id of a protein sequence

Returns The Sequence corresponding to the prot_seq_id.

Return type Bio.SeqRecord object

__iter__()

Returns a generator which iterate on the protein seq_id which constitute the contig.

Return type generator

_make_protfile()

Use *prodigal* to generate proteins corresponding to the replicon

Returns the path of the created protfile

Return type str

get_description(*gene_id*)

Parameters **gene_id** (*str*) – a protein/gene identifier

Returns The description of the protein corresponding to the gene_id

Return type *SeqDesc* namedtuple object

Raises

- **IntegronError** – when gene_id is not a valid Gembase gene identifier
- **KeyError** – if gene_id is not found in ProdigalDB instance

class integron_finder.prot_db.**GembaseDB**(*replicon*, *cfg*, *prot_file=None*)

Implements *ProteinDB* from a Gembase. Managed proteins from Proteins directory corresponding to a replicon/contig

__getitem__(*prot_seq_id*)

Parameters **prot_seq_id** (*str*) – the id of a protein sequence

Returns The Sequence corresponding to the prot_seq_id.

Return type Bio.SeqRecord object

__init__(*replicon*, *cfg*, *prot_file=None*)
Initialize self. See help(type(self)) for accurate signature.

__iter__()

Returns a generator which iterate on the protein seq_id which constitute the contig.

Return type generator

_make_protfile()
Create fasta file with protein corresponding to this sequence, from the corresponding Gembase protfile This step is necessary because in Gembase Draft One nucleic file can contains several contigs, but all proteins are in the same file.

Returns the path of the created protein file

Return type str

_parse_lst()
Parse the LSTINFO file and extract information specific to the replicon :return:

static gembase_complete_parser(*lst_path*, *sequence_id*)

Parameters

- **lst_path** (*str*) – the path of of the LSTINFO file Gembase Complet
- **sequence_id** (*str*) – the id of the genomic sequence to analyse

Returns the information related to the 'valid' CDS corresponding to the sequence_id

Return type *class*:pandas.DataFrame' object

static gembase_draft_parser(*lst_path*, *replicon_id*)

Parameters

- **lst_path** (*str*) – the path of of the LSTINFO file from a Gembase Draft
- **sequence_id** (*str*) – the id of the genomic sequence to analyse

Returns the information related to the 'valid' CDS corresponding to the sequence_id

Return type *class*:pandas.DataFrame' object

static gembase_sniffer(*lst_path*)
Detect the type of gembase :param str lst_path: the path to the LSTINFO file corresponding to the nucleic sequence :returns: either 'Complet' or 'Draft'

get_description(*gene_id*)

Parameters **gene_id** (*str*) – a protein/gene identifier

Returns The description of the protein corresponding to the gene_id

Return type *SeqDesc* namedtuple object

Raises

- **IntegronError** – when gene_id is not a valid Gembase gene identifier
- **KeyError** – if gene_id is not found in GembaseDB instance

class integron_finder.prot_db.**SeqDesc**(*id*, *strand*, *start*, *stop*)

__getnewargs__()
Return self as a plain tuple. Used by copy and pickle.

static __new__(*_cls*, *id*, *strand*, *start*, *stop*)
Create new instance of SeqDesc(id, strand, start, stop)

__repr__()
Return a nicely formatted representation string

_asdict()
Return a new OrderedDict which maps field names to their values.

classmethod _make(*iterable*)
Make a new SeqDesc object from a sequence or iterable

_replace(***kwds*)
Return a new SeqDesc object replacing specified fields with new values

id
Alias for field number 0

start
Alias for field number 2

stop
Alias for field number 3

strand
Alias for field number 1

results

The *results* module contains functions to handle the final reports.

- merging results of each sequence
- generate a summary

- or filter the calin

integron_finder.results._log = <Logger integron_finder.results (WARNING)>
utilities to manage results

integron_finder.results.**filter_calin**(*result*, *threshold=2*)
filter integron report, remove 'CALIN' integron where number of attC sites is lower than threshold.

Parameters

- **result** (pandas.dataFrame object) – the output of *integrons_report()*
- **threshold** (*int*) – the integron CALIN with less attc site than *threshold* are removed

Returns filtered integron report

Return type pandas.dataFrame object

integron_finder.results.**integrons_report**(*integrons*)

Parameters **integrons** (list of *integron_finder.integron.Integron* object.) – list of integrons used to generate a report

Returns a report off all integrons from a replicon

Return type

pandas.DataFrame object. this datafame have following columns:

"ID_integron", "ID_replicon", "element", "pos_beg", "pos_end", "strand", "evalue", "type_elt", "annotation", "model", "type", "default", "distance_2attC", "considered_topology"

integron_finder.results.**merge_results**(**results_file*)

Parameters **results_file** (*str*) – The path of the files to merge. The files can be parsed by pandas as DataFrame and have the same columns. It is used to merge the integrons files (.integrons) or summary files (.summary) from different replicons.

Returns all results aggregated in one pandas.DataFrame object. if there is no results to merge, return an empty DataFrame.

Return type a pandas.DataFrame object.

integron_finder.results.**summary**(*result*)
Create a summary of an integron report. Count the number of 'CALIN', 'In0' or 'complete' for each replicon.

Parameters **result** – the integron to summarize

Returns a `pandas.DataFrame` object. with columns 'ID_replicon', 'ID_integron', 'complete', 'In0', 'CALIN'

topology

class integron_finder.topology.**Topology**(*default*, *topology_file=None*)

Class to parse and handle replicons topologies

__getitem__(*replicon_id*)

Parameters **replicon_id** (*str*) – The id of the replicon.

Returns the topology for the replicon corresponding to the replicon_id

__init__(*default*, *topology_file=None*)

Parameters

- **default** (*str*) – the default topology
- **topology_file** – the path to the file where topology for replicon are specified

__weakref__

list of weak references to the object (if defined)

_parse(*topology_file*)

Parse a topology file where topology is specified for replicons on each line a topology is specified for a replicon the syntax of each line is

```
replicon_id topology
```

the allowed value for toplogy are 'circ', 'circular', 'lin', 'linear'

Parameters **topology_file** (*str*) – The path to the topology file

_parse_topology(*topo*)

Parse a field topology in topology file the authorized values are circular, linear or circ, lin, or in uppercase

Parameters **topo** – the field corresponding to topology in topology file

Returns the topology in "normed" format 'circ' or 'lin'

Return type str

utils

class integron_finder.utils.**FastaIterator**(*path*, *alphabet=IUPACAmbiguousDNA()*, *replicon_name=None*, *dist_threshold=4000*)

Allow to parse over a multi fasta file, and iterate over it

> **Warning: The sequences order is not guarantee.**

__init__(*path*, *alphabet=IUPACAmbiguousDNA()*, *replicon_name=None*, *dist_threshold=4000*)

Parameters

- **path** (*str*) – The path to the file containing the sequences.
- **alphabet** (*Bio.SeqIUPAC member*) – The authorized alphabet
- **replicon_name** (*str*) – The name of the replicon, if this specify all sequence.name will have this value
- **dist_threshold** (*int*) – The minimum length for a replicon to be considered as circular. Under this threshold even the provided topology is 'circular' the computation will be done with a 'linear' topology.

__len__()

Returns The number of sequence in the file

__next__()

Returns The next sequence (the order of sequences is not guaranteed).

Return type a Bio.SeqRecord object or None if the sequence is not compliant with the alphabet.

__weakref__

list of weak references to the object (if defined)

_check_seq_alphabet_compliance(*seq*)

Parameters **seq** (Bio.Seq.Seq instance) – the sequence to check

Returns True if sequence letters are a subset of the alphabet, False otherwise.

_set_topologies(*topologies*)

Parameters **topologies** (integron_finder.Topology onject) –

Returns

class integron_finder.utils.**SeqDesc**(*id*, *strand*, *start*, *stop*)

__getnewargs__()
Return self as a plain tuple. Used by copy and pickle.

static __new__(*_cls*, *id*, *strand*, *start*, *stop*)
Create new instance of SeqDesc(id, strand, start, stop)

__repr__()
Return a nicely formatted representation string

_asdict()
Return a new OrderedDict which maps field names to their values.

classmethod _make(*iterable*)
Make a new SeqDesc object from a sequence or iterable

_replace(***kwds*)
Return a new SeqDesc object replacing specified fields with new values

id
Alias for field number 0

start
Alias for field number 2

stop
Alias for field number 3

strand
Alias for field number 1

integron_finder.utils.**get_name_from_path**(*path*)

Parameters **path** – The path to extract name for instance the fasta file to the replicon

Returns the name of replicon for instance if path = /path/to/replicon.fasta name = replicon

integron_finder.utils.**log_level**(*verbose*, *quiet*)

Returns the level to apply to loggers. 0 <= level <=50

Return type int

integron_finder.utils.**make_multi_fasta_reader**(*alphabet*)
fasta generator maker

Parameters **alphabet** – the alphabet store in the fasta generator closure

Returns generator to iterate on the fasta file in the same order as in fasta file

integron_finder.utils.**model_len**(*path*)

Parameters **path** (*str*) – the path to the covariance model file

Returns the length of the model

Return type int

integron_finder.utils.**read_multi_prot_fasta**(*path*)

Parameters **path** – The path to the fasta file.

Returns The sequence parsed.

Return type Bio.SeqRecord.SeqRecord object.

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