Jun 07, 2024 Version 3

Bacterial genome annotation script using BLASTN V.3

DOI
dx.doi.org/10.17504/protocols.io.dm6gpjrb1gzp/v3

Ana Mariya Anhel[1], Lorea Alejaldre[1], Ángel Goñi-Moreno[1]

[1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA/CSIC), Madrid, Spain

Ángel Goñi-Moreno: angel.goni@upm.es

biocomp.cbgp Biocomputation Lab
Centro de Biotecnologia y Genomica de Plantas

DOI: dx.doi.org/10.17504/protocols.io.dm6gpjrb1gzp/v3

Protocol Citation: Ana Mariya Anhel, Lorea Alejaldre, Ángel Goñi-Moreno 2024. Bacterial genome annotation script using BLASTN. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.dm6gpjrb1gzp/v3**Version created by **Ana Mariya Anhel**

Protocol status: Working
We use this protocol and it's working

Created: November 20, 2023

Last Modified: June 07, 2024

Protocol Integer ID: 100178

Keywords: Genome anotation, Bacterial, P. putida, Transposon, Transposon library, E. coli

Funders Acknowledgement:
Comunidad de Madrid
Grant ID: Y2020/TCS-6555,2019-T1/BIO-14053
MCIN/AEI
Grant ID: CEX2020-000999-S,PID2020-117205GA-I00
ERC
Grant ID: 101044360

Abstract

This protocol uses a python based script and command-line BLASTn to annotate in a final table single-read sequencing results from genome amplifications, within other output files.

Its main use in our lab (https://biocomputationlab.com) is to identify the location and gene locus of transposon inserts in microbial bacterial genomes of *Pseudomonas putida KT2440*. However, this script can be used for other bacterial genomes for which its genome sequence and annotation are available.

Script was developed and tested in python 3.11.9 with blastn version 2.9.0, sickle version 1.33 and fastqc version 0.11.9

This is a description of the LAP entry LAPu-InsertsGenAnnotation-2.0.0 located in the **LAP repository**, specifically **LAPu-InsertsGenAnnotation-2.0.0** and **Github Entry LAPu-InsertsGenAnnotation-2.0.0**, 2 places that you can download directly the script used and usage examples

The major changes from previous version are:

1. **File format for -identity Argument**: Now accepts XLSX and CSV files.
2. **New Argument --summaryMap**: Added the --summaryMap argument.
3. **Enhanced -quality Argument**: When provided, the merged quality file (FastQC file) will use the file name (without extension) as the sequence identifier, which will match the *qaccver* in the *table_reads_gene_description.csv* file.
4. **Support for Numeric Identifiers**: Numeric identifiers follow the same rules as well names for locating them in a file or sequence identifier.
5. **Identifier Pattern for Sequences**: The script recognizes the last element of the pattern (+well_, +number_, _well_, _number_) as the sequence identifier for -identity and --summaryMap arguments.
6. **More read extensions accepted:** they will be treated as previously txt files are treated for all other arguments

Guidelines

This script needs min 4 arguments in the following order:

1. Directory of folder containing sequencing reads
2. Reads file type (should be FASTA format even if the extension can be anything)
3. Genome file to perform blastn alignment (FASTA format)
4. Genome annotation file (.csv)

This program, by the time this guide was developed, can only be executed with Linux and macOS systems

Materials

Software

- Linux or MacOS
- Python 3.11.9
- Python packages: pandas (v2.1.3), openpyxl (v3.1.2), os, subprocess, argpase, re and biopython (v1.81)
- BLAST+
- Sickle
- FastQC

Safety warnings

With the arguments -identity and -quality there are limitations, read carefully the instructions and specifications of those arguments

Before start

To run this script command-line blastn and python3 with the packages pandas (v2.1.3), openpyxl(v3.1.2) os, subprocess, argpase, re and biopython (v1.81)

Adquisition of files

1 **Download reference genome file**

You can download the genome of the organism that you want to compare to the reading sequences from different sources such as NCBI, GSA or even pages dedicated to the organism (for example **pseudomonas.com**).

For this script to work, genome files need to be in **FASTA format** (.fasta, .fna, .ffn, .faa, .frn). Sequence alignment is based in BLASTn which requires **FASTA format** as input.

2 **Download annotation file**

You can download the annotation of the genome from different sources, such as NCBI, KEGG or pages dedicated to the organism.

For this script to work, the annotation file needs to be in CSV format and need to have at least the following columns with these exact names (names in **bold letter**):

- **Start** - Nucleotide number that sets the beginning of the annotated region
- **End** - Nucleotide number that sets the ending of the annotated region
- **Locus Tag** - Identifiers that are systematically applied to every gene in a genome. It has to be a unique name

Note

If the annotated file does not have those specific columns, but it has the information,the user can change the name of the columns to run the script because it should have **exactly those names**

Note

In case your annotated genome is not a CSV file but either a TSV, GFF or GTF, there are software that can convert those types of files to CSV:

- **GFF to GFT**: there are several convertors, such as *gffread* and *AGAT*. Some other converters can be found at https://agat.readthedocs.io/en/latest/gff_to_gtf.html
- **GFT to CSV**: *gft2csv* (https://github.com/zyxue/gtf2csv)
- **TSV to CSV**: there are a lot of online converters and Python packages that you can use to convert TSV to CSV, such as *pandas*

3 **Download sequence files**

Sequence files should be in a folder. Depending on the sequencing company, sequence files will be in .txt, .seq, .fasta or other format. This should be specified in the arguments of the program.

Note

The sequences that companies provide usually come with other files that give the quality of those reads. These files should also be in that same folder as the sequences if we are going to provide them to the program.

If we also have these files (.ab1, .fastqc, .abi, etc), the user of this program will be able to produce results that are more trustworthy by providing these quality files to the script and trimming the regions that do not have the quality wanted by the user.

Only qual, ab1 and fastq quality files are accepted

Even if the sequence files have different extensions they must follow a FASTA format and each file should contain only 1 record

For more information about the FASTA format visit
https://www.ncbi.nlm.nih.gov/genbank/fastaformat/

We need to know from now on that the sequence id of the secuence (the id of the sequence is after the > character in the FASTA format files) does not have to be the same as the name of the file containing that sequence. For example, the name of the file could be aaaa.txt and the sequence id of that sequence >abcd, but we recommend both of them to have the same name.

For some actions the name of the file will be taken in account, like when searching for the quality files associated to that sequence; and for others the sequence identifier (SeqID) will be taken in account, like extracting the position of the well in the map indenty file.

4 **Acquisition of map of reads for annotation of variants**

The identity of each variant can be annotated by using this script if a map of the 96-well plate is provided in CSV or XLSX. It can be created by hand or can be an output file of other LAP entries, such as **LAP-PCR-1.0.0**

This map should be a CSV or XLSX (only the first sheet will be read) file that will contain the names or identities of the sequences that corresponds to the reads. It needs to have the name of the rows and columns of the plate and can only be used if the reads and map fulfill the following requirements:

1. There is only **1 map**
2. **All the reads of the directory can be tracked in that map**, i.e., the directory of reads should only include the ones in the map. An example, if the map has 12 columns and 8 rows and it is half full (48 identities), the directory of the reads cannot be more than 48 sequences.
3. **All sequences need to have an identity in the map**, i.e., the cell correspondent to a well in the map of a sequence cannot be left empty.
4. The sequence id of the reads need to have the name of the cell **between a plus and an underscore if the extension of the files is seq** (for example, *readSequence+A10_example*) or **between underscores if the extension of the reads is another one** (for example, *readSequence_A10_example*).
5. If the identities of the names on the sequences are numbers, the **numbers need to be from 1 to 96**, any other number will not be able to be tracked to any cell in the map. The numbers will be counted or tracked from top to bottom, left to right, for example, if a sequence id is *readSequence_9_example* will be tracked to the cell A2
6. If any sequence id has more than 1set of character that can be match with the expression *+number_/+well_* or *_number_/_well_*, the **last match will be the one taken as the position** that is going to be searched in the identity map

Note

The name of the well or number will be tracked in the identity of the record in the fasta file after we merge all the seuqences (the SeqID that is the name after the > character), not in the name of the file, but if you have provided the **-quality** argument, the name of the file (without the extension) will be assigned as the identity of the record, as the SeqID of that sequence.

For example, if the content of a fasta file called *ABC_A01_ABC.txt* is:
>AAAA_12_BBBB
ATCGTTTGCTGCT

The position in the map is going to be searched in AAAA_12_BBBB, which in this case is 12 that corresponds to the well D2, not the well A1 as it would be if it is searched in the name of the file

An example of this script with the map provided will be given at the end of this page

go to step #20

5 **Script**

The last script version can be found at the **Github of the repository in the LAPu entries folder** (the name of this file is the user's choice). The name of the directory should be **LAPu-**

InsertsGenAnnotation followed by the version.

You can also find the latest version of the script in **https://www.laprepo.com/repository/** with the same name as in GitHub.

Software	
LAP Repository	NAME
https://biocomputationlab.com/	DEVELOPER
www.laprepo.com	SOURCE LINK

The more updated version of this script during the development of this protocol is

ScriptAllignmentAnnotation_v200.py

Note

LAPu-InsertsGenAnnotation-2.0.0 is only available to run in Linux and MacOS systems

Preparing System

6 If you are using a **Windows system** you can install a Windows Subsystem for Linux (WSL) and run the script in that subsystem the same way a Linux user would do, but be aware of the nomenclature and path of the files

There are other ways to run a Unix system in Windows, like a virtual machine (**https://www.virtualbox.org/**)

You can find instructions to install a WSL on the following Windows page: https://learn.microsoft.com/en-us/windows/wsl/install

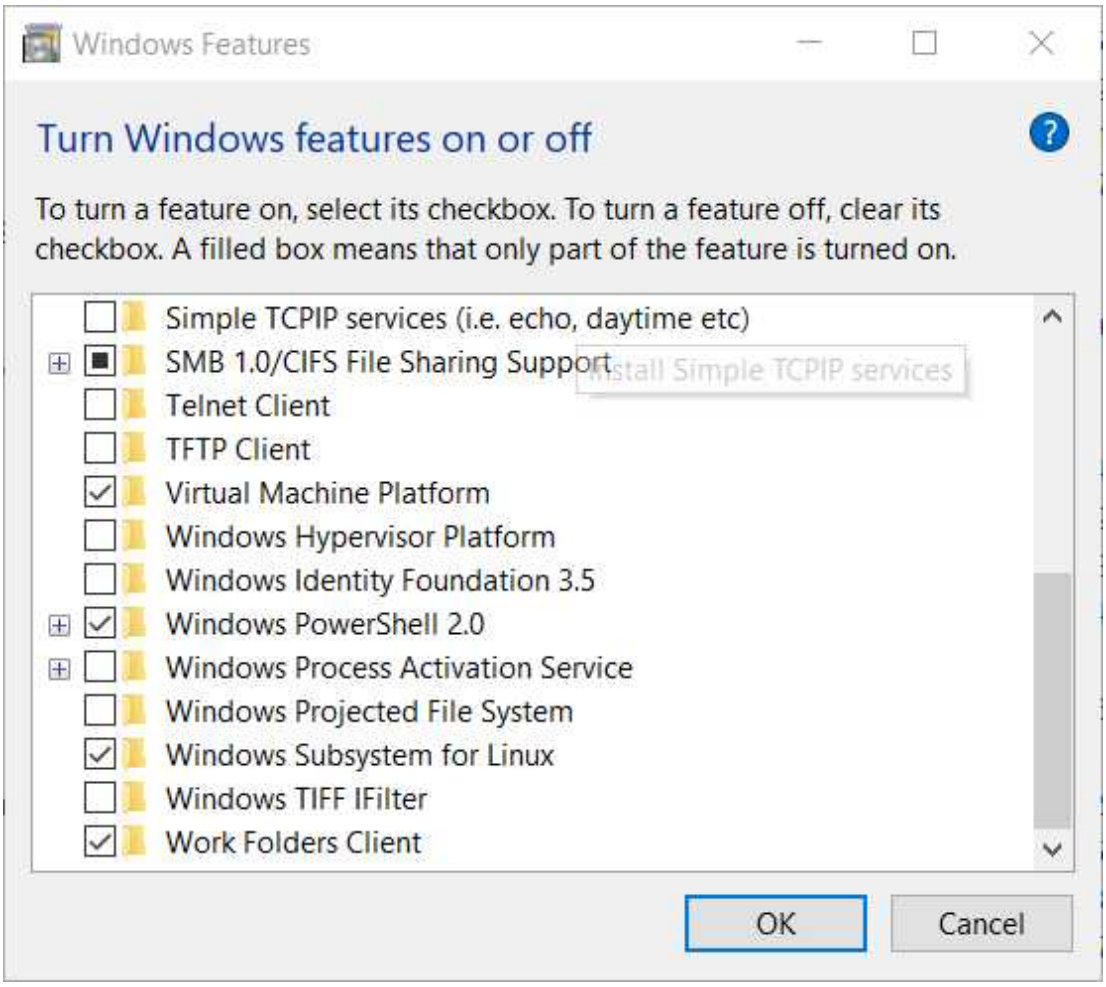
In the WSL, you can install the different requirements needed for the script to run

I will provide an example of how to install a WSL in the following sub-steps

6.1 *Make sure the Windows Subsystem in Linux Feature is activated*

Windows search bar -> Apps & Features -> (Scroll Down) Programs and Features -> Turn Windows features on or off

In that window, you will need to have a tick in the option "Windows Subsystem for Linux" you may need to restart the computer after ticking that box so changes are made in your computer



Window with the WSL (Windows Subsystem for Linux) feature ON

6.2 *Install a Linux system*

Open a window with Windows Powershell, and you can check which distributions of Linux can be installed

Command

Check (Windows 10)

```
wsl --list --online
```

To install one of the distributions, you can perform the following command by changing *Ubuntu-20.04* for the desired Linux system.

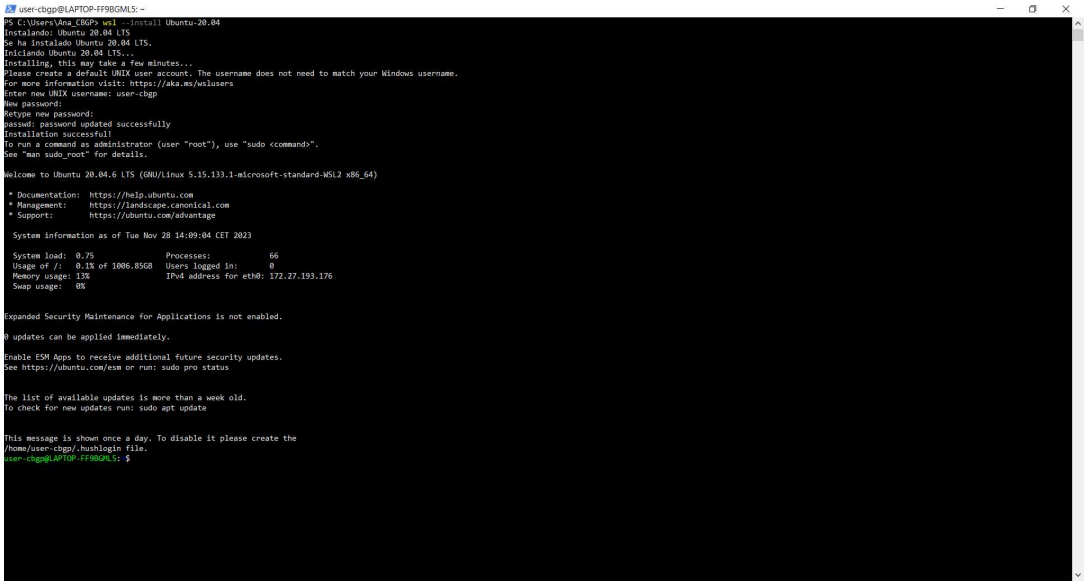
Command

Install Ubuntu 20.04 with the wsl command (Windows 10)

```
wsl --install Ubuntu-20.04
```

You enter the needed data that the system will ask you, such as the UNIX username and password

Expected result



PowerShell screen of the installation of an Ubuntu system with wsl

6.3 *Make the distribution run as default*

You can make the system installed in **step 6.2** the one that is going to run when you type the command wsl in the PowerShell by running the following command

Command

Set the distribution installed as default for wsl (Windows 10)

```
wsl --set-default Ubuntu-20.04
```

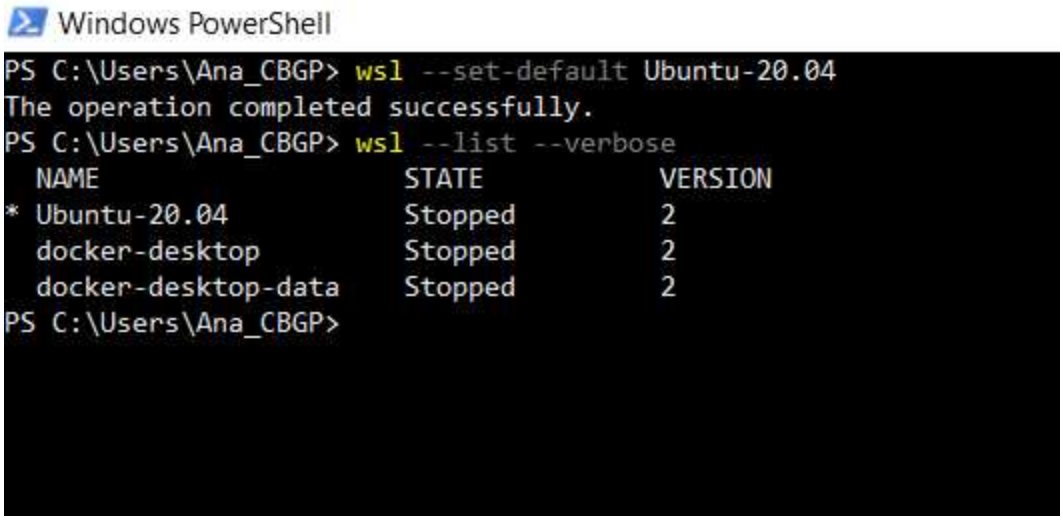
You can check which distribution is running as a default with the following command, will be the one designed or marked by an asterisk

Command

Check distributions installed WSL (Windows 10)

```
wsl --list --verbose
```

Expected result



Result of setting the Ubuntu-20.04 system as a default when running the wsl program

6.4 *Run Linux System*

To run the Linux system, in this example Ubuntu 20.04, you can type in the Windows PowerShell **wsl** and you will directly access the directory where you have typed this command, but in the Ubuntu system

Now you can perform the script and install the needed packages and programs in this system

7 Install Python

In most Unix systems, a default Python is installed, but you can always install more than 1 Python version on your computer

This script was tested with Python 3.11.9, so it cannot be guaranteed that it works as expected in previous or later ones

In the following substeps, I will show how to install Python and set it as the default one to use in case more than 1 python version is installed on the system.

7.1 *Linux systems*

You can install a specific version of this language with the following command. If you do not provide a version, the latest one accessible to sudo will be installed

Command

Install Specific Version Python (Linux)

```
sudo apt install python<version>
```

You can use this python-specific version by using the command python<version>
If you want a specific version to be the default python that will be used, you will need to change the aliases of the system.

Command

Add aliases to Linux system (Linux)

```
nano ~/.bashrc
```

Go to the last line, add the following line: **alias python='python'**, and then use *Ctrl+x* to get out. Do not forget to save before leaving.

After that, you should reload the .bashrc file by running the following command

Command

Update .bashrc file (Linux)

```
source ~/.bashrc
```

7.2 *macOS systems*

Note

We are going to use Homebrew to install it, so if you do not have it yet, you can install it by typing the following command in the command line

Command

Install Homebrew (macOS)

```
/bin/bash -c "$(curl -fsSL
https://raw.githubusercontent.com/Homebrew/install/HEAD/install.sh
)"
```

For more information about how to install this software, you can check the homebrew page (https://docs.brew.sh/Installation)

You can install a specific version of Python by typing in the command line and the following instructions. If a version is not provided, the latest python accessible in brew will be installed.

Command

Install Specific Version Python with Homebrew (macOS)

```
brew install python@<version>
```

You can use this python-specific version by using the command python<version>
If you want a specific python version to be used by default, you will need to change the aliases of the shell that you are using.

Command

Add aliases to macOS system in BASH (macOS)

```
nano ~/.bashrc_profile
```

Go to the last line and add the following line **alias python='python<version>'** and then use *Ctrl+x* to get out. Do not forget to save before leaving.

After that, you should reload the .bashrc_profile file by running the following command.

Command

Update .bashrc_profile file (macOS)

```
source ~/.bashrc_profile
```

Note

If you are using Bash, you have to change the file ~/.bashrc_profile or ~/.bashrc
In case you are using Zsh, you need to change the file ~/.zshrc

Knowing which file you need to change follow the previous commands changing the file name to the correct one.

8 **Install needed Python packages**

This script needs the following packages: *sys*, *os*, *argparse*, *re*, *subprocess*, *pandas, openpyxl* and *BioPython*.

The first 5 first packages named are installed by default in Python but the latter 3 need to be installed

To install these packages you can perform the following command

Command

Install packages that are inside of the pip packages database

```
python<version> -m pip install <name_package>
```

Note

Sometimes pip is not installed by default, you can install it with the following commands

Command

Install pip for Python 3 (Linux)

```
sudo apt install python3-pip
```

Command

Install pip for Python 3 (macOS)

```
sudo easy_install pip
```

9 **Install BLAST+**

There are different ways to install BLAST, here we provide 3 ways in the substeps

9.1 *Download the executable from NCBI*

You can download and install the BLAST from the NCBI web (**https://www.ncbi.nlm.nih.gov/**)

Download -> FTP -> blast -> executables -> blast+ -> [wanted version] -> *ncbi-blast-[version]-[system].tar.gz*

Unzip it with the following command

Command

Unzip tar.gz file

```
tar -xf [name_file].tar.gz
```

In the unzip folder, you have a bin folder that contains blastn

If you decide to install it this way, you need to run the Python script in this directory so it can be accessed by the Python file or added to the path of the system directory

9.2 *Use Anaconda*

You can install the needed commands with Anaconda if you perform the following command

Command

Install blast with Anaconda

```
conda install -c bioconda blast
```

Note

For this option, you need to have Anaconda installed in your system

Instructions to install this software can be found on the following page https://docs.anaconda.com/free/anaconda/install/index.html

9.3 *Use apt or brew install*

If you are in a Linux system, you can perform the following command to install blast+

Command

Install BLAST+ (Linux)

```
sudo apt install ncbi-blast+
```

In case you are in a macOS system, you can install it with the following instruction

Command

Install BLAST+ (macOS)

```
brew install blast
```

If you do not have Homebrew in your system go to step #7.2 Note to install it

9.4 To make sure it has been installed in your system, you can go to the command line and type the following command

Command

Check Version of BLASTn Command Line

```
blastn -version
```

If this command does not raise an error, it means that BLASTn can be used from the command line

10 **Install FastQC**

FastQC is a tool used for quality control of high-throughput sequence data. It provides a way to assess the quality of raw sequencing data

If you will not use the quality assessment of the script, there is no need to install this software

In the following sub-steps, the installation in different OS is provided

10.1 *Linux*

In Linux, you can install with apt the command line executable of fastqc. Just type in Bash the following command

Command

Install FastQC from command line (Linux)

```
sudo apt install fastqc
```

You can check if the installation has been successful if you type **fastqc --version** and you don't receive an error message

10.2 *MacOS*

In macOS you can install with homebrew the command line executable of fastqc, just type in Bash the following command

Command

Install FastQC from command line (macOS)

```
brew install fastqc
```

If you do not have Homebrew in your system go to step #7.2 Note

You can check if the installation has been successful if you type **fastqc --version** and you don't receive an error message

11 Install Sickle

Sickle is a software tool designed for quality control of high-throughput sequence data, especially for data generated by Next-Generation Sequencing (NGS) platforms. Its primary use case is trimming low-quality bases and adapter sequences from the ends of sequencing reads

If you will not use the quality assessment of the script, there is no need to install this software

In the following sub-steps, the installation in different OS is provided

11.1 *Linux*

In Linux you can install with apt the command line executable of sickle, just type in Bash the following command

Command

Install Sickle from command line (Linux)

```
sudo apt install sickle
```

You can check if the installation has been successful if you type **sickle --version** and you don't receive an error message

11.2 *MacOS*

In macOS you can install with homebrew the command line executable of sickle just type in Bash or Zhr the following command

Command

Install Sickle from command line (macOS)

```
brew install sickle
```

If you do not have Homebrew in your system go to step #7.2 Note

You can check if the installation has been successful if you type **sickle --version** and you don't receive an error message

Running Script

12 **Choose which arguments to run the script**

This script needs to be performed in a command line window using Python.

Depending on the provided arguments, the program will perform more or less actions such as a quality trimming or variant name annotation.

The script requires a minimum of 4 arguments, which we will call from now on the **positional arguments**. In addition, the program's behaviour can be changed by providing **optional arguments**. Finally, there is another type of argument that the script can accept, which is for **information usage**.

In the following sub-steps, we will define the behaviour, needs and different kinds of arguments.

12.1 *Positional arguments*

These arguments should be provided in this specific order

directoryReads

Absolute or relative path to the folder that will have the file(s) of the different sequences with a FASTA format and, optionally, the quality files of those reads.

Both files, sequence and quality should have the same name but with a different extension. More files can be in that folder but they will be ignored.

extensionReads

Extension of the files (without the dot, i.e txt or seq) in the path provided in directoryReads that correspond to the sequences in FASTA format that you want to be aligned to the genome provided in *genomeSequence.*

Ensure they are the only files with this extension in that directory.

genomeSequence

Absolute or relative path to the file that will contain the genome sequence or DNA material that will be aligned with the files inside of *directoryReads.*

genomeAnnotation

Absolute or relative path to the file that contains the genome provided in *genomeSequence* annotated and needs to be in a CSV format with the characteristics noted in *Step 2.*

12.2 *Optional arguments*

These arguments do not need to be provided in this order, but some of them need to be provided together.

-out

Absolute or relative path to where the final files will be stored. If not provided, the output files will be stored in a directory called *results_annotation* in the place or directory where the script has been run.

If the directory already exists, the program will display a warning message allowing you to stop the program from running. If you choose the program to continue, this directory will be overwritten.

-f, -filesOut

Depending on the arguments provided, you will obtain different types and numbers of files in the -out directory.

With this argument, you can control whether you obtain a **SAM file** coming from the alignment or not.

This argument only can take 2 values:

- *all* - you will obtain the SAM file and the table provided from annotating the alignments. This is the value as the default
- *table* - you will only obtain the table. No SAM file will be provided in the results directory

-t, --thresholdRange

This script takes into account the bitscore to give you the best hit.

An additional column will provide other hits in case there is a multiple alignment of the query sequence with your genome. These will be provided only if the hits are in the range of this argument, i.e, if the hit has a value minor than *(1-thresholdValue)*Bit Score of the best hit*, this value will not be included in the multiple hit column. If more than 1 alignment has the same best score and turns out that this score is the best one, the hit provided as the best one will be randomly assigned between these top hits.

By default, this value is 0.01

Note

An example of the effects of this value

We have a sequence (seq_1) which best hits with locus_1 and a bit score of 50.
seq_1 has 3 other hits, with locus_2, locus_3 and locus_4, with bit scores of 50, 45 and 10, respectively.

If we have **-t 0.01**, the **minimum bit score** that a hit needs to have to be considered a hit and added to the multiple alignment column **would be 49.5**. With this value of -t, only locus_2 would be considered as a hit.

If we have **-t 0.1**, the **minimum bit score** that a hit needs to have to be considered a hit and added to the multiple alignment column **would be 45**. With this value of -t, only locus_2 and locus 3 would be considered as a hit

If we have **-t 0.5,** the **minimum bit score** that a hit needs to have to be considered a hit and added to the multiple alignment column **would be 25**. With this value of -t, only locus_2 and locus_3 would be considered as a hit.

If we have **-t 1,** the **minimum bit score** that a hit needs to have to be considered a hit and added to the multiple alignment column **would be 0**, so all hits would be considered and locus_2, locus_3 and locus_4 would be added to the multiple alignment column.

-identity

The absolute or relative path of the file that has the names that want to be associated with the final rows that indicate the alignments.

This file should be a CSV or XLSX file and needs to have the requirements or characteristics described in Step 4.

-cb, --columnsBLAST

This variable will be the absolute or relative path of the file with the names of the columns that will be reflected in the final table that are taken from the BLASTn output alignment. There

should be 1 name of column per row in the file, and the name should be the same as will be named in the BLAST software, for example, *nident*.

The final table combines columns that are in the annotation file and columns that we obtain from the BLASTn alignment. With this variable, you can control which columns will be taken from the final alignment result file and will be written on the end table.

The default columns that are going to be taken have the following names: *qaccver*, *saccver*, *pident*, *length*, *mismatch*, *gapopen*, *qstart*, *qend*, *sstart*, *send*, *evalue*, *bitscore* and *sstrand*
The meaning of these names can be found at
https://www.metagenomics.wiki/tools/blast/blastn-output-format-6

-ca, --columnsAnnotation

This variable will be the absolute or relative path of the file with the names of the columns that will be reflected in the final table that are taken from the file statted in the argument *genomeAnnotation*. There should be 1 name of column per row in the file.

The final table combines columns that are in the annotation file and columns that we obtain from the BLASTn alignment. With this variable, you can control which columns will be taken from the annotation file and written on the end table.

The default columns will be taken with the following names: *Locus Tag*, *Feature Type*, *Start*, *End*, *Strand*, *Gene Name*, *Product Name* and *Subcellular Localization [Confidence Class]*.

If your file does not contain 1 of these columns, you need to provide which columns you want to have in the end file with this argument, always considering the considerations provided in Step 2.

-quality

Extension (without dot i.e ab1) of the quality files attached to the sequence files in the directory given in *directoryReads*. By providing this argument, a quality check and consequent trimming of the sequences will take place before doing the alignment between reads and genome.

Only sequences that come from Sanger, Solexa or Illumina sequencing can be provided to the program. In addition, only single-end reads with *fastqc*, *ab1*, *abi*, or *qual* formats can be analyzed.
If we provide this argument, we must also provide the *-seq* argument.

Providing this argument will allow you to trim the sequences to only align with high-quality nucleotides with the genome in BLASTn. For that, a FastQC analytic HTML will be provided, and

the user will decide the Q (**quality**) and length for trimming. To provide these 2 variables, you can type the numbers directly in the command window.

By default, both values would be 20 (if nothing is typed in the window and only enter is pressed)

When this argument is provided a file with all the reads merged before and after the quality check and correspondent trimming will be created and the sequence id of each read is going to be the name of the file without the extension.

-seq

Method of sequencing with which the sequences in the directory *directoryReads* have been sequenced.

The following arguments are accepted: *illumina*, *sanger* or *solexa*

-sm, --summaryMap

If this argument is given a map displaying the main locus tag of the best hit identified by BLAST will be created. When processing BLAST results, if a sequence has multiple hits, only the best hit (main locus tag) will be displayed.

To correctly use the script, the read names must follow specific formats based on the type of sequence:

- *For sequences with the extension seq*, the well name or index should be embedded in the sequence identification of the read between a plus sign (+) and an underscore (_). For example, read+A1_sequence or read+1_sequence indicates that A1 is the name of the well.
- *For other sequence extensions*, the well name should be between two underscores (_). For example, read_A1_sequence or read_1_sequence indicates that A1 is the name of the well.

If the read names use numeric identifiers instead of well names, the identifiers are mapped to wells as follows: numbers 1 to 96 correspond to wells in a 96-well plate, ordered from top to bottom and left to
right. For example, identifier 1 corresponds to well A1, identifier 2 corresponds to well B1, and identifier 96 corresponds to well H12.

This expression will be looked in the sequence id of the read, not in the file name. If you have provided the argument -quality this sequence id will be the same as the file because of how the script works.

This script supports reads coming specifically from a 96-well plate. The column number must be between 1 and 12, and the row letter must be between A and H.

The final summary, which includes the locus tag and well information, will be saved in the output directory along with other results in the -out directory

12.3 *Information usage*

These arguments give you information but do not change the behaviour of the program.

-h, --help

This argument should be provided alone, without any other arguments.

If you provide this argument information about the program will be displayed, including how to use it and what arguemnts are available.

-q, --quiet

If this argument is given, minimum information will be displayed in the window while running the script.

This argument is incompatible with -v

-v, --verbose

If this argument is given, more information will be displayed in the window while performing the script than in a run where this argument is not given.

This argument is incompatible with -q

13 Running script

The final command should look like the following one

Command

Command to run blastn annotation script

```
python alignment_and_annotation_blastn.py [directory of sequencing reads] [type of file] [genome file in fasta format] [annotation file in csv format]
```

14 **Interact with the script**

Depending on the input and the state of the folder where the program has been executed, the program can ask for different kinds of interactions:

- Ask if you want to replace the final result folder
- Ask for the quality of trimming
- Ask for the length of trimming

15 **See results**

Depending on the input, context of the run and arguments, the program can give different outputs:

- *genomeSequence DB files (.nhr, .nin and .nsq)* - In case the organism database needed to perform a BLAST is not in the path where the genomeSequence file is, these files will be created in the same directory as the genome sequence provided is located
- *-out directory (by default results_script_blast)* - folder where the results will be stored, and it can contain the following folders and files:

1. **reads_fastq** - if the -quality argument is provided, this folder will have the fastq files of the sequences provided in the directoryReads. If it is not provided, this folder will be empty.
2. **all_reads_merged.fasta** - All the sequences provided in directoryReads merged in a FASTA format.
3. **all_reads_merged_quality.fastq** - All the sequences provided in directoryReads with their respective quality merged in a FASTQ format. For more information on the FASTQ format, you can start reading the **dedicated entry in wikipedia** which provides a good starting explanation of the file.
4. **all_reads_merged_quality_fastqc.html** and **all_reads_merged_quality_fastqc.zip** - Quality analysis report of the sequences provided in directoryReads done with the software FastQC

and should be checked before assigning the Q and length of trimming that will guide the dynamic trimming of the sequences done by Sickle. Both files have the same information.

5. **all_reads_merged_quality_trimmed.fastq** - Sequences contained in all_reads_merged_quality.fastq trimmed based on the Q and length trimming variables provided by the user with their respective quality.
6. **all_reads_merged_trimmed.fasta** - Sequences contained in all_reads_merged_quality.fastq trimmed based on the Q and length trimming variables provided by the user.
7. **all_seq_aligned.sam** - SAM format output file of the BLAST done with the sequences provided. For more information about the SAM output, you can check the following page https://www.metagenomics.wiki/tools/samtools/bam-sam-file-format
8. **all_seq_aligned.tsv** - Tabular output file of the BLAST done with the sequences provided with the name of each column and an alignment for each row.
9. **table_reads_gene_description.csv** - Final table that will combine the columns selected from the annotation file and the columns selected in the BLAST output file in addition to columns providing info if there is a multiple alignment of that sequence to the genome and the hits (considering the value in the rangeThreshold argument). If the -map argument is provided, another 2 columns will be added with the position that the sequence holds in the map and the Identity that this has. In other words, it will hold the value that is in the cell on the file provided in the argument -map.
10. **summary_locus_grid_map.csv** - A table with the layout of a 96-well plate in which in each cell there is the best hit between the sequence in that position and the reference genome

Example 1

12m

16 **Annotation of sequencing results from *P. putida KT2440 with only positional arguments***

This has been done in a Windows 10 with a WSL Ubuntu 20.04

17 **Acquisition of files** 4m

17.1 *Script* 1m

Downloaded the script from the entry **LAPu-InsertsGenAnnotation-2.0.0** from **LAP repository** and re-named *annotation_DNAinserts.py*

annotation_DNAinserts.py

17.2 *Genome and Annotation Files* 2m

Both files have been obtained from **Pseudomonas Genome DB**, specifically from one fo the **entries of the Pseudomonas putida KT2440 organism**

Pseudomonas_putida_KT2440_110.fna 6MB

Pseudomonas_putida_KT2440_110.... 970KB

17.3 *Reads* 2m

Files return from the Sanger sequencing in the company **Stab Vida** of a plate coming from another protocol from this page "**High-throughput workflow for the genotypic characterization of transposon library variants**"

sequencing_results.zip

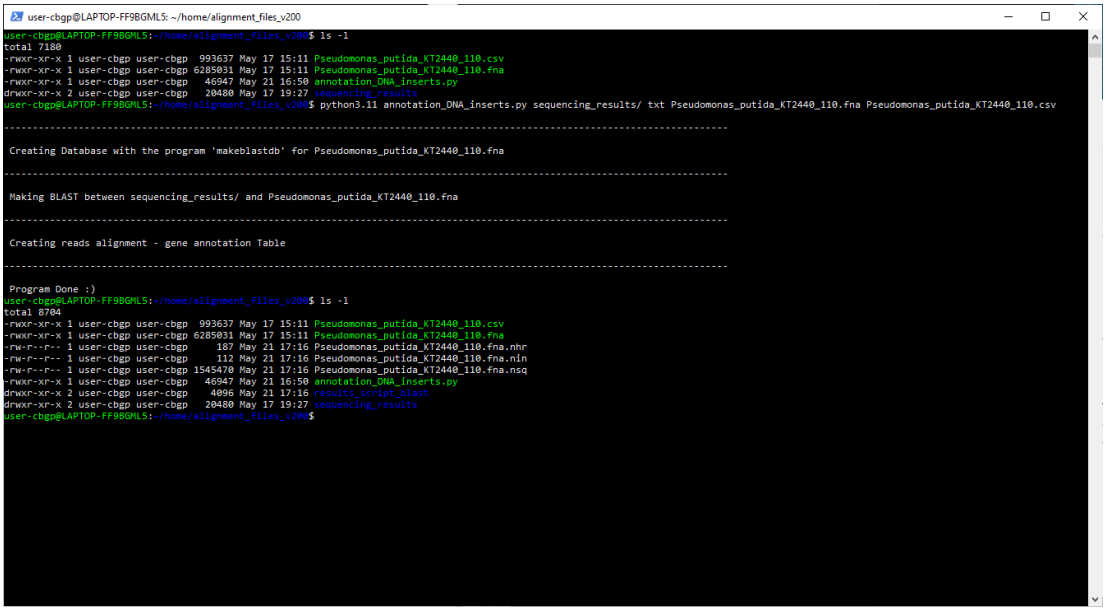
18 **Choose arguments to give** 5m

We are going to leave all the optional arguments with the default values and just give the positional ones, in other words, the minimal amount of inputs

19 **Run script** 2m

We are going to run the script in the same folder as every file that we are going to give to the script, so we are going to give relative paths as we can see in the image as we can see the files that the folder contained before and after executing the script with the following command

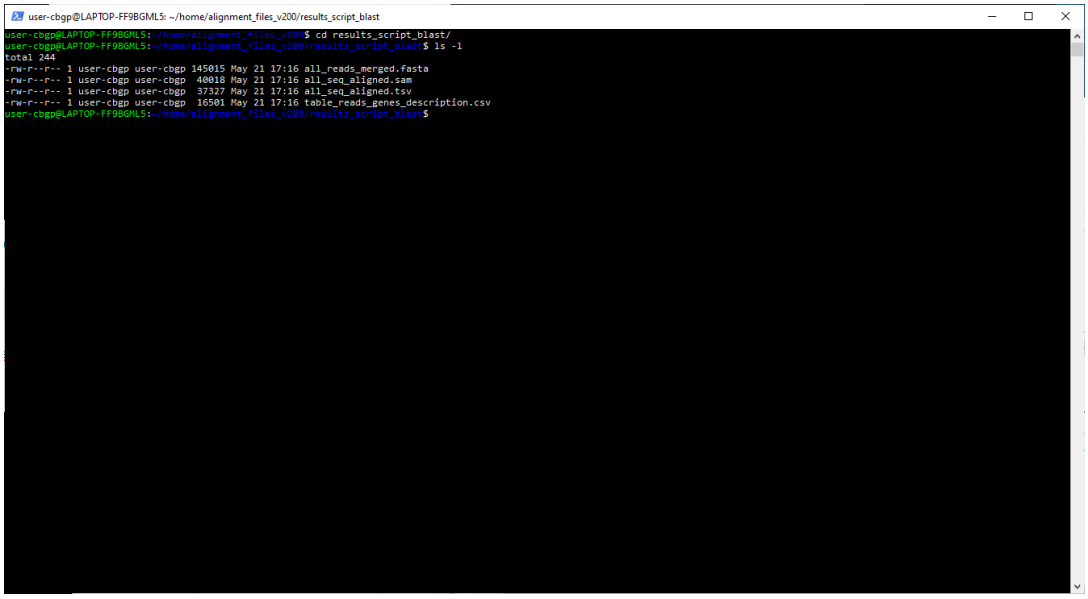
python3.11 annotation_DNA_inserts.py sequencing_results/ txt Pseudomonas_putida_KT2440_110.fna Pseudomonas_putida_KT2440_110.csv



Commands that show the state of the folder (ls -l) and the run of the Python script

Expected result

As we can see in the figure, the files for the database to do the alignments were created, and *results_script_blast* folder was created as well



Content of the folder results_script_blast created by the alignment program

- all_reads_merged.fasta
- all_seq_aligned.sam
- all_seq_aligned.tsv
- table_reads_genes_description.csv

Example 2

16m

20 **Annotation of sequencing results from *P. putida KT2440* with positional and optional arguments**

This has been done in a Windows 10 with a WSL Ubuntu 20.04

21 **Acquisition of files**

8m

21.1 *Script*

1m

Downloaded the script from the entry **LAPu-InsertsGenAnnotation-2.0.0** from **LAP repository** and re-named *annotation_DNAinserts.py*

annotation_DNAinserts.py

21.2 *Genome and Annotation Files*

2m

Both files have been obtained from **Pseudomonas Genome DB**, specifically from one of the **entries of the Pseudomonas putida KT2440 organism**

Pseudomonas_putida_KT2440_110.fna 6MB

Pseudomonas_putida_KT2440_110.... 970KB

21.3 *Reads*

1m

Files return from the Sanger sequencing in the company **Stab Vida** of a plate coming from another protocol from this page "**High-throughput workflow for the genotypic characterization of transposon library variants**"

reads_optional_arguments_ex.zip

These reads have quality files attached to each sequence/read which will allow us to enusre that only the part of the sequence that is of high quality gets alligned with the reference genome if we give the argument -quality

21.4 *Map*

1m

A file returned from the running of another protocol from this page, "**OT-2 PCR sample preparation protocol**"
This map corresponds to the same plate sent to the sequencing company and the files obtained from it.

map_identity_ex.xlsx

21.5 *Files with columns selected* 3m

In the final table, we want to have the following columns

- **From annotation file:** Locus Tag, Start, End, Strand, Gene Name, Molecular Weight (predicted)
- **From BLAST output file:** qaccver, evalue, bitscore

columns_selected_annotation.txt 0B

columns_selected_blast.txt 0B

22 **Choose optional arguments to give** 5m

We are going to give different optional arguments to achieve our objective:

- want to have a verbose screen output (*-v*)
- the result directory to be called results_example2 (*-out results_example2*)
- we want only the table, not the SAM file (*-f table*)
- we want customized columns in the final table (*-ca columns_selected_annotation.txt -cb columns_selected_blast.txt*)
- we want that the range threshold on the bit score is 0.2 (*-t 0.2*)
- we want to do a quality trimming (*-quality ab1 -seq sanger*)
- we want the summary map of position - best hit (*-sm*)
- we want the alignments to be tracked to the map of samples (*-identity map_identity_ex.xlsx*)

23 **Run script** 3m

We are going to run the script in the same folder as every file that we are going to give to the script, so we are going to give relative paths as we can see in the image as we can see the files that the folder contained before and after executing the script with the following command

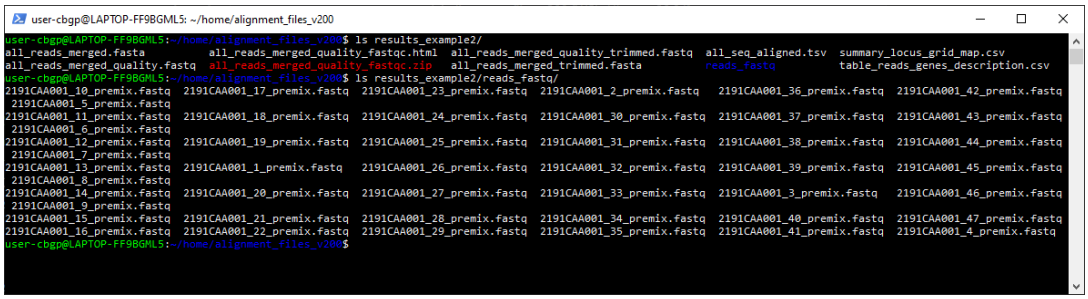
python3.11 annotation_DNA_inserts.py -v reads_optional_arguments_ex fasta Pseudomonas_putida_KT2440_110.fna Pseudomonas_putida_KT2440_110.csv -out results_example2 -f table -ca columns_selected_annotation.txt -cb columns_selected_blast.txt -t 0.2 -quality ab1 -seq sanger -sm -identity map_identity_ex.xlsx

```
user-cbgp@LAPTOP-FF9BGML5: ~/home/alignment_files_v200/results_example2
user-cbgp@LAPTOP-FF9BGML5:~/home/alignment_files_v200$ ls -l
total 7284
-rwxr-xr-x 1 user-cbgp user-cbgp  993637 May 17 15:11 Pseudomonas_putida_KT2440_110.csv
-rwxr-xr-x 1 user-cbgp user-cbgp 6285031 May 22 11:12 Pseudomonas_putida_KT2440_110.fna
-rwxr-xr-x 1 user-cbgp user-cbgp   46947 May 21 16:50 annotation_DNA_inserts.py
-rwxr-xr-x 1 user-cbgp user-cbgp      70 May 22 11:12 columns_selected_annotation.txt
-rwxr-xr-x 1 user-cbgp user-cbgp      25 May 22 11:12 columns_selected_blast.txt
user-cbgp@LAPTOP-FF9BGML5:~/home/alignment_files_v200$ python3.11 annotation_DNA_inserts.py -v reads_optional_arguments_ex fasta Pseudomonas_putida_KT2440_110.fna Pseudomonas_putida_KT2440_110.csv -out
 results_example2 -f table -ca columns_selected_annotation.txt -cb columns_selected_blast.txt -t 0.2 -quality ab1 -seq sanger -sm -identity map_identity_ex.xlsx

--------------------------------------------------------------------------------------------------------------------------------


    GENERAL INFORMATION RUNNING PROGRAM

        Reads file directory reads_optional_arguments_ex with the fasta extension
        The genome sequence and gene annotation will be extracted from Pseudomonas_putida_KT2440_110.fna and Pseudomonas_putida_KT2440_110.csv
        The alignments final table will only consider hits that have 0.8*best hit score for each read
        Output files will be stored at results_example2

--------------------------------------------------------------------------------------------------------------------------------


--------------------------------------------------------------------------------------------------------------------------------


 Creating Database with the program 'makeblastdb' for Pseudomonas_putida_KT2440_110.fna


Building a new DB, current time: 05/22/2024 11:14:25
New DB name:   /home/user-cbgp/home/alignment_files_v200/Pseudomonas_putida_KT2440_110.fna
New DB title:  Pseudomonas_putida_KT2440_110.fna
Sequence type: Nucleotide
Keep MBits: T
Maximum file size: 10000000000B
Adding sequences from FASTA; added 1 sequences in 0.037816 seconds.
--------------------------------------------------------------------------------------------------------------------------------


Started analysis of all_reads_merged_quality.fastq
Analysis complete for all_reads_merged_quality.fastq
--------------------------------------------------------------------------------------------------------------------------------
Chech the FastQC file and decide the threshold for trimming!
Enter the Q value for trimming (by default 20): 20
Enter the length (by default 20): 19

SE input file: results_example2/all_reads_merged_quality.fastq

Total FastQ records: 47
FastQ records kept: 45
FastQ records discarded: 2


--------------------------------------------------------------------------------------------------------------------------------


 BLAST command (s) that are going to be performed:
        - Tabular output command
                blastn -query results_example2/all_reads_merged_trimmed.fasta -db Pseudomonas_putida_KT2440_110.fna -out results_example2/all_seq_aligned.tsv -outfmt '6 qaccver evalue bitscore sstart'

 Final Headers that we are going to obtain in the tabular output:
        qaccver evalue  bitscore        sstart


--------------------------------------------------------------------------------------------------------------------------------


 Adding Identity Columns to table with the information in map_identity_ex.xlsx
 Volumes set in -Identity are going to be introduced and, in case the argument -sm is set, the map is also created and filled

--------------------------------------------------------------------------------------------------------------------------------


        FINAL ALIGNMENT-ANNOTATION TABLE GENERAL INFORMATION

                Dimension                                                       13 Columns and 40 Rows
                Number of reads with multiallignments (within the threshold established)  7
                PositionSeqPlate - Identity                                     True

--------------------------------------------------------------------------------------------------------------------------------


 Program Done :)
user-cbgp@LAPTOP-FF9BGML5:~/home/alignment_files_v200$ cd results_example2/
user-cbgp@LAPTOP-FF9BGML5:~/home/alignment_files_v200/results_example2$ ls
all_reads_merged.fasta          all_reads_merged_quality_fastqc.html  all_reads_merged_quality_trimmed.fastq  all_seq_aligned.tsv  summary_locus_grid_map.csv
all_reads_merged_quality.fastq  all_reads_merged_quality_fastqc.zip   all_reads_merged_trimmed.fasta          reads_fastq          table_reads_genes_description.csv
user-cbgp@LAPTOP-FF9BGML5:~/home/alignment_files_v200/results_example2$
```

Commands that show the state of the folder (ls) and the run of the Python script

As we can see, because we have given the argument -quality so we need to interact with it. After opening the file all_reads_merged_quality_fastqc.html it is decided to put as a threshold Q = 20 and length = 19, so 2 reads have been discarded from the sequencing.

Expected result



Content of the folder *results_example2 and reads_fastq*

- all_reads_merged.fasta
- all_reads_merged_quality.fastq
- all_reads_merged_quality_fastqc.html
- all_reads_merged_quality_fastqc.zip
- all_reads_merged_quality_trimmed.f…
- all_reads_merged_trimmed.fasta
- all_seq_aligned.tsv
- summary_locus_grid_map.csv
- table_reads_genes_description.csv
- reads_fastq.zip (It is a zip here for uploading purposes, but the output is a directory, not compressed)

Protocol references

https://doi.org/10.1093/synbio/ysad012
https://doi.org/10.1021/acssynbio.3c00397