Fundamentals of computational biology

Lecture notes

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Preface

> **Danger**
>
> This book is a work in progress

We started this book with the aim of compiling the lectures of the course Fundamentals of Computational Biology offered at Universidad EAFIT for undergrad students in Biology. The course has been taught from different perspectives from its creation, yet the last iteration was divided into five introductory modules to i) Unix, ii) sequence analysis, iii) genomics, iii) metagenomics iv) structural biology.

Lectures are focused on a theoretical-practical approach where basic concepts from biology, bioinformatics and computer science and interleave with the practice to solve challenges. Exercised or challenges are designed to improve students' abilities that are likely to be involved in real-life problems in computational biology.

Learning features

> **Note**
>
> Sometimes other fields might add interested value to the understanding of the computational biology area. This feature remarks some of them and aim to explain these intersections.

> **Tip**
>
> As you move forward in the computational biology field you will find that there are several tips and tricks (mainly from the command line) as well as some random CLI programs that can leverage your daily workflow as a researcher. Using this feature we highlight some of those that appeared

to linger on the field.

! Important

To help you consolidate your understanding we end most chapters with important messages or concepts that help you evaluate yourself as you move forward on the lessons.

Danger

Caution

When experimenting with the CLI and many other computational tools it is common to face several known errors and drawbacks. Then, we present some of them and how to sort them out.

Warning

Challenges

Since focused on a competences learning approach we have highlighted several real-life (but basic) *challenges* a researcher faces when approaching computational biology problem (from tool selection, usage and result analysis). Therefore the book section *challenges* presents a selection of these problems that will later be approached by a computational biology strategy (mainly from the CLI).

File format

As many analysis specialize on data analysis, many formats arise that optimize the processing steps or the data storing steps. Some of these formats are keystones of bioinformatic analyses. We present examples of some formats an describe its main elements.

Introduction

Here we present a course centered book of the Fundamentals of Computational Biology. We will cover several topics, from using the unix tools, the importance of package manager systems (such as homebrew and conda), sequencing technologies, sequence alignments, molecular phylogenetics, genome assembly and annotation, and variant calling analysis.

Inlcude a section, maybe an appendix about how to handle errors Include s section about the windows subsistem for linux WSL and the ease of use for windows users

Part I

The command line

Chapter 1

The command line

In this chapter we will explore the fundamentals of the command line interface (aka CLI). We will distinguish the differences between Unix, CLI, Bash and Terminal and other concepts from the computer sciences.

As you will see the CLI is composed of several programs enabling the interaction with the machine, we will discuss some of the basics to navigate your machine, and some advance one that enable complex operations and automating tasks.

1.1 Getting started with the command line

Before landing into the CLI let us consider the Unix concept. The first question that comes in this section is: what is Unix? It simply is an operating system (OS). In other words, it is a set of programs that inter-operate with each other to let you communicate with the machine. A very important variant (or clone) of Unix is the very well known OS Linux, which was created by Linus Torvalds from scratch. The most important idea behind Unix based systems is the idea that we can use it to access information and hardware programmatically. Other main feature from Unix-like OS systems is the fact that data is usually stored as text files and the interface by which users communicate with the machine is also text-based (TUI: text user interface as opposed to GUI, graphic user interface).

Almost every computer has a way to interact with or access to the inner elements of the computer. Such interface is called the the command-line-interface Fig. 1.1.

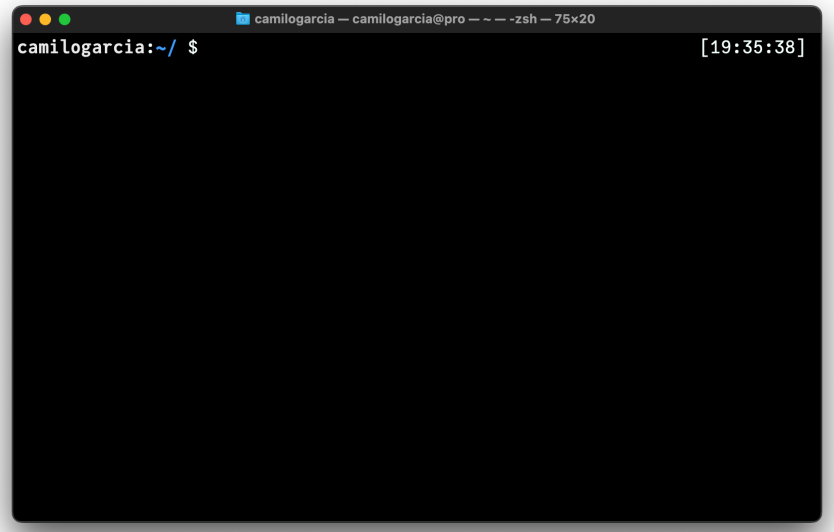


Figure 1.1: A **terminal** app displaying common features of the command line interface

1.2 File paths

Programs, files and directories on every machine (with Unix-like OS) display hierarchical paths (routes), starting out from the **root** (represented by the back-slash character `/`). The **root** represents the beginning of all the software installed in the machine. And many other files are nested from there forming a tree-like structure for the paths Fig. 1.2

> **Tip**
>
> You can inspect the paths of a nested directory tree using `tree` command in you cli:
>
> ```
> tree -d -L 1
> ```

There are basically **two** ways to explore or navigate your file system. If you always represent it from the root, then you are presenting an **absolute path**. For instance the absolute path to my desktop is (`/Users/camilogarcia/Desktop`).

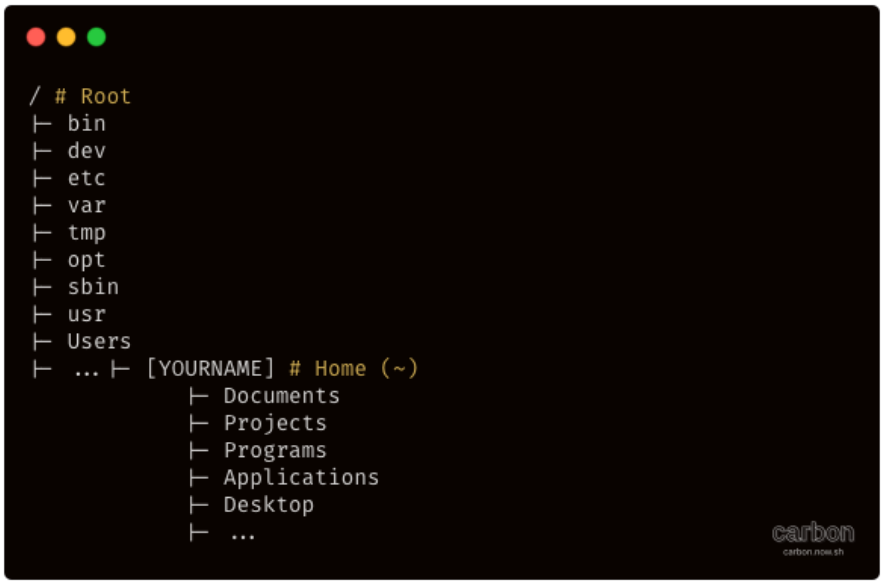


Figure 1.2: A figure displaying tree-like structure of the programs in a machine with macOS

1.3 Basic Unix commands

Given that the vast majority of file systems are organized in file paths, the first question when starting with the CLI is "Where am I?". So Unix tool system is equipped with a bunch of commands but its basic ones are pretty much oriented to answer that question and navigating this text-based interface of files. The following three commands (`pwd`, `cd`, `ls`) will help you conquer the CLI.

1.3.1 Printing your working directory

To know where you are you can see your current location, that is to *print your working directory* using the `pwd` command.

```
pwd
```

1.3.2 Change to other directory

```
cd test-dir
```

> **Tip**
>
> Some basic arguments to navigate across your terminal:
>
> ```
> cd .. # change backwards
> cd ~  # change to the home
> cd /  # change to the root
> cd -  # change to previous dir
> ```

1.3.3 Listing files

```
ls
```

> **Tip**
>
> You can navigate your executed commands by typing ↑ or ↓.

1.3.4 Making new directories

```
mkdir test-dir
```

1.3.5 Creating a file

A simple command to create any file inside your terminal is `touch` it just create a file, but do not allow any editing.

```
touch new-file.txt
```

The `new-file.txt` is empty and created on your current location unless you assign another path when creating it. We suggest to take a look at Allison Horst, especially on how to name files depending on the *case* see **?@fig-naming-files**

1.3.6 Printing files to the screen

```
cat new-file.txt
```

> **Tip**
>
> When using the CLI at first its common to feal quite slow. Then, a very useful tip to boost the productivity from the command line is the autocompletion of commands by hitting `<tab>` after the initial command.

1.3.7 Removing files or directories

```
rm
```

> **Tip**
>
> When having a long command, it becomes practically to go to the beginning or to the end of it. To do so you can use the key combination `Ctrl + A` and `Ctrl + E` respectively.

```
rmdir
```

1.4 Anatomy of a command

There is still many conventions by which the parts of a command line might be called, yet a very standard convention is presented in Fig. 1.3

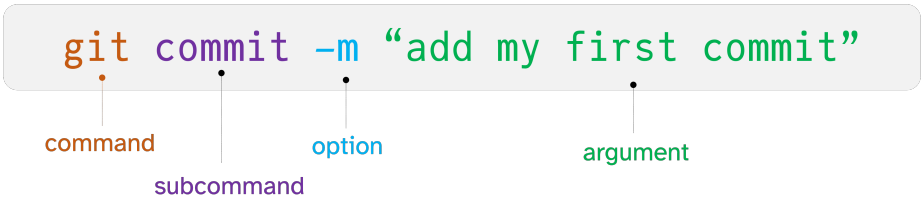


Figure 1.3: A simple command and a convention to call its main components

Some other for instance also tend to call the `option` as `flag`. This conventions are powerful because almost any command line interface display this structure (complex one add some other features and simple one tend to lack subcommands).

> **Challenge**
>
> Bacterial defense mechanisms to avoid bacteriophage infections are abundant. One of these is the restriction-modification system (RM-System), which works by targeting a specific site called *motif*, shared by the phage and bacteria, with methylations. Motifs are commonly represented as a *motif logo* which is a probabilistic representation of the nucleotides in a given position. Find the number of times the motif from **?@fig-motif** appears on *B. tequilensis* EA-CB0015 genome using a command. Assume that probabilities are equal when multiple bases appeared at one site.

1.5 intermediate Unix commands

```
grep
```

```
sed
```

```
tr
```

1.6 Some great operators/ Metacharacteres in Bash

For more explanations on the basic commands in the command line we suggest to visit the first chapters of *Computing skills for biologist* from Allesina and Wilmes (2019)

Chapter 2

Version control

As its name suggests, a version control system (VCS) allow you to keep record of the changes happening while working files and directories. Several VCSs have been created but the most popular is git. It is characterized by being a **distributed** VCS, which means that changes history are recorded locally (whether in a user laptop or user account) in contrast to other **centralized** VCSs that changes are saved on a shared machine or server.

So, why bother to learn a VCS in bioinformatics? Well there are many reasons, but to highlight some of them: i) Since VCSs allow you to record changes, you can always trace back the steps made in ana analysis, which is nice for the **reproducibility** of your work. ii) a system like git could be coupled with a shared-centralized server as it is GitHub (we'll talk about it later and then one could **share and collaborate**, expanding the extent of your research and iii) following the structures and command from git its at first overwhelming and demands consistency and order, then when scaling a project it will payoff this stepping curve of learning by keeping the **efficiency** of your work.

2.1 Git installation and configuration

Installation could proceed from the official page of git. If working from WSL it has the binaries preinstalled, so you can jump directly to the configuration. The second step is to configure your user name and an email. with the following lines:

```
git config --global user.name "Your Name"
git config --global user.email user@eafit.edu.co
```

You could always user the preferred e-mail. More configurations are available, for instance the preferred editor to work with and so on, you can explore by asking for help `git config --help` or `git config --list`.

2.1.1 The basics

There are at least **six** basic commands. Three of them allow recording local changes (`git init`, `git add` and `git commit`) and the other three help you to inspect the state of the changes (`git status`, `git diff` and `git log`), we will dive into the detail in the following lines. So, to start recording changes in a directory you must **initialize** the directory (which will now be called repository) using `git init`. This is a one-time command to get started.

2.1.2 The local workflow

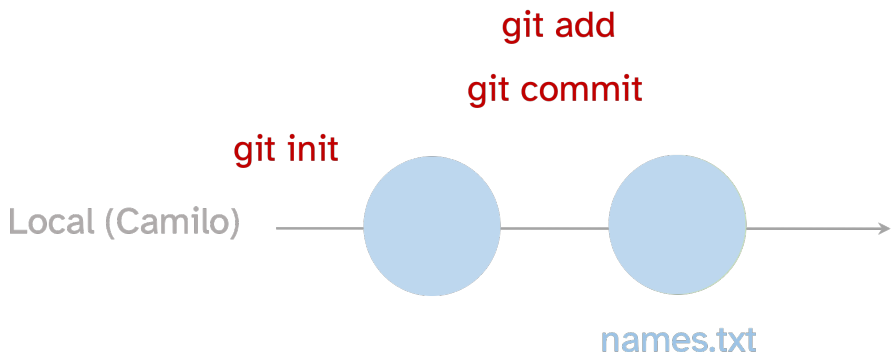


Figure 2.1: Local workflow of a git project adding and committing changes

2.1.3 Ignoring files

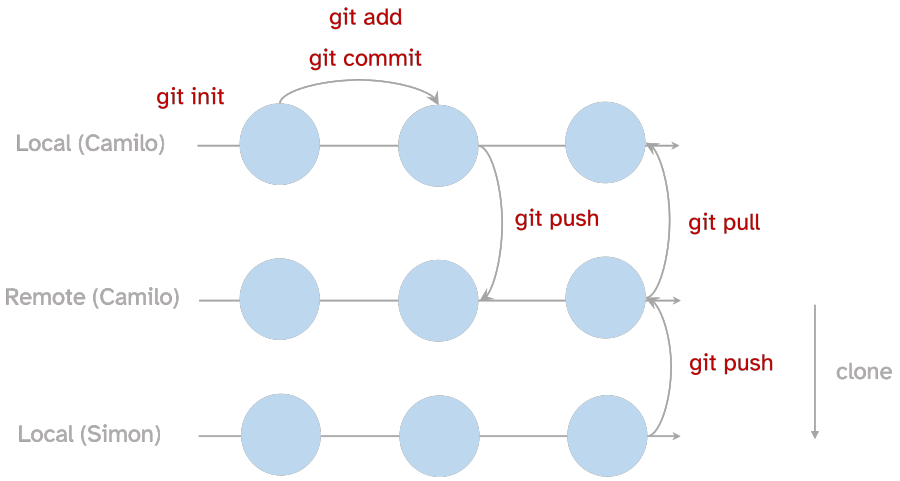
the `.gitignore` file.

2.2 Exploring GitHub

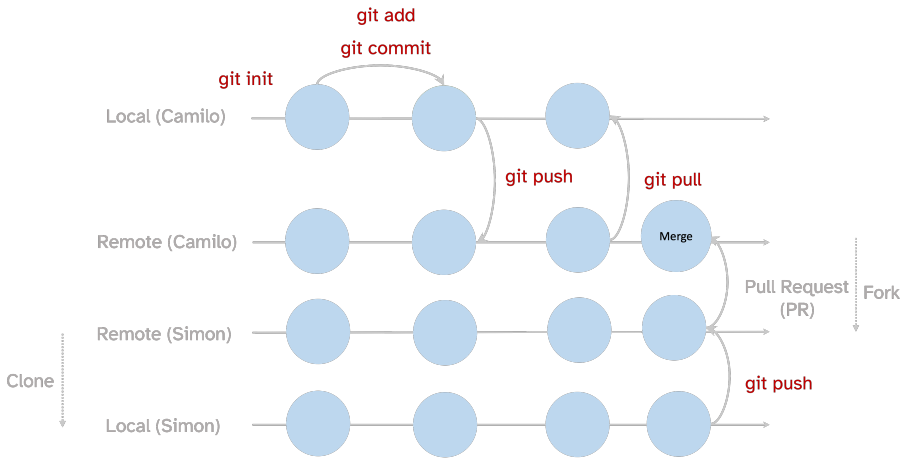
GitHub home

2.3 From local to remote

2.3.1 Cloning workflow



2.3.2 Forking and collaborate



2.3.3 Branching and merging

Chapter 3

Package managers

3.1 What is the importance of package managers

3.2 Conda environments and other package managers

3.3 Creating enviroments with Conda

3.4 Package managers for OS

There are several package managers handling general purpose packages and apps. For MacOS the famous one is Homebrew and for Windowos several could be used such as Chocolatey and Scoop.

Chapter 4

Notions of HPC

4.1 What is HPC

4.2 Some important concepts of the hardware

See Jakob's blog

4.3 Using the Apolo cluster wirh Slurm

Part II

Sequence analysis

Chapter 5

Sequence analysis

In this chapter we will discuss several about several points of view about bioinformatics and computational biology, we will further consider several biological concepts that appear central to understand the manipulation of biological data.

5.1 Endless debate: bioinformatics vs. computational biology

5.2 The duality of DNA

5.3 The central ~~dogma~~ theory of molecular biology extended

5.4 Sequencing strategies

5.5 Sequencing over time

5.6 Some insights from sequencing genomes

Chapter 6

Sanger analysis

This is a section about the first gen sequencing tech

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6.2 Sanger sequencing methods

6.2.1 The chain termination method

6.2.2 Sanger with capillary electrophoresis

6.2.3 Strengths and limitations of Sanger methods

6.3 Files from Sanger

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6.5 The 16S rRNA and its relevance for sequencing

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7.1 Why do we align sequences?

In search of homology and identy

7.2 What is homology

7.3 Pairwise alignments algorihtms

7.3.1 Hamming distance

7.3.2 Edit distance

7.3.2.1 Dynamic programming

7.3.3 Needleman-Wunsch (global alignment)

7.3.4 Smith-Waterman (local alignment)

7.4 The genetic code and Scoring matrices

7.5 BLAST and its families

psi-blast? true homologs, recurrent blast to polish scoring matrix during several generations to generate true homologs

7.6 Multiple sequence alignments

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8.2 Mehtods for phylogenetic reconstruction

8.3 Building a phylogenetic reconstruction

8.3.1 Evolutionary substitution model

8.3.2 Maximum likelihood

8.3.3 Bayesian inference

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9.1 Platforms yields

9.2 Reads main differences

9.3 Illumina principle (sequencing by synthesis)

9.3.1 The fastq format

9.3.2 Quality assesment of Illumina

9.4 PacBio principle (sequencing by incorporation)

9.4.1 Throughput evolution

9.4.2 Quality assesment of PacBio

9.5 Oxford Nanopore Technology (ONT) principle

9.5.1 Platforms

9.5.2 The fast5 file format

Part III

Genomics

Chapter 10

Genome assembly

10.1 The problem of assembling genomes

10.2 Main algorithms for genome asssembly

10.2.1 Overlay, Layout, Consensus (OLS)

10.2.2 De Bruijn graphs

10.3 Main concepts of an assembly

10.3.1 Contigs, Unitigs, Scaffolds

10.4 A complete workflow for assembling genomes

10.5 Assessing genomes

10.5.1 Inspecting genome graphs

10.5.2 Genome completeness

10.6 Understanding genome difficulties

- End of chromosomes
- Erros
- Lack of coverage
- Heterozigozity

- repeats

Chapter 11

Genome annotation

11.1 *ab initio* annotation

11.2 Homology annotation

11.3 Annotation files

11.3.1 the GBK and GBFF

11.3.2 The GFF specifications

11.4 Visualizing genomes and annotations

Chapter 12

Variant calling analysis

12.1 Common mutations

12.2 Structural variants

12.3 Genome rearrangements

12.4 Read mapping algorithms and programs

12.4.1 Burrow-Wheeler-Alignment

12.4.2 BWA-MEM2

12.4.3 Minimap2

12.4.4 SAM, BAM and CRAM formats

12.5 Identifying mutations

12.5.1 Freebayes and Snippy

12.5.2 The VCF file

Part IV

Structural bioinformatics

Chapter 13

Structural bioinformatics

Structural bioinformatics is a multidisciplinary area enriched by chemistry, physics, computer science and many others. Although, it could be focused on different biological macro-molecules, here the emphasis will be focused on proteins.

One of the first protein structure elucidated was myoglobin and it triggered the study of the role of the structure of proteins and its biological functions

Identify a protein related to your study that could be further analyzed.

13.1 Protein structures

Difference between the levels of protein structure primary structure is the basic linear representation of aminoacids. Natural aminoacids and modified or rare aminoacids display physico-chemical rich information and could be represented by letters. Therefore in the genetic code we could find the one-letter code.

Secondary structures result from the spatial arrangement of aminoacids that interact with each closer neighbors. There are some remarkable secondary structures such as β-sheets, α-helix, coils (flexible) and others.

Tertiary structure informs about the structural disposition of the secondary structures that fold between each other due to hydrophobic interactions, disulfure bonds, and other chemical interactions forming a globular and dynamic structure. Thus, proteins could display multiple structural states depending on the physical and energy stability (see the Levinthal's paradox).

Quaternary structures result from interaction of multiple tertiary structures. *The structure, therefore dictates the protein function.* This basic concept have triggered more recently a boom on the analysis of the structure of proteins.

13.2 Identifying or predicting protein structures

Xray crystallography, nuclear magnetic resonance (NMR) allows to encapsulated dynamic information of the protein in time Electron micrography (EM) and Cryo-EM. These experiment rely on highly specialized set ups and there are other drawbacks

To date, helium is scarce around the world, so labs all around are having trouble to get this element.

Modelling the structure whether *ab initio* or by *homology* also allow structure prediction. However these strategies

Recently AlphaFold

The protein database (PDB)

Protein topologies resulting from the folding: horshoe, beta-barrel and other could be identified

Structural classification of proteins (SCOP) when analyzing a new protein classification by class, architecture, topology (fold-family), homologous superfamily and sequence family

Importance of Gene Ontology

13.2.1 Secondary structure prediction

Secondary structures could also be represented in one letter (e.g.)

Functional domains could be predicted by sequence alignment and allow structural inference. Main predictions are based heavily on machine learning and are frequently accepted under a consensus of multiple tools.

There is a group of protein called intrinsically-disordered proteins that change in its tertiary structures quite frequently, therefore it prediction could be troublesome.

> ⚠ Exercise 01
>
> Submit 5lWM (JAK3) from the PDB on FASTA format on the JPRED and PSIPRED and compared with the experimentally predicted version of the protein. Analyze the predictions and tell are there differences between predictions? Which one is more accurate?

13.3 PDB database introduction

The PDB database is one of the most important and ancient open biological database where all new protein structures are submitted. It is an international consortium where several regions work together to curate information.

The PDB in Europe (PDBe) is not only for proteins but form many other *experimentally predicted* macro-molecules (protein-protein interactions, peptides, RNA and so on).

Protein structures are registered using a unique code of four characters.

X-ray crystallography: is a chemical state of the macro-molecule where it is immobilized, therefore information correspond to one state of the structure, then crystal protein is submitted to an x-ray beam to generate a diffraction pattern.

NMR spectroscopy: captures dynamic information of the protein, but generally resolves small proteins. The principle?

Electron microscopy adapted to cryo-preservation allow proteins visualization.

Part V

Challenges

Chapter 14

Motif search

Challenge

Bacterial defense mechanisms to avoid bacteriophage infections are abundant. One of these is the restriction-modification system (RM-System), which works by targeting a specific site called *motif*, shared by the phage and bacteria, with methylations. Motifs are commonly represented as a *motif logo* which is a probabilistic representation of the nucleotides in a given position. Find the number of times the motif from **?@fig-motif** appears on *B. tequilensis* EA-CB0015 genome using a command. Assume that probabilities are equal when multiple bases appeared at one site.

The first thing we must do is download the sequence of interest. In Ch 1 the genome of *Bacillus tequilensis* EA-CB0015 is proposed. There are several ways to do so. A simple way is to search in the NCBI databases for the entire genome and download as `FASTA` file (we cover the properties of this file format in Ch 5).

When inspecting the file we see the following lines at the top

```
>NZ_CP048852.1 Bacillus tequilensis strain EA-CB0015 chromosome, complete genome
ATGGAAAATATATTAGACCTGTGGAATCAAGCCCTTGCTCAAATCGAAAAAAAGTTGAGCAAACCGAGTTTTGAGACTTG
```

```
GATGAAATCCACCAAAGCCCACTCACTGCAGGGAGATACACTGACGATCACGGCTCCCAATGAATTTGCCAGAGACTGGC
TGGAGTCCAGATACTTGCATCTGATTGCAGATACTATATATGAATTAACCGGGGAAGAATTGAGCATTAAGTTTGTCATT
CCTCAAAATCAAGATGTTGAGGACTTTATGCCAAAGCCGCAAGTCAAAAAAGCGGTTAAAGAAGATACATCTGATTTTCC
```

The file is about 4 Mb and it is heavy to be opened in any editor. Therefore when looking for a pattern it becomes more useful the CLI.

A first attempt to do is simply by using `grep` for the pattern presented on **?@fig-motif**. So a first problem you will find is to represent multiple characters in one position of the pattern. There is a fantastic tool to so and its called *regular expressions* (regex). a regex is a way to encode groups of characters using single characters. For instance the character `*` (wildcards) represents any character, so it groups all characters and it is used broadly on the CLI. There are many regex dialects however and for `grep` the dot `.` represents any character and when the pipe `|` represents an special operator in the CLI, for grep regex it represents and `OR` operator.

So, the second step is to create our regular expression. Note the there are six characters in the expression that can adopt different characters. So our first regex is `TAAA(T|A)(.)(T|A)(G|A)(.)(C|A)GGT`. In the expression the parenthesis allow groups of characters.

Now lets see if we can search for that pattern using `grep` and the appropriate option for using regex, which in this case is `-E`. Another simply way to use the `grep -E` combination is to use simple `egrep` command plus the `-c` option to count the number of *lines* where the pattern appears.

```
egrep -c "TAAA(T|A)(.)(T|A)(G|A)(.)(C|A)GGT" data/Bteq-genome.fasta
```

```
43
```

Is this the solution? One might think it is solved. However, there are several issues to be aware. First `egrep -c` is counting the lines, what if in one line there are multiple matches? Then, the approach will showing a value under the actual number of motifs. So a way to solve that is to capture and extract the matches and print them out. A way to do that is to use the option `-o` which actually prints only the matching part of the lines, and then count them.

```
egrep -oc "TAAA(T|A)(.)(T|A)(G|A)(.)(C|A)GGT" data/Bteq-genome.fasta
```

```
43
```

The outcome is the same tough. So is it solved? Every NCBI genome that is downloaded display a distinct character besides de nucleotide sequence. Every several number of nucleotides there is a new line `\n` that enables the wrapping file into multiple lines. Therefore some motifs could be shopped by the `\n` character so they will never appear with the regex used. A way to circumvent this issue is deleting the `\n` in the entire file. We can easily do that with the translate `tr` command:

```
tr -d '\n' < data/Bteq-genome.fasta
```

```
>NZ_CP048852.1 Bacillus tequilensis strain EA-CB0015 chromosome, complete genomeATGGAAAATATATTAGA
```

The genome now lies in an entire and unique line along with its title. A way to chain the commands without creating intermediate files is by using the pipe `|` character. At the end we will use `wc -l` to count the occurrence (although `grep` has its own count option `\c` it is inconvenient because it will count the lines and now the lines of the exact matches provided by the `-o` option and as we have just one line, then only will count to 1).

```
tr -d '\n' < data/Bteq-genome.fasta |
    egrep -o "TAAA(T|A)(.)(T|A)(G|A)(.)(C|A)GGT" |
    wc -l
```

```
50
```

Are there yet? Well it turns out that the genome file contains only the 5'-3' strand of the genome. What about the other strands patterns? To search them we need to generate the reverse-complement (RC) of the motif so we are sure that they will appear when looking the 5'-3' strand. The RC regex will be `ACC(T|G)(.)(T|C)(T|A)(.)(T|A)TTTA"` and adding this regex to the search willl be:

```
tr -d '\n' < data/Bteq-genome.fasta |
    egrep -o "TAAA(T|A)(.)(T|A)(G|A)(.)(C|A)GGT|ACC(T|G)(.)(T|C)(T|A)(.)(T|A)TTTA" |
    wc -l
```

```
94
```

References

Allesina, Stefano, and Madlen Wilmes. 2019. “Computing Skills for Biologists,” January. https://doi.org/10.2307/j.ctvc77jrc.