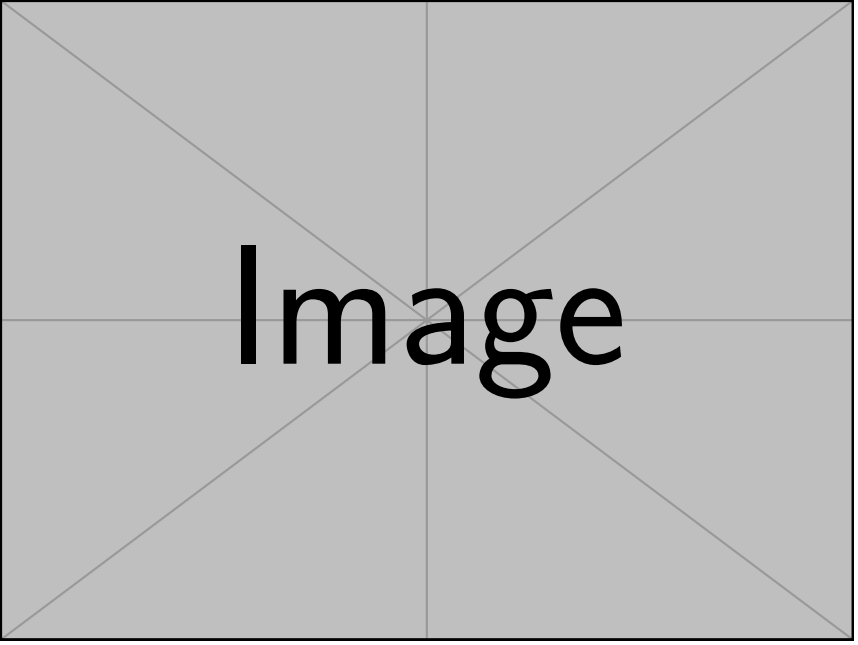


Baby Logic

Lecture Notes

作者： Duōyú Rén

时间： 01 April 2023, 03:19:42 +08:00

Table of contents

Preface

「邏輯」有很多種，「邏輯學」也有很多種。不同的人學習邏輯學有不同的**目的**。本筆記中的「邏輯學」知識主要服務於語言、思維的分析，不追求邏輯學在其他領域的功能。

本筆記大致列出邏輯基礎學習階段的主要材料，主要參考（chāoxí）以下課本攢集而成：

Bibliography

1. 徐明, 2008. **符号逻辑讲义** [M]. 武汉: 武汉大学出版社.
2. 胡龙彪, 黄华新, 2006. **逻辑学教程** [M]. 杭州: 浙江大学出版社.
3. 黄华新, 张则幸, 2011. **逻辑学导论（第二版）** [M]. 杭州: 浙江大学出版社.
4. 安德鲁 · 辛普森, 2005. **离散数学导学** [M]. 冯速, 译. 北京: 机械工业出版社.
5. Tidman P, Kahane H, 2002. ***Logic and Philosophy : A Modern Introduction[M]***. Ninth. Boston : Cengage Learning.
6. Smith N J J, 2012. ***Logic : The Laws of Truth[M]***. New Jersey : Princeton University Press.
7. Copi I M, Cohen C, Rodych V, 2018. ***Introduction to Logic[M]***. New York : Routledge.
8. Bergmann M, Moor J, Nelson J, 2014. ***The Logic Book[M]***. Sixth. New York : McGraw-Hill.

其中，直接取自《符號邏輯講義》的材料最多，取自《邏輯學導論》較多，素樸集合論、表列演算則直接取自完全開源的 Open Logic Project 項目源碼。

This book is a work in progress. If you find issues, typos or **relevant information** to share with, anything is *welcome and appreciated*.

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.

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.

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.

Notation

Mathematical notations

Introduction

The present book gathers the lecture notes of the undergrad course in Fundamentals of Computational Biology that takes place in EAFIT University. The first part will focus on how to use the the command line interface (aka CLI). It includes a long-format chapter about the Unix tools and concepts that is taught during the first four class lessons. The second chapter of covers the basics of git and the principal workflows to work daily on a collaborative manner this is actually the second lesson of the course. The third lessons, highlights the importance of package manager systems (such as homebrew and conda or scoop) and briefly introduce the main concepts and relevant commands. Later, in a fourth lesson, we introduce important concepts about how computers handle the tasks or jobs, and the parallelization of them. We talk about the computer architectures, the cluster architectures and the job scheduling software called Slurm, which is used on our local HPC cluster.

The second part is dedicated to sequence analysis and will cover several topics related to sequencing technologies, sequence alignments. All this topics are covered from the biological perspective, mathematical notions and the practical computing approach. Some of the main bioinformatics file extensions are covered and the git

Third part is focused genomics. It will cover main algorithms for genome assembly and some relevant programs. Also the biological nuances of gene calling and gene annotation, and finally will variant calling analysis, which introduces the gene mapping concept and some of the most important bioinformatics file extensions.

Next parts are currently optionals for the course per-se and some iterations may only include one of those or some combinations. They are mainly dedicated to introduce metagenomics, differential gene expression analysis and structural bioinformatics.

Bioinformatics vs. Computational biology

The extent of computational biology

There so many fields on bioinformarics Fig. 1, that sometimes its hard to focus on the fundamentals. But this is also an opportunity to discuss the main aspects and differences across the fields.

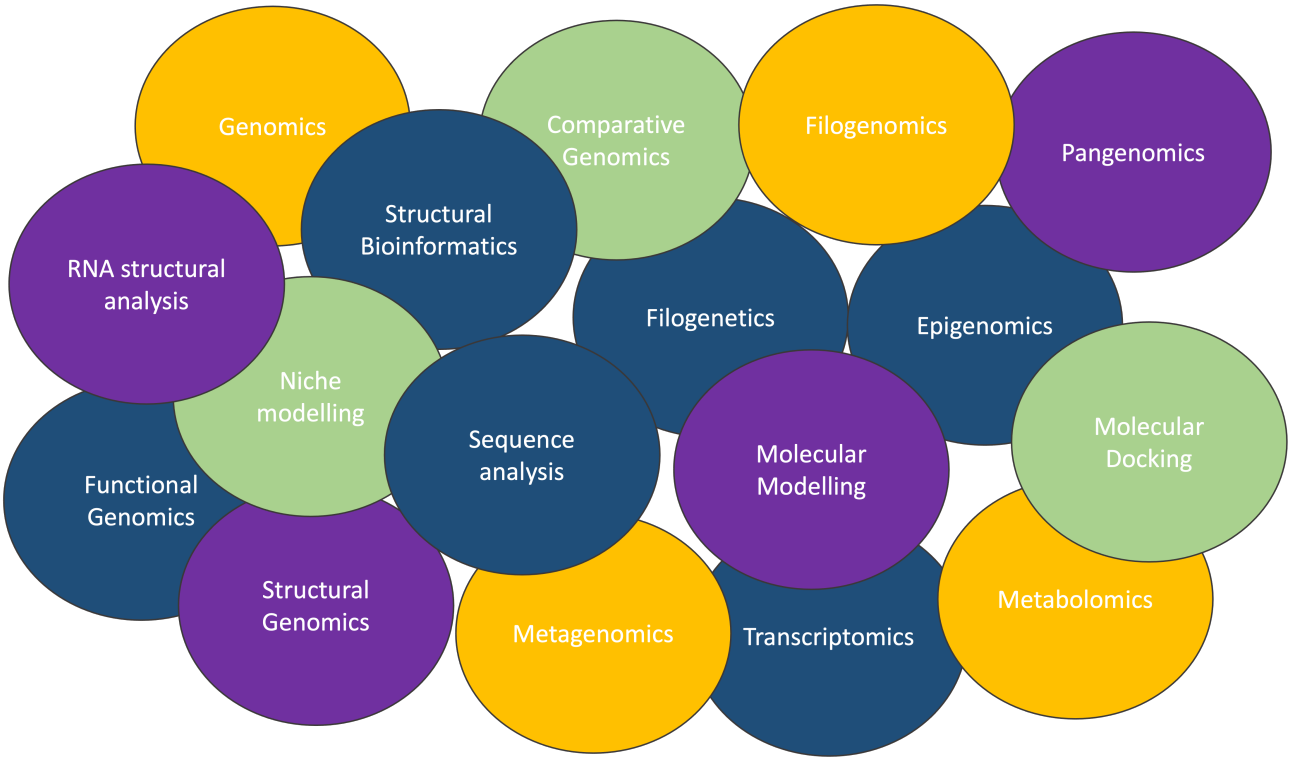


Figure 1: Different areas on bioinfomatics

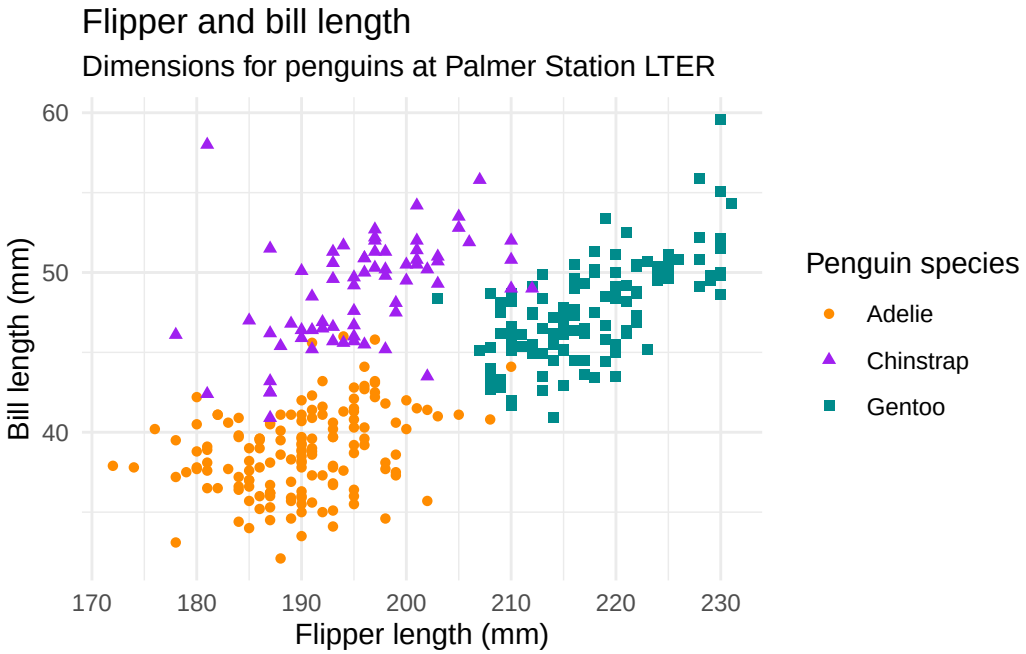
第一部分

Propositional

第 1 章 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 Command line basics

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

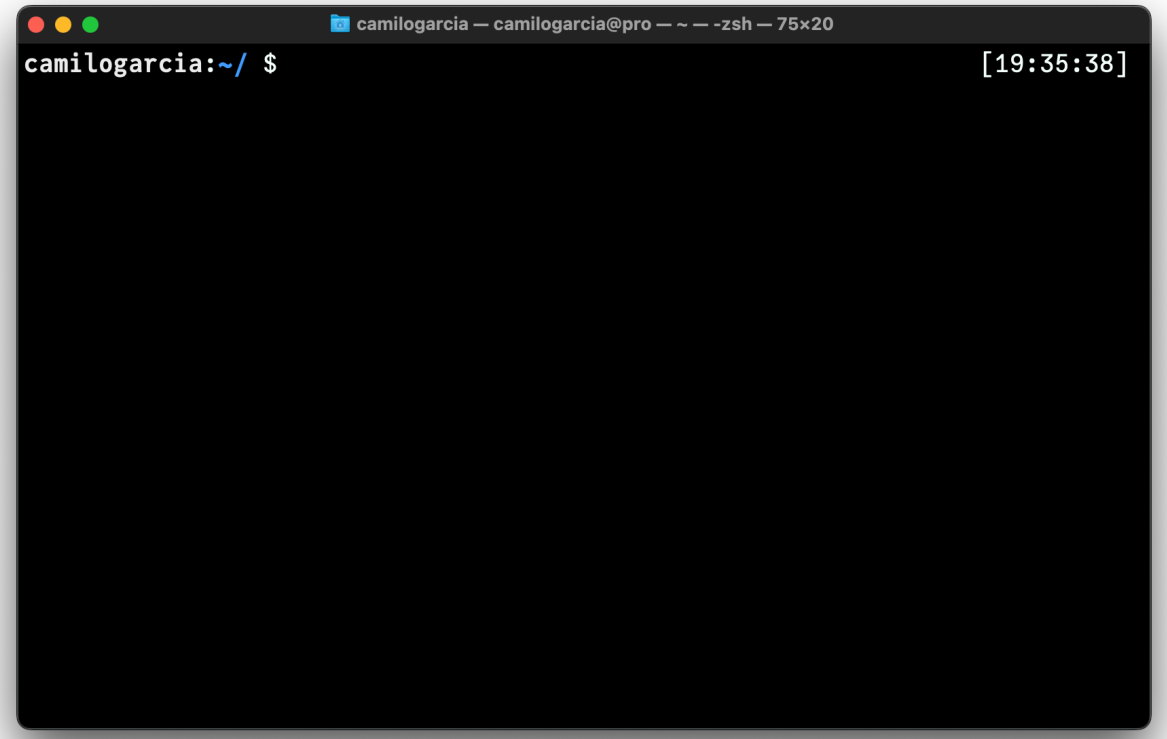


Figure 1.1: A **terminal** app displaying common features of the command line interface

computer. Such interface is called the the command-line-interface Fig. 1.1.

1.1.1 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 terminal displaying tree-like structure of the programs in a machine with macOS

1.1.2 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.

1.1.2.1 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
```

1.1.2.2 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
> ```

1.1.2.3 Listing files

```
ls
```

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

1.1.2.4 Making new directories

```
mkdir test-dir
```

1.1.2.5 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 illustrations, especially on how to name files depending on the *case* see **?@fig-naming-files**

1.1.2.6 Printing files or inputs

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

```
some
lines
that
were
written
```

```
echo "This will be printed"
```

```
This will be printed
```

1.1.2.7 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
```

1.1.3 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

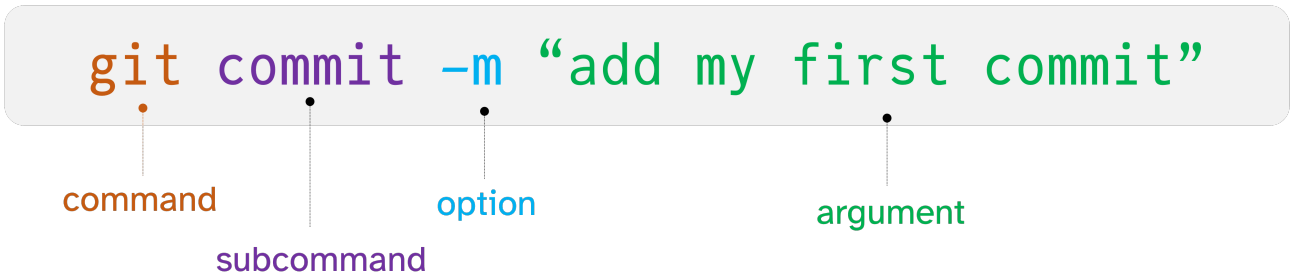


Figure 1.3: A simple command and a convention to call its main components

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 sites called *motifs*, shared by the phage and bacteria, with methylations. Motifs are commonly represented as a :sequence logo which is a probabilistic representation of the nucleotides at each position. The challenge consists of finding the number of times the motif from Fig. 1.4 appears on *B. tequilensis* EA-CB0015 genome using a command. Assume that probabilities are equal when multiple bases appeared at one site.

Figure 1.4: A RM-system motif logo

Before diving into an :answer take your time to think and solve it by your own.

1.2 Most important skills

When facing the CLI several issues or problems will arise. As for any other unintuitive challenge, a complete text interface Handling errors. Getting help Patience

1.3 Intermediate Unix

1.3.1 Special operators or metacharacters

Some operator or metacharacters have special functions in bash. For instance the `*` or *wildcard* is a regular expression character (sometimes called as a placeholder) that will turn in *any character, many times*, similarly the `?` represents *any character, once*. Whereas the `$` (dollar sign or operator) is intended for an special task : call *environmental variables* which

means that once a variable is defined (e.g., `var=1`) this variable can be called via the `$` operator anytime `echo $var` will get us `1` as the standard output

1.3.2 Intermediate commands

```
wc
```

```
tr
```

```
grep
```

```
sed
```

1.3.3 Unix flows

> **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.

1.3.3.1 Redirection

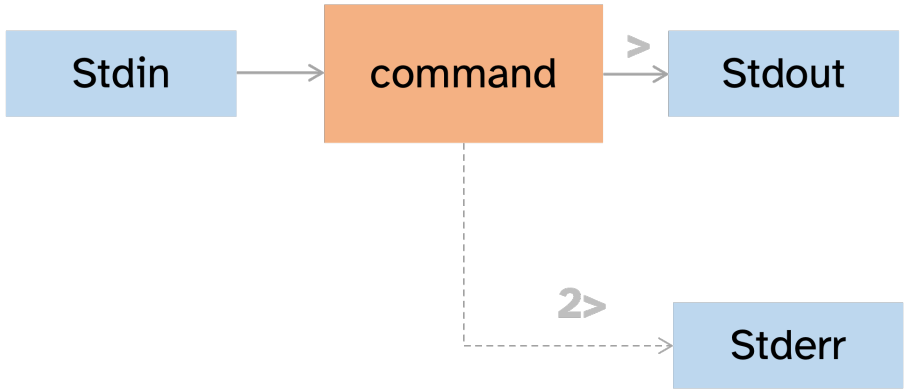


Figure 1.5: Redirecting flow

1.3.3.2 Pipe

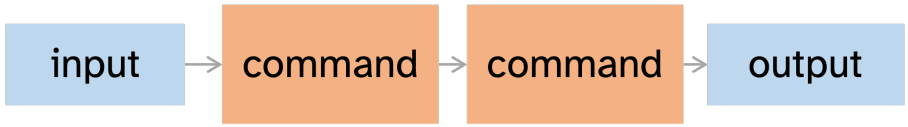


Figure 1.6: Pipe flow

Tip

When having a long command, it is also useful to jump by lines instead of character by character. To do so you can use the key combination `Alt + <-` and `Alt + ->` respectively.

1.3.4 loops, conditionals and script variables

Challenge

A second part of this challenges consists of create a script out from r the motif-search one-line command that recursively search the motifs in all genomes from a zip file that contains 10 bacterial genomes. The script should include the shebang, loops, conditionals and environmental variables.
See a possible script that solve the challenge :here

1.4 Advance Unix stuff

1.4.1 System permissions

1.4.2 Aliasing

1.4.3 The `.bashrc`

1.4.4 `awk` snippet

Challenge

:Restriction endonucleases (RE) cleave the DNA by digesting the :phosphodiester bond between two nucleotides. Many RE are directed to specific DNA motifs normally palindromic. There are mainly three types according to it digestive mechanism. RE have been widely used in molecular biotechnology because its specificity and versatility to carry out different experiments.
One of the main uses of RE is to generate a pattern of restricted fragments from different organisms so that samples of organisms, sequences or genes could be distinguished, as long as they display differences in the number of recognition motifs. This is normally done in the lab, where an RE is mixed with a DNA sample and later an :electrophoresis gel is run to see a separation pattern according to the fragments size.

> Professor Javier has sequenced the genome of a sampled SARS-CoV2 and want to see the band pattern that the genome would display if it were digested with the RE EcoRV. He has asked you to help him with this problem. The expected output is a text file with the sizes of the fragments, where the size is the number of nucleotides of each fragment.

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 & Wilmes (2019)

A list of reading for this section :

Dudley & Butte (2009)

Perkel et al. (2021)

Brandies & Hogg (2021)

第 2 章 Version control

Here we will first address what is version control, its importance and basics on the local workflow.

Later we will introduce GitHub and explore more advanced commands for the collaboration workflow

2.1 Version control systems

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.

2.2 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`.

2.2.1 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.

2.2.2 The local workflow

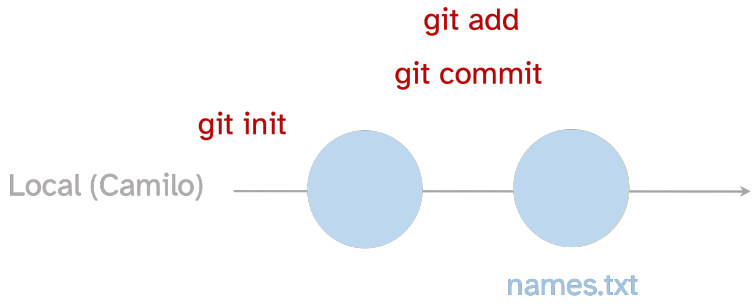


Figure 2.1: Local workflow of a git project adding and committing changes

#local-workflow

2.3 Exploring GitHub

As mention before, one of the great advantages that git can later achieve is to collaborate. However, to get into that a sharable server must allow users to have a common ground, and this is what GitHub allow. GitHub is a web platform where the local repositories become public and any user can access to the controlled versions of an image of your repo. The famous pet is the octocat Fig. 2.2

Figure 2.2: The octocat from GitHub

To enable the communication with a remote repo, git has encoded many specific commands, once the repos are **cloned** a simple workflow from the own local and remote repos is made possible thanks to two simple commands `git push` and `git pull`.

There a several ways to starting out a remote project, whether it starts from a local folder or whether it starts from a remote repo. The second strategy is sometimes easier as you just need to later `git clone` the remote into a local folder. To do so every repo has its own code-icon </> to later copy the repo link and later hit `git clone <https...>` on the desired folder. Now you got a linked copy of the remote on your machine.

Caution

But before working on a remote and pushing your first commits, it is common to find an error regarding the remote branch (also called *origin*). There are several ways to avoid this caveat, but a very anticipated way is to configure git

```
git config --global push.autoSetupRemote true
```

This will save you from every time typing `git push --set-upstream origin <main>` when working on a new remote repo.

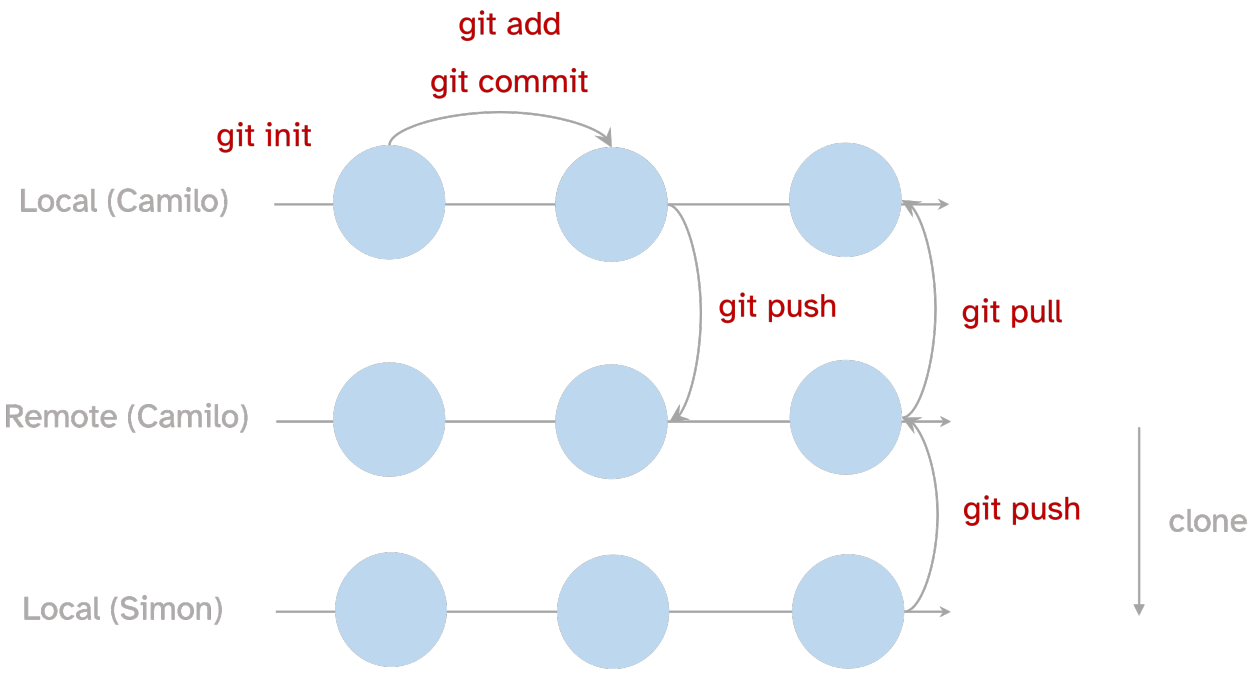


Figure 2.3: The cloning workflow in brief

Cloning is therefore the process of creating a local copy of remote repository, that is a machine version of the remote repo, later all the common local workflow is carried out normally Fig. 2.3

Tip

If you want to keep a file out of synchronization git provides a simple way to do so by creating a `.gitignore` file having the the paths to the files to keep in your local machine

2.3.1 Forking and collaborate

Basic collaboration on an open repository is a three-step process. First you need to `fork` the repo, this will create a mirror copy of the repo in your GitHub profile. In a second step, a

simple `clone` of the repo will generate a copy of the forked repo on your local machine, so you can freely work and make your mistakes and push them to your forked repo that belongs to your account. In a third step, once they are on the remote repo you will have to create a `pull request` (PR), as its name suggest : you are **asking** the owners of the original to consider your changes Fig. 2.4.

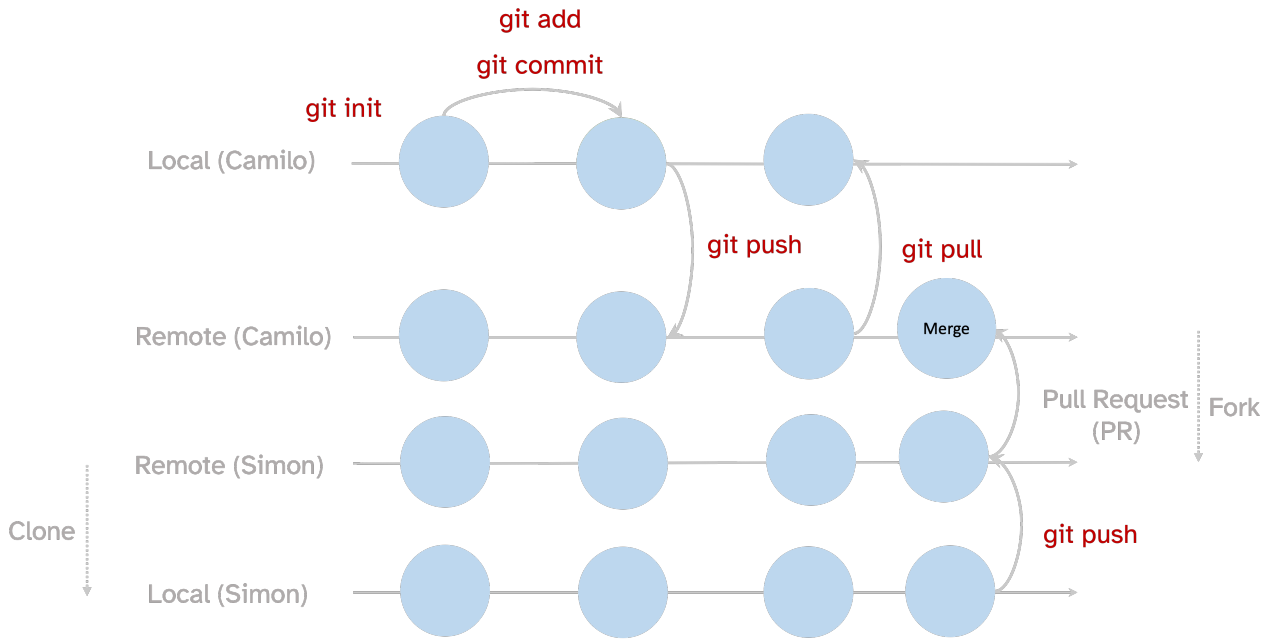


Figure 2.4: A common collaborative workflow from GitHub, using fork, clone and pull requests

2.3.2 Branching and merging

第 3 章 Package managers

3.1 What is the importance of package managers

3.2 Conda environments

What is conda :

To install conda (the command), miniconda (the lighter distribution).

For Linux x86_64 machine

```
curl -O https://repo.anaconda.com/miniconda/Miniconda3-latest-Linux-x86_64.sh
```

For Mac (Intel, will also works on M1) :

```
curl -O https://repo.anaconda.com/miniconda/Miniconda3-latest-MacOSX-x86_64.sh
```

Once downloaded, the next step is to run the installation script.

```
bash Miniconda3-latest-Linux-x86_64.sh
```

After installation is completed, it is recommended to add the following channels to your conda configuration. Conda channels are…

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

After this, conda should be installed, you may need to restart your terminal.

> **Tip**
>
> A faster implementation of the `conda` command is `mamba`. To install it simply run `conda install mamba` and then every time you will install a program just substitute `conda` for `mamba`

3.3 Package managers for OS

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

第 4 章 Notions of HPC

4.1 Monitoring my computer

What is computer doing ?

`htop`

4.2 Serial computing

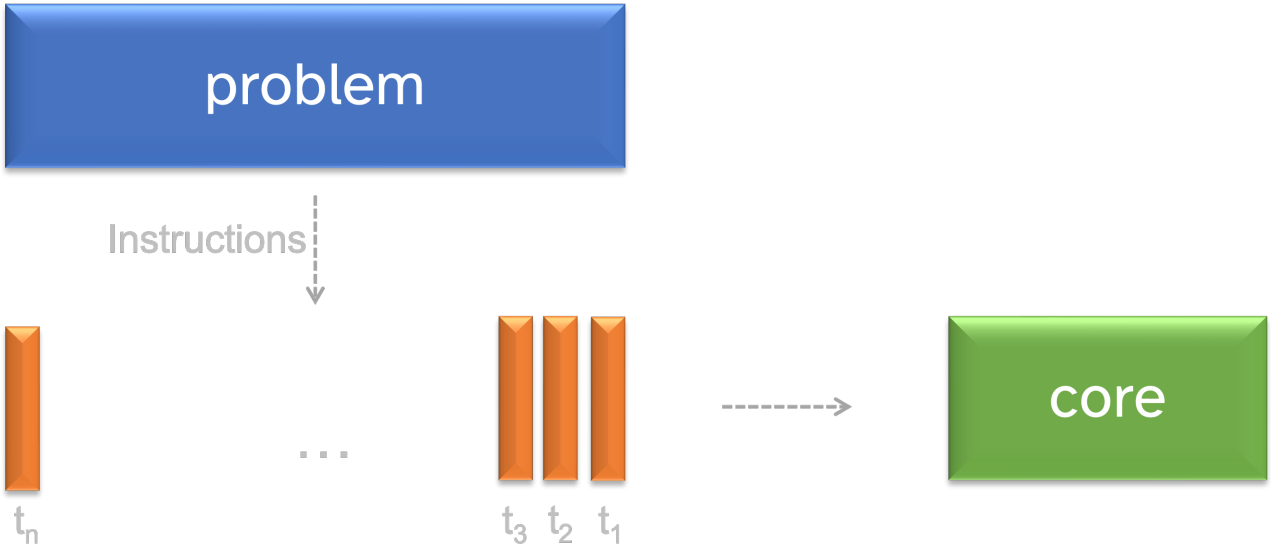
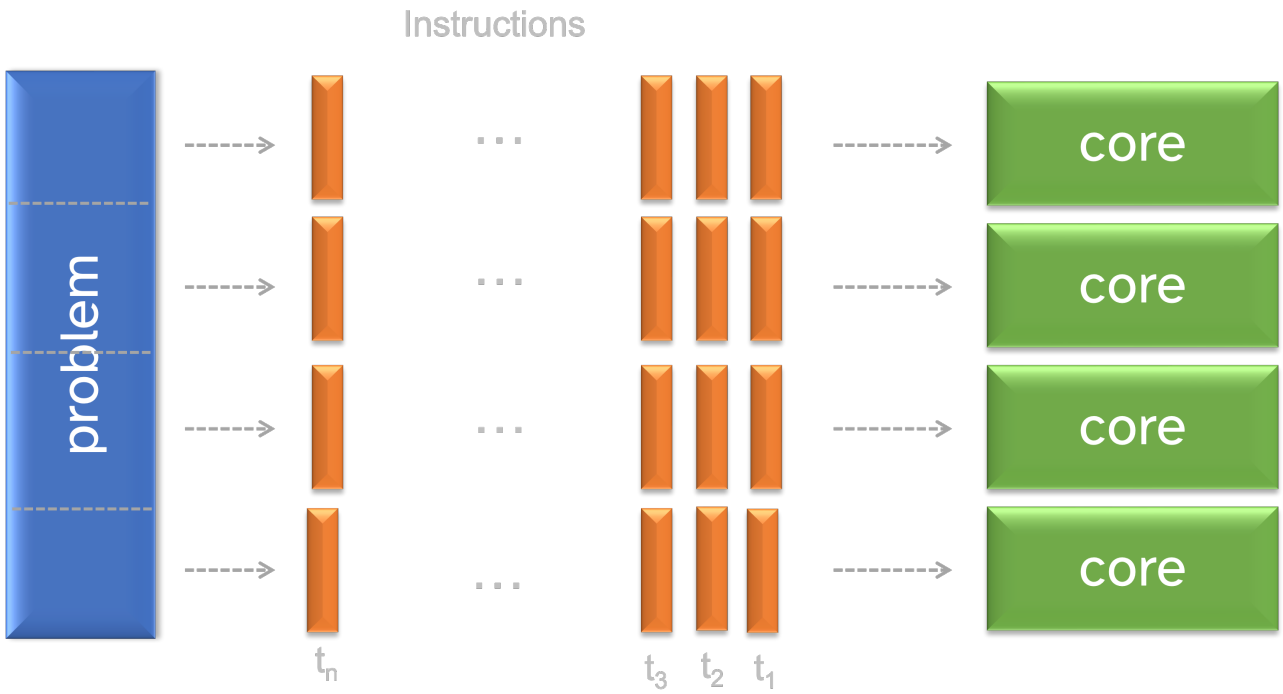


Figure 4.1: serial computing

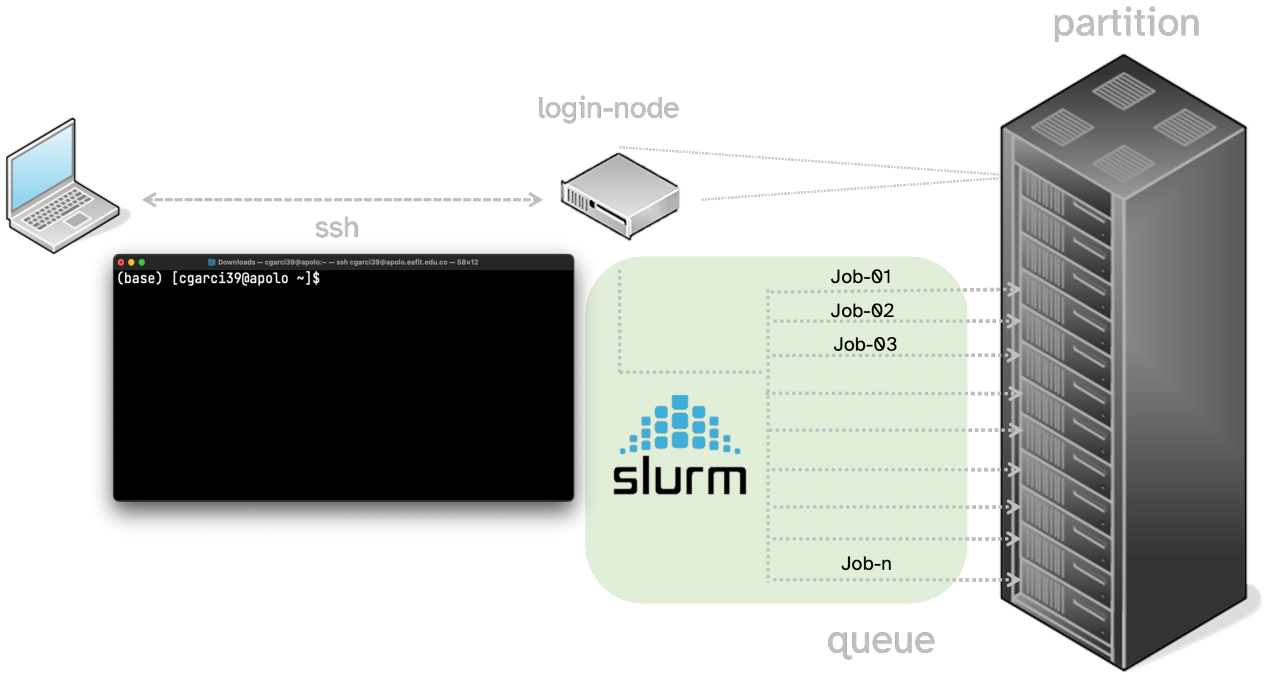
4.3 Parallel computing



4.4 Computer architectures

Figure 4.2: Laptop processor architecture

4.5 Using Apolo cluster with Slurm



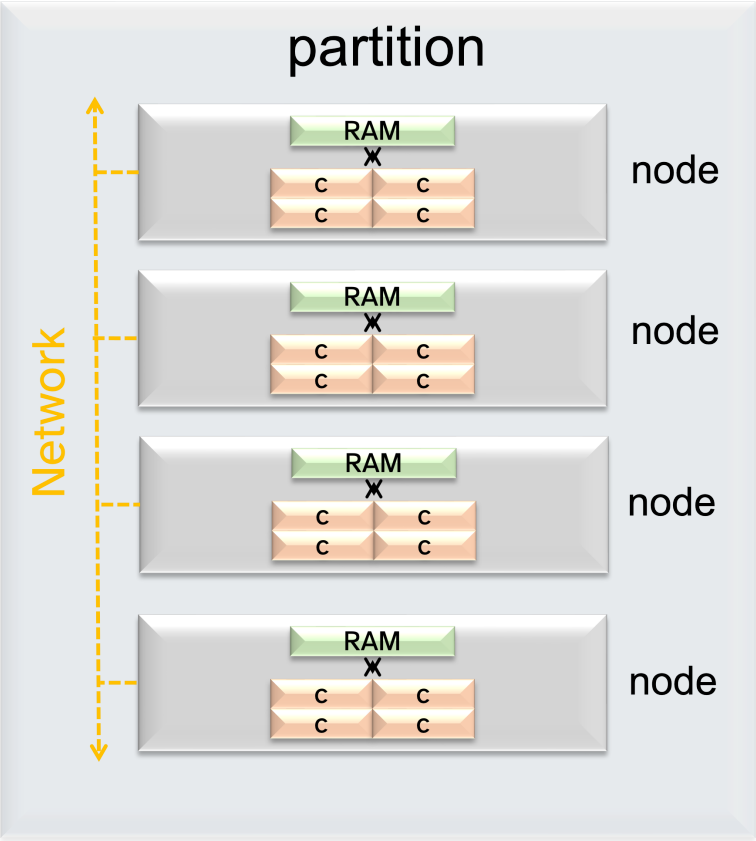


Figure 4.3: HPC architecture

Slurm is a job manager scheduler. It brings order to the jobs sent by users of the clusters and facilitate the process of check the state of the job in the cluster and the actual state of the cluster itself. To do so it has basically three main commands. The first one is `sinfo`

4.6 The Slurm instructions

To send then a job into the computer cluster several instructions should be specified so that the appropriate scheduling take part Fig. 4.4

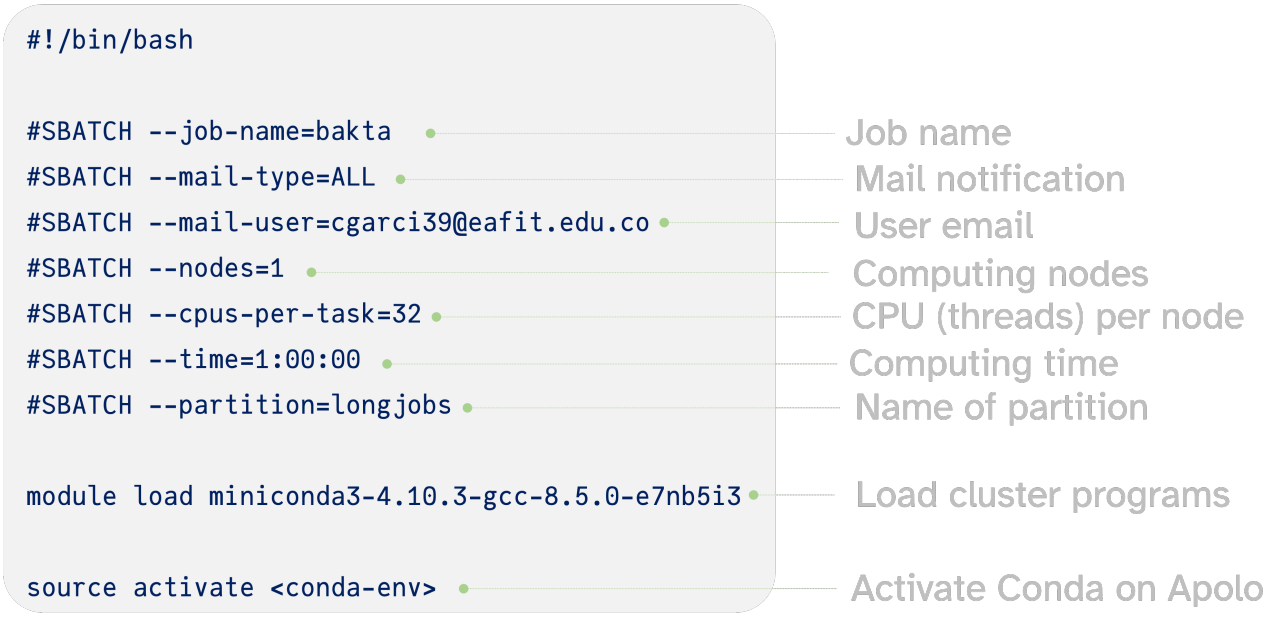


Figure 4.4: Slurm specifications to send a job into the cluster service

第二部分

Predicate

第 5 章 Algorithm thinking

5.1 What is an algorithm

5.2 Solving a problem step by step

5.3 Algorithms in computing

5.4 Relevance in biology

5.5 Pseudocode and notations

第 6 章 Algorithm techniques

6.1 Complexity

6.2 Exhaustive search

6.3 Branch and bound

6.4 Dynamic programming

6.5 Greedy algorithm

第 7 章 Data structures

7.1 Accessing data

7.2 Basic data structures

7.2.1 Hash tables

7.2.2 Binary trees

7.3 Ubiquitous bioinformatic data structures

7.3.1 Graphs

7.3.2 Strings

第三部分

Modal

第 8 章 Sequence analysis

In this chapter we we will consider several biological concepts that appear central to understand the manipulation of biological data.

We are also covering the transition of a biological (organic) information to digital bits.

We will explore some databases in which biological information is stored.

8.1 Biological information

Since the origin, organisms (or molecules) have been the result of different selective processes. An emergent property of successive iterations of survival/decease was the ability of molecules to keep a record of its past in a very stable manner, so that it will pass generation to generation. Although this might not be the first property or molecule to ever exist (see the :origin of life), the innovation of organisms to pack information of previous events became so advantageous that almost any form of living organism has this property : that is DNA.

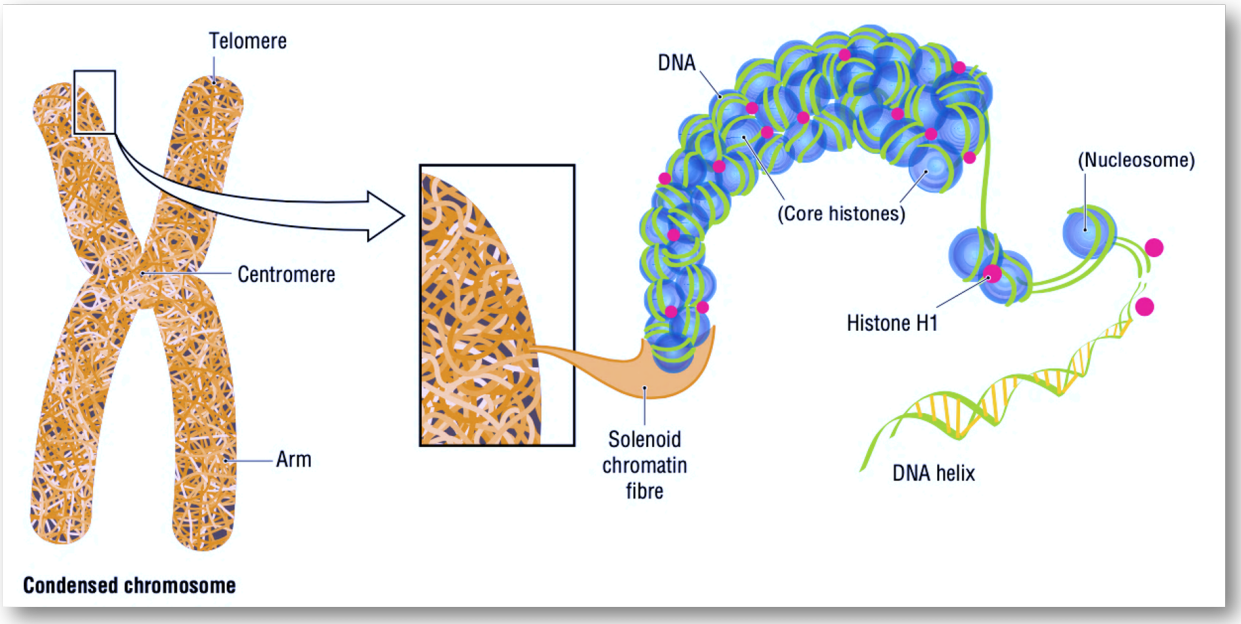


Figure 8.1: The different scales of information packed in a biological fashion. Figure adapted from Meneely et al. (2017) book showing how *DNA in a eukaryote is packaged with chromosomal proteins to form the much more compact chromatin. Chromatin is then folded into higher-order structures and eventually into the chromosome*

Almost any living organism harbor DNA in a nucleus or chromosome, which is a very compacted structure that could be actually seen to naked eye in some species. It so arranged or condensed Fig. 8.1 that it is the result of multiple compacting strategies.

8.2 The duality of DNA

DNA is an organic molecule of :nucleic acids that is mainly build out of nitrogen-containing compounds (:nucleobases) or just bases.The four bases are adenine (A), cytosine (C), guanine (G) and (T) and they bind strictly as $A = T$ and $C \equiv G$ where each of the lines represents an hydrogen bond.

DNA nitrogenous bases of are arranged in a very compacted helix structure and too much can be understood from its molecular nature. It is of course governed by the physicochemical properties of the atoms, therefore DNA is a physicochemical entity. But those common bricks have also a very beautiful emergent property that comes out from their order (mainly), and that is to keep the instructions that build the organism from which it belongs. These instructions are nothing else than information, which also follow some informational rules. Then DNA is also an informational entity.

Figure 8.3: A phosphodiester bond bind two nitrogenous bases as in the backbone of the DNA helix

The description of DNA bases helped the elucidation of the helicoidal structure as well as the elucidation of the base pairing rules of the nucleotides Fig. 8.4, also called the Chargaff's rules of base pairing. The first outstanding feature of the DNA structure was indeed highlighted by Watson and Crick in its famous paper about a subtle mechanism of replication. The

This idea of the duality of the DNA (and of course of other biological molecules as well) is the source of the study of many bioinformatic fields. But a question remains open : how do we capture the informational nature of a DNA sequence so that we end up with a sequential file of characters `ATCGCTATC...`. This is not a trivial question in fact.

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

So far we have addressed that DNA is a very stable molecule that stores biological (i.e., evolutionary) information. Also, that DNA could represent a sequential object of characters as in a computer digital object or file as well. But how is it that this order has an underlying biological sense? This was a very though question that required the accumulation of many experimental discoveries and the meeting of genetics and molecular biology.

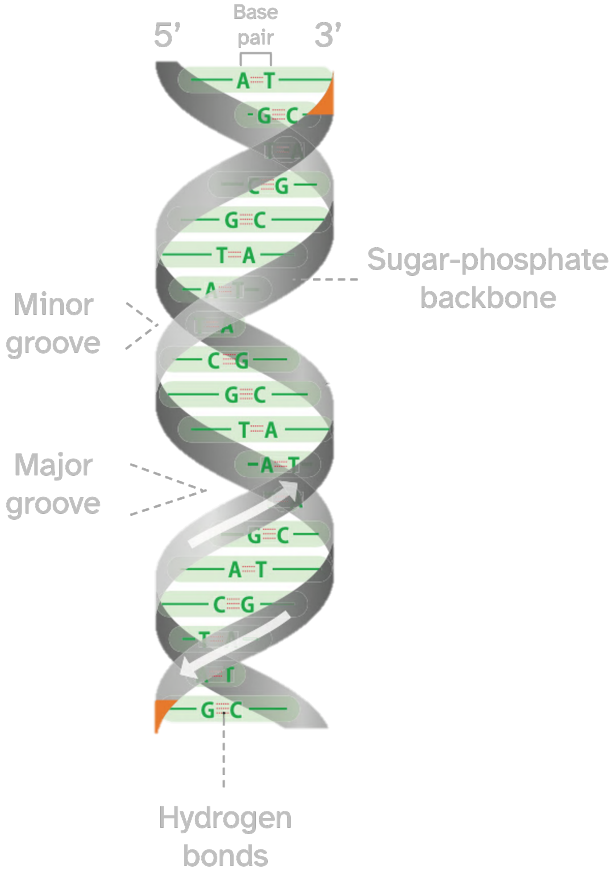
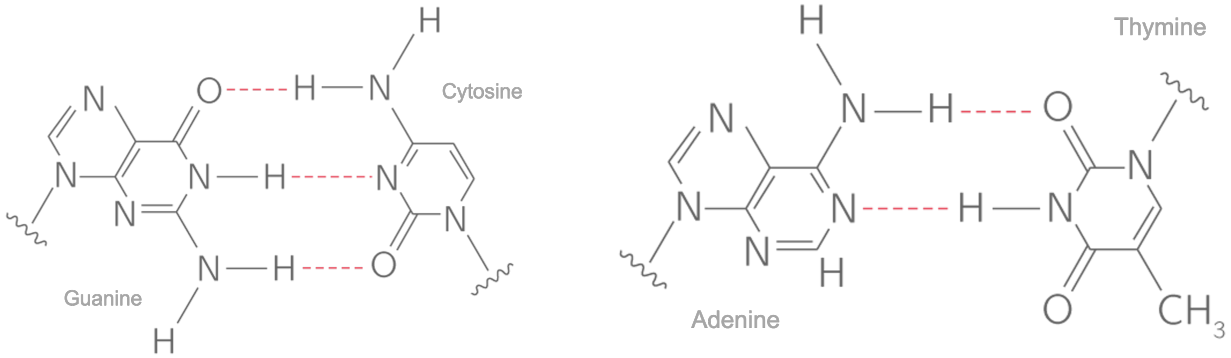


Figure 8.2: DNA double helix structure and main characteristics. Image adapted from Brown (2018) showing the structure with the *sugar–phosphate backbone of each polynucleotide drawn as a gray ribbon with the base pairs in green*



(a) Guanine and cytosine base pair

(b) Adenine and thymine base pair

Figure 8.4: Most common nucleotides present in DNA and the chemical interactions that bond each pair.

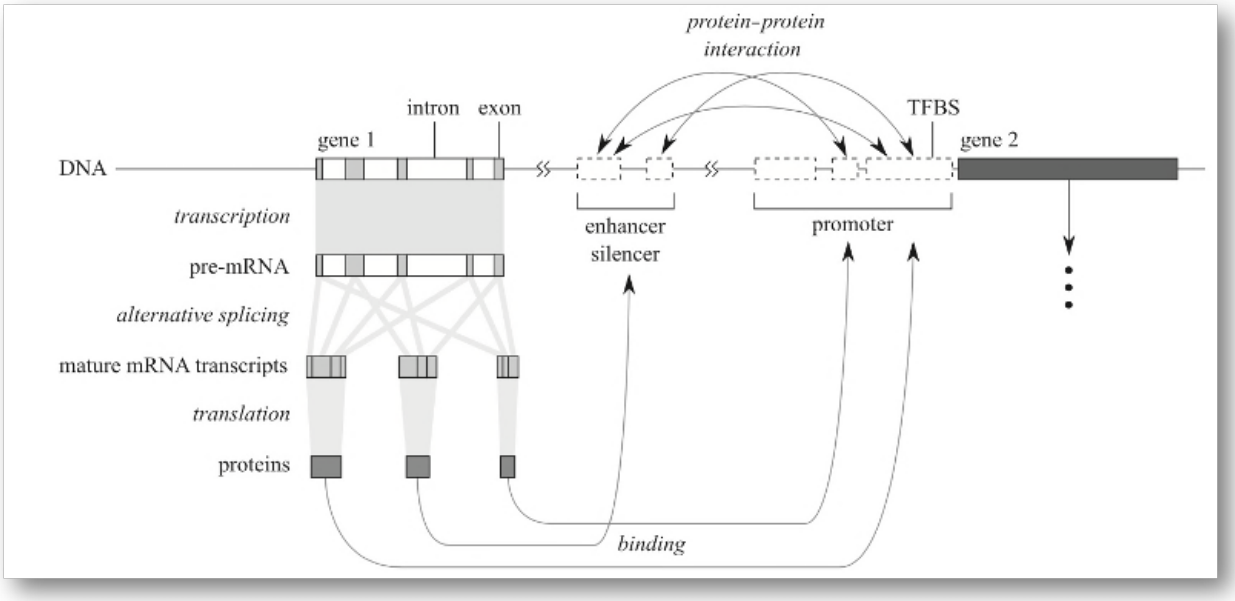


Figure 8.5: An extended representation of the central theory of molecular biology. Image from Mäkinen et al. (2015)

8.4 Sequencing strategies

8.5 Sequencing over time

8.6 Some insights from sequencing genomes

8.7 Biological information databases

8.8 Retrieving data from NCBI

Inforation or databases inside the NCBI comes in many different ways. Perhaps the most used file format in bioinformatics is the `FASTA` format. It is a very simple format that consist of two lines, the header which is basically noted by starting with the `>` symbol and is always preceding the sequence, that is present in the second line.

FASTA

```
>sequence
ATCCCTAGCTAGCTCGCTATCGATCATCCCTAGCTAGCTCGCTATCGATCATCCCTAGCTAGCTCG...
```

8.8.1 The manual way

8.8.2 Entrez direct

We first need to install the Entrez Direct command line utilities. This could be done in several ways, one suggested by the manual and a very simple one using conda (mamba).

```
mamba create -y -n entrez entrez
```

This line will create a new conda environment called *entrez* and will automatically install all dependencies. We later activate the environment with `conda activate entrez` and then we got the entire Entrez CLI utilities.

There are several commands and its function is to interact with the NCBI databases system. It is, however, a very huge set of utilities and commands to cope with at first, so the learning curve is somehow steppe. Here is a way to download the *Bacillus tequilensis* EA-CB0015 genome with a line of code that interleave three of the main commands of Entrez :

```
esearch -db assembly -query GCF_012225885.1 |
elink   -target nucleotide -name assembly_nuccore_refseq |
efetch  -format fasta > GCF_012225885.1.fasta
```

8.8.3 Simpler download programs

Since the manual way is clumsy when scaling and automating, and the `entrez-direct` utilities are somehow hard at first hand, some bioinformaticians have already thought ways to ease users the hard of knowing the NCBI internal database structure and to resume many of the utlities to just a 'download' command out from what in entrez is distributed in search, filter, and download commands.

A very nice command to download genomes is the `bit-dl-ncbi-assemblies` from Mike Lee, who has also made a remarkable work to bring many bioiformatic knowledge to newcomers. Similarly and quite more robust is the `ncbi-genome-download` CLI (or abbreviated as `ngd`) developed by Kai Blin

Using `ngd` to download a complete genome using the refeseq or genbank accession numbers, would be as easy as :

```
ngd --format fasta --flat-output -A GCF_002055965.1 bacteria
```

The one-line will download the refeseq genome of the *Bacillus tequilenis* EA-CB0015 in a

When navigating the NCBI databases some data is being curated and continuously updated

fasta nucleotide format. the `--flat-option` from the script will handle out extra metadata of the search and will download strictly the data.

It is important to highlight that these simpler scripts are focused on genomic information, so that direct downloads of genes, proteins, transcripts, and many other kind of data must be done with `entrez` or other programs. Some recent efforts have been made to create a more robust CLI to interact with different databases, but in a very easy and intuitive way, such as the `gget` project. It is more focused on vertebrate databases, but also has a port to NCBI, UniProt and other databases. And is very simple. Here is an example to download all the isoforms in a multi-fasta format of the cancer-related gene BRCA2 in a human genome (GRCh38.p13) :

```
gget seq -iso -o BRCA2.fasta ENSG00000139618
```

It is establishing a connection with the Ensemble database, which is focused on vertebrate genome and transcriptome data.

Challenge

Professor Camilo is interested on knowing how many complete genomes of *Bacillus subtilis* are there in the NCBI databases. He asks you later to count the number of features (genes, CDS, ncRNA, rRNA, etc.) in the genome of *Bacillus subtilis* NCIB 3610 (GCF_002055965.1). And tell you to document each of the steps and how did you end up with the answer. Saving the file with your initials (e.g., CG-activity01.md)

第 9 章 Sanger analysis

This is a section about perhaps one of the first generation sequencing technology, that is Sanger sequencing and its principles and evolution, strengths and limitations

This chapter will also cover the bioinformatics side of the method : what kind of files we get out Sanger, how we process them and an important paradigm that came out from this sequence method.

Finally we will dive into a study case that its very common in microbiology : the use of the 16S rRNA gene for taxonomy identification

9.1 The first sequencing methods

Back in 70s there two different methods aiming to determine the base sequences of DNA and they basically operated at the level of base per base reconstruction of the information. The most used during those days was so called :Maxam-Gilbert method and the second one was precisely the chain termination method or dideoxy sequencing or more commonly nowadays :Sanger sequencing. Both methods named after their creators. It was, however the Sanger method the one that gradually became the facto method and continue to be the standard for the next thirty years. In fact, assembled genomes before the 2000s were sequenced with Sanger method, which included the first draft of the Human genome and many other organism Brown (2018).

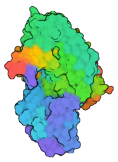


Figure 9.1: A globular structure of a bacterial DNA polymerase

https://www.youtube.com/embed/Wgv9QhjLTC8

A step-by-step of the Sanger sequencing method

9.1.1 The chain termination method

To under stand the chain termination method is important have a clear view of the DNA structure and chemical reaction that enable its polymerization. Whe the

9.1.2 Sanger with capillary electrophoresis

9.1.3 Strengths and limitations of Sanger methods

9.2 Files from Sanger sequencing

Several files are generated from a modern Sanger sequencing project.

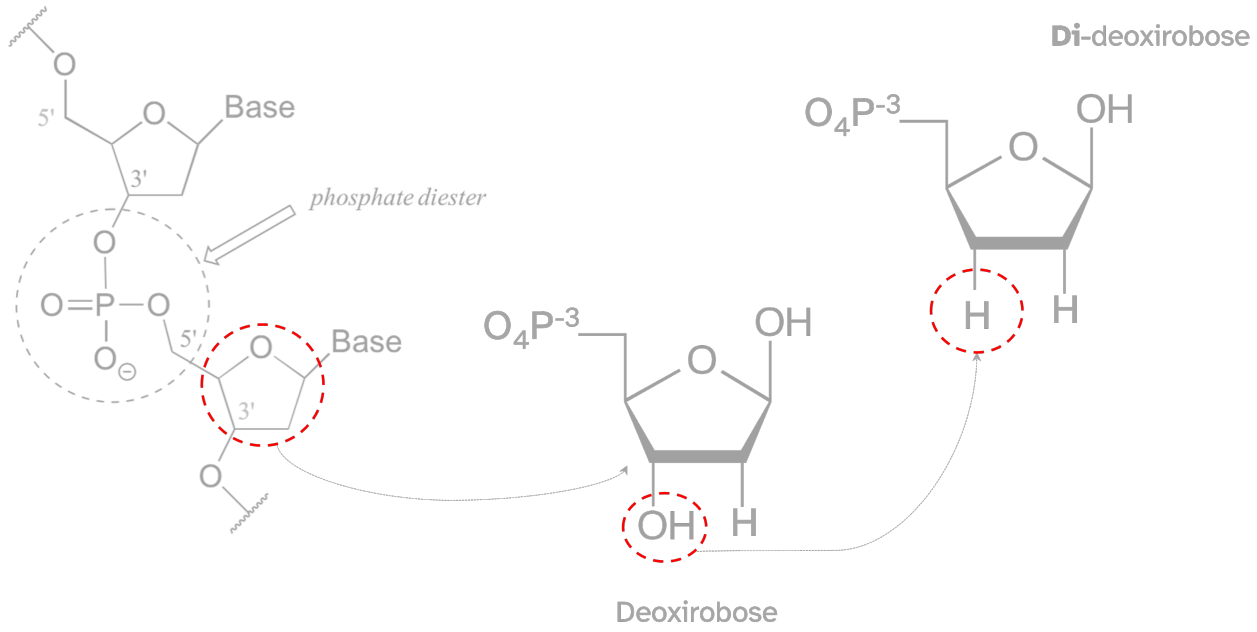


Figure 9.2: The deoxy-ribose and dideoxy-ribose and consequences on the polymerization of a DNA strand

PHD.1

```
BEGIN_SEQUENCE H200824-010_A11_455-WT_spo0B_spo0B-R

BEGIN_COMMENT

CHROMAT_FILE: H200824-010_A11_455-WT_spo0B_spo0B-R
BASECALLER_VERSION: KB 1.4.0
TRACE_PROCESSOR_VERSION: KB 1.4.0
QUALITY_LEVELS: 99
TIME: Mon Aug 24 22:05:23 2020
TRACE_ARRAY_MIN_INDEX: 0
TRACE_ARRAY_MAX_INDEX: 18141
TRIM: -1 -1 -1.000000e+000
TRACE_PEAK_AREA_RATIO: -1.000000e+000
CHEM: term
DYE: big

END_COMMENT

BEGIN_DNA
```

```
C 7 3
C 2 17
T 3 44
...
END_DNA

END_SEQUENCE
```

However the most used file is the binary version `AB1` which could be red out from different programs including cutepeaks. It displays the DNA sequence along with its quality peak in each position Fig. 9.3

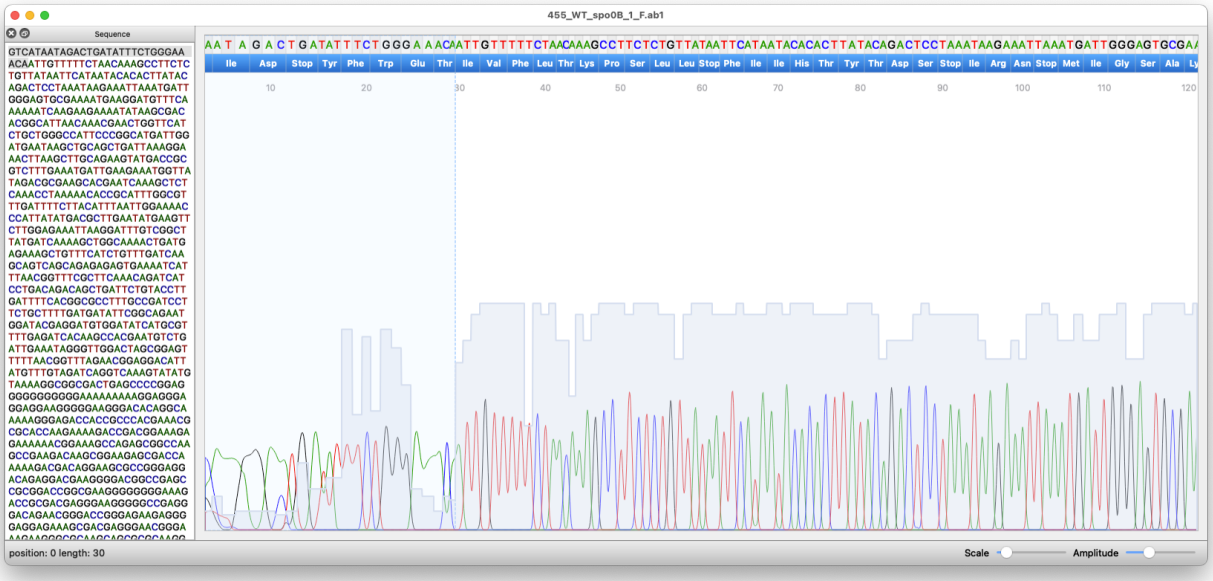


Figure 9.3: A graphic of the `AB1` file format from a Sanger sequencing project displayed in cutepeaks software

9.3 Sanger processing workflow

Despite being one of the oldest sequencing methods, Sanger sequencing has some remarkable strengths that are enable thanks to the automation era. For instance modern sequencers working in parallel can read almost 384 different sequences (of 750bp) in 1h (7Mb in 24h per machine), clearly automation introduces a second advantage and that is the low-price of sequencing small fragments Brown (2018). It is important to highlight some limitations as well like the necessity of round-the-clock technical support (this also leverages a little bit from robotic operators though). The error-free Sanger sequencing requires at least 5X of sample coverage, which could be seen as disadvantage too Brown (2018).

9.4 The 16S rRNA and its relevance for sequencing

Figure 9.4: *Bacillus subtilis* subsp. *subtilis* str. 168 bacterial SSU 16S rRNA

Challenge

Professor Valeska gives you a compressed file containing two genes (spo0B and rpoB) from three different strains (302, 321, 455). These genes were sequenced using pair end Sanger method, then the compressed file has the two raw sequences per gene. She tells you to process and analyse them using the Sanger sequencing pipeline analyis. Since she doesn't know from which species they belong, she ask you to identify the organism to whom it belongs by using the resulting consensus sequence. She finally reminds you to document each step of the process including the identification step

See the solution :here

第 10 章 Sequence alignments

10.1 Why do we align sequences ?

In search of homology and identity

10.2 What is homology

10.3 Pairwise alignments algorithms

10.3.1 Hamming distance

10.3.2 Edit distance

10.3.2.1 Dynamic programming

10.3.3 Needleman-Wunsch (global alignment)

10.3.4 Smith-Waterman (local alignment)

10.4 The genetic code and Scoring matrices

10.5 BLAST and its families

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

10.6 Multiple sequence alignments

> ⚠ Challenge
>
> Your professor is working with species from genus Bacillus and want to align an orthologous gene from 10 genomes of different isolates. He gives you the GenBank accession number of these isolates and ask you to select one orthologous gene (Nucleotide seq) that you consider might be useful to differentiate the bacterial isolates and ask you to align those

genes as you better consider. He finally ask you to document each step and send him the sequence alignment file in FASTA format along with the sequence alignment general stats in a TXT file (length, number of each nucleotides and other stats you consider important). Accessions : GCA_012225885.1, GCA_000196735.1, GCA_000742895.1, GCA_001584335.1, GCA_000007825.1, GCA_000832905.1, GCA_000008425.1, GCA_000507105.1, GCA_000832605.1, GCA_900186955.1

第 11 章 NGS and TGS : principles

11.1 Platforms yields

11.2 Reads main differences

11.3 Illumina principle (sequencing by synthesis)

11.3.1 The fastq format

11.3.2 Quality assesment of Illumina

11.4 PacBio principle (sequencing by incorporation)

11.4.1 Throughput evolution

11.4.2 Quality assesment of PacBio

11.5 Oxford Nanopore Technology (ONT) principle

11.5.1 Platforms

11.5.2 The fast5 file format

第四部分

Intuitionistic

第 12 章 Phylogenetics

12.1 What is a phylogenetic tree

12.2 Mehtods for phylogenetic reconstruction

12.3 Building a phylogenetic reconstruction

12.3.1 Evolutionary substitution model

12.3.2 Maximum likelihood

12.3.3 Bayesian inference

第 13 章 Phylogenomics

第五部分

Meta

第 14 章 Genome assembly

14.1 The problem of assembling genomes

14.2 Main algorithms for genome asssembly

14.2.1 Overlay, Layout, Consensus (OLS)

14.2.2 De Bruijn graphs

14.3 Main concepts of an assembly

14.3.1 Contigs, Unitigs, Scaffolds

14.4 A complete workflow for assembling genomes

14.5 Assessing genomes

14.5.1 Inspecting genome graphs

14.5.2 Genome completeness

14.6 Understanding genome difficulties

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

第 15 章 Genome annotation

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15.2 Homology annotation

15.3 Functional annotations

15.3.1 Gene Ontology

15.3.2 Cluster of orthologous genes

15.3.3 Enzyme commission

KEGG enrichment

15.4 Annotation files

15.4.1 the GBK and GBFF

15.4.2 The GFF specifications

15.5 Visualizing genomes and annotations

第 16 章 Variant calling analysis

16.1 Common mutations

16.2 Structural variants

16.3 Genome rearrangements

16.4 Read mapping algorithms and programs

16.4.1 Burrow-Wheeler-Alignment

16.4.2 BWA-MEM2

16.4.3 Minimap2

16.4.4 SAM, BAM and CRAM formats

16.5 Identifying mutations

16.5.1 Freebayes and Snippy

16.5.2 The VCF file

第 17 章 Comparative genomics

17.1 Genome mapping

17.2 Whole genome comparisons

mummer mauve mmseq

arrow diagrams

第六部分

Application

第 18 章 Metagenomic sequencing

Metagenomes is the study of whole DNA of a biological communities.

Metagenomic of a single gene (16S, ITS, etc.) is also called metataxonomic (amplicon sequencing). In contrast all the genes/ genomes in the sample is actually metagenomics.

Some differences :

Metagenomic | Metataxonomic expensive | cheap more samples| less specific regions | random regions

First step is to design an experimental design

See the paper from Torsten Seeman, Gilbert, Meyer - A guide from sampling data analysis

18.1 Designing the experiment

Stablish the basic experimental unit and the appropriate replicates, control, randomization !

It is important to have metadata of the experiment in a plant microbial community is common to have pH, geo-reference, temperature, etc.

In metataxonomic we analyses hypervariable regions (V3-V4) and compare relative abundance to identify which is the most informative (abundance, richness)

18.2 DNA extraction and Sequencing (Illumina)

Followed common sequencing is also important to make quality check of the data

18.3 Denoising

There are several way to define OTUs (97-99 % of similarity) for instance :

- It is defined operational unit of species or species groups (be careful to assign a sequence as an species)
- Taxonomic level of sampling selected in the study… individual, population, species,…

Pipelines for OTUs or ASVs are Quimme and Silva and deNBI

18.4 Chimera

Reads that result from combinations in the sequencing

18.5 Taxonomic annotation

Using Silva or deNBI

第七部分

Appendix

History

Edition 0.0.11 (2023-01-19)

- Adding some quarto code annotations !
- New chapter on algorithms
- Add a challenge on genome assembly
- Rendering with Q v.1.3

Edition 0.0.10 (2022-12-17)

- Update book with new quarto and extension versions
- Challenge on sequence alignment
- Experimenting new covers
- Some typos cleaning
- Change giscus theme

Edition 0.0.9 (2022-09-08)

- Starting the Sanger chapter
- More on Seq. analysis
- Keep history on book and add dates
- Change the cover
- Add new parts and chapters outlines (phylogenetics and metagenomics)
- New challenges on seq analysis solved

Ed. 0.0.8 (2022-08-29)

- New render w/ Quarto 1.1
- Adding more on sequence analysis
- Including new extension for videos
- Improve some images
- Book has now Giscus to enable discussions
- New info on download genomics data

- New foot page with license and more info

Ed. 0.0.7 (2022-08-19)

- Changes in git chapter
- More images in different chapters
- Start using new filters : lightbox for images and nutshell for expandable explanations
- Some images in HPC chapter
- Some outlines in Package manager chapter
- Some paragraphs on the sequence analysis chapter
- Additional challenges

Ed. 0.0.6 (2022-08-09)

- More updates to CLI chapter.
- Update the Version Control chapter.
- Decoupled genome annotation section into separate chapter.
- Add a motif search challenge
- Add cover image
- Many typos corrected.

Ed. 0.0.5 (2022-07-28)

- Update CLI chapter.
- Decoupled Seq analysis and Genomics into separate chapters.
- Chapters were reorganized in its own dirs to make easier navigation. They also were renamed for later easier addition of other chapters.
- New learning features using Quarto callouts. This will hopefully improve learning and enjoy the book.
- Chapters sections will have numbering.

Ed. 0.0.4 (2022-04-24)

- Minor typos and outline for the structural biology section
- Start improving crossreferences

Ed. 0.0.3 (2022-04-18)

- Citation and reference of the book is now almost complete

Ed. 0.0.2 (2022-04-17)

- This Ed. includes more information of the book and author
- Adds DOI and Zenodo
- Includes Social commenting

Ed. 0.0.1 (2022-04-12)

- This preliminary Ed. contains the basic outline of the book
- Contains the first draft solutions to challenges demos

References

Allesina, S., & Wilmes, M. (2019). *Computing skills for biologists*. https://doi.org/10.2307/j.ctvc77jrc

Brandies, P. A., & Hogg, C. J. (2021). Ten simple rules for getting started with command-line bioinformatics. In *PLoS Computational Biology* (No. 2; Vol. 17, p. e1008645). Public Library of Science San Francisco, CA USA.

Brown, T. A. (2018). *Genomes 4*. Garland science.

Dudley, J. T., & Butte, A. J. (2009). A quick guide for developing effective bioinformatics programming skills. In *PLOS computational biology* (No. 12; Vol. 5, p. e1000589). Public Library of Science San Francisco, USA.

Mäkinen, V., Belazzougui, D., Cunial, F., & Tomescu, A. I. (2015). *Genome-scale algorithm design*. Cambridge University Press.

Meneely, P. M., Hoang, R. D., Okeke, I. N., & Heston, K. (2017). *Genetics: Genes, genomes, and evolution*. Oxford University Press.

Perkel, J. M. et al. (2021). Five reasons why researchers should learn to love the command line. *Nature*, *590*(7844), 173–174.