

BScBI-CG

# Practicals Report

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## Exercise 00

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# 1 Introduction

We are going to reuse a set of existing **MarkDown** report templates to define the protocols to be run on each practical session. This first document will allow us to describe the different sections it contains, highlighting the major blocks its made of. We will also use it to provide applied examples of the **MarkDown** and **L<sup>A</sup>T<sub>E</sub>X** syntax; for both we will introduce new features on each new practical session. You can read the [MarkDown syntax guide](#), as well as from the manual pages (just type `man pandoc` and/or `man pandoc_markdown` if you have installed this software tool).

## 1.1 Objectives

- We will practice how to report the commands and the results required to solve the practical exercises, using a text file, this report of course, and the **MarkDown** syntax. Some enhancements using **L<sup>A</sup>T<sub>E</sub>X** will be also illustrated.
- The main goal is to understand the structure and logic of the report document, what can be found on each section, how code blocks are defined, and how to process it for submission to the [Computational Genomics Virtual Campus at ESCI](#).
- This exercise will be useful to check that all required packages and libraries are working properly in order to generate the practical reports for the continued assessment of the subject.
- At the same time, if those commands can run on our computers, we will have some commands already installed and available for the forthcoming practical sessions.

## 1.2 Prerequisites

### 1.2.1 Installing required software

For this practical we must ensure that at least `pandoc` and `pdflatex` commands are running smoothly over our report files. You probably may need to install the following packages before working on anything else.

**1.2.1.1 Linux users:** You must submit an exercise report for each practical as two single files, a **MarkDown** text file and a PDF compiled from that **MarkDown** file. In order to run such procedure, we must ensure that we have the software tools and the corresponding dependencies. This has to be done for the first exercise only, as you will have those tools already available for future compilations of the other exercises. The command below will install those tools:

```
sudo apt-get install pandoc \
    texlive-latex-recommended \
    texlive-latex-extra \
    texlive-fonts-recommended

# getting the latest version from repository
# https://github.com/jgm/pandoc/releases/latest
# for a Debian-based linux distribution you can run those two commands:
wget https://github.com/jgm/pandoc/releases/download/3.1.8/pandoc-3.4-1-amd64.deb
sudo dpkg -i pandoc-3.4-1-amd64.deb
```

When using `pandoc` version greater than 2.x we will be able to apply further macros and tags on our report file (like embed **LaTeX** blocks). In case you want to play with **L<sup>A</sup>T<sub>E</sub>X**, I will recommend you to install the complete set with this command (it takes some time to download them though, as you may get the packages and many dependencies):

```
sudo apt-get install texlive-full \
    texlive-fonts-recommended \
    texlive-fonts-extra
```

You can also install optional packages, such a text editor with programming facilities and extensions, like `emacs` or `geany` (you can also use `sublime`, `atom`, `gedit`, ...):

```
sudo apt-get install emacs geany vim vim-gtk
```

Another example of downloading source code and compiling it (**NOT NEEDED if you are using CONDA ENVIRONMENT**).

```
git clone https://github.com/kimrutherford/EMBOSS.git
cd EMBOSS
./configure --prefix=/usr/local/install/EMBOSS
make
make check
make install
```

Further instructions will be given on the templates in case a practical requires that you install further software...

**1.2.1.2 MacOS users:** MacOS users can try to install ports for the software tools from `homebrew` or `conda` repositories. Here we have a brief summary of such commands. `Homebrew` is a package manager for **MacOS**; it also has a port for **Linux**, known as `Linuxbrew`. To install this package manager, just copy the following command and paste it to a Terminal window:

```
# setting up brew tool and its repositories
/bin/bash -c "$(curl -fsSL https://raw.githubusercontent.com/Homebrew/install/master/install.sh)"
# OLD ruby installer:
## /usr/bin/ruby -e "$(curl -fsSL https://raw.githubusercontent.com/Homebrew/install/master/install)"
```

A list of all the available packages (known as *formulae*) is found at [formulae.brew.sh](https://formulae.brew.sh). Examples of `brew` command to install the `EMBOSS` or the `pandoc` are shown here:

```
# Getting EMBOSS installed with brew
brew search emboss
# homebrew/science/emboss #<-- check the output of the previous command to use in your system
brew install homebrew/science/emboss

# Getting pandoc installed with brew
brew install pandoc
# this is optional
brew install pandoc-citeproc
# you need this to typeset PDFs with LaTeX
brew install librsvg python homebrew/cask/basictex

# You can also download the pandoc MacOS instaler from the following URL:
# https://github.com/jgm/pandoc/releases/download/3.4/pandoc-3.4-x86_64-macOS.pkg
```

**1.2.1.3 Using conda/mamba environments:** Yet another way to install the software required to complete the exercises is to use `conda` environments. You can install `conda` following the instructions from [this link](#); you can also use `mamba` instead, which is a compact and faster implementation of `conda`, from the instructions at [this link](#). Once you have one of those environment managers installed, you can follow the commands in the next code block to create the `BScBI-CG2425_exercises` environment and activate it.

```
#
# If you have conda instead of mamba already installed on your system
# you can just replace 'mamba' by 'conda' on the commands below:
#
mamba env create --file environment.yml

# Now you can run the tools installed on that environment by activating it:
mamba activate BScBI-CG2425_exercises

# Remember that each time you deactivate a conda environment
# all shell variables defined inside will be lost
# (unless they were exported before activating the conda environment).
# Anyway, you can reload project vars with:
source projectvars.sh

# To return to the initial terminal state, you must deactivate the environment:
mamba deactivate
```

You can review the contents of the environment YAML file at the Appendices (see section [4.2.1](#) on page [12](#)),

**1.2.1.4 Using docker containers:** If you have a MacOS, a MS-Windows OS, or you cannot deal with the software install commands from the previous section, you can even use the container we provide for the course, which has all those tools already packed and ready to be used.

```
# You must install docker server to run containers in your system.
# Follow the instructions from the URLs below
#   https://docs.docker.com/
#   https://docs.docker.com/install/linux/docker-ce/debian/
#   https://docs.docker.com/docker-for-mac/install/
#   https://docs.docker.com/docker-for-windows/install/

# Download the tarball from the course materials (take care, it's a 3.5GB file)
wget https://compgen.bio.ub.edu/~jabril/teaching/BScBI-CG2425/MScGGBIA_docker.tar.gz

# Then, get the tarball unzipped on your machine:
gunzip -vc '/mounted_device_path/MScGGBIA_docker.tar.gz' > ~/MScGGBIA_docker.tar

# Upload the container to your docker server:
docker load -i ~/MScGGBIA_docker.tar

# Change directory on the current terminal/shell to the exercise folder.

# Run this docker with:
docker run --rm -ti -e WD="$PWD" -v /home:/home mscggbia # on a Linux machine
docker run --rm -ti -e WD="$PWD" -v /Users:/Users mscggbia # on a Mac machine
docker run --rm -ti -e WD="$pwd" -v c:/Users:/Users mscggbia # on a Windows machine

# Now you will be inside the container on the root folder,
# so you have to change to the working directory:
cd $WD
# Then you are ready to play with commands at the exercise folder.
source projectvars.sh
runpandoc
```

## 1.2.2 Initializing the main report files

In a bash command-line terminal, create a folder for all the practicals on this subject and change working dir to that folder:

```
mkdir practicals
cd practicals
```

Then, we need to download the exercise tarball (the \*.tgz file) from the [Computational Genomics Virtual Campus at ESCI](#) into that folder, unpack such file, modify the files accordingly to the user within the exercise folder, and set it as the current working directory for the rest of the practical session. . .

```
# Uncompress and unpack the exercise files from tarball
#
# NOTE: If you are reading this, you probably have already done this step.
#
tar -zxvf BScBI-CG2425_exercise_00.tgz

# Move into the new extracted folder
cd exercise_00

# Rename the Markdown README_*.md file by replacing NAME and SURNAME strings
# with your "NAME" and "SURNAME" with the following command
mv -v README_BScBICG2425_exercise00_SURNAME_NAME.md \
    README_BScBICG2425_exercise00_YourSurname_YourName.md

# Open exercise files using your text editor of choice
```

```
# (for instance vim, emacs, gedit, sublime, atom, ...);
emacs projectvars.sh \
    README_BScBICG2425_exercise00_YourSurname_YourName.md

# Fix "NAME" and "SURNAME" placeholders on those files
# and save the changes before continuing.

# Load the bash definitions from projectvars.sh
source projectvars.sh
# for instance the variable WDR was set to the absolute path
# to current exercise working directory
echo $WDR

# Now you are ready to start the practical by looking at
# the MarkDown file for further instructions to run
# the corresponding code blocks.

# Each time you include your answers/code/results
# on the README file, you can compile it into PDF.
# So that, let's tests if we can compile the modified MarkDown document.
# You probably must install some dependencies yet...
runpandoc
```

Again, remember to submit both files, the MD and the PDF, to the [Computational Genomics Virtual Campus at ESCI](#), once errors/warnings have been fixed, all the requested task have been completed, and you have discussed your results on the corresponding sections.

If you have succeeded on the software installation step, then you can start with the analyses provided on the next section... May the shell be with you...

## 2 Genome properties Comparison

As an example of analysis we can record in a **Markdown** report file, we are going to plot the distribution of lengths for a set of eukaryotic organisms.

### 2.1 Datasets

We already have a file in tabular format in the **stats** folder, where we have saved some whole-genome properties of several eukaryotic species. The initial **genomes.csv** file was downloaded from the [Genome Information by Organism online resource](#) at [NCBI-Genomes database division](#). The file has info about species, their taxonomic group, the genome size in mega-basepairs (Mbp), number of completed chromosomes and organelles, as well as the number of available assemblies at NCBI, for a total of 47,345 species.

### 2.2 Basic data checks from command-line

We can take a look to the **genomes.csv** file contents with some basic **Unix** commands. We will filter out some fields and records, then we will upload the processed table into an R shell and we will transform the data into few summary plots.

```
## listing directory contents
ls -alFhrt stats/
# drwxr-xr-x 2 jabril users 4.0K Sep 28 14:21 ./
# drwxr-xr-x 6 jabril users 4.0K Sep 28 14:13 ../
# -rw-r--r-- 1 jabril users 5,9M Sep 28 14:15 genomes.csv

## counting number of lines, words and chars of a file
wc stats/genomes.csv
# 72068 245486 6143707 stats/genomes.csv
# this means that the file has 72068 lines;
# the first one defines the column names,
# thus there are 72067 records with genomes information

## looking at the first two lines of the tabular file in csv format
head -2 stats/genomes.csv
# #Organism Name,Organism Groups,Size(Mb),Chromosomes,Organelles,Plasmids,Assemblies
# "'Brassica napus' phytoplasma","Bacteria;Terrabacteria group;Tenericutes",0.743598,0,0,0,1

## filtering out relevant information
gawk 'BEGIN{ FS=","; OFS="\t"; }
{ gsub("/"," ", $2);
  split($2, t, ";");
  print $1, t[1], t[2], $3, $4;
}' stats/genomes.csv \
> stats/genomes.tbl

## looking at the first two lines of the new tabular file in tsv format
head -2 stats/genomes.tbl
# #Organism Name      Organism Groups      Size(Mb)      Chromosomes
# "'Brassica napus' phytoplasma"      Bacteria      Terrabacteria group 0.743598      0

# just focus on animal genomes
gawk 'BEGIN{ FS=OFS="\t"; }
$2 == "Eukaryota" && $3 == "Animals" {
  print $0;
}' stats/genomes.tbl \
> stats/genomes.animals_only.tbl

# yet another counting step
wc stats/genomes.*
# 72068 245486 6143707 stats/genomes.csv
# 72068 514828 4445887 stats/genomes.tbl
# 4748 28521 236574 stats/genomes.animals_only.tbl
```

## 2.3 Visualizing the analysis

Let's explore the distribution of genome lengths in animal genomes. By running R command, we enter in the R shell interpreter, which understands R commands of course.

```
R
#
# R version 4.1.2 (2021-11-01) -- "Bird Hippie"
# Copyright (C) 2021 The R Foundation for Statistical Computing
# Platform: x86_64-pc-linux-gnu (64-bit)
#
# R is free software and comes with ABSOLUTELY NO WARRANTY.
# You are welcome to redistribute it under certain conditions.
# Type 'license()' or 'licence()' for distribution details.
#
# R is a collaborative project with many contributors.
# Type 'contributors()' for more information and
# 'citation()' on how to cite R or R packages in publications.
#
# Type 'demo()' for some demos, 'help()' for on-line help, or
# 'help.start()' for an HTML browser interface to help.
# Type 'q()' to quit R.
```

Now, we must load the tabular data into a variable.

```
# if we have an uncompressed tabular file
DATA <- read.table("stats/genomes.animals_only.tbl",
  header=FALSE, comment.char='#',
  col.names=c("SpeciesName", "Superkingdom", "TaxonGroup",
    "GenomeSize", "ChromNum"));

# just checking the data structure
head(DATA, 4)
#           SpeciesName Superkingdom TaxonGroup GenomeSize ChromNum
# 1   Abisara bifasciata   Eukaryota   Animals    332.742         0
# 2   Abramis brama       Eukaryota   Animals    1067.400        25
# 3  Abrostola tripartita   Eukaryota   Animals    381.057        31
# 4  Abscondita terminalis   Eukaryota   Animals    499.653         0
```

Let's calculate some stats on the dataset.

```
options(width=180)
summary(DATA)
# SpeciesName      Superkingdom      TaxonGroup      GenomeSize      ChromNum
# Length:4748      Length:4748      Length:4748      Min.   : 0.0      Min.   : 0.000
# Class :character Class :character Class :character 1st Qu.: 285.2    1st Qu.: 0.000
# Mode  :character Mode  :character Mode  :character Median : 663.2    Median : 0.000
#                                     Mean  : 935.3     Mean  : 5.006
#                                     3rd Qu.: 1126.7   3rd Qu.: 0.000
#                                     Max.   :40054.3   Max.   :99.000
```

Now, let's make an histogram of total lengths for the animal genomes:

```
png(file="images/genome_length_animals.png");
hist(DATA$GenomeSize);
dev.off();
```



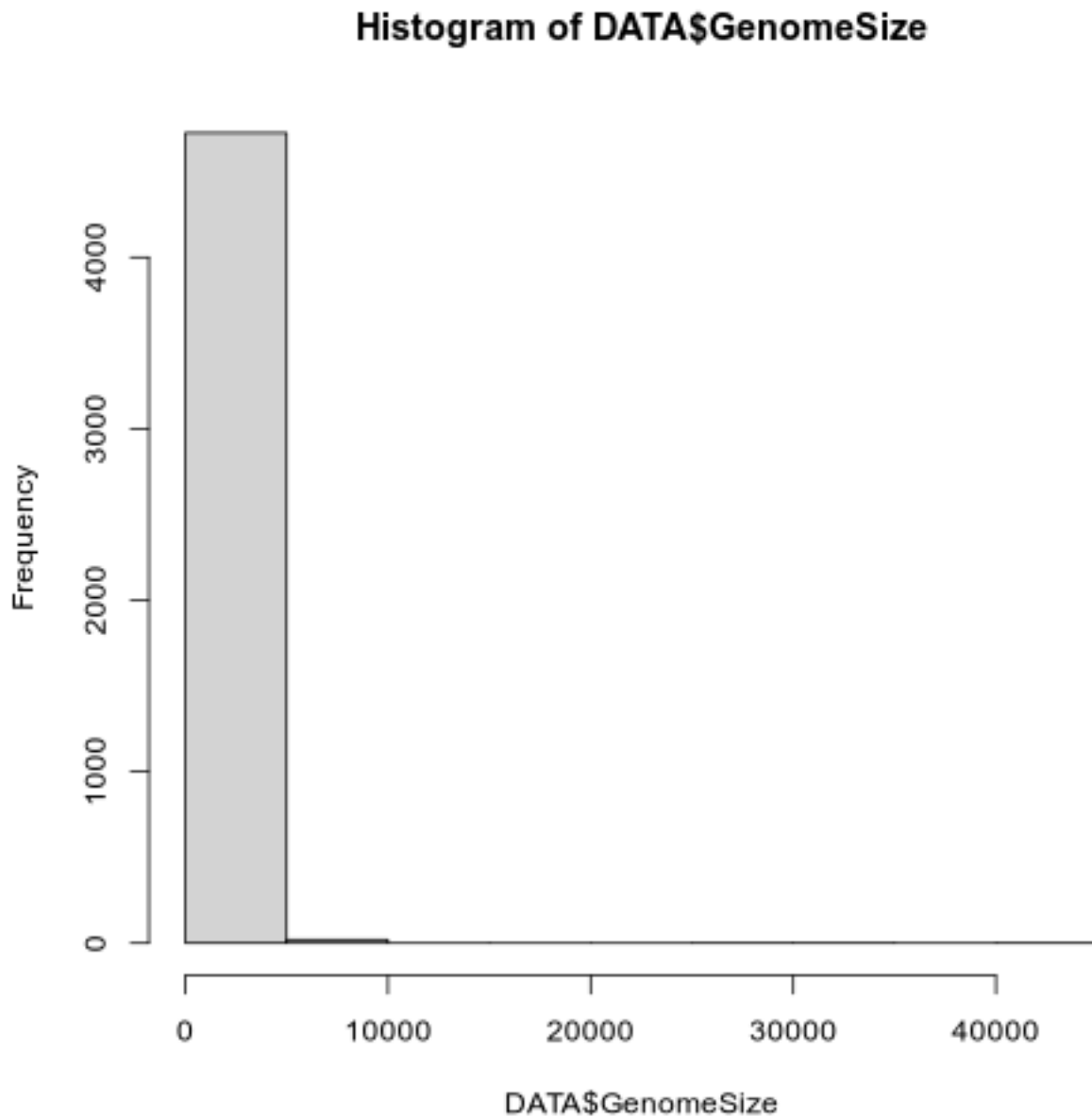


Figure 1: Showing animals genome length distribution

```
bacteria <- read.table("stats/genomes.bacteria.tbl",
header=FALSE, comment.char='#', col.names=c("SpeciesName", "Superkingdom", "TaxonGroup", "Genome-Size", "ChromNum"));
```

## 2.4 Further analyses

**IMPORTANT** You can provide here the **bash** and **R** commands to generate the genome sizes histograms for plants, bacteria (archaea not included), and viruses. If you were able to perform and complete that task, you can play with the data after that and provide box-plots by taxonomic group showing the distribution of the genome sizes side-by-side.

*# Your shell commands here*

*#so we create a .tbl for each case first*

*# just focus on plant genomes*

```
gawk 'BEGIN{ FS=OFS="\t"; }
      $2 == "Eukaryota" && $3 == "Plants" {
        print $0;
      }' stats/genomes.tbl \
    > stats/genomes.plants.tbl
```

*# just focus on bacteria genomes*

```
gawk 'BEGIN{ FS=OFS="\t"; }
      $2 == "Bacteria"{
        print $0;
      }' stats/genomes.tbl \
    > stats/genomes.bacteria.tbl
```

*#Terrabacteria group -> Terrabacteria\_group*

```
cut -f3 -d$'\t' stats/genomes.bacteria.tbl |grep " "
```

*#find pattern of " "group and unclassified" " among many others, use find and replace to set \_group and un*

*# just focus on virus genomes*

```
gawk 'BEGIN{ FS=OFS="\t"; }
      $2 == "Viruses"{
        print $0;
      }' stats/genomes.tbl \
    > stats/genomes.virus.tbl
```

```
cut -f3 -d$'\t' stats/genomes.virus.tbl |grep " "
```

*# Your R commands here*

```
animal <- read.table("stats/genomes.animals_only.tbl",
                    header=FALSE, comment.char='#',
                    col.names=c("SpeciesName", "Superkingdom", "TaxonGroup",
                               "GenomeSize", "ChromNum"));
```

*#first we load the three tables into the R environment*

```
plant <- read.table("stats/genomes.plants.tbl",
                  header=FALSE, comment.char='#',
                  col.names=c("SpeciesName", "Superkingdom", "TaxonGroup",
                              "GenomeSize", "ChromNum"));
png(file="images/genome_length_plants.png", width=800, height=600, res=150);
hist(plant$GenomeSize);
dev.off();
```

```
bacteria <- read.table("stats/genomes.bacteria.tbl",
                    header=FALSE, comment.char='#',
                    col.names=c("SpeciesName", "Superkingdom", "TaxonGroup",
                               "GenomeSize", "ChromNum"));
```

*#As there were parts that created errors, we used a bash command to fix the data*

```
png(file="images/genome_length_bacteria.png", width=800, height=600, res=150);
hist(bacteria$GenomeSize);
dev.off();
```

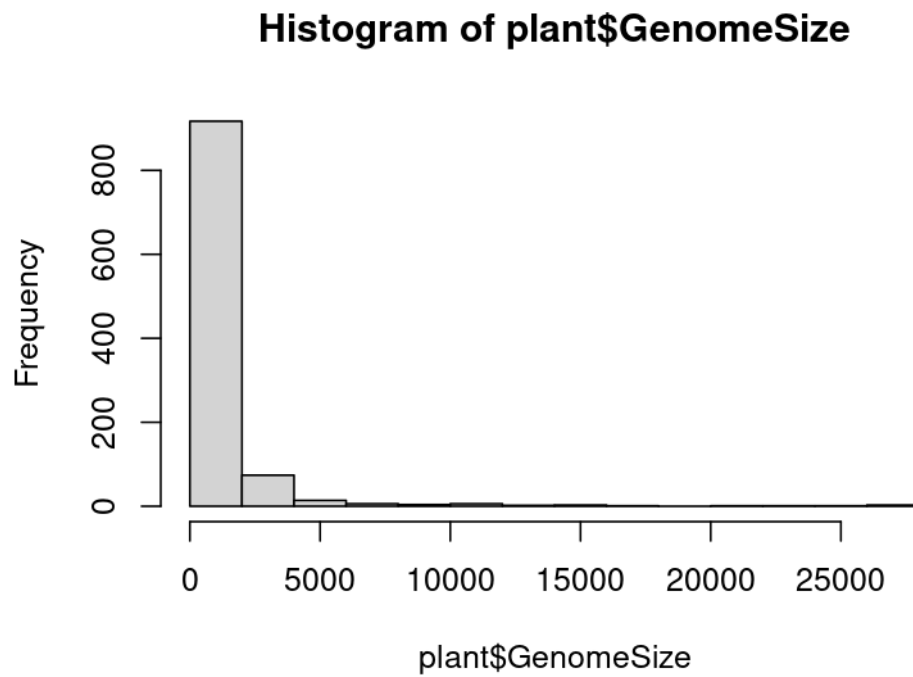


Figure 2: Showing plants genome length distribution

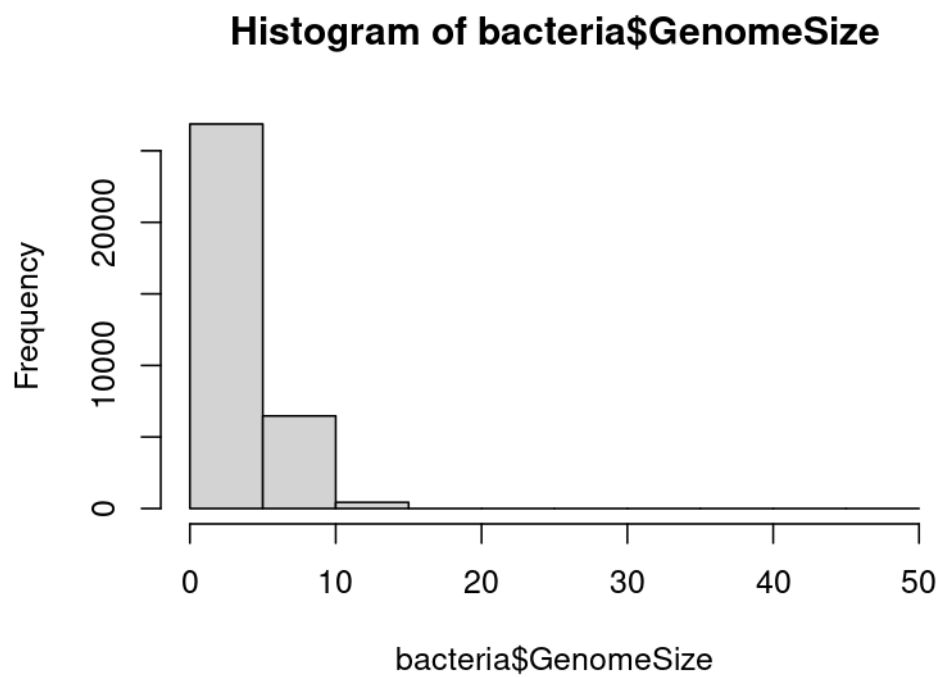


Figure 3: Showing plants genome length distribution

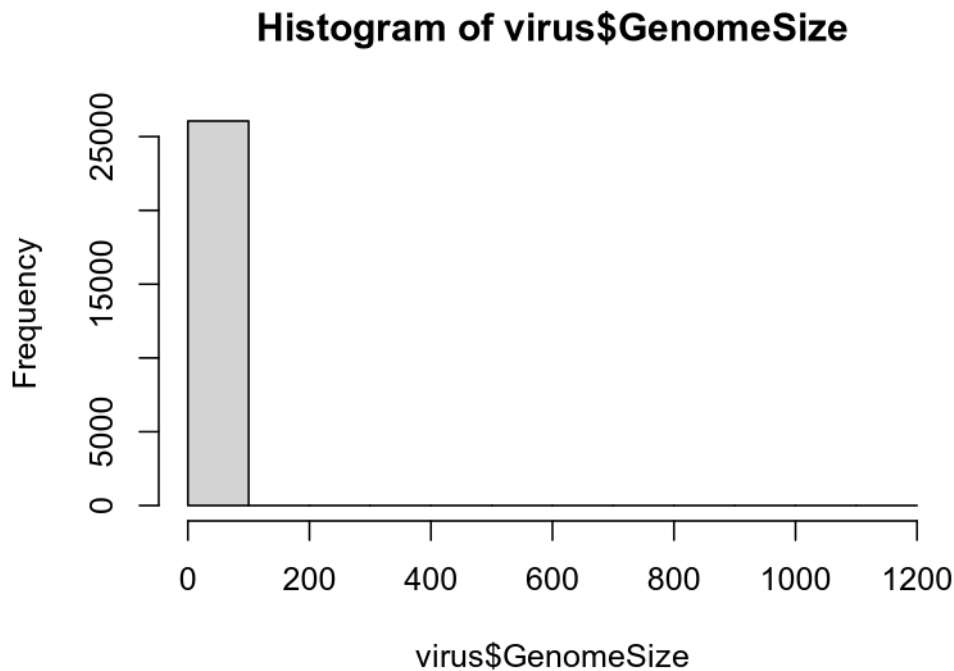


Figure 4: Showing plants genome length distribution

```
#Virus genome plots
virus <- read.table("stats/genomes.virus.tbl",
                    header=FALSE, comment.char='#',
                    col.names=c("SpeciesName", "Superkingdom", "TaxonGroup",
                               "GenomeSize", "ChromNum"));

#As there were parts that created errors, we used a bash command to fix the data
png(file="images/genome_length_virus.png", width=800, height=600, res=150);
hist(virus$GenomeSize);
dev.off();

#now the boxplot
library(dplyr)
library(ggplot2)
animal$Database<-"Animals"
plant$Database<-"Plants"
bacteria$Database<-"Bacteria"
virus$Database<-"Virus"
data_joint <- bind_rows(animal, plant, bacteria, virus)
ggplot(data=data_joint, aes(x=Database, y=GenomeSize, fill=Database))+
  geom_boxplot()+ggtitle("Genome sizes across organism groups")+theme(plot.title = element_text(hjust = 0.5))
ggsave(file="images/genome_boxplot_comparison.png", width = 9, height = 7)
```

### 3 Discussion

**IMPORTANT** Discuss your results here (around 300 words). And remember to include in the Appendices section (see page 12), any extra script you wrote from this exercise bin folder using the `loadfile` macro.

:: All of the organism groups have a high frequency of small genome sizes, as can be seen from the histograms of each individual group. We can also see in the box plots that the genome sizes of eukaryotes, or plants and animals, are larger than those of bacteria and viruses. It is also evident that, of all the groups, plants have the highest frequency of large genomes; nevertheless, animals have the largest genome sizes. ::

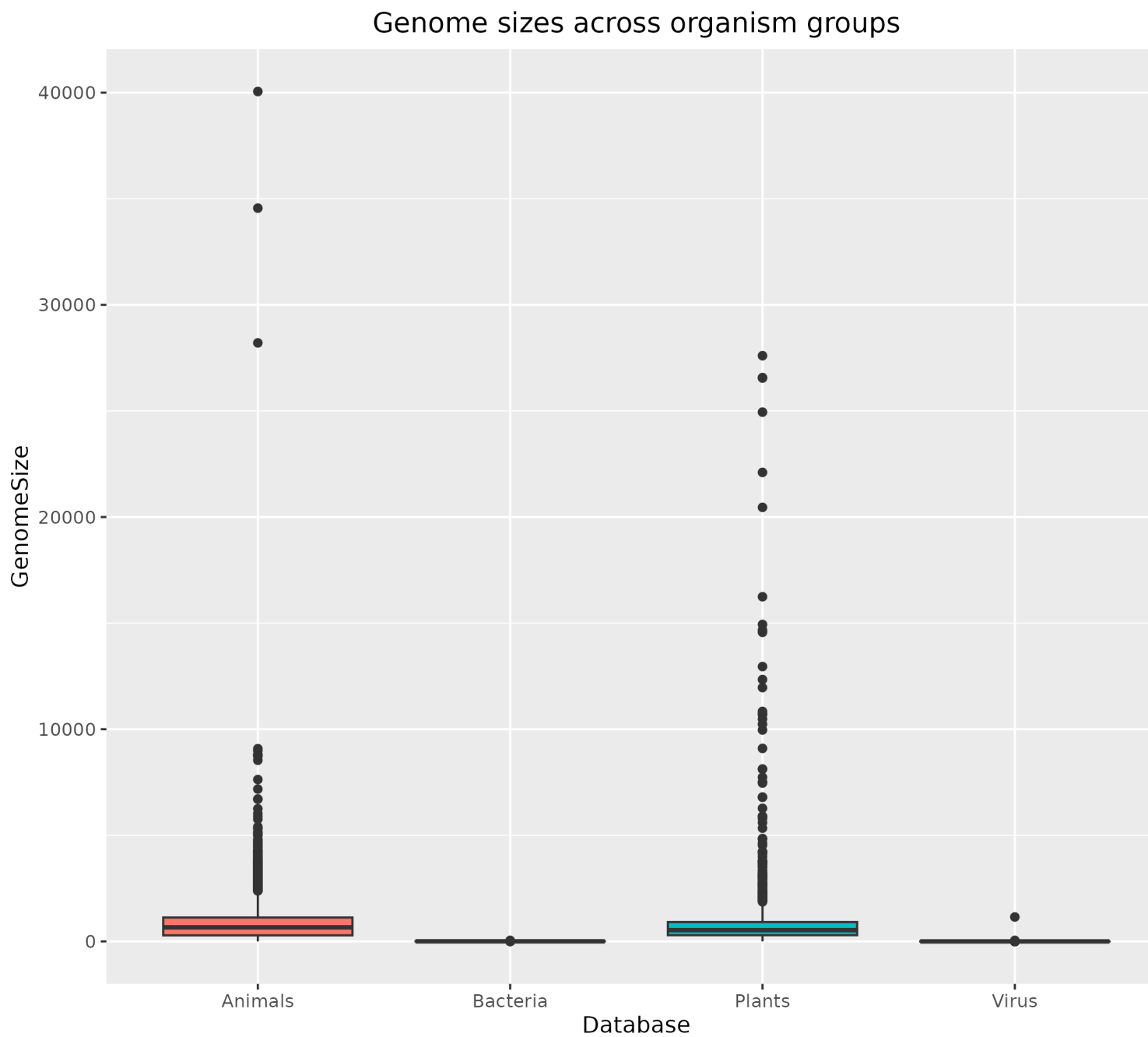


Figure 5: Showing plants genome length distribution

## 4 Appendices

### 4.1 Software

We have used the following versions:

```
uname -a
# Linux aleph 5.15.0-117-generic #127-Ubuntu SMP
# Fri Jul 5 20:13:28 UTC 2024 x86_64 x86_64 x86_64 GNU/Linux

R --version
# R version 4.3.1 (2023-06-16) -- "Beagle Scouts"
# Copyright (C) 2023 The R Foundation for Statistical Computing
# Platform: x86_64-conda-linux-gnu (64-bit)

wget --version
# GNU Wget 1.21.2 built on linux-gnu.

pandoc --version
# pandoc 3.1.3
# Features: +server +lua
# Scripting engine: Lua 5.4

mamba --version
# mamba 1.4.2
# conda 23.3.1
```

### 4.2 Supplementary files

#### 4.2.1 conda environment dependencies for the exercise

environment.yml

```
#
## #####
##
## environment.yml
##
## Defining conda/mamba software dependencies to run BScBI-CG practical exercises.
##
## #####
##
##          CopyLeft 2024 (CC:BY-NC-SA) --- Josep F Abril
##
## This file should be considered under the Creative Commons BY-NC-SA License
## (Attribution-Noncommercial-ShareAlike). The material is provided "AS IS",
## mainly for teaching purposes, and is distributed in the hope that it will
## be useful, but WITHOUT ANY WARRANTY; without even the implied warranty
## of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE.
##
## #####
#
# To install software for the exercise use the following command:
#
#   conda env create --file environment.yml
#
# then run the command below to activate the conda environment:
#
#   conda activate BScBI-CG2425_exercises
#
name: BScBI-CG2425_exercises
channels:
  - bioconda
  - conda-forge
  - defaults
dependencies:
  - htop
  - vim
  - emacs
  - gawk
```

```
- perl
- python
- biopython
- wget
- curl
- gzip
- r-ggplot2
- texlive-core
- pandoc
- pandocfilters
```

## 4.2.2 Project specific scripts

an\_script\_example.pl

```
#!/usr/bin/perl
#
# an_script_example.pl - just a silly example for the MarkDown template
#
use strict;
use warnings;
#

print STDOUT "\n";

for (my $i = 0; $i < 15; $i++) {

    printf STDOUT "\r\tHi, this loop example has iterated %02d times already...", $i + 1;

    sleep(1);

} # for $i

print STDOUT "\n... Bye!!!\n\n";

exit(0);
```

## 4.2.3 Shell global vars and settings for this project

projectvars.sh

```
## #####
##
## projectvars.sh
##
## A BASH initialization file for BScBI-CG practical exercise folders
##
## #####
##
## Copyright 2024 (CC:BY-NC-SA) --- Josep F Abril
##
## This file should be considered under the Creative Commons BY-NC-SA License
## (Attribution-Noncommercial-ShareAlike). The material is provided "AS IS",
## mainly for teaching purposes, and is distributed in the hope that it will
## be useful, but WITHOUT ANY WARRANTY; without even the implied warranty
## of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE.
##
## #####

#
# Base dir
export WDR=$PWD; # IMPORTANT: If you provide the absolute path, make sure
# that your path DOES NOT contains white-spaces
# otherwise, you will get weird execution errors.
# If you cannot fix the dir names containing such white-space
# chars, you MUST set this var using the current folder '.'
# instead of '$PWD', i.e: export WDR=.;

export BIN=$WDR/bin;
export DOC=$WDR/docs;

#
# Formating chars
export TAB=$'\t';
```

```

export RET='${\n}';
export LC_ALL="en_US.UTF-8";

#
# pandoc's vars
NM="SURNAME_NAME";           #-> IMPORTANT: SET YOUR SURNAME and NAME ON THIS VAR,
RB="README_BScBICG2425_exercise00"; #-> MUST FIX ON MARKDOWN README FILE
                                   #-> FROM TARBALL (AND INSIDE TOO)

RD="${RB}_${NM}";
PDOCFGLS='markdown+pipe_tables+header_attributes';
PDOCFGLS=${PDOCFGLS}+raw_tex+latex_macros+tex_math_dollars';
PDOCFGLS=${PDOCFGLS}+citations+yaml_metadata_block';
PDOCTPL=${DOC}/BScBI_CompGenomics_template.tex;
export RD PDOCFGLS PDOCTPL;

#
### IMPORTANT ###
#
# MacOSX users may need to remove /usr/bin/ from below shell functions,
# just try first if that path works anyway...
#
function ltx2pdf () {
    RF=$1;
    /usr/bin/pdflatex $RF.tex;
    /usr/bin/bibtex $RF;
    /usr/bin/pdflatex $RF.tex;
    /usr/bin/pdflatex $RF.tex;
}

function runpandoc () {
    /usr/bin/pandoc -f $PDOCFGLS \
        --template=${PDOCTPL} \
        -t latex --natbib \
        --number-sections \
        --highlight-style pygments \
        -o $RD.tex $RD.md;
    ltx2pdf $RD;
}

#
# add your bash defs/aliases/functions below...

```

### 4.3 About this document

This document was be compiled into a PDF using `pandoc` (see `projectvars.sh` from previous subsection) and some `LaTeX` packages installed in this linux system. `synaptic`, `apt-get` or `aptitude` can be used to retrieve and install those tools from linux repositories. As the `raw_tex` extension has been provided to the `markdown_github` and `tex_math_dollars` formats, now this document supports inline `LATEX` and inline formulas!

You can get further information from the following links about the [Mark Down syntax](#), as well as from the manual pages (just type `man pandoc` and/or `man pandoc_markdown`).