

# D I F F R N A : A RNA-Seq analysis pipeline

Izem Mouhoubi      Théo Roncalli      Gustavo Magaña López  
Alex Westbrook

2021-12-10

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# Chapter 1

## Prerequisites

### 1.1 Hardware and Operating System

The pipeline was developed and tested on Ubuntu 20.04.3 LTS on top of the (GNU/Linux 5.4.0-88-generic x86\_64) kernel. The output of the commands `uname` and `neofetch` are provided to further detail our configuration.

```
$ uname -a
```

```
Linux machine329a7396-059f-41aa-94c7-4c41b4ec8290 5.4.0-88-generic #99-Ubuntu SMP Thu Sep 23 17:2
```

```
$ neofetch
```

```
      .-/+oossssoo+/-.  
      `:+ssssssssssssssss+:`  
      -+ssssssssssssssssyyssst+-  
      .ossssssssssssssssdMMMMyssso.  
      /sssssssssshdmNNmyNMMMHssssss/  
      +sssssssshmydMMMMMMNdddyssssssst+  
      /ssssssshNMMMyhhyyyhmNMMNhssssssss/  
      .sssssssdMMMNhssssssssshNMMMdssssssss.  
      +sssshhhyNMMNysssssssssssyNMMMyssssssst+  
      ossyNMMMNyMMhssssssssssshmmhssssssso  
      ossyNMMMNyMMhssssssssssshmmhssssssso  
      +sssshhhyNMMNysssssssssssyNMMMyssssssst+  
      .sssssssdMMMNhssssssssshNMMMdssssssss.  
      /ssssssshNMMMyhhyyyhdNMMNhssssssss/  
      +sssssssdmydMMMMMMNdddyssssssst+  
      /ssssssssshdmNNNNmyNMMMHssssss/  
      .ossssssssssssssssdMMMMyssso.  
      -+ssssssssssssssssyyssst+-  
      `:+ssssssssssssssss+:`
```

```
ubuntu@machine3f9ae3dc-6a4d-46b7-8131-04f00a1be146
```

```
-----  
OS: Ubuntu 20.04.3 LTS x86_64  
Host: OpenStack Compute 18.2.1-1.el7  
Kernel: 5.4.0-88-generic  
Uptime: 13 hours, 14 mins  
Packages: 719 (dpkg), 4 (snap)  
Shell: bash 5.0.17  
Theme: Adwaita [GTK3]  
Icons: Adwaita [GTK3]  
Terminal: /dev/pts/0  
CPU: Intel (Haswell, no TSX, IBRS) (16) @ 2.294GHz  
GPU: 00:02.0 Cirrus Logic GD 5446  
Memory: 635MiB / 64323MiB
```

```
.-/+oossssoo+/-. 
```

This configuration was actually a virtual machine hosted on Biosphere’s RAIN-Bio a cloud service maintained by the French Institute of Bioinformatics (*Institut Français de Bioinformatique*).

## 1.2 BioPipes: a Biosphere-commons app

The instance of the virtual machine we used is called *BioPipes*. It provides the most notable bioinformatics pipeline tools:

- nextflow
- snakemake
- cwltool

## 1.3 Software

Our pipeline is built on top of `nextflow`, which was installed via the conda package manager.

```
$ conda --version
conda 4.11.0
$ nextflow -v
nextflow version 21.10.0.5640
```

## Chapter 2

# Introduction

You can label chapter and section titles using `{#label}` after them, e.g., we can reference Chapter 2. If you do not manually label them, there will be automatic labels anyway, e.g., Chapter 4.

Figures and tables with captions will be placed in `figure` and `table` environments, respectively.

```
par(mar = c(4, 4, .1, .1))  
plot(pressure, type = 'b', pch = 19)
```

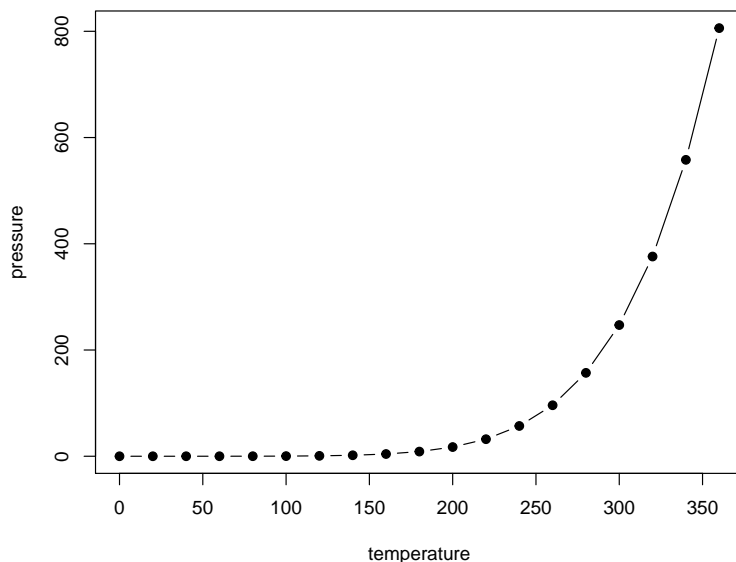


Figure 2.1: Here is a nice figure!

Table 2.1: Here is a nice table!

Sepal.Length	Sepal.Width	Petal.Length	Petal.Width	Species
5.1	3.5	1.4	0.2	setosa
4.9	3.0	1.4	0.2	setosa
4.7	3.2	1.3	0.2	setosa
4.6	3.1	1.5	0.2	setosa
5.0	3.6	1.4	0.2	setosa
5.4	3.9	1.7	0.4	setosa
4.6	3.4	1.4	0.3	setosa
5.0	3.4	1.5	0.2	setosa
4.4	2.9	1.4	0.2	setosa
4.9	3.1	1.5	0.1	setosa
5.4	3.7	1.5	0.2	setosa
4.8	3.4	1.6	0.2	setosa
4.8	3.0	1.4	0.1	setosa
4.3	3.0	1.1	0.1	setosa
5.8	4.0	1.2	0.2	setosa
5.7	4.4	1.5	0.4	setosa
5.4	3.9	1.3	0.4	setosa
5.1	3.5	1.4	0.3	setosa
5.7	3.8	1.7	0.3	setosa
5.1	3.8	1.5	0.3	setosa

Reference a figure by its code chunk label with the `fig:` prefix, e.g., see Figure 2.1. Similarly, you can reference tables generated from `knitr::kable()`, e.g., see Table 2.1.

```
knitr::kable(
  head(iris, 20), caption = 'Here is a nice table!',
  booktabs = TRUE
)
```

You can write citations, too. For example, we are using the **bookdown** package (Xie, 2021) in this sample book, which was built on top of R Markdown and **knitr** (Xie, 2015).

## Chapter 3

# Literature

Here is a review of existing methods.

# Chapter 4

## Methods

We describe our methods in this chapter.

Math can be added in body using usual syntax like this

### 4.1 math example

$p$  is unknown but expected to be around  $1/3$ . Standard error will be approximated

$$SE = \sqrt{\left(\frac{p(1-p)}{n}\right)} \approx \sqrt{\frac{1/3(1-1/3)}{300}} = 0.027$$

You can also use math in footnotes like this<sup>1</sup>.

We will approximate standard error to  $0.027^2$

---

<sup>1</sup>where we mention  $p = \frac{a}{b}$

<sup>2</sup> $p$  is unknown but expected to be around  $1/3$ . Standard error will be approximated

$$SE = \sqrt{\left(\frac{p(1-p)}{n}\right)} \approx \sqrt{\frac{1/3(1-1/3)}{300}} = 0.027$$



## Chapter 5

# Applications

Some *significant* applications are demonstrated in this chapter.

### 5.1 Example one

### 5.2 Example two

## Chapter 6

# Final Words

We have finished a nice book.

# Bibliography

Xie, Y. (2015). *Dynamic Documents with R and knitr*. Chapman and Hall/CRC, Boca Raton, Florida, 2nd edition. ISBN 978-1498716963.

Xie, Y. (2021). *bookdown: Authoring Books and Technical Documents with R Markdown*. <https://github.com/rstudio/bookdown>, <https://pkgs.rstudio.com/bookdown/>.