

A Minimal Book Example

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Chapter 1

Prerequisites

This is a *sample* book written in **Markdown**. You can use anything that Pandoc's Markdown supports, e.g., a math equation $a^2 + b^2 = c^2$.

The **bookdown** package can be installed from CRAN or Github:

```
install.packages("bookdown")  
# or the development version  
# devtools::install_github("rstudio/bookdown")
```

Remember each Rmd file contains one and only one chapter, and a chapter is defined by the first-level heading #.

To compile this example to PDF, you need XeLaTeX. You are recommended to install TinyTeX (which includes XeLaTeX): <https://yihui.name/tinytex/>.

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, 2020) 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.

Chapter 5

Applications

Some *significant* applications are demonstrated in this chapter.

5.1 Example one

5.2 Example two

Chapter 6

Histological Protocols

6.1 Alizarin Staining

6.1.1 PURPOSE: Alizarin staining of fixed adult stickleback.

6.1.2 MATERIALS:

- 0.5% Alizarin red S Stock: To make 50 mls add 0.25g alizarin red S powder to 50 ml water.
- 0.025% Alizarin Stain: To make 100 mls: Add 500µl 0.5% alizarin red S (stock) to 99.5ml 1% KOH
- 1 Liter: Add 5ml 0.5% alizarin red S (stock) to 9950ml (1 liter) 1%KOH

3% H2O2/0.5%KOH: {#h2O20.5koh .subhead2}

Mix and keep at 4C; Before using, bring to room temperature to hold down introducing bubbles under the skin: 0.5ml 6%H2O2 & 0.5ml 1%KOH.

MESAB: {#mesab .subhead2}

Tricaine: 3-amino benzoic acid ethyl ester from Sigma (Cat # A-5040). Mix in fish safe container with a stir bar:

- 400 mg tricaine powder
- 800 mg Na2HPO4 (anhydrous)
- 100 ml glass distilled water

Adjust to ~pH 7 with a drop at a time of 1N NaOH or 1N HCl if needed but it's usually right if you weigh the sodium phosphate carefully and measure the water with a graduated cylinder.

For storage: Aliquot into 6 x 25 ml fish safe plastic bottles and store at 4C. Label with date made and use within a couple of weeks.

8% PFA: {#pfa .subhead2}

- 8 g Pelleted PFA (Ted Pella, Inc.; cat# 18501)
 - 90 ml dH₂O
 - 25 drops 1N NaOH
1. Heat at very low heat and stir until solution clears.
 2. Add 25 drops 1N HCl. pH should be 7.0-7.2.
 3. Filter and store at 4C not more than 1 week.
 4. Use as 4% PFA: dilute 1:1 with 2X PBS, do not store solution more than a few hours.

2X PBS {#x-pbs .subhead2}

- 1.6% NaCl
- 0.04% KCl
- 0.04 M PO₄ pH 7.0- 7.3

6.1.3 Procedure:

Day

Step

Time for Step

Date and Time

1

2h-8h at R/T depending on size on shaker.

1h or longer at R/T on shaker.

Without agitation and with lid open until eyes start to lighten and all skin pigment is gone (usually about an hour or more)

2

2 h to O/N at R/T on shaker

2 h to overnight O/N at R/T on shaker.

Check for bone staining.

R/T on shaker until excess stain in tissue is gone.

Without agitation

Wild caught specimens are put in 100% EtOH in the field and then rehydrated and put into 4% when back in the lab.

Bibliography

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

Xie, Y. (2020). *bookdown: Authoring Books and Technical Documents with R Markdown*. R package version 0.21.