

Cresko Laboratory Procedures and Protocols

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How to use this book

This is a Quarto book that contains all of the Procedures and Protocols for the Cresko Laboratory in the Institute of Ecology and Evolution at the University of Oregon.

The book is organized into major sections that contain

- General Laboratory Protocols or the lab
- More detailed Laboratory Protocols
- Husbandry protocols for vertebrate animals primarily stickleback and pipefish, but also zebrafish
- Husbandry protocols for *Daphnia*
- Bioinformatic protocols including how to get on to **Talapas**

You can scroll through the book using the index on the left, but also use the search field to find all relevant protocols.

There are also useful appendices at the end, as well as a section for the references cited throughout the book.

This book was written in Markdown using Quarto. To learn more about Quarto books visit <https://quarto.org/docs/books>.

1 Introduction

This is a book created from markdown and executable code.

See Knuth (1984) for additional discussion of literate programming.

```
1 + 1
```

```
[1] 2
```

Part I

General Laboratory Protocols

2 Twenty Gallon Aquarium Cleaning

2.1 Introduction

- **Purpose:** This procedure describes how to clean 20 gallon glass tanks.
- **Procedure Type:** Husbandry
- **Species:**
 - Threespine stickleback, (*Gasterosteus aculeatus*),
 - Gulf pipefish (*Syngnathus scovelli*)

Schedule for Cleaning

Tank cleaning is to be done ONLY Monday - Friday

2.2 Materials:

- Scrub pad or sponge
- Cart (you may or may not want to use)
- Old clothes (this can be messy)
- Personal protection equipment (Splash proof glasses or face shield).

2.3 Solutions:

- **Bleach solution:** Make a 10% bleach solution in a 2 gallon bucket. Add 4.5 L of water. Add 0.5 L of bleach and gently stir.
- **Sodium thiosulfate:** Make a 3% solution of sodium thiosulfate in a separate 2 gallon bucket. Add 5 L of water (to line) and 150g (marked on dispenser) of sodium thiosulfate. Mix

Note: When using bleach and/or sodium thiosulfate. Eye protection is required. Please use splash proof glasses or a face shield when using bleach and sodium thiosulfate.

2.4 Procedure:

- Complete bleaching and cleaning of tank. This needs to be done to each tank every 2 months.
- Remove fish from tank and put them into a clean tank. Tanks that are emptied of fish need to be cleaned and sterilized before another batch of fish can be introduced.
- Drain the tank and remove it from the rack. Clean air diffuser as instructed below.
 - Clean the tank and all parts thoroughly with a scrub pad, taking care not to damage the silicon water seals on the inside (algae should be left if very gentle rubbing will not remove it).
 - Squirt about 10 – 20 mls of bleach into the tank. Wash the bleach water thoroughly around the inside of the tank by hand using a pad or sponge exposing all inside portions of the tank to bleach.
 - Rinse the tank thoroughly with hot tap water. Rinse the tank with sodium thiosulfate, and then rinse it again with hot water. Put a few thiosulfate crystals into the tank and leave it.
 - Reassemble the tank and put it back on the rack. Fill with system water and allow water to recirculate for about 30 minutes before adding fish. Watch fish for 15min to look for any signs of distress.
 - Using a dry erase marker record date/time on the front of the tank when system water is turned back on.
- Initial the check list that you have completed the tank cleaning.

2.5 Air difuser cleaning:

- Remove dirty air diffusers from tanks and rinse with tap water to remove excess algae and debris.
- Place in 10% bleach solution for 15-30 minutes.
- Rinse the corner filters with hot water for 5 and then place into 3% sodium thiosulfate for 5 minutes.
- Rinse with hot water for 5 minutes.
- When cleaned air diffusers are placed back into aquaria, observe fish for 15 min for signs of distress.

Part II

Molecular Protocols

3 cDNA basic

3.1 Introduction

- **Purpose:** This procedure describes how to synthesis cDNA for use with PCR.
- **Procedure Type:** Molecular
- **Species:** N/A

3.2 Materials:

- 2 µl Oligo d(T)23 VN (50 µM, NEB; anchored-dT primer)*
- X µl up to 5 µg total RNA
- 1 µl 10 mM dNTP
- water
- 2 µl 10x RT buffer (Invitrogen)
- 4 µl 25 mM MgCl₂
- 2 µl 0.1 mM DTT – Invitrogen
- 1 µl RNase inhibitor – e.g., RNaseOUT (Invitrogen)
- 1 µl Superscript III reverse transcriptase (200 u/µl – Invitrogen)

3.3 Solutions:

NONE

3.4 Procedure:

3.4.1 First strand synthesis

Combine:

- 2 µl Oligo d(T)23 VN (50 µM, NEB; anchored-dT primer)*
- X µl up to 5 µg total RNA

- 1 μ l 10 mM dNTP mix
- Water (if necessary) to bring total to 10 μ l

Heat to 65°C for 5 min., then ice

Collect contents at bottom of tube by brief centrifugation.

Add:

- 2 μ l 10x RT buffer (Invitrogen)
- 4 μ l 25 mM MgCl₂
- 2 μ l 0.1 mM DTT – Invitrogen
- 1 μ l RNase inhibitor – e.g., RNaseOUT (Invitrogen)
- 1 μ l Superscript III reverse transcriptase (200 u/ μ l – Invitrogen)

Mix by gentle aspiration

- 25°C for 5 min.

3.4.2 Reaction can be scaled up to accommodate more starting RNA

Synthesis: Incubate at 50°C for 50 min.

Inactivation: 85°C for 5 min. Chill on ice, collect contents to bottom by short spin.

Destroy RNA template: 1 μ l RNase H (2 u/ μ l), incubate at 37°C for 20 min.

Proceed to PCR. Depending on expression level, may be able to use a dilution of cDNA as template – try 1:50 dilution in EB, use 2 μ l as template in a 20 μ l reaction. Don't dilute your entire amount of cDNA, as some products may require a higher concentration of template.

4 Placeholder_Molecular

4.1 xxx

XXXX

4.1.1 xxx

XXXXX

Part III

Vertebrate Husbandry

5 Twenty Gallon Aquarium Cleaning

5.1 Introduction

- **Purpose:** This procedure describes how to clean 20 gallon glass tanks.
- **Procedure Type:** Husbandry
- **Species:**
 - Threespine stickleback, (*Gasterosteus aculeatus*),
 - Gulf pipefish (*Syngnathus scovelli*)

Schedule for Cleaning

Tank cleaning is to be done ONLY Monday - Friday

5.2 Materials:

- Scrub pad or sponge
- Cart (you may or may not want to use)
- Old clothes (this can be messy)
- Personal protection equipment (Splash proof glasses or face shield).

5.3 Solutions:

- **Bleach solution:** Make a 10% bleach solution in a 2 gallon bucket. Add 4.5 L of water. Add 0.5 L of bleach and gently stir.
- **Sodium thiosulfate:** Make a 3% solution of sodium thiosulfate in a separate 2 gallon bucket. Add 5 L of water (to line) and 150g (marked on dispenser) of sodium thiosulfate. Mix

Note: When using bleach and/or sodium thiosulfate. Eye protection is required. Please use splash proof glasses or a face shield when using bleach and sodium thiosulfate.

5.4 Procedure:

- Complete bleaching and cleaning of tank. This needs to be done to each tank every 2 months.
- Remove fish from tank and put them into a clean tank. Tanks that are emptied of fish need to be cleaned and sterilized before another batch of fish can be introduced.
- Drain the tank and remove it from the rack. Clean air diffuser as instructed below.
 - Clean the tank and all parts thoroughly with a scrub pad, taking care not to damage the silicon water seals on the inside (algae should be left if very gentle rubbing will not remove it).
 - Squirt about 10 – 20 mls of bleach into the tank. Wash the bleach water thoroughly around the inside of the tank by hand using a pad or sponge exposing all inside portions of the tank to bleach.
 - Rinse the tank thoroughly with hot tap water. Rinse the tank with sodium thiosulfate, and then rinse it again with hot water. Put a few thiosulfate crystals into the tank and leave it.
 - Reassemble the tank and put it back on the rack. Fill with system water and allow water to recirculate for about 30 minutes before adding fish. Watch fish for 15min to look for any signs of distress.
 - Using a dry erase marker record date/time on the front of the tank when system water is turned back on.
- Initial the check list that you have completed the tank cleaning.

5.5 Air difuser cleaning:

- Remove dirty air diffusers from tanks and rinse with tap water to remove excess algae and debris.
- Place in 10% bleach solution for 15-30 minutes.
- Rinse the corner filters with hot water for 5 and then place into 3% sodium thiosulfate for 5 minutes.
- Rinse with hot water for 5 minutes.
- When cleaned air diffusers are placed back into aquaria, observe fish for 15 min for signs of distress.

Part IV

Daphnia Husbandry

6 Placeholder_Daphnia

6.1 xxx

XXXX

6.1.1 xxx

XXXXX

Part V

Bioinformatic

7 A field guide to base R

7.1 Introduction

To finish off the programming section, we're going to give you a quick tour of the most important base R functions that we don't otherwise discuss in the book. These tools are particularly useful as you do more programming and will help you read code you'll encounter in the wild.

This is a good place to remind you that the tidyverse is not the only way to solve data science problems. We teach the tidyverse in this book because tidyverse packages share a common design philosophy, increasing the consistency across functions, and making each new function or package a little easier to learn and use. It's not possible to use the tidyverse without using base R, so we've actually already taught you a **lot** of base R functions: from `library()` to load packages, to `sum()` and `mean()` for numeric summaries, to the factor, date, and POSIXct data types, and of course all the basic operators like `+`, `-`, `/`, `*`, `|`, `&`, and `!`. What we haven't focused on so far is base R workflows, so we will highlight a few of those in this chapter.

After you read this book, you'll learn other approaches to the same problems using base R, `data.table`, and other packages. You'll undoubtedly encounter these other approaches when you start reading R code written by others, particularly if you're using StackOverflow. It's 100% okay to write code that uses a mix of approaches, and don't let anyone tell you otherwise!

In this chapter, we'll focus on four big topics: subsetting with `[]`, subsetting with `[[` and `$`, the apply family of functions, and `for` loops. To finish off, we'll briefly discuss two essential plotting functions.

7.1.1 Prerequisites

This package focuses on base R so doesn't have any real prerequisites, but we'll load the tidyverse in order to explain some of the differences.

```
library(tidyverse)
```

7.2 Selecting multiple elements with `[]`

`[]` is used to extract sub-components from vectors and data frames, and is called like `x[i]` or `x[i, j]`. In this section, we'll introduce you to the power of `[]`, first showing you how you can use it with vectors, then how the same principles extend in a straightforward way to two-dimensional (2d) structures like data frames. We'll then help you cement that knowledge by showing how various dplyr verbs are special cases of `[]`.

7.2.1 Subsetting vectors

There are five main types of things that you can subset a vector with, i.e., that can be the `i` in `x[i]`:

1. **A vector of positive integers.** Subsetting with positive integers keeps the elements at those positions:

```
x <- c("one", "two", "three", "four", "five")
x[c(3, 2, 5)]
```

```
[1] "three" "two"   "five"
```

By repeating a position, you can actually make a longer output than input, making the term “subsetting” a bit of a misnomer.

```
x[c(1, 1, 5, 5, 5, 2)]
```

```
[1] "one"  "one"  "five" "five" "five" "two"
```

2. **A vector of negative integers.** Negative values drop the elements at the specified positions:

```
x[c(-1, -3, -5)]
```

```
[1] "two"  "four"
```

3. **A logical vector.** Subsetting with a logical vector keeps all values corresponding to a TRUE value. This is most often useful in conjunction with the comparison functions.

```
x <- c(10, 3, NA, 5, 8, 1, NA)

# All non-missing values of x
x[!is.na(x)]
```

```
[1] 10 3 5 8 1
```

```
# All even (or missing!) values of x
x[x %% 2 == 0]
```

```
[1] 10 NA 8 NA
```

Unlike `filter()`, NA indices will be included in the output as NAs.

4. **A character vector.** If you have a named vector, you can subset it with a character vector:

```
x <- c(abc = 1, def = 2, xyz = 5)
x[c("xyz", "def")]
```

```
xyz def
5 2
```

As with subsetting with positive integers, you can use a character vector to duplicate individual entries.

5. **Nothing.** The final type of subsetting is nothing, `x[]`, which returns the complete `x`. This is not useful for subsetting vectors, but as we'll see shortly, it is useful when subsetting 2d structures like tibbles.

7.3 Summary

In this chapter, we've shown you a selection of base R functions useful for subsetting and iteration. Compared to approaches discussed elsewhere in the book, these functions tend to have more of a “vector” flavor than a “data frame” flavor because base R functions tend to take individual vectors, rather than a data frame and some column specification. This often makes life easier for programming and so becomes more important as you write more functions and begin to write your own packages.

This chapter concludes the programming section of the book. You've made a solid start on your journey to becoming not just a data scientist who uses R, but a data scientist who can *program* in R. We hope these chapters have sparked your interest in programming and that you're looking forward to learning more outside of this book.

8 Summary

In summary, this book has no content whatsoever.

`1 + 1`

[1] 2

References

Knuth, Donald E. 1984. “Literate Programming.” *Comput. J.* 27 (2): 97–111. <https://doi.org/10.1093/comjnl/27.2.97>.

A Appendix 1

B

C Appendix 2

Hah Hah