Cresko Laboratory Procedures and Protocols

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Table of contents

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 section 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.

Part I

General Laboratory Protocols

1 Contact Information

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Part II

Molecular Protocols

2 cDNA basic

2.1 Introduction

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

2.2 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)

2.3 Solutions:

NONE

2.4 Procedure:

2.4.1 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 µl 10 mM dNTP mix
- Water (if necessary) to bring total to 10 µl

Heat to 65°C for 5 min., then ice

Collect contents at bottom of tube by brief centrifugation.

Add:

- 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)

Mix by gentle aspiration

- 25°C for 5 min.

2.4.2 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 µl RNase H (2 u/µl), 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 µl as template in a 20 µl reaction. Don't dilute your entire amount of cDNA, as some products may require a higher concentration of template.

3 2x Turbo

3.1 Introduction

- **Purpose**: This procedure describes how to create 2x Turbo PCR mix.
- **Procedure Type**: Molecular
- **Species**: N/A

3.2 Materials:

- 33,000 µl npH2O
- 2000 µl MgSO4 (100mM)
- 1600 µl 1M Tris-HCl (pH 8.6)
- 800 µl 1M KCl
- 800 µl 1M (NH4)2SO4
- 800 µl Triton-X 100 (10%)
- 400 µl DMSO (100 %)
- 120 µl dATP (100mM)
- 120 µl dGTP (100mM)
- 120 µl dTTP (100mM)
- 120 µl dCTP (100mM)
- 80 µl 100mg/ml BSA

Total = 40 ml of buffer

3.3 Solutions:

NONE

3.4 Procedure:

- Mix above reagents together
- Place in 1.5 ml ependorph tubes
- Store at -20C

Part III

Vertebrate Husbandry

4 Twenty Gallon Aquarium Cleaning

4.1 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

4.2 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).

4.3 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.

4.4 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.

4.5 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

5 Placeholder_Daphnia

5.1 xxx

xxxx

5.1.1 xxx

xxxxx

Part V

Bioinformatic

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

6 A field guide to base R

6.1 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.

6.1.1 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)
```

6.2 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 `[`.

6.2.1 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 `NA`s.

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.

6.3 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.

7 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 - Sbf1 Barcodes in 96 Well Plate

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
A1	AAACGG	SbfI-AAACGG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAACGG	1	SbfI-AAACGG-bot	/5Phos/ACCGTTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A2	AACGTT	SbfI-AACGTT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACGTT	2	SbfI-AACGTT-bot	/5Phos/AACGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A3	AACTGA	SbfI-AACTGA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACTGA	3	SbfI-AACTGA-bot	/5Phos/TCAGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A4	AAGACG	SbfI-AAGACG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGACG	4	SbfI-AAGACG-bot	/5Phos/CGTCTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A5	AAGCTA	SbfI-AAGCTA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGCTA	5	SbfI-AAGCTA-bot	/5Phos/TAGCTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A6	AATATC	SbfI-AATATC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATATC	6	SbfI-AATATC-bot	/5Phos/GATATTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A7	AATGAG	SbfI-AATGAG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATGAG	7	SbfI-AATGAG-bot	/5Phos/CTCATTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A8	ACAAGA	SbfI-ACAAGA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAAGA	8	SbfI-ACAAGA-bot	/5Phos/TCTTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A9	ACAGCG	SbfI-ACAGCG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGCG	9	SbfI-ACAGCG-bot	/5Phos/CGCTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A10	ACATAC	SbfI-ACATAC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACATAC	10	SbfI-ACATAC-bot	/5Phos/GTATGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
A11	ACCATG	SbfI-ACCATG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-ACCATG-bot	/5Phos/ACATGGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
A12	ACCCCC	SbfI-ACCCCC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-ACCCCC-bot	/5Phos/AGGGGGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B1	ACTCTT	SbfI-ACTCTT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-ACTCTT-bot	/5Phos/AAGAGTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B2	ACTGGC	SbfI-ACTGGC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-ACTGGC-bot	/5Phos/AGCCAGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B3	AGCCAT	SbfI-AGCCAT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-AGCCAT-bot	/5Phos/ATGGCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B4	AGCGCA	SbfI-AGCGCA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-AGCGCA-bot	/5Phos/TGCGCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B5	AGGGTC	SbfI-AGGGTC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-AGGGTC-bot	/5Phos/GACCCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B6	AGGTGT	SbfI-AGGTGT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-AGGTGT-bot	/5Phos/ACACCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B7	AGTAGG	SbfI-AGTAGG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-AGTAGG-bot	/5Phos/CCTACTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B8	AGTTAA	SbfI-AGTTAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-AGTTAA-bot	/5Phos/TTAACTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B9	ATAGTA	SbfI-ATAGTA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-ATAGTA-bot	/5Phos/TACTATGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B10	ATCAAA	SbfI-ATCAAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-ATCAAA-bot	/5Phos/TTTGATGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	
B11	ATGCAC	SbfI-ATGCAC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	5PG6-ATGCAC-bot	/5Phos/GTGCATGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA	

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
B12	ATGTTG	SbfI-ATGTTG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	B12	SbfI-ATGTTG-bot	/5Phos/ACAACATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C1	ATTCCG	SbfI-ATTCCG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C1	SbfI-ATTCCG-bot	/5Phos/ACGGAATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C2	CAAAAA	SbfI-CAAAAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C2	SbfI-CAAAAA-bot	/5Phos/ATTTTTGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C3	CAATCG	SbfI-CAATCG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C3	SbfI-CAATCG-bot	/5Phos/ACGATTGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C4	CACCTC	SbfI-CACCTC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C4	SbfI-CACCTC-bot	/5Phos/AGGTGATGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C5	CAGGCA	SbfI-CAGGCA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C5	SbfI-CAGGCA-bot	/5Phos/TGCCTGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C6	CATACT	SbfI-CATACT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C6	SbfI-CATACT-bot	/5Phos/AGTATGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C7	CCATTT	SbfI-CCATTT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C7	SbfI-CCATTT-bot	/5Phos/AAATGGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C8	CCCGGT	SbfI-CCCGGT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C8	SbfI-CCCGGT-bot	/5Phos/ACCGGGGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C9	CCCTAA	SbfI-CCCTAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C9	SbfI-CCCTAA-bot	/5Phos/TTAGGGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C10	CCGAGG	SbfI-CCGAGG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C10	SbfI-CCGAGG-bot	/5Phos/ACCTCGGAGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C11	CCGCAT	SbfI-CCGCAT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C11	SbfI-CCGCAT-bot	/5Phos/ATGCCGGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC
C12	CCTAAC	SbfI-CCTAAC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	C12	SbfI-CCTAAC-bot	/5Phos/GTTAGGATCGATGCGGAAGAGCACACGTCTGAACTCCAGTCAC

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
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D2	CGCAGA	SbfI-CGCAGA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	D2	SbfI-CGCAGA-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTCTGCGAGATGCGCAG
D3	CGCGTG	SbfI-CGCGTG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	D3	SbfI-CGCGTG-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTCACGCGAGATCGCAG
D4	CGGTCC	SbfI-CGGTCC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	D4	SbfI-CGGTCC-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTCGGACCGAGATGCAG
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D8	CTCGCC	SbfI-CTCGCC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	D8	SbfI-CTCGCC-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTCGGCGAGATGCGCAG
D9	CTGCGA	SbfI-CTGCGA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	D9	SbfI-CTGCGA-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTCGCAGGATGCGCAG
D10	CTGGTT	SbfI-CTGGTT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	D10	SbfI-CTGGTT-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTAACCAGGATCGCAG
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E1	GAAATG	SbfI-GAAATG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	E1	SbfI-GAAATG-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTCATTTCCGATCGCAG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
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E3	GACGAC	SbfI-GACGAC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	E3	SbfI-GACGAC-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTACCTGCAGG
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E9	GCCGTA	SbfI-GCCGTA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	E9	SbfI-GCCGTA-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTACCTGCAGG
E10	GCGACC	SbfI-GCGACC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	E10	SbfI-GCGACC-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTACCTGCAGG
E11	GCGCTG	SbfI-GCGCTG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	E11	SbfI-GCGCTG-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTACCTGCAGG
E12	GCTCAA	SbfI-GCTCAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	E12	SbfI-GCTCAA-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTACCTGCAGG
F1	GGACTT	SbfI-GGACTT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F1	SbfI-GGACTT-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTACCTGCAGG
F2	GGCAAG	SbfI-GGCAAG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F2	SbfI-GGCAAG-bot	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTGGTACCTGCAGG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
F3	GGGCGC	SbfI-GGGCGC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F3	SbfI-GGGCGC-bot	/5Phos/ACGCCCGCGTGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F4	GGGGCG	SbfI-GGGGCG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F4	SbfI-GGGGCG-bot	/5Phos/ACGCCGCGTGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F5	GGTACA	SbfI-GGTACA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F5	SbfI-GGTACA-bot	/5Phos/ATGTACCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F6	GGTTTG	SbfI-GGTTTG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F6	SbfI-GGTTTG-bot	/5Phos/ACAAACCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F7	GTAAGT	SbfI-GTAAGT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F7	SbfI-GTAAGT-bot	/5Phos/ACTTACCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F8	GTATCC	SbfI-GTATCC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F8	SbfI-GTATCC-bot	/5Phos/AGGATACCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F9	GTCATC	SbfI-GTCATC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F9	SbfI-GTCATC-bot	/5Phos/ATGATCCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F10	GTGCCT	SbfI-GTGCCT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F10	SbfI-GTGCCT-bot	/5Phos/AGGCACCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F11	GTGTAA	SbfI-GTGTAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F11	SbfI-GTGTAA-bot	/5Phos/ATTACACTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
F12	GTTGGA	SbfI-GTTGGA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	F12	SbfI-GTTGGA-bot	/5Phos/ATCCAACTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
G1	TAAGCT	SbfI-TAAGCT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	G1	SbfI-TAAGCT-bot	/5Phos/AGCTTAGCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
G2	TAATTC	SbfI-TAATTC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	G2	SbfI-TAATTC-bot	/5Phos/AGAATTAGCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
G3	TACACA	SbfI-TACACA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	G3	SbfI-TACACA-bot	/5Phos/ATGTGTAGCTGCATGCAGGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
G4	TACGGG	SbfI-TACGGG-top	ACACTCTTTC[illegible]TACACG[illegible]	G4	SbfI-TACGGG-bot	/5Phos/[illegible]
G5	TAGTAT	SbfI-TAGTAT-top	ACACTCTTTC[illegible]TACACG[illegible]	G5	SbfI-TAGTAT-bot	/5Phos/[illegible]
G6	TATCAC	SbfI-TATCAC-top	ACACTCTTTC[illegible]TACACG[illegible]	G6	SbfI-TATCAC-bot	/5Phos/[illegible]
G7	TCAAAG	SbfI-TCAAAG-top	ACACTCTTTC[illegible]TACACG[illegible]	G7	SbfI-TCAAAG-bot	/5Phos/[illegible]
G8	TCCTGC	SbfI-TCCTGC-top	ACACTCTTTC[illegible]TACACG[illegible]	G8	SbfI-TCCTGC-bot	/5Phos/[illegible]
G9	TCGATT	SbfI-TCGATT-top	ACACTCTTTC[illegible]TACACG[illegible]	G9	SbfI-TCGATT-bot	/5Phos/[illegible]
G10	TCGCCA	SbfI-TCGCCA-top	ACACTCTTTC[illegible]TACACG[illegible]	G10	SbfI-TCGCCA-bot	/5Phos/[illegible]
G11	TCGGAC	SbfI-TCGGAC-top	ACACTCTTTC[illegible]TACACG[illegible]	G11	SbfI-TCGGAC-bot	/5Phos/[illegible]
G12	TCTCGG	SbfI-TCTCGG-top	ACACTCTTTC[illegible]TACACG[illegible]	G12	SbfI-TCTCGG-bot	/5Phos/[illegible]
H1	TCTTCT	SbfI-TCTTCT-top	ACACTCTTTC[illegible]TACACG[illegible]	H1	SbfI-TCTTCT-bot	/5Phos/[illegible]
H2	TGAACC	SbfI-TGAACC-top	ACACTCTTTC[illegible]TACACG[illegible]	H2	SbfI-TGAACC-bot	/5Phos/[illegible]
H3	TGACAA	SbfI-TGACAA-top	ACACTCTTTC[illegible]TACACG[illegible]	H3	SbfI-TGACAA-bot	/5Phos/[illegible]
H4	TGCCCG	SbfI-TGCCCG-top	ACACTCTTTC[illegible]TACACG[illegible]	H4	SbfI-TGCCCG-bot	/5Phos/[illegible]

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
H5	TGCTTA	SbfI-TGCTTA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H5	SbfI-TGCTTA-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTGCTTAATCGGTGTAGGGAAAGAGTGTGCA
H6	TGGGGA	SbfI-TGGGGA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H6	SbfI-TGGGGA-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTCCCCATCTCGTGTAGGGAAAGAGTGTGCA
H7	TTATGA	SbfI-TTATGA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H7	SbfI-TTATGA-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTTATAATCTCGTGTAGGGAAAGAGTGTGCA
H8	TTCCGT	SbfI-TTCCGT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H8	SbfI-TTCCGT-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTTCCGATCTCGTGTAGGGAAAGAGTGTGCA
H9	TTCTAG	SbfI-TTCTAG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H9	SbfI-TTCTAG-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTTCTAGATCGTGTAGGGAAAGAGTGTGCA
H10	TTGAGC	SbfI-TTGAGC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H10	SbfI-TTGAGC-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTTGAGCATCGTGTAGGGAAAGAGTGTGCA
H11	TTTAAT	SbfI-TTTAAT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H11	SbfI-TTTAAT-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTTTAATATCGTGTAGGGAAAGAGTGTGCA
H12	TTTGTC	SbfI-TTTGTC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	H12	SbfI-TTTGTC-bot	/5Phos/ATCGGAAGAGCACGTCTGAACTCCAGTCACTTTGTCATCGTGTAGGGAAAGAGTGTGCA

B Appendix 2

Hah Hah