Cresko Laboratory Procedures and Protocols

Cresko Laboratory

Wednesday, December 6, 2023

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

This section of the book contains general protocols for working in the laboratory.

1 Contact Information

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Part II

Molecular Protocols

This section of the book contains common protocols used for molecular biology and genomics in the laboratory. These include standard protocols such as setting up creating reagents, setting up PCRs and running gels, as well as advanced protocols such as creating constructs.

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:

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.

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

4 Paraformaldehyde

4.1 Introduction

- **Purpose**: This procedure describes how to make 8% paraformaldehyde. This protocol is the one I have used and makes use of pH to get the PFA into solution relatively quickly - then you readjust the pH. It's for 8% - then you can add 1:1 2x PBS.
- **Procedure Type**: Molecular
- **Species**: N/A

4.2 Materials:

- xxx

Total = 40 ml of buffer

4.3 Solutions:

NONE

4.4 Procedure:

> ⚠ HUMAN HEALTH WARNGING
>
> Paraformaldehyde can be hazardous to your health - make sure you prepare in the fume hood.

- Add 40 g Paraformaldehyde to 450 ml distilled water (or scale for desired final volume).
- Add 1 ul of 10 N NaOH per ml of water (i.e. 500 ul for 500 ml).
- Apply medium heat while stirring at medium speed to dissolve - approx 15-20 min.
- Solution should not go above 60º C.

- Eventually, granules will fully dissolve and the solution will become translucent.

> **DO NOT LET THE SOLUTION STIR BEYOND THIS POINT**
>
> It will form a fuzzy precipitate that reduces the solution strength after filtering.

- Once the granules have dissolved and the solution clears, turn off the heat and equilibrate to pH 7.4 with approx 1.5 ml of 20% HCl (or scale, depending on target volume).
- Bring volume to 500 ml (or scaled volume) with distilled water.
- Filter while still warm to 0.45 um (or 0.2 um). Aliquot and store at -20^{o} C.

Part III

Bioinformatic

This ection of the book contains protocols for basic bioinformatic skills such as using our laboratory cluster 'Genome', as well as our account Nereus on the UO supercomputer Talapas.

Note that there are several appendices that contain greater details and training on things such as the use of command line, R and Python, markdown and literature programming, and documentation using Quarto and Jupyter notebooks.

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

5 Placeholder_Molecular

5.1 xxx

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xxxxx

Part IV

Vertebrate Husbandry

This section of the manual contains protocols for the safe and ethical husbandry and use of vertebrate animals, particular the fish models stickleback, zebrafish and syngnathids.

6 Twenty Gallon Aquarium Cleaning

6.1 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

6.2 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).

6.3 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.

6.4 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.

6.5 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 V

Daphnia Husbandry

This section of the manual contains protocols for the safe and ethical husbandry and use of invertebrate animals, particular the nematode worm *C. remanei* and water fleas of the genus *Daphnia*

7 Placeholder_Daphnia

7.1 xxx

xxxx

xxx

xxxxx

Part VI

Bioinformatic

This ection of the book contains protocols for basic bioinformatic skills such as using our laboratory cluster 'Genome', as well as our account Nereus on the UO supercomputer Talapas.

Note that there are several appendices that contain greater details and training on things such as the use of command line, R and Python, markdown and literature programming, and documentation using Quarto and Jupyter notebooks.

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

8 A field guide to base R

8.1 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.

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)
```

8.2 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 `[`.

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.

8.3 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.

9 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 Sbf1 Barcodes in 96 Well Plate

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
A1	AAACGG	SbfI-AAACGG-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTAAACGG	A1	SbfI-AAACGG-bot	/5Phos/CCGTTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A2	AACGTT	SbfI-AACGTT-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTAACGTT	A2	SbfI-AACGTT-bot	/5Phos/AACGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A3	AACTGA	SbfI-AACTGA-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTAACTGA	A3	SbfI-AACTGA-bot	/5Phos/TCAGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A4	AAGACG	SbfI-AAGACG-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTAAGACG	A4	SbfI-AAGACG-bot	/5Phos/CGTCTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A5	AAGCTA	SbfI-AAGCTA-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTAAGCTA	A5	SbfI-AAGCTA-bot	/5Phos/TAGCTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A6	AATATC	SbfI-AATATC-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTAATATC	A6	SbfI-AATATC-bot	/5Phos/GATATTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A7	AATGAG	SbfI-AATGAG-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTAATGAG	A7	SbfI-AATGAG-bot	/5Phos/CTCATTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A8	ACAAGA	SbfI-ACAAGA-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTACAAGA	A8	SbfI-ACAAGA-bot	/5Phos/TCTTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A9	ACAGCG	SbfI-ACAGCG-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTACAGCG	A9	SbfI-ACAGCG-bot	/5Phos/CGCTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A10	ACATAC	SbfI-ACATAC-top	ACACTCTTTACCTACACGACGCTCTTCCGATCTACATAC	A10	SbfI-ACATAC-bot	/5Phos/GTATGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
A11	ACCATG	SbfI-ACCATG-top	ACACTCTTTACCCTACACGACGCTCTTCCGATCTNNNNNNACCATG	A11	SbfI-ACCATG-bot	/5Phos/ATGGTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
A12	ACCCCC	SbfI-ACCCCC-top	ACACTCTTTACCCTACACGACGCTCTTCCGATCTNNNNNNACCCCC	A12	SbfI-ACCCCC-bot	/5Phos/GGGGTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B1	ACTCTT	SbfI-ACTCTT-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNACTCTT	B1	SbfI-ACTCTT-bot	/5Phos/AAGAGTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B2	ACTGGC	SbfI-ACTGGC-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNACTGGC	B2	SbfI-ACTGGC-bot	/5Phos/GCCAGTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B3	AGCCAT	SbfI-AGCCAT-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNAGCCAT	B3	SbfI-AGCCAT-bot	/5Phos/ATGGCTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
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B5	AGGGTC	SbfI-AGGGTC-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNAGGGTC	B5	SbfI-AGGGTC-bot	/5Phos/GACCCTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B6	AGGTGT	SbfI-AGGTGT-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNAGGTGT	B6	SbfI-AGGTGT-bot	/5Phos/ACACCTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B7	AGTAGG	SbfI-AGTAGG-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNAGTAGG	B7	SbfI-AGTAGG-bot	/5Phos/CCTACTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B8	AGTTAA	SbfI-AGTTAA-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNAGTTAA	B8	SbfI-AGTTAA-bot	/5Phos/TTAACTNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B9	ATAGTA	SbfI-ATAGTA-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNATAGTA	B9	SbfI-ATAGTA-bot	/5Phos/TACTATNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
B10	ATCAAA	SbfI-ATCAAA-top	ACACTCTTTBCCCTACACGACGCTCTTCCGATCTNNNNNNATCAAA	B10	SbfI-ATCAAA-bot	/5Phos/TTTGATNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
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C1	ATTCCG	SbfI-ATTCCG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNCGGAATCTCGTATGCCGTCTTCTGCTTG	C1	SbfI-ATTCCG-bot	/5Phos/NNNNNNCGGAATCTCGTATGCCGTCTTCTGCTTG
C2	CAAAAA	SbfI-CAAAAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNTTTTTGCTCGTATGCCGTCTTCTGCTTG	C2	SbfI-CAAAAA-bot	/5Phos/NNNNNNTTTTTGCTCGTATGCCGTCTTCTGCTTG
C3	CAATCG	SbfI-CAATCG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNCGATTGCTCGTATGCCGTCTTCTGCTTG	C3	SbfI-CAATCG-bot	/5Phos/NNNNNNCGATTGCTCGTATGCCGTCTTCTGCTTG
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C5	CAGGCA	SbfI-CAGGCA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNTGCCTGCTCGTATGCCGTCTTCTGCTTG	C5	SbfI-CAGGCA-bot	/5Phos/NNNNNNTGCCTGCTCGTATGCCGTCTTCTGCTTG
C6	CATACT	SbfI-CATACT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNAGTATGCTCGTATGCCGTCTTCTGCTTG	C6	SbfI-CATACT-bot	/5Phos/NNNNNNAGTATGCTCGTATGCCGTCTTCTGCTTG
C7	CCATTT	SbfI-CCATTT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNAAATGGCTCGTATGCCGTCTTCTGCTTG	C7	SbfI-CCATTT-bot	/5Phos/NNNNNNAAATGGCTCGTATGCCGTCTTCTGCTTG
C8	CCCGGT	SbfI-CCCGGT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNACCGGGCTCGTATGCCGTCTTCTGCTTG	C8	SbfI-CCCGGT-bot	/5Phos/NNNNNNACCGGGCTCGTATGCCGTCTTCTGCTTG
C9	CCCTAA	SbfI-CCCTAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNTTAGGGCTCGTATGCCGTCTTCTGCTTG	C9	SbfI-CCCTAA-bot	/5Phos/NNNNNNTTAGGGCTCGTATGCCGTCTTCTGCTTG
C10	CCGAGG	SbfI-CCGAGG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNCCTCGGCTCGTATGCCGTCTTCTGCTTG	C10	SbfI-CCGAGG-bot	/5Phos/NNNNNNCCTCGGCTCGTATGCCGTCTTCTGCTTG
C11	CCGCAT	SbfI-CCGCAT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNATGCGGCTCGTATGCCGTCTTCTGCTTG	C11	SbfI-CCGCAT-bot	/5Phos/NNNNNNATGCGGCTCGTATGCCGTCTTCTGCTTG
C12	CCTAAC	SbfI-CCTAAC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGTTAGGCTCGTATGCCGTCTTCTGCTTG	C12	SbfI-CCTAAC-bot	/5Phos/NNNNNNGTTAGGCTCGTATGCCGTCTTCTGCTTG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
D1	CGAGGC	SbfI-CGAGGC-top	ACACTCTTTCCCTACACGAC	D1	SbfI-CGAGGC-bot	/5Phos/GTCTTCGGACATCG
D2	CGCAGA	SbfI-CGCAGA-top	ACACTCTTTCCCTACACGAC	D2	SbfI-CGCAGA-bot	/5Phos/ATCTGCGGAGATCG
D3	CGCGTG	SbfI-CGCGTG-top	ACACTCTTTCCCTACACGAC	D3	SbfI-CGCGTG-bot	/5Phos/ACTGCGCGATCG
D4	CGGTCC	SbfI-CGGTCC-top	ACACTCTTTCCCTACACGAC	D4	SbfI-CGGTCC-bot	/5Phos/AGGACCGGATCG
D5	CGTCTA	SbfI-CGTCTA-top	ACACTCTTTCCCTACACGAC	D5	SbfI-CGTCTA-bot	/5Phos/ATAGACGTATCG
D6	CGTGAT	SbfI-CGTGAT-top	ACACTCTTTCCCTACACGAC	D6	SbfI-CGTGAT-bot	/5Phos/ATCACGGAATCG
D7	CTACAG	SbfI-CTACAG-top	ACACTCTTTCCCTACACGAC	D7	SbfI-CTACAG-bot	/5Phos/CTGTAGGATCG
D8	CTCGCC	SbfI-CTCGCC-top	ACACTCTTTCCCTACACGAC	D8	SbfI-CTCGCC-bot	/5Phos/AGGCGAGATCG
D9	CTGCGA	SbfI-CTGCGA-top	ACACTCTTTCCCTACACGAC	D9	SbfI-CTGCGA-bot	/5Phos/ATCGCAGATCG
D10	CTGGTT	SbfI-CTGGTT-top	ACACTCTTTCCCTACACGAC	D10	SbfI-CTGGTT-bot	/5Phos/AACCAGGATCG
D11	CTTATG	SbfI-CTTATG-top	ACACTCTTTCCCTACACGAC	D11	SbfI-CTTATG-bot	/5Phos/ATACATGATCG
D12	CTTTGC	SbfI-CTTTGC-top	ACACTCTTTCCCTACACGAC	D12	SbfI-CTTTGC-bot	/5Phos/AGCAAAGATCG
E1	GAAATG	SbfI-GAAATG-top	ACACTCTTTCCCTACACGAC	E1	SbfI-GAAATG-bot	/5Phos/ACATTTCAATCG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
E2	GAACCA	SbfI-GAACCA-top	ACACTCTTTCCCTACACGAC	E2	SbfI-GAACCA-bot	/5Phos/ATGTTACCGATGG
E3	GACGAC	SbfI-GACGAC-top	ACACTCTTTCCCTACACGAC	E3	SbfI-GACGAC-bot	/5Phos/GTCGTACGAATGG
E4	GACTCT	SbfI-GACTCT-top	ACACTCTTTCCCTACACGAC	E4	SbfI-GACTCT-bot	/5Phos/AGAGTACGATGG
E5	GAGAGA	SbfI-GAGAGA-top	ACACTCTTTCCCTACACGAC	E5	SbfI-GAGAGA-bot	/5Phos/TCTCTACGAATGG
E6	GATCGT	SbfI-GATCGT-top	ACACTCTTTCCCTACACGAC	E6	SbfI-GATCGT-bot	/5Phos/ACGATACGATGG
E7	GCAGAT	SbfI-GCAGAT-top	ACACTCTTTCCCTACACGAC	E7	SbfI-GCAGAT-bot	/5Phos/ATCTGCGAATGG
E8	GCATGG	SbfI-GCATGG-top	ACACTCTTTCCCTACACGAC	E8	SbfI-GCATGG-bot	/5Phos/CCATGCGAATGG
E9	GCCGTA	SbfI-GCCGTA-top	ACACTCTTTCCCTACACGAC	E9	SbfI-GCCGTA-bot	/5Phos/TACGGCGATGG
E10	GCGACC	SbfI-GCGACC-top	ACACTCTTTCCCTACACGAC	E10	SbfI-GCGACC-bot	/5Phos/GGTCGCGATGG
E11	GCGCTG	SbfI-GCGCTG-top	ACACTCTTTCCCTACACGAC	E11	SbfI-GCGCTG-bot	/5Phos/CAGCGCGATGG
E12	GCTCAA	SbfI-GCTCAA-top	ACACTCTTTCCCTACACGAC	E12	SbfI-GCTCAA-bot	/5Phos/TTGAGCGATGG
F1	GGACTT	SbfI-GGACTT-top	ACACTCTTTCCCTACACGAC	F1	SbfI-GGACTT-bot	/5Phos/AAGTCCGATGG
F2	GGCAAG	SbfI-GGCAAG-top	ACACTCTTTCCCTACACGAC	F2	SbfI-GGCAAG-bot	/5Phos/CTTGCCGATGG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
F3	GGGCGC	SbfI-GGGCGC-top	ACACTCTTTCCCTACACGAC	F3	SbfI-GGGCGC-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F4	GGGGCG	SbfI-GGGGCG-top	ACACTCTTTCCCTACACGAC	F4	SbfI-GGGGCG-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F5	GGTACA	SbfI-GGTACA-top	ACACTCTTTCCCTACACGAC	F5	SbfI-GGTACA-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F6	GGTTTG	SbfI-GGTTTG-top	ACACTCTTTCCCTACACGAC	F6	SbfI-GGTTTG-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F7	GTAAGT	SbfI-GTAAGT-top	ACACTCTTTCCCTACACGAC	F7	SbfI-GTAAGT-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F8	GTATCC	SbfI-GTATCC-top	ACACTCTTTCCCTACACGAC	F8	SbfI-GTATCC-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F9	GTCATC	SbfI-GTCATC-top	ACACTCTTTCCCTACACGAC	F9	SbfI-GTCATC-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F10	GTGCCT	SbfI-GTGCCT-top	ACACTCTTTCCCTACACGAC	F10	SbfI-GTGCCT-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F11	GTGTAA	SbfI-GTGTAA-top	ACACTCTTTCCCTACACGAC	F11	SbfI-GTGTAA-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
F12	GTTGGA	SbfI-GTTGGA-top	ACACTCTTTCCCTACACGAC	F12	SbfI-GTTGGA-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
G1	TAAGCT	SbfI-TAAGCT-top	ACACTCTTTCCCTACACGAC	G1	SbfI-TAAGCT-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
G2	TAATTC	SbfI-TAATTC-top	ACACTCTTTCCCTACACGAC	G2	SbfI-TAATTC-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
G3	TACACA	SbfI-TACACA-top	ACACTCTTTCCCTACACGAC	G3	SbfI-TACACA-bot	/5Phos/ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
G4	TACGGG	SbfI-TACGGG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACGGG	G4	SbfI-TACGGG-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
G5	TAGTAT	SbfI-TAGTAT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGTAT	G5	SbfI-TAGTAT-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTATACTAAAGAGTGT
G6	TATCAC	SbfI-TATCAC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATCAC	G6	SbfI-TATCAC-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTAGTGATAAGAGTGT
G7	TCAAAG	SbfI-TCAAAG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCAAAG	G7	SbfI-TCAAAG-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTACTTTGAAAGAGTGT
G8	TCCTGC	SbfI-TCCTGC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCTGC	G8	SbfI-TCCTGC-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTAGCAGGAAAGAGTGT
G9	TCGATT	SbfI-TCGATT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGATT	G9	SbfI-TCGATT-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTAATCGAAAGAGTGT
G10	TCGCCA	SbfI-TCGCCA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGCCA	G10	SbfI-TCGCCA-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTATGGCGAAGAGTGT
G11	TCGGAC	SbfI-TCGGAC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGGAC	G11	SbfI-TCGGAC-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTAGTCCGAAAGAGTGT
G12	TCTCGG	SbfI-TCTCGG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCGG	G12	SbfI-TCTCGG-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTACCGAGAAAGAGTGT
H1	TCTTCT	SbfI-TCTTCT-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTCT	H1	SbfI-TCTTCT-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTAAGAAAAGAGTGT
H2	TGAACC	SbfI-TGAACC-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAACC	H2	SbfI-TGAACC-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTAGGTTCAAAGAGTGT
H3	TGACAA	SbfI-TGACAA-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACAA	H3	SbfI-TGACAA-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTATTGTCAAAGAGTGT
H4	TGCCCG	SbfI-TGCCCG-top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCCCG	H4	SbfI-TGCCCG-bot	/5Phos/ACTCTAAGAGATCGGAAGAGCGTCGTGTACGGGCAAAGAGTGT

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
H5	TGCTTA	SbfI-TGCTTA-top	ACACTCTTTCCCTACACGAC	H5	SbfI-TGCTTA-bot	/5Phos/ATCGGAAGAGCACACGTCTGAACTCCAGTCACTGCATATCGG
H6	TGGGGA	SbfI-TGGGGA-top	ACACTCTTTCCCTACACGAC	H6	SbfI-TGGGGA-bot	/5Phos/ATCTCGTATGCCGTCTTCTGCTTGAAAATACTCCCCATCCG
H7	TTATGA	SbfI-TTATGA-top	ACACTCTTTCCCTACACGAC	H7	SbfI-TTATGA-bot	/5Phos/ATCTCGTATGCCGTCTTCTGCTTGAAAATACTTATGATCG
H8	TTCCGT	SbfI-TTCCGT-top	ACACTCTTTCCCTACACGAC	H8	SbfI-TTCCGT-bot	/5Phos/ATCTCGTATGCCGTCTTCTGCTTGAAAATACTTCCGTATGG
H9	TTCTAG	SbfI-TTCTAG-top	ACACTCTTTCCCTACACGAC	H9	SbfI-TTCTAG-bot	/5Phos/ATCTCGTATGCCGTCTTCTGCTTGAAAATACTTCTAGATCG
H10	TTGAGC	SbfI-TTGAGC-top	ACACTCTTTCCCTACACGAC	H10	SbfI-TTGAGC-bot	/5Phos/ATCTCGTATGCCGTCTTCTGCTTGAAAATACTTGAGCATCG
H11	TTTAAT	SbfI-TTTAAT-top	ACACTCTTTCCCTACACGAC	H11	SbfI-TTTAAT-bot	/5Phos/ATCTCGTATGCCGTCTTCTGCTTGAAAATACTTTAATATCG
H12	TTTGTC	SbfI-TTTGTC-top	ACACTCTTTCCCTACACGAC	H12	SbfI-TTTGTC-bot	/5Phos/ATCTCGTATGCCGTCTTCTGCTTGAAAATACTTTGTCATCG

B Appendix 2

Hah Hah